Increases in whole brain grey matter associated with long-term Sahaja Yoga Meditation: a detailed area by area description

Sergio Elías Hernández[1*], Roberto Dorta[2], José Suero[3], Alfonso Barros-Loscertales[4], José Luis González-Mora[5], Katya Rubia[6]

[1] Department of Ingeniería Industrial, Universidad de La Laguna, Tenerife, Spain.

[2] Department of Matemáticas, Estadística e Investigación Operativa, Universidad de La Laguna, Tenerife, Spain.

[3] Centro de Salud Jazmín, Sermas, Madrid, Spain.

[4]Department of Psicología Básica, Clínica y Psicobiología, Universitat Jaume I, Castellón, Spain

[5] Department of Fisiología, Universidad de La Laguna, Tenerife, Spain.

[6] Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom

* Corresponding author

E-mail: sehdez@ull.edu.es

Abstract

Objectives: Our previous study showed that long-term practitioners of Sahaja Yoga Meditation (SYM) had around 7% larger grey matter volume (GMV) in the whole brain compared with healthy controls; however, when testing individual regions, only 5 small brain areas were statistically different between groups. Under the hypothesis that those results were statistically conservative, with the same dataset, we investigated in more detail the regional differences in GMV associated with the practice of SYM, with a different statistical approach.

Design: Twenty-three experienced practitioners of SYM and 23 healthy non-meditators matched on age, gender and education level, were scanned using structural Magnetic Resonance Imaging. Their GMV were extracted and compared using Voxel-Based Morphometry. Using a novel ad-hoc GLM model, statistical comparisons were made to observe if the GMV differences between meditators and controls were statistically significant.

Results: In the 16 lobe area subdivisions, GMV was statistically significantly different in 4 out of 16 areas: Right hemispheric temporal and frontal lobes, left frontal lobe and brainstem.

In the 116 AAL area subdivisions, GMV difference was statistically significant in 11 areas. The GMV differences were statistically more significant in right hemispheric brain areas.

Conclusions: The study shows that long-term practice of SYM is associated with larger GMV overall, and with significant differences mainly in temporal and frontal areas of the right hemisphere and the brainstem. These neuroplastic changes may reflect emotional and attentional control mechanisms developed with SYM. On the other hand, our statistical ad-hoc method shows that there were more brain areas with statistical significance compared to the traditional methodology which we think is susceptible to conservative Type II errors.

Introduction

Meditation is a general term that includes a large variety of practices that mainly focus on the inner observation of the body and the mind. The western goal of most meditation techniques is to achieve an improved control of attention and emotions in order to live a more balanced, stress-free and healthier life. On the other hand, yoga includes many different techniques among which meditation (dhayana in classical yoga) has a main role. If we travel back to the origins of yoga, the first known treaty “The yoga sutras of Patanjali” mentions that “Yoga is the suppression of the modifications of the mind” [1, 2]. In ancient yoga, a higher state of consciousness called Nirvichara Samadhi was described, in today’s words Nirvichara could be translated as “mental silence” or “thoughtless awareness”. In this state, the mind has none thoughts and there is inner calm in a state of inner pure joy and the attention is focused on each present moment. Sahaja Yoga Meditation (SYM) shares the goals of Patanjali’s Yoga Sutras to achieve the state of Nirvichara or mental silence.

SYM, presumably through the regular achievement of the state of mental silence, has shown health benefits in disorders that are often associated with recurrent or repetitive negative thoughts, such as: depression, stress, anxiety, and attention-deficit/hyperactivity disorder [2-7]. Other studies on SYM has shown beneficial effects in treating physiological and neurological diseases such as asthma [8], high blood pressure [6], menopause [9] and epilepsy [10-12], for a meta-analysis see [8]. Furthermore, the frequency with which the practitioners perceive the state of mental silence has been shown to be associated with better physical and mental health [13].

Neuroplasticity is one of the most commonly used terms in today’s neuroscience to express the capacity of our human brain to change permanently. One of the key insights over the past 2 decades of neuroimaging research has been that the human brain, even in adulthood, is not static, but on the contrary is a dynamic system that has the ability to shape itself. One of the key fascinating questions that researchers try to answer is hence: how can we improve our brain structure and function? One potential non-pharmacological way to shape our brain could be through meditation [14].

Neuroplasticity can be measured by changes in grey matter volume (GMV). Many studies have shown that brain areas that are more utilized through practice of a particular skill for example, in music [15] , or high performance sports [16, 17], can become enlarged. It has even been shown that relatively short periods of training of a particular skill, such as 3 months of training to juggle or 3 months of studying for an exam in

students can lead to transient changes in the relevant brain areas such as visual-spatial perception regions for juggling [18, 19] or the hippocampus and parietal lobe for memory storage in medical students preparing for an exam [19, 20].

Voxel Based morphometry VBM is the most used automated technique to measure GMV by means of MRI scans. In most cases researchers follow the steps provided by the VBM authors of the technique [21-24]. VBM has evolved [21] and the different steps like segmentation and normalization has been improved within each new software version [24, 25].

In most cases, the statistical path followed to compare GMV mean differences between groups has been throughout ANCOVAs, were typically total intracranial volumes (TIV), gender and age are treated as nuisance covariates. This statistical method is based on random field theory [21, 26].Another important point to consider is that structural images display local variation in smoothness, which implies that cluster-level corrections should be applied using Random Field Theory and non-stationary correction [27].

In our previous structural MRI study, we showed that 23 long-term practitioners of SYM compared to healthy controls had 6.9 % significantly larger GMV in the whole brain [28] which represent, as far as we know, the highest GMV difference shown between groups of healthy volunteers. However, this significant whole brain difference was correlated with only two relatively small areas showing statistical significance located at right insula and right inferior temporal gyrus with respective volumes of 564 and 739 mm3.

Considering the concern of incurring in Type II errors (false negatives or conservative assumptions), the aim of our study was to analyze in more detail how the GMV differences are distributed across the whole brain. This new study is based in two key issues: 1) The development of an ad-hoc statistical GLM method that adapts itself on each brain area depending on the significance of covariates of that particular area; and 2) The parcellation of the human brain using 2 different methods i. Based on the human brain lobes: frontal, temporal, etc.… that gives rise to 16 different brain areas and ii. Using the more specific automated anatomical labelling (AAL) of 116 brain areas [29, 30]. The key question for this analysis was whether there were any areas that differed between long-term meditators and healthy controls which were overlooked in our previous paper [28] due to type II error correction effect.

Materials and methods

Participants

Forty-six white Caucasian, right-handed, healthy volunteers, between 21 and 63 years participated in this study. Twenty-three of them were long-term expert practitioners of SYM (17 females and 6 males) while the other 23 (also 17 females and 6 males) were non-meditators matched on gender, education degree, body mass index and age (see Table 1). All volunteers informed that they had no physical or mental illness, no history of neurological disorders, and no addiction to alcohol, nicotine or drugs.

