Indian J Med Res 148, August 2018, pp 190-199
DOI: 10.4103/ijmr.IJMR_194_17

Imaging & neuropsychological changes in brain with spiritual practice: A pilot study

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Received February 1, 2017

***Background & objectives*: Some studies have systematically assessed the effects of spiritual practice (SP) on the brain using combined neuropsychological testing and functional imaging. The objective of the present study was to compare imaging and neuropsychological changes in healthy individuals after SP and those with only physical exercise.**

***Methods*: Healthy adult male volunteers, aged 25-45 yr were randomized into two groups. Group 1 (SP group) underwent the SP and group 2 (controls) did brisk walk for 30 min daily. Detailed neuropsychological evaluation, resting-state functional magnetic resonance imaging (fMRI) and brain ^{99m}Tc ethyl cysteinate dimer single-photon emission computed tomography (SPECT) were carried out for both groups before and three months after intervention.**

***Results*: Post-intervention, resting state fMRI showed increased connections of left precuneus (in the posterior cingulate cortex area of default mode network) in group 1 and increased left frontal connections in group 2. The neuropsychological tests showed significant improvement in 'Speed of Processing' (Digit Symbol Test) in group 1 and in Focused Attention (Trail Making A) in group 2. The SPECT data in group 1 showed significant improvement in perfusion of the frontal areas, with relatively lesser improvement in parietal areas. Group 2 showed significant improvement in perfusion predominantly in parietal areas, as compared to frontal areas. In addition, significantly improved mood was reported by group 1 and not by group 2.**

***Interpretation & conclusions*: This pilot study shows important functional imaging and neuropsychological changes in the brain with SP.**

Key words Magnetic resonance imaging - neuropsychological evaluation - single-photon tomography - spiritual practice

Spiritual practice (SP)-induced spiritual transformation (ST) has various definitions[1]. SPs include varied methods such as meditation, prayer and other processes to optimize spiritual health that can lead to ST. In 1998, the World Health Organization executive board proposed an amendment to the 1946 definition of health and re-defined health as 'a dynamic state of complete physical, mental, spiritual and social well-being and not merely the absence of disease or infirmity'[2].

SP-induced ST experiences have shown effects on autonomic, electrophysiological, hormonal, immunological systems[3] and various illnesses[4,5]. Cognitive changes after different types of meditation have been described[6] but no study has looked at changes in the brain using a combination of cognitive tests, functional neuroimaging techniques and single-photon emission computed tomography (SPECT) brain perfusion. The aim of this pilot study was to evaluate the changes in brain network connectivity, brain perfusion, cognitive functioning and mood in healthy individuals, after specific spiritual processes and compare it with a control group doing only physical exercises.

Material & Methods

The study was conducted in the department of Radiology/MRI, P.D. Hinduja Hospital, Mumbai, India, during April 2015 to April 2016. For this pilot study, participants were enrolled from April to September 2015, until a total of 14 healthy vegetarian, males, 25-45 yr of age who had not followed any previous spiritual routines or had any current psychopathology and/or history of any psychiatric morbidity, volunteered to participate in the study, who were then randomly segregated into two groups. The neuroimaging and psychometric analysis was done in a hospital setting. Spiritual processes were done under guidance at an institute by a spiritual therapist.

After obtaining written informed consent a detailed proforma was filled by each participant. This included clinical information and screening for the presence of any psychopathology using the Millon Clinical Multiaxial Inventory-III (MCMI III)[7]. Detailed neuropsychological evaluation, resting-state functional magnetic resonance imaging (rs-fMRI) and ^{99m}Tc ethyl cysteinate dimer SPECT (^{99m}Tc ECD SPECT) brain study were also done. The study protocol was approved by the institutional ethics committee of P.D. Hinduja Hospital, Mumbai.

