Use of a Functional Neuroimaging Battery for the Evaluation of a Meditation Retreat

1. General Information

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Use of a Functional Neuroimaging Battery for the Evaluation of a Meditation Retreat

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1.6 Physician Responsible for Trial Site Related Medical Decisions

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2. Background Information

2.1 Test Article

The test article used in this study is DaTSCAN (GE Medical) which is an approved product for SPECT imaging and will be utilized in the same manner, dose, and route of administration.

2.2 Findings from Non Clinical and Clinical Trials

Serotonin Transporters.

Serotonin, or 5-hydroxytryptophan (5-HT), is a neurotransmitter found in the brain, spinal cord, and enteric nervous system. Serotonin transporters (5-HTT) are macromolecular complexes which are designed to remove serotonin from the synaptic cleft and move it intact back into the neuronal cytoplasm where it can be repackaged for re-use or metabolized. Serotonin transporter sites are located on 5-HT nerve terminals and on somatodendritic sites on 5-HT cell bodies. They are widely distributed throughout the brain, but particularly dense on GABAergic and peptidergic neurons. Human studies have indicated that the distribution can be very heterogeneous. Animal studies using [^{3}H]cyanoimipramine, a radioligand developed at the University of Pennsylvania (Kovachich 1992; Gurevich 1996) demonstrated a high density of serotonin transporter sites a number of regions including the thalamus, hypothalamus and portions of the amygdala. There are also patches of high density in the ventral striatum and nucleus accumbens. In the cortex, relatively high binding is observed in cingulate gyrus and in the occipital lobe. There are apparently no serotonergic receptors in the cerebellum. These findings show that serotonin transporters are found in some of the most strategic regions of the primitive and neolimbic system. Their localization on the amygdala, which mediates rage, and the nucleus accumbens, which participates in the mediation of euphoria, clearly implicate the

serotonergic system in the modulation of normal as well as abnormal emotion. Their localization in the hypothalamus and nucleus accumbens suggests that serotonin plays a role in normal drive states and the abnormal drive states that characterize mood disorders. In fact, the evidence implicating a role for serotonin in the pathogenesis of depression is now considerable. The evidence includes reduced CSF concentrations of 5-HIAA, the principal metabolite of 5-HT; reduced concentrations of 5-HT and 5-HIAA and an increased density of 5-HT2 receptors in post-mortem samples from suicide victims; a decreased density of serotonin transporter sites (5HTT) in post-mortem brain samples from suicide victims (Arango 1995), a reduced level of 5HTT in postmortem brain sections of patients with depression (Perry 1983; Mann 1998); preliminary SPECT studies in depressed patients (Malison 1998); and a relapse in depression when patients who have initially responded to serotonergic antidepressants undergo a tryptophan depletion protocol (Moreno 1999), tryptophan being the precursor for 5-HT.

There is a growing body of knowledge in literature that neurotransmitter systems in the brain cannot be considered in isolation. Alterations in one neurotransmitter system will frequently lead to alterations in other neurotransmitter systems. This has been particularly well documented for serotonin and dopamine. Dopamine neurons in both the VTA and the substantia nigra receive serotonergic input from the raphe nuclei (Bonhomme and Esposito, 1998). In addition, limbic forebrain areas, containing serotonin terminals are linked to the nucleus accumbens via glutaminergic afferents (Cador et al., 1991). Electrophysiological experiments have shown that serotonin exerts an inhibitory influence on mesolimbic dopamine function and that this is mediated via 5-HT2 (probably 5-HT2C) receptors (Prisco et al., 1994). In keeping with these findings acute administration of SSRIs reduces the spontaneous activity of dopaminergic neurons in the VTA (Di Mascio et al., 1998). On repeated administration, 5-HT2 receptors become desensitized and SSRIs no longer inhibit the activity of VTA dopamine neurons. It has been suggested that repeated administration of SSRIs leads to an increased function of the mesolimbic dopaminergic system via an inhibitory effect to its serotonergic input and that it is this effect that is responsible for antidepressant activity (Bonhomme and Esposito, 1998).

Dopamine Transporters

Imaging the dopamine transporter in vivo has become the primary means of assessing the dopaminergic system in controls and several patient populations including movement disorders and depression. Transporter concentrations can now be visualized non-invasively with several radiopharmaceuticals. Investigators from many different institutions have shown that transporter ligands can differentiate patient populations from controls with very high degrees of sensitivity. Quantitative measurements of transporter concentrations appear strongly correlated with several clinical features of the disease. We have developed [Tc-99m] TRODAT-1, a transporter imaging agent with several highly advantageous features. Our research using [Tc-99m] TRODAT-1, demonstrated that drug free uptake values are significantly related to age and several neuropsychological task performance scores in both patients and controls. Unlike other transporter imaging agents, the radiopharmaceutical [Tc-99m] TRODAT-1, is labeled with Technetium-99m, which gives it the potential to be used in conventional clinical settings.