Table 1 Demographic characteristics of the groups

	Meditators Mean (SD)	**Controls Mean+ (SD)**	**t(df=44)**	**p-value***
Volunteers Nº	23	23		
Age (years)	46.5 (11.4)	46.9 (10.9)	-0.13	0.89
Age range (years)	20.3 – 63.1	21.3 – 63.3		
Education degree, 0 to 6	3.78 (1.2)	4.04 (1.36)	0.69	0.50
Height (cm)	167.0 (8.8)	167.2 (7.6)	0.09	0.93
Weight (Kg)	69.5 (14.6)	71.7 (14.5)	0.53	0.60
Body mass index	24.9 (4.5)	25.5 (3.9)	0.54	0.60

*p-values represent group differences between meditators and controls using two-tailed independent samples t-tests.

Meditators had more than 5 years of daily meditation practice in SYM (mean 14.1 SD (6.1) years); the daily average time dedicated to meditation was 84.7 (32.2) minutes.

Before their participation in this research, all volunteers filled in different questionnaires to validate their individual health status, education and age. Additionally, meditators filled in other questionnaire that asked about their experience in SYM, including: average time dedicated to meditation per day, frequency of the perception of the state of mental silence, total hours of meditation and years of practice of SYM.

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

MRI Acquisition

All images were obtained on a 3T MRI Scanner, using an echo-planar-imaging gradient-echo sequence and an 8-channel head coil. A high-resolution T1-weighted three-dimensional inversion recovery spoiled gradient echo sequence was used to image the whole brain and the brainstem. A 3D fast spoiled-gradient-recalled pulse sequence was obtained with the following parameters: TR=8.761 ms, TE=1.736 ms, flip angle=12º, matrix size= 256 x 256 pixels, spacing between slices and slice thickness = 1 mm, voxel resolution=0.98 x 0.98 x 1 mm. Total acquisition time was 13 minutes.

Voxel-Based Morphometry

Voxel-based morphometry (VBM) [21] with DARTEL was conducted using the SPM12 software package (Statistical Parametric Mapping software: http://www.fil.ion.ucl.ac.uk/spm/). Processing steps were performed as suggested by the method's author [31]. VBM with DARTEL has been shown to be more sensitive than standard VBM [24] and provides results comparable to those achieved with manual segmentation [32].

The procedure followed these steps: 1. All T1-weighted anatomical images were displayed to screen to verify they were free from gross anatomical abnormalities. 2. For better registration, the T1 images were manually centred at the anterior commissure and reoriented according to the anterior–posterior commissure line. 3. Using the New Segment procedure in SPM12, images were segmented into: Grey matter (GM), White matter (WM) and Cerebrum Spinal Fluid (CSF), a segmentation that provides acceptable substitute for labour intensive manual estimates [25]. 4. The DARTEL routine inside SPM12 was used to spatially normalize the segmented images [24]. The image intensity of each voxel was modulated by the Jacobian determinants to ensure that regional differences in the total amount of GMV were conserved. 5. The registered images were then transformed to the Montreal Neurological Institute (MNI) space using affine spatial normalization. 6. Finally, the normalized modulated GMV images were smoothed with a 4-mm full-width at half-maximum (FWHM) isotropic Gaussian kernel to increase the signal to noise ratio.

For each individual, total GM, WM and CSF were obtained with the Matlab script 'get_totals.m' [33] and used to calculate the individual Total Intracranial Volume (TIV) by summing the volumes of the three already mentioned components (GM, WM, CSF).

Regional GMV extractions.

The WFU Pickatlas [29] was used to generate ROI masks of the selected brain areas in MNI space. Among the different brain areas subdivision generated by WFU Pickatlas, we chose the lobar atlas, and the AAL subdivisions. The lobar ROI subdivisions were as follows: right/left frontal lobe, right/left temporal lobe, right/left parietal lobe, right/left occipital lobe, right/left limbic system, and right/left sublobar area (internal cerebrum: summation of basal ganglia, thalamus, insula, and callosum), right/left brainstem and right/left cerebellum, the AAL subdivision es the 116 area parcellation by Rolls et al. [30]. To automatically extract the GMV at each ROI for each subject, we programmed a Matlab script based on the MATLAB code "get_totals" [33]. The output of the ad-hoc program was the regional GMV data for each volunteer at each ROI. Similar or equivalent procedures to extract regional GMV have been used in previous studies [17, 34, 35] To verify the truthfulness of the results obtained by the MATLAB "get_totals.m" script, several comparisons were made with the equivalent Marsbar toolbox (available at https://www.nitrc.org/projects/marsbar/). We verified that both tools provided the same results but because "get_totals" was easier to implement inside our ad-hoc program we used this method.

Statistical Analysis

Differences in GMV between meditators and controls at each zone/area were analysed by conducting an ad-hoc general linear model (AH-GLM) - ANCOVA that adapts it-self to every area's statistical specificities. The AH-GLM had the following terms eq.(1): the dependent variable (DV) at each area Grey Matter Volume (GMV) *;* the factor Meditator (Med) with two levels (control Med=0 and meditator Med=1); two covariates, the volunteer's age (Age) and the volunteer's Total Intracranial Volume (TIV); and two interactions, the factor with each covariate: $(Med \times TIV)$ and $(Med \times Age)$ notice that the interactions could be significant only when the associated covariate was significant. At eq. (1) each volunteer is represented by the subscript j and i represents each level of Meditator factor.

$$GMV_{ij} = \beta_0 + Med_i + \beta_1 \cdot Age_{ij} + \beta_2 \cdot TIV_{ij} + \beta_3 \cdot (Med \times TIV)_{ij} + \beta_4 \cdot (Med \times Age)_{ij} + \varepsilon_{ij} \quad (1)$$

Each brain area classification into zones from Zone 1 till Zone 3D was dependent on the statistical significance of each covariates (Age, TIV) and the corresponding interactions $(Med \times Age)$ and $(Med \times TIV)$. Covariates Age and TIV were considered significant at a threshold of *p<0.05*, having a Pearson's correlation coefficient with GMV of *r>0.4*. The interactions $(Med \times Age)$ and $(Med \times TIV)$ were considered significant when their associated covariate was significant and the interaction had *p <0.05*. This way we differentiated zones starting from the simplest Zone 1 where none of the covariates was significant, see eq. (2), to the zone 3D where all covariates and interactions were significant represented by the full model eq. (1).

$$\mathrm{GMV_{ij}} = \beta_0 + \mathrm{Med_i} + \varepsilon_{ij} \qquad (2)$$

Gender was not included into the AH-GLM because one of the conditions to be able to carry out an ANCOVA is that there is no effect of the factors on the covariates that are included in the model. When studying whether there is an effect of gender on the covariate *TIV* it was verified that this effect was highly significant *p < 0.0001*, because males had significant larger *TIV* than females. Therefore, including *TIV* in the model intrinsically controls for the gender factor.

