Connectomic Analysis in Dementia with Lewy Bodies: A Potential Diagnostic Imaging Biomarker

Short Title: DLB rsfMRI

Protocol Number: Version 5

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CONFIDENTIALITY STATEMENT

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1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	Connectomic Analysis in Dementia with Lewy Bodies: A Potential Diagnostic Imaging Biomarker
IRB Number:	A#_21-037_____
Study Description:	This is a cross-sectional study in patients with Lewy Body Dementia (DLB) with two comparison groups (AD and cognitively normal controls) drawn from a publicly available database called ADNI-2/3. We intend to study dysfunctional large scale brain networks (LSBNs) in DLB by comparing resting state functional MRI (rs-fMRI) imaging data. We propose using a novel cloud-based automated imaging software processing program that identifies abnormal brain networks or connectomes using rs-fMRI and data from the Human Connectome Project (HCP).
Specific Aims:	Specific Aim #1: Evaluate the role of rs-fMRI and connectomic analysis as a diagnostic imaging biomarker for LSBN deterioration in DLB by comparing a clinic population of DLB patients with pre-existing data from an ADNI 2/3 cohort of CN and MCI/mild AD subjects Specific Aim #2: Correlate cross-sectional dysfunction between LSBN parcellations with specific neuropsychological abnormalities in DLB patients
Outcomes:	Aim 1 Outcomes: Functional connectivity (FC) scores between pairs of LSBN parcellations. Aim 2 Outcomes: Functional connectivity (FC) scores between pairs of LSBN parcellations vs Neurophsychological test scores
Study Population:	Inclusions: 1) Age 40- 90 years, 2) Established DLB diagnosis (McKeith Criteria 2017), 3) MMSE>15 Exclusions: 1) Other forms of dementia including, but not limited to Alzheimer's dementia, frontotemporal dementia, vascular dementia, NPH, etc, 2) Inability to tolerate brain fMRI, 3) Risk of brain fMRI due to implants or metal. Sample Size: We plan to enroll 19 DLB patients. 38 AD and 38 CN age and sex-matched subjects will be selected from the ADNI-2/3 database.
Description of Sites/Facilities Enrolling Participants:	Participants will be enrolled at HealthPartners Neuroscience Center, located at 295 Phalen Blvd., St. Paul, MN 55130.
Study Duration:	The duration of this study is 1.5 years.
Participant Duration:	Participants should take about 3 hours - total time for all the visits, that may be conducted within 6 weeks

2 INTRODUCTION

2.1 BACKGROUND & STUDY RATIONALE

Dementia with Lewy bodies (DLB) is a common neurodegenerative condition characterized by parkinsonism, hallucinations, and cognitive fluctuations. This disease represents the second most common cause of dementia, affecting 1.4 million individuals (www.lbda.org). In comparison to Alzheimer's disease (AD), DLB is a more rapid dementia that not only results in progressive cognitive decline, but also leads to a variety of non-cognitive symptoms that include motoric impairment, REM sleep behavioral disorder, psychosis, and autonomic dysfunction. Disease progression is predicated on the propagation of alpha-synuclein aggregates from peripheral nervous system locations such as the olfactory cortex and enteric nervous system to central nervous system structures that include the brainstem and temporal cortex, thus resulting in progressive deterioration of LSBNs (H Braak J Neurol 200).