Dopamine participates in the mediation of cognition, emotion, and movement (McHugh 1989; Alexander 1990). Dopaminergic circuits help regulate and coordinate the execution of many complex neuropsychological functions (Leibowitz 1979a, 1979b; Silverstone 1980; Geary 1985; Smith 1988). The dopamine transporter is one of the primary regulators of dopaminergic

tone. The reuptake of intact dopamine molecules from the synaptic cleft is performed by a macromolecular complex which is embedded in the axonal membrane (Giros 1993). It includes a dopamine receptor portion that is exposed to the intrasynaptic milieu. When free dopamine in the intrasynaptic space binds the receptor portion, a conformational change occurs in the structure of the transporter complex which allows dopamine to be physically moved, or "transported", across the axonal membrane against a concentration gradient. The rate at which dopamine is removed from the synaptic cleft may be the primary mechanism for maintaining constant dopaminergic tone. Once removed from the synapse and transported inside the axonal bouton, structurally intact dopamine molecules can then be conserved by being repackaged within the storage vesicles. Alternatively, the level of dopamine, and hence the tone of the dopaminergic system, can be reduced by oxidative enzymes in the cytoplasm that make it unavailable for another transmission.

The presynaptic dopamine transporters are located at the distal-most terminals of dopaminergic axons that originate from neuronal cell bodies located in relatively distant regions of the brain. Transporter concentrations are thought to reflect the vitality of these distant neurons. However, many symptoms produced by the dopaminergic degenerative diseases are attributable to dysfunction in the otherwise healthy neurons they enervate. Reciprocal dopaminergic fibers enervate the basal ganglia, the midbrain, hippocampus, amygdala, mammillary bodies, cingulate gyrus, and the thalamus. This may explain why most Parkinsonian syndromes are characterized by disorders of cognition and mood as well as motor dysfunction (Mayeux 1986; Standaert 1989; Stern 1993; Vingerhoets 1994a, 1994b; Caparros-Lefebvre 1995). Similarly, this may explain why transient dysregulation of the system by dopaminergic drugs like amphetamine and cocaine affects cognition and emotion as well as psychomotoric behavior (Imperato 1992; Wiess 1994). Chronic blockade of the extra-striatal transporters may also contribute to the dysphoric affects and cognitive impairment that are associated with cocaine withdrawal (Goeders 1983; Feldman 1984; Hurd 1989; Kuhar 1991; Robertson 1991). Neuroimaging studies have consistently shown that the concentration of dopamine transporters is decreased in most elderly volunteers when compared to the levels in young adults (Martin 1989; Sawle 1990; Shimuzu 1991; Kaufman 1993; Volkow 1994; Cordes 1994).

DaTSCAN

DaTSCAN is a currently approved radiopharmaceutical that enables measurement of both the serotonin and dopamine transporter in a single scan. DaTscan binds to both the serotonin transporter (SERT) and dopamine transporter (DAT) so that we can measure both neurotransmitter systems with a single scan. DaTscan requires preadministration of Lugol's solution which helps to block the thyroid gland from exposure to the I-123 isotope that is part of DaTscan. This is standard protocol for diagnostic studies of this type. DaTscan is injected intravenously and then SPECT imaging is performed approximately 3 hours post injection. The images can then be quantitatively analyzed to determine SERT and DAT binding by analyzing the midbrain and basal ganglia respectively. SERT and DAT activity has been widely evaluated in a number of disease states such as Parkinson's disease and depression. DaTscan has been one of the major radioactive tracers that has been involved in these studies. DaTscan is therefore an excellent tracer for its cost, availability, and ability to quantify changes in SERT and DAT binding in association with a meditation retreat.

Functional Brain Imaging Studies of Meditation

We are aware of a growing number of reports utilizing functional brain imaging techniques for studying subjects practicing meditation. In two studies, positron emission tomography (FDG-PET) was used to measure changes in glucose metabolism in subjects undergoing Yoga meditative relaxation (Herzog 1990-1991; Lou 1999). A third, functional magnetic resonance imaging was used to measure similar changes in subjects practicing a yoga relaxation technique (Lazar 2000). We have conducted a study of cerebral blood flow utilizing SPECT imaging (see below). One study utilized FDG PET to evaluate the Dopaminergic system during meditation. This latest study demonstrated significant increases in dopaminergic tone in the basal ganglia associated with meditation.

Herzog et al (1990-1991) utilized FDG-PET to measure regional glucose metabolism in subjects undergoing Yoga Meditative Relaxation. In this study of eight meditators there was a significant increase in the frontal: occipital ratio of cerebral metabolism. Specifically, there was only a mild increase in the frontal lobe, but marked decreases in metabolism in the occipital and superior parietal lobes. However, the subjects did not experience deep meditative states and the measurement of cerebral metabolism did not evaluate specified regions were not evaluated. Another study utilized [^{15}O]H_2O PET to evaluate subjects practicing Yoga Meditative Relaxation by following a meditation tape (Lou 1999). These subjects demonstrated relative decreases in the posterior brain structures, but no significant increase in the frontal structures. It may be that this "passive" type of meditation was associated with a lack of change in the frontal regions since subjects were following verbal instructions rather than actively concentrating on the meditation. A study of word generation demonstrated that internally generated words resulted in activation of the prefrontal cortex while repeating words did not (Crosson 2001). We suspect that a similar following of instructions for meditation may not have the same effect as actually performing the practice volitionally. However, other changes in cerebral activity may be similar if the ultimate subjective experience is similar. This appears to be the case since several studies demonstrated increased activity in the limbic structures (in those studies that evaluated these regions). A study utilizing functional magnetic resonance imaging (fMRI) of subjects performing a similar yoga relaxation technique designed to bring about the "relaxation response" demonstrated relative increases in cerebral blood flow in the frontal lobes as well as the limbic system (Lazar 2000).