Standardized residuals for the GMV and for the overall model at each zone ε_{ij} were normally distributed, as assessed by Shapiro-Wilk's test ($p > 0.05$). There was homogeneity of variances, as assessed by visual inspection of a scatterplot and Levene's test of homogeneity of variance ($p < 0.05$). There were no outliers in the data, as assessed by no cases with standardized residuals greater than ±*3* standard deviations. These models require compliance with two other assumptions: 1. To verify the existence of a non-zero linear relationship between the DV and the covariates in all groups together. If there is no such relationship, conducting an ANCOVA does not make sense, so a unifactorial ANOVA should be conducted alternatively; 2. To check the homogeneity of regression slopes; that is, to ensure that the linear relationship of the DV and the covariate is the same in all groups.

The multiple comparison problem was solved by controlling the false discovery rate (FDR), which manages the expected proportion of false positive findings among all the rejected null hypotheses [36], by means of the q-values estimated by Storey and Tibshirani's method [37] implemented in neuroscience research by Takeda et al [38]. We should consider that the q value is similar to the p value, with the exception that it is a measure of significance in terms of the false discovery rate rather than the false positive rate. From the distribution of p-values obtained from the multiple comparison, the q-values were provided by means of the

Bioconductor´s q-value package [3] from R software (3.6.1, R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was indicated by a false discovery rate (FDR) q-value <0.05 or p-value<0.05 when corresponds.

Results

Our previous paper [28] reported two main results: 1. The whole brain was statistically significant larger GMV in meditators compared to controls. 2. There were 5 cluster areas with larger GMV in meditators compared to controls: 2 from the direct VBM statistical results and 3 from a priori hypothesised regions with more lenient threshold.

Here we show in Table 2, that the summation of the differences of GMV between meditators vs. controls on the above mentioned 5 clusters reflect only around 1.0% of the total GMV difference found at the whole brain: 429.5 mm^3 GMV difference at the 5 clusters and 42354.2 mm^3 GMV difference in the whole brain, 611.005 (74.633) mm^3 controls whole brain GMV versus 653.374 (86.971) mm^3 meditators.

Table 2. Summary of previous results [28]

	R_Insula, vmOFC	R_Inf. Temporal, Fusiform Gyrus	R_Angular Gyrus	L_anterior insula	L_VLPFC	Summation of 5 Clusters	whole brain
p-value	0.023*	0.037*	0.069*	0.04 **	0.04 **		0.002
Vol cluster mm^3	563.6	739.1	475.9	543.4	239.6	2561.6	610961.2
% Diff larger in meditators	12.6	19.6	20.0	11.2	24.0	17.5***	6.9
Vol diff (Med-Controls) mm^3	70.8	145.2	95.0	61.0	57.6	429.5	42354.2

* Non-stationary cluster-level correction based on family wise error
** A priori hypothesised regions with more lenient threshold
*** Average of the 5 clusters percentages

Lobes area subdivision

In the 16 lobes area subdivision, GMV was statistically significantly larger in meditators compared to non-meditators (FDR q < *0.05*) in 4 out of 16 areas: R. temporal, R. frontal, R. brainstem and L. frontal. (See Table 3 and Fig 1).

Table 3. Statistics of GMV differences between groups in the different lobes (16 areas).

Area	Zone model	F	Nom. p-value	FDR q-value	GMV Controls (mean ± std) mL	GMV Medit (mean ± std) mL	*Relat dif %
R. temporal	3A	10.52	0.002	0.016	46.65 ± 5.92	50.86 ± 7.28	9.02
R. frontal	3A	10.44	0.002	0.016	78.35 ± 11.61	85.68 ± 12.95	9.36
R. brainstem	1	9.82	0.003	0.016	1.67 ± 0.28	2.00 ± 0.42	19.68
L. frontal	3A	9.3	0.004	0.016	76.57 ± 11.35	83.48 ± 13.4	9.02
**L. limbic	3A	5.82	0.02	0.064	25.45 ± 2.82	27.11 ± 3.36	6.52

*Relat dif % = (GMV Medit - GMV Controls) x 100 / GMV Controls.
** trend-level significance

Fig 1. Axial slices of the lobes areas with different GMV between groups, in the order of 1 to 5, following statistical significance. Z coordinates are shown in mm from the anterior-posterior commissure. The right side of the image corresponds to the right side of the brain.

In the two hemispheres GMV was statistically significantly (FDR q < *0.05*) larger in meditators relative to non-meditators, see Table 4.

Table 4. Statistics of GMV differences between groups in the hemispheres and whole brain.

Area	Zone model	F	Nom. p-value	FDR q-value	GMV Controls (mean ± std) mL	GMV Medit (mean ± std) mL	*Relat dif %
R.Hemisph.	3A	9.31	0.004	0.007	284.92 ± 35.02	304.95 ± 39.76	7.03
L.Hemisph.	3A	7.94	0.007	0.007	276.62 ± 33.46	295.22 ± 39.9	6.72
Whole brain GMV	3A	9.02	0.005	0.007	611 ± 74.63	653.37 ± 86.97	6.93

*Relat dif % = (GMV Medit - GMV Controls) x 100 / GMV Controls.

The relative GMV difference between meditators and controls showed both extreme cases at brainstem in meditators. On average, the difference in GMV considering all lobes areas was 6.8 ± 3.8 % larger in meditators. A similar difference was shown for both hemispheres where the relative difference was always larger GMV for meditators: 7,03% in the right hemisphere and 6,72% in the left hemisphere (Table 4). In the whole brain the difference was 6.93 %, which was already shown on our previous paper [28].

If we consider the reported GMV differences at lobes from Table 3 we see that the summation of the lobes GMV differences between groups was 20,44 mL or 20440 mm^3; this represent a 48,2 % of the total GMV difference reported at the whole brain that was 42354.2 mm^3. In the same way the reported GMV difference at the right hemisphere 20,03 mL represents a 47,3 % of the whole brain difference while the left hemisphere difference 18.60 mL represents a 43,9 %.

AAL area subdivision

In the 116 AAL area subdivision, GMV was statistically significant (FDR q < *0.05*) larger in meditators relative to non-meditators in 11 out of the 116 AAL areas: Right Middle temporal gyrus (MTG.R), Right Paracentral lobule (PCL.R), Right Inferior frontal gyrus opercular part (IFGoperc.R), Right Precentral gyrus (PreCG.R), Right Inferior temporal gyrus (ITG.R), Right Inferior frontal gyrus orbital part (IFGorb.R), Left Postcentral gyrus (PoCG.L), Left Precentral gyrus (PreCG.L), Left Middle frontal gyrus (MFG.L) , Left Olfactory cortex (OLF.L), Right Middle frontal gyrus orbital part (MFGorb.R), see Table 5 and Fig 2. In 59 AAL areas, the FDR *q*-value was between 0.05 and 0.1.

Table 5. Statistic of GMV differences between groups through significant AAL brain areas.