The participants were randomized into two groups. Group I: SP group and Group II: Exercise group. Randomization was done on the Research Randomizer, a computer based 'random number generator' (*https://www.randomizer.org/*). SP group individuals visited *Shanti Kshetra Premgiri Ashram*, Raigad, where they underwent a SP called '*ShaktiPravaah*', by the spiritual therapist (by a special intervention, in our study this being *ShaktiPravaah*). During *ShaktiPravaah,* the transfer of energies was done by touching the head of the subject, who received it passively over a three minute period and these processes were conducted four times during the three months period with a minimum of one month gap between the two processes. During the interim period, individuals were asked to do the meditation process taught to them for 30 min every day. Participants in the control group were asked to walk for 30 min every day, at any convenient time, for three months.

Blinding & Post-intervention assessment: Single blinding without cross-over was done. The psychological tests were done by the same person (blinded) before and after the procedure. All the rs-fMRI and SPECT scans were divided into four groups: Pre- and post-intervention groups in both the SP and exercise groups. The radiologists were blinded to the groups and also as to whether the scan was pre- or post-intervention. At the end of the three months, all individuals again underwent neuropsychiatric evaluation, rs-fMRI and ^{99m}Tc ECD SPECT study.

Resting state rs-fMRI evaluation: MRIs were performed on 3.0T Philips ingenia, using a 32-channel head coil. rs-fMRI sequence was performed using Blood-Oxygen-Level Dependent (BOLD)/Echo-Planar Images (EPI) image acquisition covering the whole brain, with the anterior commissure-posterior commissure (AC-PC) line in 250 dynamics with the following parameters: BOLD sequence: (No of Volume=250, TR=2034 ms, TE=35 ms, field of view=220/220 mm, 38 slices, slice thickness=4 mm, Flip angle 90°, matrix - 72×96 voxel size-3 mm×3 mm×4 mm). A T1-weighted three-dimensional (3D) Magnetization-Prepared Rapid Acquisition Gradient Echo (MP RAGE) sequence was performed (TR=8.2 ms, TE=3.8 ms, flip angle=80, slice thickness=1 mm, voxel size 1×1×1 mm, 200 contiguous 1 mm slice acquired with 256×256 mm matrix). Each participant, following the localizer first underwent rs-fMRI BOLD sequence, with eyes open, followed by 3D - T1-weighted sequence, where eyes were kept closed. The images were inspected immediately after the scan acquisition to ensure the absence of gross movement.

rs-fMRI data analysis was performed using the FSL, FMRIB software library tool (*www.fmrib.ox.ac.uk/fsl*), with many of the steps below as described previously[8]. Pre-processing of rs-fMRI was carried out using the FEAT (FMRI Expert Analysis tool)[8]. The first five functional images were discarded to allow magnetization equilibrium and for the individual to adjust to the environment. Non-brain removal was done using brain extraction tool (BET)[9] and motion correction was conducted using MCFLIRT (*www.fmrib.ox.ac.uk/fsl*). Spatial smoothening using the Gaussian kernel with full width at half maximum (FWHM) was 5 mm. Registration was done using both high resolution main structural image and standard space Montreal Neurological Institute (MNI) template MNI 152_T1 2 mm brain image with 12 DOF (degree of freedom)[8]. The main structural image was subjected to high-resolution structural Image extraction with BET. Independent component analysis (ICA) was carried out using FSL's Multivariate Exploratory Linear Decomposition into Independent Components (MELODIC)[8]. The single-subject ICA was carried out in FEAT MELODIC data exploration, and all components were further visually inspected and noise component was removed using the command 'reg filt'. Multivariate group probabilistic ICA (PICA) was then carried out using FSL MELODIC[8] to derive maximum spatially ICs across all datasets, which were temporally concatenated. The dataset was decomposed into 18 sets of independent vectors using the Fast ICA algorithm (*www.fmrib.ox.ac.uk/fsl*).