Our preliminary study of cerebral blood flow utilizing SPECT imaging during Tibetan Buddhist meditation demonstrated a number of complex changes including relatively increased cerebral blood flow (CBF) in the prefrontal cortex, the orbitofrontal cortex, and cingulate gyrus, and relatively decreased CBF in the superior parietal region. The results from this study supported the basic hypotheses in this proposal in that there was increased activity in the structures underlying attention and memory processes (Frith 1991). Furthermore, a study by Lazar et al (2005) demonstrated that experienced practitioners of Insight meditation demonstrated increased cortical thickness in the prefrontal cortex when compared to controls. One potential implication is that meditation practice induces long term changes in the brain, but this has not been widely studied on a functional level. Furthermore, functional changes in metabolism and neurotransmitters should theoretically precede structural changes.

The above described functional imaging studies of meditation suggest that there is a network associated with such practices that includes changes in the attentional system of the brain including the prefrontal cortex, cingulate gyrus, and superior parietal lobes. There also appears to be changes of activity in the limbic areas such as the amygdala, hippocampus, and thalamus. An analysis of functional changes will have implications for understanding complex neurocognitive and affective tasks. Such findings can also assist toward focusing future clinical

studies of meditation techniques on specific disorders known to be associated with the same nervous system structures.

We have chosen fMRI imaging of cerebral blood flow for several reasons. We have increasingly utilized fMRI in our studies and fMRI can provide multiple scans on subjects in the same session. This allows for the ability to compare meditation to baseline as well as to other control conditions.

2.3 Risks and Benefits

Potential Risks of Radiation Exposure: The I-123 label on I-123 DaTSCAN is radioactive, which results in some exposure to ionizing radiation. The amount is acceptable for human research in subjects who will not benefit from the test.

Pharmacological Risks: No pharmacological effects have been encountered in animal models or in human subjects for DaTSCAN.

Special Risk Factors: There are no significant additional risks.

Risks of venous cannulation. Venous cannulation is a routine clinical procedure that carries minimal risks when performed by trained personnel. It is possible that bruising could occur in some subjects. There is a theoretical risk of phlebitis or infection which is very remote.

Blood loss. The maximum amount of blood that will be drawn from each subject will be less than 4 Tablespoons (< 1 fluid ounces). This is less than half the amount of blood that volunteers donate to the Red Cross (450 mls). Additional screening will only be performed if not already performed for clinical purposes.

Teratogenic Effects: Pregnancy Category C

Animal reproduction studies have not been conducted with I-123 DaTSCAN. It is not known whether DaTSCAN injection can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Therefore, DaTSCAN injections should not be administered to a pregnant woman unless the potential benefit justifies the potential risk to the fetus.

Nursing Mothers

The effects of DaTSCAN injection on human breast milk are unknown.

We will not enroll a woman who is nursing or pregnant into the study.

MRI Risk: The fMRI scan does not involve any radiation exposure. Due to the strength of the magnetic field of the MRI, there is a risk of being injured if an unsecured metal object flies into the MRI scanner. In order to minimize this risk, you will be asked to remove all metal objects from your person. Also, all metal objects will be cleared from the area prior to the scan. This is

the standard practice when subjects undergo MRI exams. It is important when discussing the study that you inform the staff if they have any of the following:

- Surgically implanted electrical devices
- Pacemaker
- Surgically placed metallic clips (aneurysm clips)
- Ear implants
- Any history of metal fragments in the eye
- Subjects will be asked to complete a standardized Metals Screening From prior to receiving an MRI. If an MRI is contraindicated the subject will not receive an MRI or fMRI.

Subjects may participate in other studies for research or medical diagnosis involving exposure to radiation. However, they must inform the investigators of their participation in other studies.

Incidental Findings: Any abnormal incidental findings from the scans or other procedures may cause stress and anxiety in the subjects. However, the incidental findings will be discussed with the subject by the study PI or a designated physician. The subject will be counseled on the findings and provided information on how to follow up with his or her neurologist or primary care physician. Any information will be made available to the subject's primary care physician so that it can be properly addressed.

Benefit: The subjects will obtain no medical benefit from this study unless some neurological disease is detected during the study. Subjects will be informed of objective evidence of changes in their brain function using SPECT or fMRI scans. It is anticipated that this study poses minimal risk to the involved subjects as the methods have been extensively used on medical patients and healthy controls.

2.4 Justification for Route, Dose, and Period of Administration

The amount of radioactivity administered will be according to the recommended dosage in the package insert and will be111 to 185 MBq (3 to 5 mCi) for each DaTscan.
Each subject will receive a single DaTscan injection at baseline, and then after a meditation program.

2.5 Compliance

This study will be conducted in compliance with Good Clinical Practices and applicable regulations 21 CFR 50 and 56 and 312, and will be approved by the Internal Review Board.

2.6 Population

Subjects will be healthy controls.

2.7 References

Please see end of protocol for references.