Area	Zona	F	Nom p-value	FDR q-value	GMV Controls (mean) mm³	GMV Controls (std) mm³	GMV Medit (mean) mm³	GMV Medit (std) mm³	Relat dif %
MTG.R	3A	11.84	0.001	0.0291	14.34	1.93	15.77	2.26	9.97
PCL.R	3A	11.00	0.002	0.0291	4.15	0.40	4.32	0.52	4.10
IFGoperc.R	3A	10.47	0.002	0.0291	3.68	0.60	4.12	0.67	11.96
PreCG.R	3A	9.75	0.003	0.0291	5.92	1.06	6.75	1.23	14.02
ITG.R	3A	9.30	0.004	0.0291	12.22	1.62	13.41	1.91	9.74
IFGorb.R	3A	9.08	0.004	0.0291	4.31	0.65	4.76	0.89	10.44
PoCG.L	3A	8.13	0.007	0.0382	7.70	1.25	8.42	1.22	9.35
PreCG.L	3A	7.90	0.007	0.0382	7.17	1.26	7.97	1.42	11.16
MFG.L	3A	7.52	0.009	0.0393	13.34	2.07	14.57	2.34	9.22
OLF.L	3A	7.45	0.009	0.0393	1.04	0.13	1.13	0.16	8.65
MFGorb.R	3A	6.88	0.012	0.0477	2.61	0.54	2.94	0.60	12.64

*Relat dif % = (GMV Medit - GMV Controls) x 100 / GMV Controls

Fig 2. Horizontal slices of AAL areas with different GMV between groups, in the order of 1 to 11, following statistical significance. Z coordinates are shown in mm distance from the anterior-posterior commissure. The right side of the image corresponds to the right side of the brain.

The GMV difference between meditators and controls ranged from +15.3% larger GMV at Right Parahippocampal gyrus to 0.0%, almost equal, at Right Lenticular nucleus - Pallidum. On average the difference in GMV considering all AAL areas was a 6.7 ± 3.0 % larger in Meditators.

If we consider the 11 AAL areas with significant GMV differences, similar to the calculation for the lobe areas, the summation of the difference in GMV between groups on those 11 areas was 6,25 mL which represents a 14.8 % of the total GMV difference at the whole brain.

Discussion

Discussion of the ad-hoc statistical method

As previously mentioned in the results section, the GMV differences between groups in the 5 clusters reported in our previous paper represent only 1 % of the total significant GMV difference at the whole brain (see Table 2). Out of the 5 reported clusters, the most significant one, in right insula-vmOFC had a corrected p-value of 0.027 while the whole brain p-value was 0.002, which is ten times more significant (no need of correction at the whole brain analysis because it was a single comparison).

The analysis conducted in this study shows that 11 out of the 116 AAL areas were significantly larger in meditators which represents a 14.8% of the total GMV difference at the whole brain (see Table 5). Five out of 16 lobes areas were statistically different in GMV between meditators and non-meditators and represent a 20.4% of the GMV differences reported at the whole brain; the left and right hemisphere GMV differences previously reported represent, respectively, 43.9% and 47,3 % of the GMV difference reported at the whole brain.

What these data seem to show is that the larger the number of area subdivisions tested the smaller the amount of GMV with statistical significance between groups. A possible explanation is the dilution of significant differences at the whole brain with subsequent brain partitions, presumably due to Type II error due to conservative assumptions.

This conservative bias may occur in other cross-sectional between-group studies where the whole brain GMV is significantly different between groups, in which case the use of an ad-hoc GLM method like the one here presented could be a possible solution to deal with the Type II error that the standard VBM statistical method seems to produce in these situations.

Based on our ad-hoc GLM method we present here a more sensitive and detailed examination that reveals significantly different areas that were not detected with the statistical VBM standard procedure. The acknowledgment of these areas will allow to better understand the neuroplastic mechanisms associated with the practice of SYM and its inherent consciousness state of mental silence, discussed in the next section.

Discussion of the VBM results

The 3 lobe areas with the largest significant GMV differences were in the right hemisphere: R. temporal, R frontal and R brainstem. Furthermore, the 6 AAL areas with the largest significant GMV differences were also in the right hemisphere: in mid and inferior temporal lobe, in inferior and orbital frontal cortices, and in para- and precentral lobes (Tables 3 and 5, Figs 1 and 2.)

This prevalence of larger differences in GMV in areas of the right hemisphere is in concordance with our previous publications of functional and structural MRI associated with the long-term practice of SYM [28, 39] where we found larger neuronal activation of right hemispheric regions of right inferior frontal cortex and superior temporal lobe in long-term SYM during their meditation and significantly larger GMV in areas mainly of the right hemisphere in anterior insula, inferior temporal gyrus and angular gyrus. It is also in line with a study that tested only 4 weeks of SYM training and found an enlargement in right inferior frontal cortex in the Meditators [40].

The frontal lobes are crucial for higher order executive functions and emotion control[41, 42]. The inferior frontal lobes are crucial for executive functions such as sustained attention, working memory, switching and inhibitory self-control [43]. The finding of larger GMV in these regions is in line with previous VBM studies of other meditation techniques that also found larger frontal lobe volumes in long-term Meditators, in particular in inferior frontal regions [44]. A recent study found that novices to meditation after only 4 weeks of SYM training developed larger GMV in right inferior frontal lobe compared to a control group [40]. The findings suggest that long-term meditation leads to enlargement of inferior frontal lobe regions possibly due to the fact that meditation which teaches the practitioner to inhibit unwanted thoughts and control their attention is a powerful attention and self-control training which may lead to the enlargement of areas that mediate attention and inhibitory self-control [45-48]. This would be in line with several studies that have shown that long-term Meditators have better performance in tasks of executive functions, in particular in tasks of sustained attention and inhibitory self-control [2, 49, 50]. Meditation, however, also has shown to lead to better emotional detachment [51] and emotional self-control which is mediated by the orbitofrontal and ventromedial frontal regions[42]. In fact, the orbitofrontal cortex was already been shown to be enlarged in our previous more strongent VBM analysis of these data [52].

The enlargement in the temporal lobe is also interesting. The middle and inferior temporal lobes are closely connected to the limbic system and form crucial part of the emotion control network [53-55].

The enlargement in the brainstem is of particular interest, as previous studies have found increased GMV in long-term meditators relative to controls in the brainstem [56, 57]; in a longitudinal study of mindfulness meditation this increase of GMV in the brainstem in the meditators was associated with better well-being [58]. The brainstem contains several production areas of several modulatory neurotransmitter pathways, such as those arising from the raphe nuclei (serotonergic; associated with modulation of mood and cognitve functions), ventral tegmental area (dopaminergic; associated with motivation and attention) and locus coeruleus (noradrenergic; associated with arousal and attention) [58, 59]. The state of mental silence has been described subjectively in meditation scriptures as a state of enhanced alertness, attention and arousal [1, 2].