Statistical analysis: The analysis between groups was performed using an FSL dual regression and set of spatial maps from the group-mean analysis (GroupICs) was used to generate subject-specific-spatial maps and associated time series[8]; and finally, the default mode network (DMN) component maps were collected across participants into single 4D file and tested voxel wise for significant difference between groups 1A and 1B and groups 2A and 2B using FSL's randomize nonparametric permutation testing tool (*www.fmrib.ox.ac.uk/fsl*) with 5000 permutation[8]. To estimate the group differences different contrasts were used in general linear model (GLM), and resultant statistical maps were at threshold *P*<0.05 [FEW (family wise error) corrected].

Neuropsychological evaluation

Cognitive assessment: The neuropsychological protocol included standardized tests examining different cognitive domains (Table I)[10-15]. The key domains included were attention, speed of processing and working memory.

Table I. Neuropsychological protocol with standardized tests examining different cognitive domains

Cognitive domains	Tests	Measures
Attention		
Immediate auditory	Digit span forward[10]	Number of digits
Immediate visual	Spatial span forward[11]	Number of digits
Focused	Trails A & B[15]	Time
Sustained	Digit vigilance[12]	Time
Speed of processing		
	Coding[10]	Time
	Symbol search[10]	Number correct
Working memory		
	Digit span backwards[10]	Number of digits
	Spatial span backwards[11]	Digits correct
	N Back 1& 2[13]	Number of hits
	Paced Authority Serial Addition Task (PASAT)[14]	Number correct

Superscript numerals denote reference numbers

Daily spiritual experience scale (DSES)[16]: The Daily Spiritual Experience Scale (DSES) is a standardized self-report measure, which consists of 16 items that evaluates perceived ST in daily life experiences.

Subjective changes report: All individuals completed self-report forms to describe changes they perceived after the intervention. These changes were under the heads of cognitive, interpersonal relationships, mood, occupational functioning and general health.

The statistical analyses for the neuropsychological data (cognitive test scores and DSES scale scores) were carried out with two levels - within subject and between subject analyses. First, the normality of the distribution was checked for, and then a paired sample *t* test was carried out to compare pre-to-post intervention changes in cognitive measures and the scale score within the experimental group and the control group individually. An independent samples *t* test was also conducted to compare the effect of the intervention on the cognitive measures and spirituality scale between the two groups. For this, the difference score of the pre-test and post-test data were calculated for each participant, in both groups, and then, these scores were compared between the two groups.

For the mood and behaviour evaluation, the subjective report was analyzed using the qualitative methodology of 'Thematic Analysis'[17] to draw out the key themes in the written narratives of the participants. Thematic analysis is a qualitative method of data analyses that is frequently used with psychological data. In this study, a qualitative thematic analysis was carried out to identify patterns of perceived change across participants between the two groups. The recurring themes across the participants that resonated for the majority (>50%) were selected as significant.

SPECT brain analysis: Brain perfusion scans with ^{99m}Tc ECD SPECT were performed for all participants. All images were interpreted and scored. Images were processed with reorientation of the 3D volume of each brain study and raw data corrected for lateral deviations and reoriented by defining the vertical anterior and PC lines. Inter- and intra-observer variations were also minimized. In addition to qualitative interpretation, NeuroGam™ software (Segami-Corporation, USA) was applied on the reconstructed data, for semi-quantitative evaluation of brain perfusion in frontal and parietal areas of both hemispheres. This software applies an affine anatomical co-registration by blocks of data defined in the Talairach space. The software programme automatically calculates mean and standard deviation (SD) of each cortical area's perfusion level voxel-by-voxel and inter-subject comparisons of ^{99m}Tc ECD uptake in brain cortex are done. Perfusion values in brain areas were expressed as mean (±SD) percentage of mean perfusion of the cerebellum.

Since previous studies (discussed later) reported changes mainly in the frontal and parietal areas, perfusion in these two important areas was compared before and after intervention in both groups. We excluded other areas since our study involved normal participants and standardized database was unavailable for these areas.

The analysis of individual study utilizing the NeuroGam™ software created a z-score map. Subtle changes appreciated qualitatively in more than one area with minimal though not significant respective change on quantitative method were not considered as disagreement. Visual interpretation images appear to be more representative for the areas under study hence provided.