3. Trial Objectives and Purpose

1. To determine if various neuropsychological states related to a meditation retreat program are associated with changes in the serotonin and dopaminergic systems.

2. To determine if there will also be changes in cerebral blood flow using fMRI of that are associated with the dopamine and serotonin transporter activity.
3. To analyze and compare pre and post imaging and neuropsychological changes in healthy controls who participate in a meditation retreat program.

4. Trial Design

4.1 Endpoints

DaTSCAN binding changes, and CBF changes, associated with the pre and post meditation program states.

4.2 Trial Design

Overall:

This study will initially utilize DaTSCAN and fMRI in healthy subjects within one month of entering a meditation retreat program. In addition, they will complete a variety of questionnaires and scales to assess their psychological status. They will then undergo the retreat and will return within one week of completing the retreat to repeat the scans and questionnaires. Thus, they will receive 2 DaTSCAN scans and 2 fMRI scans over the course of the study.

Approximate Timeline for DaTSCAN SPECT

Time	Procedure
09:00 a.m.	Subject arrival. Review informed consent procedures.
09:30 a.m.	beta-HCG (if necessary)
09:45 a.m.	SSKI (Lugol's solution)
10:00 a.m.	Insert i.v. catheter
10:15 a.m.	Inject DaTSCAN
11:00 a.m.	Subject may take a break for 4 hours during which several qualitative scales will be performed
01:15 p.m.	Begin SPECT imaging
02:15 p.m.	SPECT scan completed
02:30 p.m.	Study completed

Medical assessment procedures. A trained physician will take a detailed medical history on each subject and perform a standardized review. Structured physical, neurological, and psychological examinations will be performed. A set of clinical laboratory studies may be used to screen potential subjects with benign histories and normal physical examinations. A urine test for pregnancy will be performed in all women of childbearing potential. Female subjects with negative pregnancy tests will be enrolled.

Quantifying subjective phenomena. This protocol will add the administration of a number of neuropsychological tasks, psychological inventories, and spiritual scales at the time of imaging. These tests and questionnaires should take approximately two hours to complete, and many have already been widely used in both control and patient populations. These well established tests

were originally validated in large populations of people, and have since been used extensively in both clinical and research settings. Our lab has its own normative data base, which has been generated for people of all age ranges. The battery may include the following tests:

The Speilberger State Trait Anxiety Inventory (STAI) contains a total of 40 questions, half of which relate to the way subjects are feeling at the moment and half of which ask them to describe how they usually feel. The Profile of Moods Scale (POMS) will be administered. The Beck Depression Inventory (Beck 1972) is a standard 21 item questionnaire probing cognitive and somatic symptoms of depression. Additional scales may include: Short Form-12; Hood Mysticism Scale (Lifetime); Brief Multidimensional Measure of Religiousness/Spirituality; Gratitude Questionnaire; Daily Spiritual Experience Scale; ASPIRES (self-rated and observer-rated transcendence); Index of Core Spiritual Experiences (INSPIRIT); FACIT-Sp-12; Cloninger Self-Transcendence Scale, and Five Facet Mindfulness Questionnaire.

Women and minorities. While this is a limited study in terms of subject numbers, all efforts will be made to select volunteers that reflect the diverse demographics of the local urban community will be recruited for this project. It should be possible to enroll essentially equal numbers of men and women. All of our previous studies have suggested that it will be feasible to recruit a racially mixed population of subjects that includes African Americans and people whose families originated from the Pacific Rim.

Imaging Procedures.

a) OVERVIEW – All subjects will receive a DaTSCAN SPECT scan and an fMRI scan within 7 days of each other and within one month of beginning the meditation retreat program. fMRI scans will be performed within the same period of time, ideally on the DaTSCAN imaging day. Subjects repeat the neuroimaging battery and neuropsychological tests to evaluate for change after the retreat program. Subjects may engage in various activities or practices in between the scans in order.

b) DaTSCAN SPECT ACQUISITION, PROCESSING, AND ANALYSIS – Subjects will be asked to arrive at the Nuclear Medicine SPECT Center in the morning. A signed consent form will be documented after all questions have been answered. Women of child-bearing potential *must* have a negative pregnancy test within 48 hours *before* proceeding with the SPECT study. Subjects will be given 16 drops of concentrated Lugol's solution in order to block the thyroid. An intravenous catheter will be inserted and capped. Subjects will spend 20 minutes lying supine on an examination table before the radiopharmaceutical is administered to allow them to recover 'physiologically' from the stress of the catheter insertion. 111 to 185 MBq (3 to 5 mCi) ^{123}I DaTSCAN will then be administered intravenously. After injection of the ^{123}I DaTSCAN, the subject will have the intravenous catheter removed. They will be able to take a break for lunch. Subjects will then return to the nuclear medicine imaging room 3 hours after ^{123}I DaTSCAN administration for a 60 minute brain scan. The reason for this delay is that kinetic modeling data performed on ^{123}I DaTSCAN has demonstrated that estimation of the binding potential can be made without arterial sampling at this time period (Acton 1998). The subject can be moved from the imaging table after the SPECT study is completed.