Table 1: Criteria for Dementia with Lewy Bodies

Table 1	Revised[1,2] criteria for the clinical diagnosis of probable and possible dementia with Lewy bodies (DLB)
Essential for a diagnosis of DLB is dementia, defined as a progressive cognitive decline of sufficient magnitude to interfere with normal social or occupational functions, or with usual daily activities. Prominent or persistent memory impairment may not necessarily occur in the early stages but is usually evident with progression. Deficits on tests of attention, executive function, and visuoperceptual ability may be especially prominent and occur early.	
Core clinical features (*The first 3 typically occur early and may persist throughout the course.*)	
Fluctuating cognition with pronounced variations in attention and alertness. Recurrent visual hallucinations that are typically well formed and detailed. REM sleep behavior disorder, *which may precede cognitive decline.* One or more spontaneous cardinal features of parkinsonism: these are bradykinesia (defined as slowness of movement and decrement in amplitude or speed), rest tremor, or rigidity.	
Supportive clinical features	
Severe sensitivity to antipsychotic agents; postural instability; repeated falls; syncope or other transient episodes of unresponsiveness; severe autonomic dysfunction, e.g., constipation, orthostatic hypotension, urinary incontinence; hypersomnia; hyposmia; hallucinations in other modalities; systematized delusions; apathy, anxiety, and depression.	
Indicative biomarkers	
Reduced dopamine transporter uptake in basal ganglia demonstrated by SPECT or PET. Abnormal (low uptake) ^{123}iodine-MIBG myocardial scintigraphy. Polysomnographic confirmation of REM sleep without atonia.	
Supportive biomarkers	
Relative preservation of medial temporal lobe structures on CT/MRI scan. Generalized low uptake on SPECT/PET perfusion/metabolism scan with reduced occipital activity ± the cingulate island sign on FDG-PET imaging. Prominent posterior slow-wave activity on EEG with periodic fluctuations in the pre-alpha/theta range.	

Detection rates of DLB in clinical practice are suboptimal (Jones & O'brien, 2014). To facilitate the diagnosis of this relatively common dementia, revised criteria have been developed based on core clinical features, supportive clinical features, and indicative biomarkers **(table 1)** (McKeith et al., 2017). The diagnosis of probable DLB (greatest degree of certainty in the absence of confirmatory brain pathology) can be made when a patient has either **2 core clinical features** or **one clinical feature** accompanied by **one indicative biomarker**. In contrast to most other neurological diseases, the indicative biomarkers in DLB measure indirect effects of the disease: reduced dopamine transporter update on a DaT scan indicates parkinsonism, abnormal cardiac uptake on a ^{123}iodine-MIBG myocardial scintigraphy suggests autonomic dysfunction, and tonic contractions during REM sleep during polysomnography supports REM sleep behavioral disorder. Limitations of these tests include a lack of specificity for this diagnosis (findings can be seen in Parkinson's disease or multiple system atrophy), relatively higher costs, and lack of insurance coverage as in the case of the DaT scan.

Further complicating matters, there are no universal imaging tools that directly show the impact of DLB on the CNS. Structural brain MRI is relatively less helpful for diagnostic classification, described as a "supportive biomarker" meaning that it has a less critical role in determining diagnosis. MRI will support a DLB diagnosis when there is an absence of medial temporal lobe atrophy, commonly found in Alzheimer's disease. However, the sensitivity and specificity of this diagnostic test is suboptimal: a

multisite study with autopsy confirmation showed that MRI was 64% sensitive and 68% specific for differentiating DLB from AD. Fluorodeoxyglucose positive emission tomography (FDG-PET), a functional biomarker that has been used to support a DLB diagnosis, shows occipital hypometabolism with relatively sparing of the posterior cingulate although these findings likewise lack sensitivity (83%) and specificity for DLB (80%) (Minoshima et al., 2001; Yamamoto et al., 2007).

The role of brain rs-fMRI, an imaging tool that maps interactions between brain regions during a resting state and produces a blood-oxygen-level-dependent signal, has been only preliminarily evaluated in patients with DLB. Currently, there is no defined pattern of LSBN deterioration in this condition. Certain investigations have failed to demonstrate characteristic differences between connectivity in DLB versus AD whereas others have demonstrated subtle difference (eg. increased precuneus-medial frontal connectivity (Galvin, Price, Yan, Morris, & Sheline, 2011). A study consisting of 18 DLB, 18 AD, and 15 CN subjects showed that posterior cingulate cortex connectivity was lower in AD than in DLB (Franciotti et al., 2013). Another study evaluated rs-fMRI functional connectivity in 15 DLB, 13, AD, and 40 controls, showing that functional connectivity was lower in DLB in the default mode, salience, and executive networks compared to the other groups (Lowther, O'Brien, Firbank, & Blamire, 2014) whereas a second study failed to show significant differences in LSBN connectivity between AD and DLB (Schumacher et al., 2018).