Results

Resting state functional magnetic resonance imaging (rs-fMRI) data: The changes in the connectivity of various components of Default Mode Network (DMN), post-intervention, are summarized in Table II. Group regression analysis of the group 1 before and after spiritual process revealed significant (FWE P=0.05) decreased connections of the right cuneus (Fig. 1A) and increased connections of the left precuneus (Fig. 1B). Analysis of group 2 before and after physical exercise revealed significantly increased connections along anterolateral aspect of the left frontal lobe (Fig. 2).

Neuropsychology data: Comprehensive analysis of the entire neuropsychological data of both groups is

Table II. Change in connectivity of various components of default mode network (DMN), on resting state functional magnetic resonance imaging (rs-fMRI) post-intervention, in both groups

Components of DMN	ST group	Control group
Medial prefrontal cortex	No change	No change
PCC (precuneus)	Increased (left)	No change
Cuneus	Decreased (right)	No change
Amygdala/hippocampus	No change	No change
Lateral parietal	No change	No change
Lateral frontal	No change	Increased (left)
Other areas of DMN	No change	No change

ST, spiritual transformation; PCC, posterior cingulate cortex

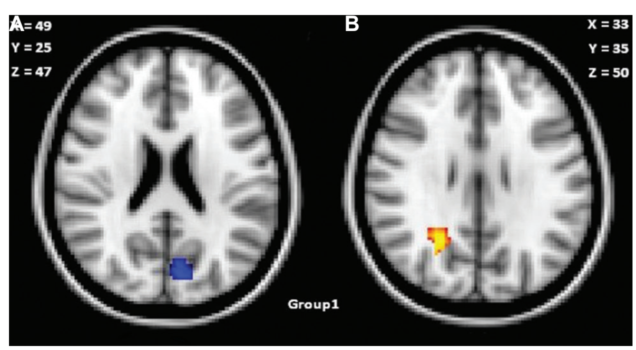

Fig. 1. Resting state functional magnetic resonance imaging (rs-fMRI) group regression analysis in group 1 on an FSL template, showing decreased connections of the right cuneus (in blue in **A**) and increased connections of the left precuneus (in orange in **B**) after intervention. (FSL template is opposite to radiologic convention and left side of the brain is to the left side of the reader).

shown in Table III. Among all these tests, a significant difference between baseline and post-test scores was observed for the domain of 'Speed of Processing' (Digit Symbol Test) in group 1 and significant difference between baseline and post-test scores for Focused Attention (Trail Making A) was seen in group 2.

Subjective change report (thematic analysis): In group 1, 100 per cent of the participants reported improvement in their general well being and feeling relaxed/calm/balanced and had reduced anxiety. More than 70 per cent in this group also reported improvements in interpersonal relationships, occupational functioning and attention. They also experienced positive energy and had a positive attitude to life. In group 2, 57 per cent had improved attention and 43 per cent also reported improved general health. No significant mood changes were reported in this group.

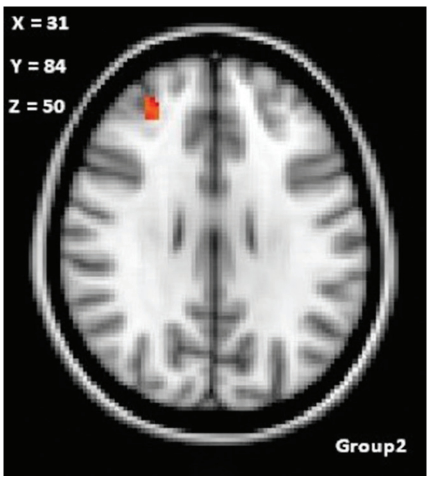

Fig. 2. Resting state functional magnetic resonance imaging (rs-fMRI) group regression analysis in group 2 visualised on an FSL template, increased connections along anterolateral aspect of the left frontal lobe (in orange) is noted after exercise. (FSL template is opposite to radiologic convention and left side of the brain is the left side of the reader).