The autonomic nervous system, brainstem and cortical systems are closely interconnected in their mediation of the regulation of behaviour and cognition [60]. The enlargement of the brainstem in long-term Meditators is therefore potentially a consequence of the long-term practice of achieving the state of thoughtless awareness which leads to enhanced alertness and arousal. It may also be related to the activation of the autonomic nervous system during meditation [61] that is closely interconnected with brainstem regions. Given that the brainstem is closely interconnected with frontal regions. It is also of note that brainstem and the two frontal lobes were increased in GMV in long-term Meditators.

The 6,9% larger GMV in meditators at the whole brain with a p-value of 0.002 constitutes as far as we know the largest difference in GMV between healthy groups of similar age and conditions. No other meditation technique or practice has shown such a large statistical difference in GMV at the whole brain. One of the assumptions of SYM is the spontaneous (Sahaja = spontaneous) awakening of the Kundalini energy [62] during the meditation which allows the practitioners to perceive the achievement of yoga (yoga=union) and the state of mental silence, which is felt like a cool breeze of energy on top of the head. It is possible that this experience, which is specific to SYM, may be related to the enlargement of VBM and this needs to be further tested.

Conclusions

In our previous paper where we used the standard statistical model for VBM, only 5 relatively small brain areas were statistically different in GMV between groups. These 5 areas represented only around 1% of the total 6.9% larger GMV difference shown at the whole brain in meditators compared with non-meditators. Hence the possibility of a type I error or conservative results was considered. In this paper, with an ad-hoc

statistical method, we have shown in more detail how this 6,9 % larger GMV in meditators, the largest GMV difference in healthy groups of similar age and conditions in the literature so far, is distributed in the meditator's brain subregions. The larger GMV in meditators is focused in particular in the right hemisphere in frontal and temporal brain areas related with attention and emotional control.

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References

[1] A. Kokodoko, "The Yoga Sutra of Patanjali," *Library Journal,* vol. 139, no. 6, pp. 96-96, Apr 1 2014.

[2] K. Rubia, "The neurobiology of Meditation and its clinical effectiveness in psychiatric disorders," *Biological Psychology,* vol. 82, no. 1, pp. 1-11, Sep 2009, doi: 10.1016/j.biopsycho.2009.04.003.

[3] R. Manocha, D. Black, J. Sarris, and C. Stough, "A Randomized, Controlled Trial of Meditation for Work Stress, Anxiety and Depressed Mood in Full-Time Workers," *Evidence-Based Complementary and Alternative Medicine,* pp. 1-8, 2011 2011, doi: 10.1155/2011/960583.

[4] L. Harrison, R. Manosh, and K. Rubia, "Sahaja Yoga Meditation as a family treatment program for attention deficit hyperactivity disorder children," *Journal of Clinical Psychology and Psychiatry,* vol. 9(4), pp. 479–497, 2004.

[5] K. Rubia, A. Smith, and E. Taylor, "Performance of children with attention deficit hyperactivity disorder (ADHD) on a test battery of impulsiveness," *Child Neuropsychology,* vol. 13, no. 3, pp. 276-304, May 2007, doi: 10.1080/09297040600770761.

[6] S.-C. Chung, M. M. Brooks, M. Rai, J. L. Balk, and S. Rai, "Effect of Sahaja Yoga Meditation on Quality of Life, Anxiety, and Blood Pressure Control," *Journal of Alternative and Complementary Medicine,* vol. 18, no. 6, Jun 2012, doi: 10.1089/acm.2011.0038.

[7] A. Morgan, "Sahaja Yoga: an ancient path to modern mental health?," vol. 4, Transpersonal Psychology Review ed: Transpersonal Psychology, 2001.

[8] R. Manocha, G. B. Marks, P. Kenchington, D. Peters, and C. M. Salome, "Sahaia yoga in the management of moderate to severe asthma: a randomised controlled trial," *Thorax,* vol. 57, no. 2, pp. 110-115, Feb 2002, doi: 10.1136/thorax.57.2.110.

[9] R. Manocha, B. Semmar, and D. Black, "A pilot study of a mental silence form of meditation for women in perimenopause," *Journal of Clinical Psychology in Medical Settings,* vol. 14, no. 3, pp. 266-273, Sep 2007, doi: 10.1007/s10880-007-9076-5.

[10] U. Panjwani, H. L. Gupta, S. H. Singh, W. Selvamurthy, and U. C. Rai, "Effect of Sahaja yoga practice on stress management in patients of epilepsy," *Indian journal of physiology and pharmacology,* vol. 39, no. 2, pp. 111-6, 1995-Apr 1995.

[11] U. Panjwani, W. Selvamurthy, S. H. Singh, H. L. Gupta, L. Thakur, and U. C. Rai, "Effect of Sahaja yoga practice on seizure control and EEG changes in patients of epilepsy," *Indian Journal of Medical Research,* vol. 103, pp. 165-172, Mar 1996.

[12] U. Panjwani, W. Selvamurthy, S. H. Singh, H. L. Gupta, S. Mukhopadhyay, and L. Thakur, "Effect of Sahaja yoga meditation on Auditory Evoked Potentials (AEP) and Visual Contrast Sensitivity (VCS)

in epileptics," *Applied Psychophysiology and Biofeedback,* vol. 25, no. 1, pp. 1-12, Mar 2000, doi: 10.1023/a:1009523904786.

[13] R. Manocha, D. Black, and L. Wilson, "Quality of Life and Functional Health Status of Long-Term Meditators," *Evidence-Based Complementary and Alternative Medicine,* 2012 2012, Art no. 350674, doi: 10.1155/2012/350674.

[14] C.-C. Yang *et al.*, "State and Training Effects of Mindfulness Meditation on Brain Networks Reflect Neuronal Mechanisms of Its Antidepressant Effect," *Neural Plasticity,* 2016 2016, Art no. 9504642, doi: 10.1155/2016/9504642.

[15] L. Jancke, "The plastic human brain," (in English), *Restorative Neurology and Neuroscience,* Review vol. 27, no. 5, pp. 521-538, 2009, doi: 10.3233/rnn-2009-0519.

[16] J. Haenggi, N. Langer, K. Lutz, K. Birrer, S. Merillat, and L. Jaencke, "Structural Brain Correlates Associated with Professional Handball Playing," *Plos One,* vol. 10, no. 4, Apr 27 2015, Art no. UNSP e0124222, doi: 10.1371/journal.pone.0124222.

[17] Y. Taki *et al.*, "Correlations among Brain Gray Matter Volumes, Age, Gender, and Hemisphere in Healthy Individuals," *Plos One,* vol. 6, no. 7, Jul 27 2011, Art no. e22734, doi: 10.1371/journal.pone.0022734.

[18] C. Sampaio-Baptista *et al.*, "Gray matter volume is associated with rate of subsequent skill learning after a long term training intervention," (in English), *Neuroimage,* Article vol. 96, pp. 158-166, Aug 2014, doi: 10.1016/j.neuroimage.2014.03.056.