The core symptoms of DLB (hallucinations, cognitive fluctuations, parkinsonism, and REM sleep behavioral disorder) may offer clues to the specific LSBNs impacted by this disease. To our knowledge, few studies have evaluated the networks responsible for these symptoms and then attempted to leverage findings for diagnostic purposes. The locus coeruleus, a brainstem structure containing abundant noradrenergic neurons with ascending pathways throughout the central nervous system, has been found to exhibit cell loss in post-mortem analysis of DLB patients (Del Tredici & Braak, 2013) and may be responsible for the characteristic cognitive fluctuations (O'Dowd et al., 2019). fMRI studies have further shown that patients with disorders of consciousness exhibit compromise of those connections between the locus coeruleus, intralaminar nucleus of the thalamus, and nucleus basalis of Meynert (Edlow, Claassen, Schiff, & Greer, 2020). It has also been demonstrated that neuronal loss and Lewy bodies in the nucleus basalis of Meynert are correlated with the reduction of choline acetyltransferase levels in DLB (Lippa, Smith, & Perry, 1999). Thus, we suspect that the locus coerulus and its ascending projections play a significant role in the arousal disturbances and cognitive fluctuations in DLB. Parkinsonism results from disruption of connections between the substantia nigra and basal ganglia structures (Young & Penney, 1984). Furthermore, findings of attention/executive dysfunction in DLB correlate with the basal ganglia, particularly the left caudate nucleus (Botzung, Philippi, Noblet, de Sousa, & Blanc, 2019). Finally, hallucinations in Lewy body are thought to involve dysfunction in a variety of brain areas. A systemic literature search of 56 neuroimaging studies in PD and DLB with visual hallucinations were reviewed—the main structural neuroimaging results showed grey matter loss in the frontal areas whereas functional investigations revealed parietal and temporal hypometabolism in PD with hallucinations (Pezzoli, Cagnin, Bandmann, & Venneri, 2017). Disrupted functional connectivity was also detected in the fronto-parietal regions through fMRI studies. One structure, the inferior longitudinal fasciculus (ILF), which connects the occipital lobe with the temporal lobe, has been found to be dysfunctional in DLB (Kantarci et al., 2010). We suspect that brain regions within this pathway may be compromised in DLB patients with visual hallucinations. Preliminary work from our group has shown from that area PH, a region located within the anterioinferior lateral occipital lobe and lateral to the occipitotemporal sulcus, is associated with visual hallucinations in schizophrenia and has white matter connections within the ILF. This area is a higher-level perception region of the visual system that acts as a hub of ventral stream input, integrating "place-specific" information, while showing little to no activity to objects or faces (Baker, Burks, Briggs, Milton,

et al., 2018). There are connections to other brain regions including area or hub FST, which is involved in integrating information related to detail, motion, and form. Thus, our study will focus on pairwise functional connectivity between the seven parcellations listed in **Table 2**.