Single-photon emission computed tomography (SPECT) brain data: In group 1, overall improvement was found in frontal lobe perfusion (Fig. 3) post-intervention in almost all participants, and in a few in parietal lobes (Table IVA). In group 2, overall improvement in perfusion was seen more in parietal lobes than in frontal lobes (Fig. 4 and Table IVB).

Discussion

Resting state fMRI (rs-fMRI) or functional imaging, based on BOLD is used to evaluate regional interactions and networks in the brain when an individual is not performing an explicit task[13]. Resting-state functional connectivity research has revealed a number of networks in healthy individuals. Two main networks[18-20] that have been extensively studied are (*i*) DMN, which is a network of brain regions that are active when an individual is at rest and awake but when not actively engaging with the outside world or any external task, and (*ii*) Task-Positive Network (TPN), when an individual performs an external task, which is goal oriented. The DMN gets deactivated when the TPN is active and positively correlates when the focus from outside world shifts to daydreaming, envisioning the future, retrieving memories or mind wandering.

The subsystems[6,21,22] found in DMN are parts of medial temporal lobe, mainly the hippocampus and

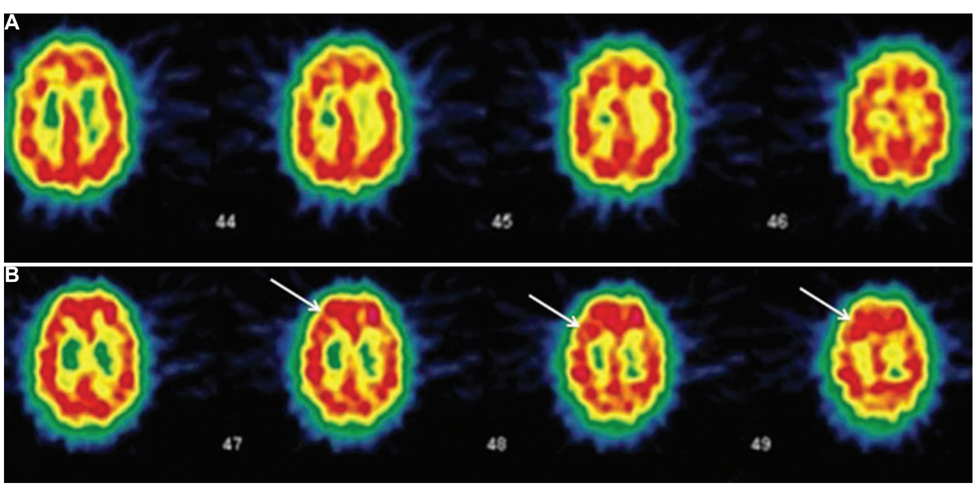

Fig. 3. Ethyl cysteinate dimer single-photon emission computed tomography (ECD SPECT) brain-group 1 before (**A**) and after three months (**B**), showing improvement in perfusion in bilateral frontal regions (arrows).

Table III. A comprehensive analysis of almost the entire neuropsychology data in both groups