All SPECT images may be coregistered with the corresponding anatomic MRI and a previously described template (Resnick 1993), focusing on selected ROIs, will be fit on each

MRI and copied onto the DaTSCAN SPECT images. These ROIs will include the basal ganglia, amygdala and hippocampus, thalamus, brain stem, and cerebellum. To reduce the effects of volume averaging in the axial direction, ROIs will not be placed on the slices that contain the upper most and lower most portions of the structures they represent. This will tend to limit the small ROIs to the central aspect of structures they represent. The primary outcome measure will be the SERT and DAT binding ratios in which the ROI is compared to a reference region (cerebellum) at 4 to 5 hours post administration, when the distribution of ^{123}I DaTSCAN has approached a transient, near equilibrium-like state that reflects the k3/k4 ratio and is related to the binding potential. This allows for a quantitative assessment of SERT binding as described in the Preliminary Studies section.

The reference region has typically been the cerebellum because this region has such low activity on ^{123}I DaTSCAN scans and no appreciable DAT or SERT receptors. However, values will be obtained for both whole brain regions above the basal ganglia and cerebellar regions in order to confirm the findings. The procedures will be implemented with a commercial statistical package (StatS, Think Point Software, Inc). The reliability of SPM will be evaluated similarly by having two different technicians run the analyses independently. We have found the reliability of the image analysis techniques to be high with intra-class correlations consistently above 0.95.

c) MR image acquisition: Subjects will be studied to evaluate the effects of the meditation retreat program on cerebral activity using fMRI. This approach will enable us to track changes and activation in cerebral activity that result from the program. This data will be correlated to standardized neuropsychological and behavioral measures of cognition, mood, and spirituality.

The fMRI images will be co-registered and comparable slices of the cerebral cortex will be examined pre and post program. We will evaluate changes in baseline brain function while the subject is at rest as well as during the meditation practice itself. Two different analysis approaches will be used since they can provide different types of information. A region of interest analysis will be performed to assess changes of activity in specific brain regions. A comparison between meditation and baseline cerebral activity will indicate which regions of the brain are activated at various stages of meditation.

Images will also be analyzed utilizing a semiautomated method such as statistical parametric mapping (SPM). This provides a voxel by voxel analysis in order to assess differences between scan states. Voxel by Voxel analysis is also referred to as voxel based morphometry (VBM). Brain morphometry starts with noninvasive brain imaging typically obtained from magnetic resonance imaging (MRI) to measure brain structures and changes in the brain. VPM helps to identify structural abnormalities that are found in persons with Alzheimer's disease and other disorders. VBM procedure involves normalizing structural (anatomic) brain images to a standard template, segmenting those into gray and white matter. In healthy subjects VPM assists in understanding the changes in the brain associated with learning and the effect of specific practices activation of brain regions**.** This method can provided a more detailed assessment, but is sometime over conservative in its results. For these reasons, we typically perform and report both types of analysis.

4.3 Minimization of Bias

Scans will not be identified by name so that the reviewer can be blinded to the subject's clinical condition. When such blinding cannot be maintained as determined by the principal investigator,

an impartial reviewer who has had no connection with the subject or the subject data will confirm the analysis.

4.4 Dosage and labeling

SSKI. Subjects will be administered 130 mEq of a potassium iodide solution before administration of the tracer. The KI will be supplied by the Hospital pharmacy as per USP, either 16 drops of a 5% wt/vol Lugol's preparation or 5 drops of a super saturated potassium iodide solution.

[I-123] Radioactivity. The dose of 111 to 185 MBq (3 to 5 mCi) for each DaTscan [I-123] will be administered for each scan and will be given as per the package insert with regard to dose and route of administration. The amount of radioactivity in each syringe containing [I-123] DaTSCAN will be measured in a Capintec CRC Radioisotope Calibrator before and after injection.

4.5 Duration of Participation

Subjects will undergo the SPECT and fMRI scan in one day and will be followed for the course of that day. Subjects will also be scanned following the meditation retreat program. Thus, subjects will undergo a second SPECT and fMRI scan and will be followed for the course of that day.

4.6 Discontinuation Criteria

Individual Subjects: Since I-123 DaTSCAN is a commercially approved diagnostic radiopharmaceutical, it is not anticipated that there should be any pharmacological response that would necessitate stopping an individual from participating in the scanning procedure. In the event that the subject cannot tolerate lying still in the scanner due either to pain or claustrophobia, then efforts will be made to allow for mild pain relief medications or sedation of the subject, as determined by their referring physician, and provided that they have a person who can transport them home after the scan, or the subject may withdraw from the study without prejudice. If the subject develops a systemic allergic reaction such as generalized hives, shortness of breath or hemodynamic compromise, the current scanning session will be terminated and the subject will no longer participate in the study. However, the subject will continue to be followed from a safety and clinical perspective at six months after the adverse event or every six months until the event resolves.

4.7 Accountability Procedures

The drug will be obtained from GE Healthcare and handled by the Nuclear Medicine department using the standard procedures for clinically approved radiopharmaceuticals.

4.8 Randomization Codes

Since this is a diagnostic study, subjects are not randomized.

4.9 Case Report Forms

All safety and scan procedural information, including radiopharmaceutical information and dose, adverse events, and subject reports will be included in the case report forms and

adverse event forms as indicated. Subject source data including exam findings, medications, and prior radiographic and scintigraphic studies when deemed necessary by the principle investigator, to assess proper use of inclusion and exclusion criteria will also be included and to ensure all criteria are met (see below).