[19] B. Draganski, C. Gaser, V. Busch, G. Schuierer, U. Bogdahn, and A. May, "Neuroplasticity: Changes in grey matter induced by training - Newly honed juggling skills show up as a transient feature on a brain-imaging scan," *Nature,* vol. 427, no. 6972, pp. 311-312, Jan 22 2004, doi: 10.1038/427311a.

[20] G. Brod, U. Lindenberger, A. D. Wagner, and Y. L. Shing, "Knowledge Acquisition during Exam Preparation Improves Memory and Modulates Memory Formation," *Journal of Neuroscience,* vol. 36, no. 31, pp. 8103-8111, Aug 3 2016, doi: 10.1523/jneurosci.0045-16.2016.

[21] J. Ashburner and K. J. Friston, "Voxel-based morphometry - The methods," *Neuroimage,* vol. 11, no. 6, pp. 805-821, Jun 2000, doi: 10.1006/nimg.2000.0582.

[22] J. Ashburner and K. J. Friston, "Why voxel-based morphometry should be used," *Neuroimage,* vol. 14, no. 6, pp. 1238-1243, Dec 2001, doi: 10.1006/nimg.2001.0961.

[23] J. Ashburner, J. G. Csernansky, C. Davatzikos, N. C. Fox, G. B. Frisoni, and P. M. Thompson, "Computer-assisted imaging to assess brain structure in healthy and diseased brains," *Lancet Neurology,* vol. 2, no. 2, pp. 79-88, Feb 2003, doi: 10.1016/s1474-4422(03)00304-1.

[24] J. Ashburner, "A fast diffeomorphic image registration algorithm," *Neuroimage,* vol. 38, no. 1, pp. 95-113, Oct 15 2007, doi: 10.1016/j.neuroimage.2007.07.007.

[25] I. B. Malone *et al.*, "Accurate automatic estimation of total intracranial volume: A nuisance variable with less nuisance," *Neuroimage,* vol. 104, pp. 366-372, Jan 1 2015, doi: 10.1016/j.neuroimage.2014.09.034.

[26] A. Mechelli, C. J. Price, K. J. Friston, and J. Ashburner, "Voxel-based morphometry of the human brain: Methods and applications," *Current Medical Imaging Reviews,* vol. 1, no. 2, pp. 105-113, Jun 2005, doi: 10.2174/1573405054038726.

[27] S. Hayasaka, K. L. Phan, I. Liberzon, K. J. Worsley, and T. E. Nichols, "Nonstationary cluster-size inference with random field and permutation methods," *Neuroimage,* vol. 22, no. 2, pp. 676-687, Jun 2004, doi: 10.1016/j.neuroimage.2004.01.041.

[28] S. E. Hernandez, J. Suero, A. Barros, J. Luis Gonzalez-Mora, and K. Rubia, "Increased Grey Matter Associated with Long-Term Sahaja Yoga Meditation: A Voxel-Based Morphometry Study," *Plos One,* vol. 11, no. 3, Mar 3 2016, Art no. e0150757, doi: 10.1371/journal.pone.0150757.

[29] N. Tzourio-Mazoyer *et al.*, "Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain," (in English), *Neuroimage,* Article vol. 15, no. 1, pp. 273-289, Jan 2002, doi: 10.1006/nimg.2001.0978.

[30] E. T. Rolls, M. Joliot, and N. Tzourio-Mazoyer, "Implementation of a new parcellation of the orbitofrontal cortex in the automated anatomical labeling atlas," *Neuroimage,* vol. 122, pp. 1-5, Nov 2015, doi: 10.1016/j.neuroimage.2015.07.075.

[31] A. J. VBM Tutorial. Available: [Online] Available: http://www.fil.ion.ucl.ac.uk/~john/misc/VBMclass10.pdf.

[32] L. Bergouignan *et al.*, "Can voxel based morphometry, manual segmentation and automated segmentation equally detect hippocampal volume differences in acute depression?," *Neuroimage,* vol. 45, no. 1, pp. 29-37, Mar 2009, doi: 10.1016/j.neuroimage.2008.11.006.