Domain	Test	Within group analyses (Wilcoxon Signed-Rank Test) Group 1 Z (asymptotic significant)	Within group analyses (Wilcoxon Signed-Rank Test) Group 2 Z (asymptotic significant)	Between group analyses (Mann-Whitney U-test) U (asymptotic significant)
Attention	Digit span forward	−0.259 (0.796)	−0.530 (0.596)	21.000 (0.646)
	LDSF	−0.414 (0.679)	−0.849 (0.396)	19.000 (0.471)
	Spatial span forward	−0.516 (0.606)	0.000 (1.000)	18.000 (0.395)
	Trail making A	−0.677 (0.498)	−2.132* (0.033)	17.500 (0.367)
	Trail making B	−0.254 (0.799)	−1.355 (0.176)	15.000 (0.223)
	Digit vigilance	−0.507 (0.612)	−0.676 (0.499)	23.000 (0.848)
Processing speed	Symbol search	−0.841 (0.400)	−1.022 (0.307)	13.000 (0.139)
	Coding	−2.366* (0.018)	−1.363 (0.173)	9.000* (0.047)
Working memory	Digit span backward	−0.638 (0.524)	−1.127 (0.260)	16.500 (0.293)
	LDSB	−0.552 (0.581)	0.000 (1.000)	21.000 (0.645)
	Digit span sequencing	−1.219 (0.223)	−1.382 (0.167)	12.000 (0.106)
	LDSS	−1.134 (0.257)	−1.414 (0.157)	11.500 (0.084)
	Spatial span backward	−0.846 (0.398)	−0.632 (1.000)	24.500 (1.000)
	N back 1	−1.414 (0.157)	−1.000 (0.317)	21.000 (0.530)
	N back 2	−0.750 (0.453)	−1.186 (0.236)	13.500 (.152)
	PASAT 3”	−1.187 (0.235)	−0.736 (0.462)	23.000 (0.848)
	PASAT 2”	−1.183 (0.237)	−1.439 (0.150)	23.500 (0.898)
	Digit span total	−0.420 (0.674)	−0.341 (0.733)	20.500 (0.607)
	Spatial span total	−0.085 (0.932)	−0.531 (0.595)	21.500 (0.697)
Scale	DSES	−1.703 (0.089)	−0.423 (0.672)	17.000 (0.337)

*P<0.05 between baseline and post-test scores. LDSF, longest digit span forward; LDSB, longest digit span backward; LDSS, longest digit span sequencing; DSES, Daily Spiritual Experience Scale; PASAT, paced auditory serial-addition task

Table IVA. Data of ^{99m}Tc ethyl cysteinate dimer single-photon emission computed tomography (ECD SPECT) result with areas of improvement in perfusion in group 1

Group	1st observer (%)	2nd observer (%)
Group I		
B/L frontal	4/7 (57.14)	6/7 (85.71)
Right frontal	2/7 (28.5)	1/7 (14.28)
Left frontal	1/7 (14.28)	
B/L parietal	2/7 (28.57)	1/7 (14.28)
Left parietal	3/7 (42.85)	4/7 (57.14)
Overall		
Frontal	6/7 (85.71)	7/7 (100)
Parietal	5/7 (71.42)	5/7 (71.42)

Inter-observer variation: Correlation coefficient κ=1.00. B/L, bilateral

Table IVB. Data of ^{99m}Tc ethyl cysteinate dimer single-photon emission computed tomography (ECD SPECT) result with areas of improvement in perfusion in group 2

Group	1st observer (%)	2nd observer (%)
Group II		
B/L frontal	4/7 (57.14)	2/7 (28.57)
Left frontal	1/7 (14.28)	
B/L parietal	5/7 (71.42)	2/7 (28.57)
Right parietal	1/7 (14.28)	
Left parietal	3/7 (42.85)	2/7 (28.57)
Overall		
Frontal	5/7 (71.42)	2/7 (28.57)
Parietal	7/7 (100)	4/7 (57.14)