Table 2. Parcellations of Interest for Each DLB Core Symptom and Associated LSBN

DLB Core Symptom	Associated Large Scale Brain Network	Parcellations of Interest
Cognitive fluctuations	Ascending brainstem norepinephrine tracts	Locus coeruleus, basal nucleus of Meynert, intralaminar nucleus of thalamus (Edlow et al., 2020)
Visual hallucinations	Inferior longitudinal fasciculus	Areas PH and FST (Pezzoli et al., 2017)
Parkinsonism	Ascending brainstem dopamine tracts	Substantia nigra, caudate nucleus (Young & Penney, 1984)

One potential challenge for functional imaging to this point is that brain parcellations (or distinct brain partitions) have been based on outdated models such as the Broadmann map from the 19th century (Zilles, 2018). In 2009, the Human Connectome Project (HCP) began one of the most ambitious neuroscientific initiatives to map the brain (Fox, 2018; Glasser et al., 2016). This project identified 379 functional areas of the brain and discovered 97 new regions that had never been previously described. This data has provided neuroscientists for the first time in history the ability to view the brain not merely as a structural entity, but as an individualized network system.

Figure 1: Infinitome Program – Removed for CT.GoV version

Omniscient, a for-profit, Sydney, Australia-based company, created the cloud-based software Infinitome, a program that utilizes data from the HCP together with machine learning to analyze diffusion tensor and resting-state fMRI imaging data from remote sites **(Figure 1)**. The foundation for this imaging tool is based upon the HCP atlas, which has also informed prior publications from our group, including the Connectomic Atlas of the Human Cerebrum (Baker, Burks, Briggs, Conner, et al., 2018). The Infinitome program creates a subject specific version of the Human Connectome Project Multimodal Parcellation (HCP-MMP1) atlas using diffusion tractography. Analytics are performed on both diffusion tensor imaging and rs-fMRI. Outlier detection using a tangent space connectivity matrix is performed by comparing results with a subset of 300 normal HCP subject fMRI samples to determine the range of normal correlations for each regions of interest in a LSBN. Abnormal connectivity is determined as a 3-sigma outlier for that correlation. The imaging protocol for rs-fMRI is limited to 15 minutes, enabling each study to be readily added on to structural MRI sequences. The program also provides automated image processing and analysis, thus simplifying the process of accessing imaging data and incorporating rs-fMRI into routine practice. By providing support for fMRI processing through a cloud-based server and incorporating normal control data from the HCP, this program addresses the challenges associated with the analysis and interpretation of rs-fMRI data. Thus, it has the potential to simplify the incorporation of rs-fMRI into clinical trials as a biomarker for neurodegeneration of vulnerable networks in DLB. Preliminary work has shown that connectomic analysis in AD subjects using this software is feasible and can detect functional anomalies involving regions of interest described by the HCP (Ren et al., 2020). Results from this proposal

will be applicable to both clinical practice and research where a diagnostic biomarker may impact either medical decision-making or enrollment into a clinical trial.

2.2 RISK/BENEFIT ASSESSMENT

2.2.1 KNOWN POTENTIAL RISKS

Study has no Intervention.

Imaging

Structural MRI is a routine diagnostic procedure at HealthPartners, and the resting state portion merely requires 15 minutes of scan time. The patient may experience claustrophobia with the narrow imaging space, and hearing loud noises while inside the machine. A MRI scan does not involve radiation like conventional X-rays. Instead, images are generated using a magnetic field and radio signals. Because an MRI scanner uses strong magnets, subjects will be screened by the NSC Imaging staff – as for a routine MRI. People with artificial heart valve, metal plate, pin, or other metallic objects in their body (including gun shot or shrapnel) will not be eligible for this study.

Cogntive Assesments

The questions on these assessments may make participants feel uncomfortable because some parts may be easy to answer, while some parts may be difficult or tiring. It may also cause individuals to feel uncomfortable or upset.

Loss of Confidentiality

There may be a slight possibility of breach of confidential information that was collected. However, the following procedures will be implemented to reduce this risk:

- Data collection and reporting tools will be developed and stored internally.
- Data collected and stored electronically will remain confidential and secure (e.g. secured server and password protected files [REDCap]).
- Study binders will be stored in a locked file cabinet within a locked office.
- After the study is closed, all subject identifiers will be destroyed.

2.2.2 KNOWN POTENTIAL BENEFITS

No known direct benefits.