[33] Ridgway, " Get_totals.m Matlab script ", ed.
[34] W. Gonoi *et al.*, "Age-related changes in regional brain volume evaluated by atlas-based method," *Neuroradiology,* vol. 52, no. 10, pp. 865-873, Oct 2010, doi: 10.1007/s00234-009-0641-5.
[35] J. E. Peelle, R. Cusack, and R. N. A. Henson, "Adjusting for global effects in voxel-based morphometry: Gray matter decline in normal aging," *Neuroimage,* vol. 60, no. 2, pp. 1503-1516, Apr 2 2012, doi: 10.1016/j.neuroimage.2011.12.086.
[36] Y. Benjamini and Y. Hochberg, "CONTROLLING THE FALSE DISCOVERY RATE - A PRACTICAL AND POWERFUL APPROACH TO MULTIPLE TESTING," *Journal of the Royal Statistical Society Series B-Statistical Methodology,* vol. 57, no. 1, pp. 289-300, 1995, doi: 10.1111/j.2517-6161.1995.tb02031.x.
[37] J. D. Storey and R. Tibshirani, "Statistical significance for genomewide studies," *Proceedings of the National Academy of Sciences of the United States of America,* vol. 100, no. 16, pp. 9440-9445, Aug 2003, doi: 10.1073/pnas.1530509100.
[38] Y. Takeda, K. Suzuki, M. Kawato, and O. Yamashita, "MEG Source Imaging and Group Analysis Using VBMEG," *Frontiers in Neuroscience,* vol. 13, Mar 2019, Art no. 241, doi: 10.3389/fnins.2019.00241.
[39] S. E. Hernandez, J. Suero, K. Rubia, and J. L. Gonzalez-Mora, "Monitoring the Neural Activity of the State of Mental Silence While Practicing Sahaja Yoga Meditation," *Journal of Alternative and Complementary Medicine,* vol. 21, no. 3, pp. 175-179, Mar 1 2015, doi: 10.1089/acm.2013.0450.
[40] A. Dodich *et al.*, "Short-term Sahaja Yoga meditation training modulates brain structure and spontaneous activity in the executive control network," *Brain and Behavior,* vol. 9, no. 1, Jan 2019, Art no. e01159, doi: 10.1002/brb3.1159.
[41] D. Badre and D. E. Nee, "Frontal Cortex and the Hierarchical Control of Behavior," *Trends in Cognitive Sciences,* vol. 22, no. 2, pp. 170-188, Feb 2018, doi: 10.1016/j.tics.2017.11.005.
[42] M. L. Dixon, R. Thiruchselvam, R. Todd, and K. Christoff, "Emotion and the Prefrontal Cortex: An Integrative Review," *Psychological Bulletin,* vol. 143, no. 10, pp. 1033-1081, Oct 2017, doi: 10.1037/bul0000096.
[43] R. Lorenz, I. R. Violante, R. P. Monti, G. Montana, A. Hampshire, and R. Leech, "Dissociating frontoparietal brain networks with neuroadaptive Bayesian optimization," *Nature Communications,* vol. 9, Mar 2018, Art no. 1227, doi: 10.1038/s41467-018-03657-3.
[44] K. C. R. Fox *et al.*, "Is meditation associated with altered brain structure? A systematic review and meta-analysis of morphometric neuroimaging in meditation practitioners," *Neuroscience and Biobehavioral Reviews,* vol. 43, pp. 48-73, Jun 2014, doi: 10.1016/j.neubiorev.2014.03.016.
[45] K. Rubia, A. B. Smith, M. J. Brammer, and E. Taylor, "Right inferior prefrontal cortex mediates response inhibition while mesial prefrontal cortex is responsible for error detection," *Neuroimage,* vol. 20, no. 1, pp. 351-358, Sep 2003, doi: 10.1016/s1053-8119(03)00275-1.
[46] Y. W. Hung, S. L. Gaillard, P. Yarmak, and M. Arsalidou, "Dissociations of cognitive inhibition, response inhibition, and emotional interference: Voxelwise ALE meta-analyses of fMRI studies," *Human Brain Mapping,* vol. 39, no. 10, pp. 4065-4082, Oct 2018, doi: 10.1002/hbm.24232.
[47] S. G. Ryman *et al.*, "Proactive and reactive cognitive control rely on flexible use of the ventrolateral prefrontal cortex," *Human Brain Mapping,* vol. 40, no. 3, pp. 955-966, Feb 2019, doi: 10.1002/hbm.24424.
[48] S. Vossel, J. J. Geng, and G. R. Fink, "Dorsal and Ventral Attention Systems: Distinct Neural Circuits but Collaborative Roles," *Neuroscientist,* vol. 20, no. 2, pp. 150-159, Apr 2014, doi: 10.1177/1073858413494269.
[49] P. Sedlmeier *et al.*, "The Psychological Effects of Meditation: A Meta-Analysis," *Psychological Bulletin,* vol. 138, no. 6, pp. 1139-1171, Nov 2012, doi: 10.1037/a0028168.
[50] R. Marciniak, K. Sheardova, P. Cermakova, D. Hudecek, R. Sumec, and J. Hort, "Effect of meditation on cognitive functions in context of aging and neurodegenerative diseases," *Frontiers in Behavioral Neuroscience,* vol. 8, Jan 2014, Art no. 17, doi: 10.3389/fnbeh.2014.00017.
[51] S. V. Pavlov, N. V. Reva, K. V. Loktev, V. V. Korenyok, and L. I. Aftanas, "Impact of long-term meditation practice on cardiovascular reactivity during perception and reappraisal of affective images," *International Journal of Psychophysiology,* vol. 95, no. 3, pp. 363-371, Mar 2015, doi: 10.1016/j.ijpsycho.2015.01.002.
[52] S. E. Hernandez, A. Barros-Loscertales, Y. Q. Xiao, J. L. Gonzalez-Mora, and K. Rubia, "Gray Matter and Functional Connectivity in Anterior Cingulate Cortex are Associated with the State of Mental Silence During Sahaja Yoga Meditation," *Neuroscience,* vol. 371, pp. 395-406, Feb 2018, doi: 10.1016/j.neuroscience.2017.12.017.

[53] V. S. Rotenberg and I. Weinberg, "Human memory, cerebral hemispheres, and the limbic system: A new approach," *Genetic Social and General Psychology Monographs,* vol. 125, no. 1, pp. 45-70, Feb 1999.

[54] M. R. Roxo, P. R. Franceschini, C. Zubaran, F. D. Kleber, and J. W. Sander, "The Limbic System Conception and Its Historical Evolution," (in English), *Thescientificworldjournal,* Review vol. 11, pp. 2427-2440, 2011, doi: 10.1100/2011/157150.

[55] J. S. S. Torres, W. J. D. Cordoba, L. F. Z. Ceron, C. A. N. Amezquita, and T. O. Z. Bastidas, "Functional correlation of the limbic system with emotion, learning and memory," (in Spanish), *Morfolia,* Review vol. 7, no. 2, pp. 29-44, 2015.

[56] B. K. Hoelzel *et al.*, "Mindfulness practice leads to increases in regional brain gray matter density," *Psychiatry Research-Neuroimaging,* vol. 191, no. 1, pp. 36-43, Jan 30 2011, doi: 10.1016/j.pscychresns.2010.08.006.

[57] P. Vestergaard-Poulsen *et al.*, "Long-term meditation is associated with increased gray matter density in the brain stem," *Neuroreport,* vol. 20, no. 2, pp. 170-174, Jan 28 2009, doi: 10.1097/WNR.0b013e328320012a.

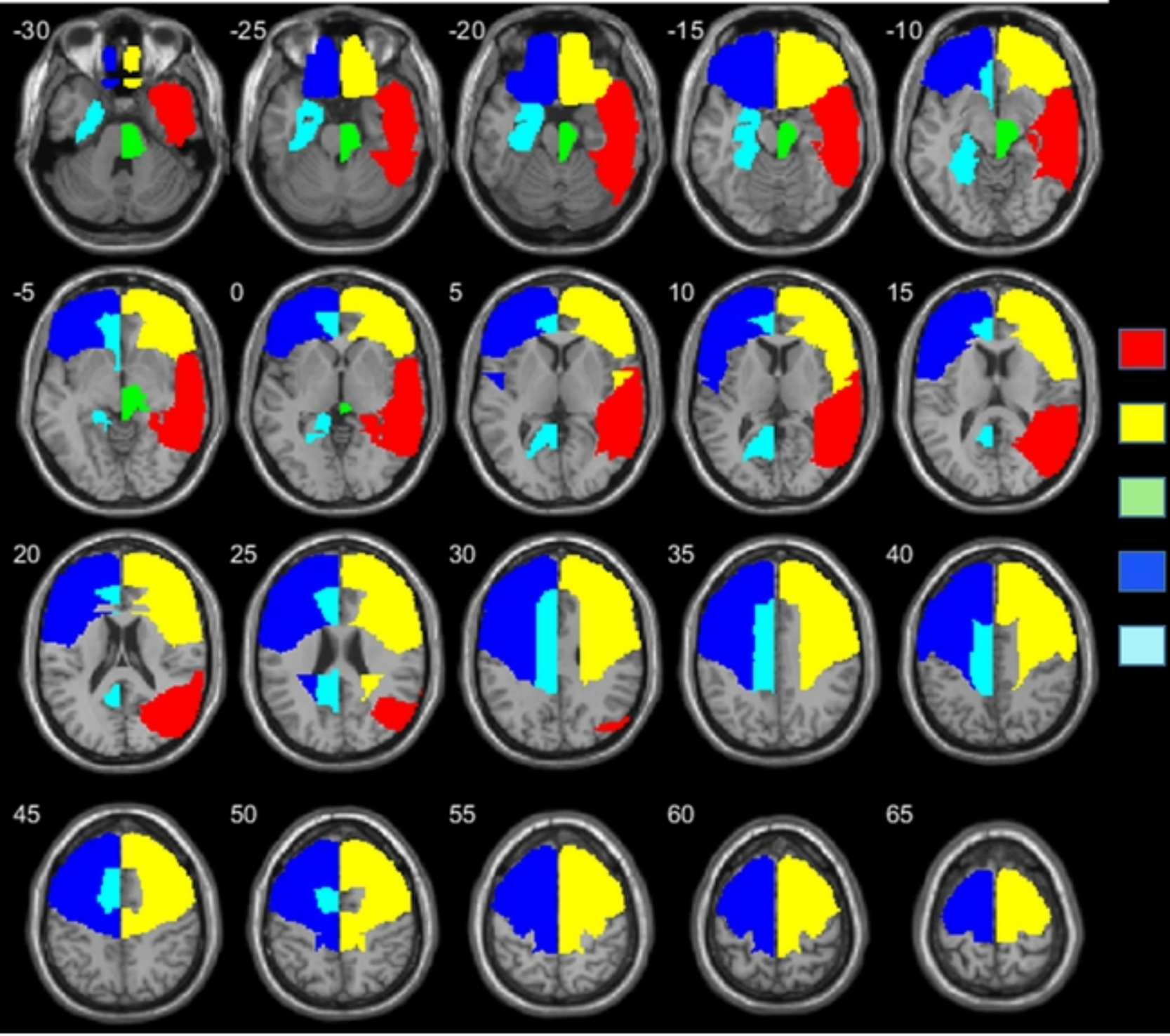
[58] O. Singleton, B. K. Hoelzel, M. Vangel, N. Brach, J. Carmody, and S. W. Lazar, "Change in brainstem gray matter concentration following a mindfulness-based intervention is correlated with improvement in psychological well-being," *Frontiers in Human Neuroscience,* vol. 8, Feb 18 2014, Art no. 33, doi: 10.3389/fnhum.2014.00033.