The DaTSCAN and fMRI scans and the qualitative and quantitative data will be kept as computer files and will not be a part of the case report forms.

5. Selection and Withdrawal of Subjects

5.1 Subject Inclusion Criteria

a. Subject selection

Subjects will be given informed consent at the Myrna Brind Center of Integrative Medicine or in the Department of Nuclear Medicine on the day of the study. After signing informed consent, all subjects will receive a physical, neurological, and psychiatric evaluation to ensure that none of the study subjects has a disorder that might affect cerebral metabolism, blood flow, or other brain functions (i.e. stroke, tumor, epilepsy, Axis I psychiatric disorders). Screening laboratory studies assessing electrolytes, complete blood cell count, liver function, thyroid function tests, pregnancy test if necessary, and urine toxicology screen may be obtained in order to confirm the subject meets inclusion criteria as determined by the PI.

Subjects will be excluded if: (1) they are taking medications that may alter cerebral blood flow as per the P.I., although supplements and medications to aid with mild dementia will be allowed if on a stable regimen, (2) they cannot lie still in the scanner due to physical or psychiatric reasons as per the P.I., or (3) they have physical, neurological or psychological disorders that might affect cerebral metabolism or blood flow as per the Principal Investigator. Female subjects of childbearing potential who are not pregnant or breastfeeding will be included in the study if they have received a negative pregnancy test (blood or urine) within 48 hours from the day of the study.

The subjects will meet the following criteria:

Inclusion criteria.

1. Able to give informed consent and willing to complete the study.
2. Willing to undergo the full imaging procedures.
3. Women of childbearing potential with a negative serum pregnancy test.

Exclusion criteria.

1. Any neurological or psychiatric disorders, including drug or alcohol abuse, that may interfere with cerebral blood flow as determined by the principle investigator.
2. Any medical conditions that may interfere with cerebral blood flow as determined by the principle investigator.
3. Currently taking medication that might affect cerebral blood flow (i.e. antidepressants, antipsychotics, anxiolytics, benzodiazepines, sedatives, antiseizure medications)
4. Subject is unable or unwilling to lie still in the scanner (i.e. due to claustrophobia or weight > 350 pounds)

5. Subject has metal in their body or other reason that they cannot undergo magnetic resonance imaging.
6. Previous brain surgery or intracranial abnormalities they may complicate interpretation of the brain scans (e.g., stroke, tumor, vascular abnormality).
7. Pregnancy.
8. Allergy to iodine or shellfish.
9. Concurrent participation in another research protocol that might affect the outcome of this study as determined by the principle investigator.

b. Sample Size

We anticipate recruiting up to 30 subjects for the study.

5.3 Subject Withdrawal Criteria

5.3.a. When and How to Withdraw Subjects

a. The subject cannot tolerate lying still in the scanner due either to pain or claustrophobia that cannot be corrected by pain medications or sedation of the subject.
b. The subject develops a systemic allergic reaction.
c. The subject experiences significant hemodynamic compromise that requires medication and hospitalization.
d. The subject is free to withdraw at any time they choose without affecting their continuing care for their illness.
e. If any of the above criteria are met, the subject will no longer undergo DaTSCAN scans. A discontinuation form will be completed and placed in the subject's binder.
f. If a subject is withdrawn, they will be requested to continue follow up only if they experienced an adverse event as per the protocol.

5.3.b. Type and Timing of Data Collection

1. All data will be collected at the time of the scanning procedure and at the time that it is determined that the subject is to be withdrawn from the study.

2. Any data from the scan will be removed from the data analysis due to possible confounding of the actual scan data related to the subject's inability to complete the scan.

3. Clinical data that can be used as part of the study can continue to be evaluated and the subject may continue with a clinical arm of the trial. However, the subject's data will not be included in any analysis of DaTSCAN or fMRI scan findings and their relationship to clinical variables.

5.3.c. Subject Replacement

Subjects that cannot or do not complete the study will be replaced in order to obtain enough data. If a subject experiences a serious adverse event directly related to the DaTSCAN or fMRI scan, then no further subjects will be recruited until the cause of the event is determined and it is ensured that no future such events will occur.

5.3.d. Follow up of Withdrawn Subjects

1. They will continue to have medical follow up as necessary.

6. Treatment of Subjects

6.1 Treatment to be Administered

DaTSCAN is not a therapeutic radiopharmaceutical. However, as a diagnostic agent, it will be administered intravenously in a single bolus.

6.2 Other Medications Permitted and Not Permitted

This is a study of healthy controls. Medications will be reviewed by the Principal Investigator prior to imaging. Subjects may not have taken Prozac for 6 weeks, SSRIs for two weeks. Vitamins, over the counter drugs and herbal supplements will also be evaluated.

6.3 Subject Compliance

Since the DaTSCAN SPECT and fMRI scans are given only in the hospital setting, all subjects will necessarily be compliant.

7. Assessment of Efficacy

7.1 Specification of Efficacy Parameters

This study will determine if there is a change in DaTSCAN related to meditation programs.

7.2 Methods and Timing for Evaluating Efficacy

We will evaluate changes in DaTSCAN and regional CBF related to the meditation programs before and after the program. The scans are performed in a single day.