With minimal risk beyond what is typically encountered in the standard evaluation of dementia, research participants will have the opportunity to review novel rs-fMRI brain imaging that depicts the human connectome as large scale brain networks.

By employing a novel and innovative cloud-based program that is based on machine learning and HCP data, this study will serve to develop rs-fMRI as a diagnostic biomarker for DLB.

2.2.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

We believe the potential risks to the participants in this study are minimal. A significant portion of the study population will come from rs-fMRI from the publically accessible ADNI 2/3 database, thus reducing the number of study subjects undergoing study procedures for this project.

The following measures will be taken to protect providers and patients from the risk of breach of confidentiality:

- A unique study ID code unrelated to the medical record number or other study subject-specific information will be assigned to each patient and used to link data from various sources and needed for analysis. The study number will be used on the RedCap database and Infinitome Program. All imaging data uploaded from the NSC research site will be de-identified and uploaded to the Infinitome cloud-based server.
- Infinitome program uses an industrial grade cyber security that is superior to what is offered at the leading medical device companies. No personal health information is stored on servers and only de-identified scans are stored on a dedicated edge node on encrypted MinIO instances. Patient data is only accessed through authenticated calls form Kubernetes pods contained in the VPC. The web service requires user authentication. Only the data related to a specific facility can be retrieved through authenticated calls and are restricted to their use.

3 OBJECTIVES AND ENDPOINTS

Aim 1: Evaluate the role of rs-fMRI and connectomic analysis as a biomarker of disease in DLB using data from an ADNI-2/3 population of MCI and mild AD subjects and a clinic population of DLB subjects

Functional connectivity (FC) scores between pairs of LSBN parcellations
• Primary pairs of interest (see background for justification of these choices):
  ○ Locus coeruleus v. Basal nucleus of Meynert
  ○ Locus coeruleus v. Intralaminar nucleus of thalamus
  ○ Basal nucleus of Meynert v. Intralaminar nucleus of thalamus
  ○ PH v. FST
  ○ Substantia nigra v. Caudate nucleus
• Exploratory pairs: Other 15 combinations of the pre-specified parcellations, other pairs of parcellations found to be anomalies by Infinitome program

Aim 2: Correlate cross-sectional and longitudinal dysfunction between LSBN parcellations with specific neuropsychological abnormalities in DLB Patients
Functional connectivity (FC) scores between pairs of LSBN parcellations (same primary pairs of interest as Aim 1).
Scores on neuropsychological tests: WMS-R Logical Memory Story A, Boston Naming Test Short, Category Fluency, WMS-R Digit Span Forward and Backward, ANART, RAVLT, Trails A/B, WMS-R Digit Symbol, Clock Drawing, NPI-Q, FAQ, ADAS-Cog 13

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is a cross-sectional study in patients with DLB with two comparison groups. The study includes analyzing resting state-functional MRI (rs-fMRI) imaging data in CN and AD subjects from the ADNI 2/3 database and in DLB subjects recruited from a multidisciplinary dementia clinic

Specific Aim #1: Evaluate the role of rs-fMRI and connectomic analysis as a diagnostic imaging biomarker for LSBN deterioration in DLB by comparing a clinic population of DLB patients with pre-existing data from an ADNI 2/3 cohort of CN and mild AD subjects

Specific Aim #2: Correlate cross-sectional dysfunction between LSBN parcellations with specific neuropsychological abnormalities in DLB patients .

4.2 OVERVIEW – STUDY PROCEDURES/DATA COLLECTION

Data will be obtained for cognivite measures and imaging data (rs-fMRI, DTI, T1) from ADNI 2/3 data base cohort subjects with AD diagnosis, CN and DLB subjects recruited from a multidisciplinary dementia clinic. See the section # for detailed study procedures/data collection. Cognitive tests were selected so as to be harmonized with those used with the ADNI-2/3 database.