Inter-observer variation: Correlation coefficient κ=1.00. B/L: bilateral

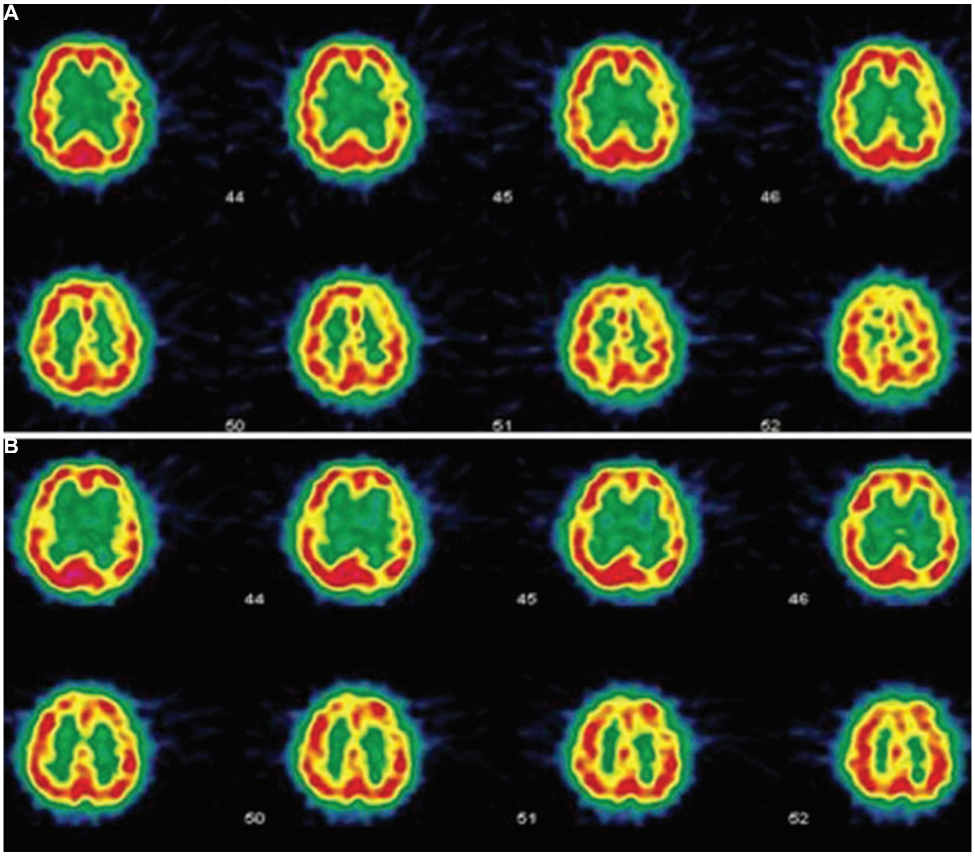

Fig. 4. Ethyl cysteinate dimer single-photon emission computed tomography (ECD SPECT) brain-group 2 before (**A**) and after three months (**B**), showing no significant changes in the frontal regions.

amygdala, (hippocampus for memory and amygdala as a source of emotional content), medial prefrontal cortex (emotional sensor, which processes social and emotional information), posterior cingulate cortex/precuneus (PCC - for emotional integration, integrates self-perception and emotionally relevant memory retrieval) and ventral precuneus, with adjacent infero-lateral parietal cortex (executive network). Some other regions such as middle frontal gyrus and middle temporal gyrus have also been described. It has been hypothesized that DMN generates spontaneous thoughts during mind-wandering and may relate to creativity. It exhibits the highest overlap in its structural and functional connectivity and appears to be activated by default[22].

In our study, increased connections of the left precuneus (part of the PCC of DMN) were found in group 1 after the intervention. The relevance of decrease in right cuneus activity in this group was uncertain. The PCC/precuneus area of DMN acts as an emotional integrator[21] and has widespread connectivity, involving higher association regions, suggesting an important role in integrating both internally and externally driven information. Cavanna and Trimble[23] have proposed that precuneus is involved in the interwoven network of the neural correlates of self-consciousness, engaged in self-related mental representations during rest. Precuneus has also been called as the 'Mind's Eye'[24]. Increase in DMN activity has also been observed in other studies with meditation[25-28], more so in the medial prefrontal cortex with some showing involvement of precuneus as well[28]. Brewer *et al*[29], however, have shown a decrease in DMN activity, especially in long-term meditators.

Both spiritual processes and physical exercises affected different areas in the brain. Group 2 individuals were found to have increased connections in the anterolateral aspect of the left frontal lobe, which is in the region of executive networks. Other studies have published similar findings where, walking increased functional connectivity between aspects of the frontal, posterior and temporal cortices within the DMN and a Frontal Executive Network[30].

Studies that have investigated the relationship between long-term mindfulness meditation and cognition have found that the meditation leads to enhanced cognitive capabilities[31,32]. More specifically, studies have shown that meditation training improves functioning in domains such as attention[33,34], working memory[35] and speed of information processing[36]. Zeidan *et al*[31] found that similar to the reported effects of a long-term meditation on attention, even four days of meditation elicited improvements in sustained attention capabilities.