8.1 Specification of Safety Parameters

Safety Assessment: DaTSCAN is a commercially approved tracer so this study is not designed to specifically evaluate safety. However, subjects will be asked if they experienced any unusual effects during the study and the debriefing period. Any adverse events will be recorded in the subject's data, either in the case report form or on the serious adverse events form.

Safety Evaluation: Adverse events will be recorded in the subject's data. Additional side effects and symptoms will be recorded in each subject's case report forms. This data will be pooled at the conclusion of the study in order to assess any additional safety concerns with the radiopharmaceutical or the imaging procedure. It should be noted that any study participant who experiences a serious adverse event directly related to the DaTSCAN will be excluded from further study using this radiopharmaceutical. For the purposes of this study, any adverse events or serious adverse events occurring within the duration of the study procedures that include the administration of DaTSCAN and the SPECT scan will be recorded and reported as indicated by regulation and IRB policy. However, events occurring after the SPECT scan dates and prior to the follow up scan dates will not be considered adverse events for the purposes of this study.

8.2 Methods and Timing for Evaluating Safety

Since all safety monitoring of DaTSCAN will be performed on the day of the scan, safety information will be available on an immediate basis. Should a significant subject reaction occur during the administration of the DaTSCAN, no additional studies will be performed until the specific batch of DaTSCAN can be fully analyzed and tested for purity, pyrogenicity, and sterility by GE Medical. If it is determined that there was not a problem with the DaTSCAN product, the subject will be withdrawn from the study and additional subjects may be injected and scanned with DaTSCAN. If however, there is a problem with the batch of DaTSCAN, no studies will be performed until the problem with the batch can be evaluated and corrected in future batches.

8.3 Procedures for Eliciting Reports and Reporting Adverse Events

Subjects will be evaluated during the injection period, immediately post injection, and prior to leaving on the day of imaging with a brief examination of the injection site and with a clinical evaluation to determine if the subject had any unusual experiences during the injection or scanning procedure. Subjects will be contacted by phone approximately one week after the scanning in order to assess for any further adverse events. For the purposes of this study, any adverse events or serious adverse events occurring on the day of administration of DaTSCAN and scan will be recorded and reported as indicated by regulation and IRB policy. After the imaging day , the subject will be considered to have completed the study and will not longer be followed.

8.4 Type and Duration of Follow-up of Subjects After Adverse Events

Subjects will be followed six months after the injection if they experienced an adverse event directly related DaTSCAN. If there is no further problem, this will terminate the follow up. However, if the subject continues to have problems directly related to the DaTSCAN, then they will continue to be followed at six months intervals until the adverse event resolves or until the subject's death.

9. Statistics

9.1 Description of Statistical Methods

Region of Interest Analysis.

The images of each of the scans will be reconstructed and resliced, using an oblique reformatting program, according to the anterior-posterior commissure line. The SPECT images will be co-registered with the anatomical MRI and comparable slices of the cerebral cortex will be examined. A previously validated template methodology consisting of regions of interest (ROI) corresponding to the major cortical and subcortical structures will be placed over the scans (Resnick 1993). For the purposes of this study, we will examine the regional CBF, serotonin transporter and dopamine transporter activity in only a selected number of ROIs which will be hypothesis driven. The ROIs to be examined based on the hypotheses described above and the preliminary findings will be the inferior frontal, superior frontal, dorsolateral prefrontal, orbitofrontal, dorsal medial cortex, inferior temporal, amygdala, hippocampus, superior parietal, inferior parietal, occipital, and sensorimotor areas, as well as the caudate, putamen, thalami, midbrain, hypothalamus, cerebellum, and cingulate gyrus regions. Each ROI will have its placement adjusted manually in order to achieve the best fit according to the atlas. The ROIs will then be copied directly onto each of the scans. This will be possible because the images will

already resliced into the same planes as described above. Counts will be obtained for each ROI and normalized to the mean whole brain counts. This will result in ratios of activity in each ROI will be compared across scans for each neuropsychological state. Other covariables such as age, and psychological measures, can also be included in the statistical analysis. The cerebral structures will be identified by their Talairach coordinates (Talairach 1988).

Statistical parametric mapping (SPM):

The traditional method for analyzing brain fMRI and SPECT images involves the ROI method described above. However, it has long been known that ROI definition, particularly on functional images, is time-consuming, subjective and prone to operator bias. Small regular ROI's are capable of resolving localized focal changes between images, although they are subject to a loss of specificity due to the problems associated with multiple comparisons. Alternatively, large ROI's encompassing an entire brain structure may dilute small activation foci, with a subsequent loss of sensitivity. Unless a study is very strongly hypothesis driven, and comparisons between images are only being made in highly specific regions, ROI analysis lacks the power to accurately distinguish regional variations between a set of images. The solution to this problem has been the development of pixel-based statistical analyses, where the regions are effectively reduced to single pixels (Acton 1998). This method can also be utilized to observe changes in structures not included as part of the ROI analysis.