This research investigation will take place at the Center for Memory and Aging at the HealthPartners Neuroscience Center (multidisciplinary dementia clinic). Potential candidates will also be referred to our site through the Park Nicollet Neurology Clinic.

4.3 END-OF-STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed study visits and assessments.

5 STUDY POPULATION

For ADNI 2/3 data, we will include CN and AD subjects who have undergone rs-fMRI images (with DTI and T1 images) and have cognitive/function testing data available. Below we describe the study population regarding clinic recruitment.

5.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

- Age 40- 90 years
- Established DLB diagnosis (McKeith et al. 2017)
- MMSE>15

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

- Other forms of dementia including, but not limited to Alzheimer's dementia, frontotemporal dementia, vascular dementia, NPH, etc,
- Inability to tolerate brain fMRI
- Risk of brain fMRI due to implants or metal.

5.3 LIFESTYLE CONSIDERATIONS

NA

5.4 SCREEN FAILURES

Pre-screening Phone Call: All potential participants will undergo a pre-screening phone or a video call to determine whether they meet the inclusion/exclusion criteria. Patients will be considered ineligible if they do not meet one or more of the inclusion/exclusion criteria during pre-screening. We will collect information on why participants are ineligible or decide not to move forward with the trial.

Screen failures are defined as participants who are considered eligible during the pre-screening phone call, but it was subsequently determined that they do not meet one or more of the inclusion/exclusion criteria. We will collect information on why participants screen fail or decide not to move forward with the trial.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Recruitment: For Alzheimer's Disease Neuroimaging Initiative (ADNI), analysis will be based on data available from the database. As such there are no recruitment or retention activities. rs-fMRI data as well as variables listed in Table 3 & 4 will be downloaded from ADNI-2/3 for age/sex-matched mild AD (with supportive biomarkers) and age/sex-matched CN patients.

Clinic Population: Potential DLB subjects will be referred to the study coordinator by clinicians in the neurology departments of HealthPartners and Park Nicollet. Participants with clinic visits will meet with research staff. Other patients will be mailed/e-mailed a letter and flyer/brochure. An informed consent form will be provided to those individuals expressing interest in the study. A video or phone encounter will be arranged to provide additional details of the study and informed consent. This study will be listed as one of the regional DLB investigations on the Alzheimer's Association Trial Match website. All referrals will be directed to study staff who will contact interested participants by phone. Participation will include one virtual visit and two in-person visits at HealthPartners Neuroscience Center.
To reach our target enrollment, we anticipate that we will need to screen 40 people, of those 19 individuals will sign the informed consent.

Remuneration: Participants will be provided gift cards totaling $100 per subject for completing certain visits of the research study. The gift cards will be provided at the end of the study for completing the cognitive and imaging visits.

5.6 PARTICIPANT WITHDRWAL

5.6.1 REASONS FOR PARTICIPANT WITHDRWAL

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may withdraw a participant from the study if:

- Any medical condition, event or situation occurs such that continued participation in the study would not be in the best interest of the subject.
- The participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.
- Significant study procedure non-compliance
- Lost-to-follow up; unable to contact subject

5.6.2 HANDLING OF PARTICIPANT WITHDRWALS

The reason for participant discontinuation or withdrawal from the study will be recorded on the relevant eCRF. Subjects who sign the informed consent form and undergo only cognitive measures, and not imaging will not have completed participation and may be replaced.

A participant will be considered lost to follow-up if he or she fails to attend any scheduled study visit and study staff are unable to contact the participant after at least 5 attempts.

The following actions must be taken if a participant fails to attend any required study visit:

- Study staff will attempt to contact the participant, reschedule the missed visit, counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, telephone calls or e-mail – if no answer leave a voicemail on the first and last attempt). These contact attempts will be documented.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

6 STUDY SCHEDULE

The sections below are only for recruitment of DLB patients for clinic. See Section 11.1 for schedule.