In our study, group 1 individuals had significant improvements in the domain of speed of processing (improved scores on the digit symbol test), an effect that was not observed in the control group. Processing speed is critical for performance on complex cognitive tasks and is the key ability that underlies and facilitates all cognitive processing.

In our study, different meditative techniques were taught to the participants in group 1, and in the subjective report, all reported feeling relaxed and calm. It has been suggested that relaxation is possibly an important mind state that enhances performance for incidental learning[37]. It may be postulated that the state of heightened awareness with relaxation due to the transformation and meditation practice has facilitated the improved performance on the digit symbol test.

Cognitive processing speed has been found to be closely related to the integrity of the white matter pathways. In one study, comparing scores of the digit symbol test with fractional anisotropy (FA), a correlation between performance on the test and FA of white matter in parietal and temporal lobes and left middle frontal gyrus was found[38]. Although diffusion tensor imaging was not a part of our study, in our fMRI data, the left medial parietal region in the region of the posterior cingulate cortex-PCC/left precuneus of the DMN, showed increased connection in group 1. Therefore, it is possible that improved performance on the speed of processing test may be related to the increased connectivity in this region of the brain. This region is also regarded as the emotional integrator in the brain, and we found improved mood in all participants in group 1.

In group 2, there was significant improvement in the scores of the focused attention test - Trails A and in the subjective report significant improvements were reported primarily in attention and to an extent in general health. The finding of specific improvement in focused attention on objective testing and its relation to the subjective report was an interesting finding. Further, the fMRI findings for the group 2 individuals showed increased connection in the left anterolateral frontal region, the region of attention and executive networks of the DMN.

There were no significant score shifts on the DSES. It is possible that the type of items in the standardized scale may not have captured the kind of positive changes experienced by the groups.

SPECT brain perfusion imaging performed in group 1 individuals suggested that the frontal cortex was activated with improvement in perfusion in frontal lobes due to SP. There were studies that hypothesized the possibility of decreased activity in the parietal lobes during meditation, which was based in part on reports that demonstrated decreased activity in parietal lobes during meditation practices[39]. It is also known that parietal regions are associated with spatial processing, and since alterations in the sense of self and spatial orientation are subjectively changed during meditation practices, changes in parietal lobes might be expected[40]. Newberg *et al*[41] studied changes in regional cerebral blood flow (rCBF) during the complex cognitive task of meditation using SPECT in eight experienced Tibetan Buddhist meditators and found increased rCBF in the cingulate gyrus, inferior and orbital frontal cortex, dorsolateral prefrontal cortex (DLPFC), and thalamus. Lutz *et al*[42] have suggested that the practice of meditation for long period can cause longer-lasting changes in the brain. In our study, we expected to observe relatively higher frontal lobe CBF in group 1 because previous studies have reported increased activity in the frontal lobe regions during active meditation[41]. In addition, the frontal lobes have been suggested as an important mediator in the sense of self and altered states of consciousness[41,42]. We expected to see relatively more parietal lobe activity in group 2 individuals since these are associated with visuospatial orientation, and perhaps regular walk is also associated with the subjective experience of personal space. Higher CBF was observed in the parietal regions in a few individuals in group 2.

In conclusion, SPs appear to have an impact on the neural networks, brain perfusion, cognitive processing and sense of well-being. Although the exact mechanisms of ST are not yet clearly delineated and fully understood, the preliminary findings are promising, and pave the way for further research to understand the role of ST in enhancing human potential and holistic health.

***Acknowledgment*:** Authors thank Dr C. Balkrishnan, P.D. Hinduja Hospital, Mumbai for technical support in writing this article.

***Financial support & sponsorship*:** Authors acknowledge P.D. Hinduja Hospital Medical & Research Centre, Mumbai for financial support.

***Conflicts of Interest*:** None.

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