[59] A. Venkatraman, B. L. Edlow, and M. H. Immordino-Yang, "The Brainstem in Emotion: A Review," *Frontiers in Neuroanatomy,* vol. 11, Mar 2017, Art no. 15, doi: 10.3389/fnana.2017.00015.

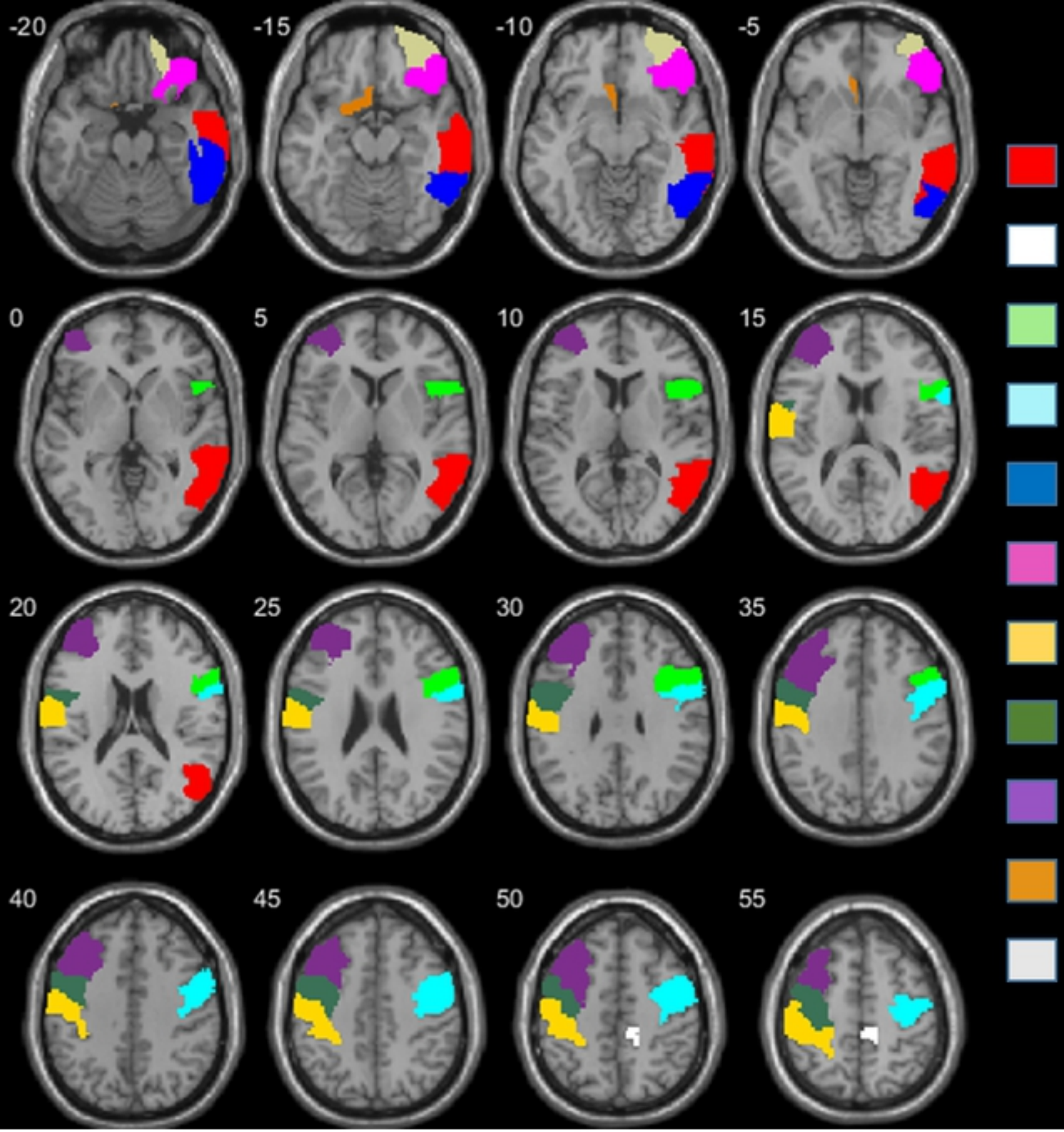
[60] A. Bellato, I. Arora, C. Hollis, and M. J. Groom, "Is autonomic nervous system function atypical in Attention Deficit Hyperactivity Disorder (ADHD)? A systematic review of the evidence," (in eng), *Neurosci Biobehav Rev,* Nov 10 2019, doi: 10.1016/j.neubiorev.2019.11.001.

[61] Y. Y. Tang *et al.*, "Central and autonomic nervous system interaction is altered by short-term meditation," *Proceedings of the National Academy of Sciences of the United States of America,* vol. 106, no. 22, pp. 8865-8870, Jun 2009, doi: 10.1073/pnas.0904031106.

[62] H. G. Coward, "JUNG AND KUNDALINI," *Journal of Analytical Psychology,* vol. 30, no. 4, pp. 379-392, 1985, doi: 10.1111/j.1465-5922.1985.t01-1-00379.x.



Figure



Figure