6.1 SCREENING

6.1.1 SCREENING/CONSENT VISIT (VISIT 1) (DAY 0)

- This visit may be a telephone/video visit
- Review, obtain and document consent from subject and caregiver (e-consent)
- Adminster MMSE
- Review medical history, demorgraphics to determine eligibility to participate
- Review history regarding any contraindications for MRI imaging
- Schedule study visits for individuals who are eligible and available for the duration of the study

- A total time of 40 minutes is anticipated for this visit

6.2 COGNITIVE VISIT (VISIT 2) (WITHIN 3 WEEKS OF VISIT 1)

- Verify inclusion/exclusion criteria
- Neuropsychological battery as mentioned in Section 7.
- Schedule Imaging visit if not scheduled
- This visit may be combined with imaging visit, but imaging can be performed only after cognitive assessments
- This visit is at the clinic (HealthPartners Neuroscience Center [NSC]), is about 60 minutes.

6.3 IMAGING VISIT (VISIT 3 – AT NSC) (WITHIN 3 WEEKS OF VISIT 2)

- Verify inclusion/exclusion criteria
- Subject will undergo MRI imaging as mentioned in Section 7.
- This visit may be combined with cogntive visit, but imaging can be performed only after cognitive assessments
- This visit is at the clinic (NSC), is about 50 minutes.

6.4 OPTIONAL RESULTS REVIEW VISIT (AFTER VISIT 3 – AT NSC/VIRTUAL)

An optional 30 minute visit may be scheduled to share the brain imaging results with with the subject and the caregiver by the PI. The participant can decide whether or not they would like to attend this visit, which can either be virtual or at NSC.

Participants will be asked to adhere to study visits and to complete study assessments. Participants will remain active unless withdrawn from the study. These will be documented in the relevant CRF.

7 STUDY ASSESSMENTS AND PROCEDURES

7.1 STUDY ASSESSMENTS FOR ENROLLED PATIENTS

7.1.1 DEMOGRAPHICS AND MEDICAL HISTORY

Demographic information will be collected, including: gender, age, race, ethnicity, height, weight, BMI, education, dementia diagnosis, co-morbidities (such as Diabetes, Hypertension) and e-mail address for consent.

7.1.2 MMSE (MINI-MENTAL STATUS EXAMINATION)

Originally developed in 1976 by Folstein, the MMSE is a paper-based test commonly used in clinical and research settings (Folstein, Folstein, & McHugh, 1975). A 30 point cognitive screening tool that assesses orientation, working memory, short term memory, visuospatial construction, and language.

7.1.3 NEUROPSYCHOLOGICAL ASSESSMENTS

All enrolled subjects will undergo the following cognitive scales. These will be adminstered by the research staff trained in these assessments using instructions specific for tests under the guidance/ supervision of the study neuropsychologist. Cognitive tests (validated) were selected so as to be harmonized with those used with the ADNI-2/3 database. Testing will take between 45-60 min. The following battery will be administered:

Table 3. Neuropsychological Battery (Visit 2)

Cognitive Test/Scale	Domain	References
WMS R Logical Memory Story A	Story Memory/Delay	(Wechsler, 1987)
ADAS-COG-13 Number Cancellation	Visual Attention	(Rosen, Mohs, & Davis, 1984)
ADAS-COG—13 Praxis	Visuoconstruction	(Rosen et al., 1984)
Boston Naming Test (Odd items)	Naming	(Kaplan, Goodglass, & Weintraub, 1983)
Verbal Fluency	Verbal Fluency	(Butters, Granholm, Salmon, Grant, & Wolfe, 1987)
WMS R Digit span F and B	Auditory Attention	(Wechsler, 1987)
ANART	Pre-morbid function	(Nelson & O'Connell, 1978)
RAVLT	List Learning/Memory/Recog	(Rey, 1964; Rosenberg, Ryan, & Prifitera, 1984)
Trails A/B	Attention/Executive Function	(Reitan, 1958)
Clock Drawing	Clock Draw	(Goodglass & Kaplan, 1983)
NPI-Q	Neuropsychiatric Symptom	(Kaufer et al., 2000)
FAQ	Functional assessment	(Pfeffer, Kurosaki, Harrah Jr, Chance, & Filos, 1982)