Therefore, SPM2 software (Wellcome Department of Cognitive Neurology, London, UK) will also be performed in which the image volumes of transverse slices will be made compatible with SPM by creation of usable headers for the images. To begin, a file will be created for each image that contains data on image size, number of slices, pixel depth, maximal pixel value, and voxel size. SPM analysis then requires 1) spatial normalization of all images into the same stereotactic space, 2) smoothing, and 3) statistical parametric comparisons. Spatial Normalization will begin by having the scans from each individual subject will be coregistered to each other, using a rigid-body transformation. To correct for global brain shape differences, the individual MRI volume acquisitions will be transformed into a standard 3D space (MNI, provided by SPM). The images will be coregistered to a MNI atlas template, using affine transformations and nonlinear warping ($7 \times 8 \times 7$ basis functions). The resulting spatially normalized images had isotropic voxels of size 2 mm. The normalized images will be smoothed by convolving with an isotropic FWHM 12 mm Gaussian kernel that allows the subsequent voxel-by-voxel analysis. This reduces the effects of image noise and conditions the data for subsequent statistical tests performed in SPM.

Groups of images will be compared voxel-by-voxel using SPM (Friston 1995). Statistically significant differences between sets of data will be assessed at each voxel with a threshold of $P < 0.001$. To correct for correlated multiple comparisons, clusters of voxels which survive this threshold will be assessed further using the theory of random Gaussian fields (Friston 1994, 1995b; Worsley 1994), which calculates the significance of clusters based on their peak height and spatial extent. With this technique, differences in blood flow between and within groups will be assessed, and statistic images generated showing significant regional differences between groups. The meditation or activation state can be modeled by a simple one-way analysis of covariance (ANCOVA) with a group effect (the activation) combined with a nuisance or confounding effect (global or reference region activity) to produce the observed data. Other covariables such as age, and psychological measures, can also be included in the statistical

analysis. The cerebral structures will be identified by their Talairach coordinates (Talairach 1988).

9.2 Number of Subjects

This is an exploratory study and thus a full power analysis will be based upon the determination of the effect size obtained from this data. However, preliminary analyses of our studies in other neuropsychological states of subjects as well as in differences in our serotonin and dopamine transporter binding differences between various populations and treatment groups, we conservatively estimate that the mean differences/changes in these values are approximately 10% with a 15% standard deviation. A sample size of 25 subjects would have 80% power to detect such an effect size using a two group t-test with a 0.05 two-sided significance level. For sample size based on correlation analysis, we typically have considered an R=0.5 to be of clinical importance and have frequently obtained such as measure in comparing various neuropsychological test results to imaging measures. A power calculation to detect a correlation between imaging and neuropsychological measures for an R=0.5 would require a sample size of 30 subjects. Thus, given our usual difference and standard deviation between pre and post studies as well as correlational analysis, a sample size of 25 subjects seems appropriate.

9.3 Level of Significance

A significance value of $p<0.05$ will be required for all statistical measures.

9.4 Criteria for Termination of the Trial

The study will be terminated only if there are serious adverse events that occur in the subjects. Otherwise, we anticipate terminating the trial after 25 subjects are recruited and studied.

9.5 Procedure for Accounting for Missing, Unused, or Spurious Data

Missing or unused data will be excluded from analysis and reported in the subject's study binder. However, other data from that subject will be included in the analysis where appropriate and also for all safety evaluations. Spurious data will be evaluated by a statistician to determine how such data should be handled from a research reporting perspective. However, any data that is spurious will be provided to the subject's physicians in the event that it provides useful clinical information.

9.6 Procedures for Reporting Deviations from the Original Statistical Plan

All statistical analyses and deviations from the original statistical plan will be reported in any presentation (i.e. publications, oral, or poster presentations) of the results from this study.

9.7 Selection of Subjects to be Included in the Analysis

All subjects entered into this study will have their data included in the imaging analysis unless there is relevant missing data or the subject drops out of the study so that critical data points are not available.

All subjects will be included for the safety analysis even if there are some missing data points (i.e. imaging or clinical).

10. Direct Access to Source Data

Study related monitoring, audits, Institutional Review Board, Institutional Ethics Committee, and regulatory inspections will all have direct access to the source data.

11. Quality Control and Quality Assurance

The principle investigator will review all data in the case report forms and this data will also be evaluated as part of the periodic monitoring plan (see attached).

12. Ethics

This study will be performed under the auspices of the Institutional Review Board (IRB) and the Office of Human Research. All protocols and consent forms will be approved by the IRB prior to performing any studies. Any ethical issues that arise during the study will be brought to the immediate attention of the principle investigator who will hold all studies until an appropriate course of action can be undertaken to ensure that the study is performed only after such ethical issues are resolved. All investigators and personnel will also have taken the Thomas Jefferson University training programs for performing Human Research.

This study will be carried out in compliance with the protocol and in accordance with the standard operating procedures. These are designed to ensure adherence to Good Clinical Practice as described in:

1. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations.
2. Declaration of Helsinki, concerning medical research in humans (recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

The investigator agrees, when signing the protocol, to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

13. Data Handling and Recordkeeping

Data and record keeping will be overseen by the sponsor/investigator. The sponsor/investigator will also maintain the individual subject's study binder which includes source data and safety data in a secure location. The sponsor/investigator will also keep all imaging studies.

14. Financing and Insurance

Financing of this study will be partially funded by a grant from the Fetzer Foundation.

15. Publication Policy

The results will be published in peer reviewed scientific journals and will not identify any subjects by name.

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