ADAS-Cog-13=Alzheimer's Disease Assessment Scale-cognitive subscale 13; WMS R=Weschler Memory Scale-Revised; ANART=American National Adult Reading Test; RAVLT: Rey Auditory Verbal Learning Test; Rey O Copy: Rey Osterrieth Figure Copy; NPI-Q: Neuropsychiatric Inventory Questionnaire; FAQ: Functional Assessment Questionnaire

7.1.4 MRI IMAGING

Subjects will undergo MRI imaging at HealthPartners Neuroscience Center. Subjects will be screened for any contraindications for MRI. Subjects will undergo a protocol based on recommendation by Omniscent (o8t MR Acquisition Recommendations). We anticipate 15 minutes of scan run time using a 3T Siemens Skyra scanner and the following images will be obtained.

1) High-resolution multi-scan directional diffusion scan, which is most similar to a diffusion tensor image (DTI) acquisition - Specifically, diffusion weighted imaging with the following acquisition parameters will be used: *2 mm x 2 mm x 2 mm voxels, FOV = 25.6 cm, matrix = 128 mm x 128 mm, slice thickness = 2.0 mm, one non-zero b-value of b = 1000, 40 directions, and gap = 0.0 mm.*

2) EPI BOLD rs-fMRI - *A resting-state fMRI as a T2-star EPI sequence, with 3 x 3 x 3-mm voxels, 128 volumes/run, a TE = 27ms, a TR = 2.8s, a field of view – 256mm, a flip angle = 90°*
3) Anatomical Scan –T1 Weighting

All images will be de-identified and uploaded to the infintome cloud-based server to be analyzed. Internal guidelines/best practices based on infinitome user manual will be developed for transfer, obtaining accounts, uploading of raw images and downloading of analyzed data.

7.1.5 CONNECTOMIC ANALYSIS

All subject images will be processed using the infitiome tool. This tool creates a machine learning-based, subject specific version of the Human Connectome Project-Multimodal Parcellation (HCP-MMP1) atlas based upon diffusion tractography structural connectivity. This will be used to conduct a connectomic analysis to identify large scale brain networks.

7.2 ADNI DATABASE

A data use agreement with ADNI will be completed prior to downloading the data for analysis from the ADNI database (ADNI Database). A user login – for research staff will be used for downloading the data.

All necessary ADNI data for MCI, mild AD patients, and cognitively normal (CN) will be downloaded to a local secure server for further analysis.

Data will be obtained regarding demographics, medical history, cognitive assessments (as mentioned in earler sections), MMSE for eligibility, biomarkers and imaging (as mentioned in the previous section). The imaging data will be uploaded and processed with the Infinitome software as stated in the previous sections.

7.3 UNANTICIPATED PROBLEMS

7.3.1 DEFINITION OF UNANTICIPATED PROBLEMS

This protocol uses the definition of Unanticipated Problems as defined by the Office for Human Research Protections (OHRP). OHRP considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

1. Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
2. Related or possibly related to participation in the research (“possibly related” means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

3. Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.3.2 UNANTICIPATED PROBLEMS REPORTING

The PI will report unanticipated problems (UPs) to the reviewing IRB. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number
- A detailed description of the event, incident, experience, or outcome
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs will be reported to the IRB as soon as possible, but no later than 10 working days after the investigator first learns of the event

7.3.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Following IRB review of any unanticipated problems, the PI will follow the IRB's recommended actions. This may include, but is not limited to, modifying the informed consent document or process, re-consenting current participants, providing information to past or current participants (e.g. whenever the information may relate to the participant's willingness to continue participants), and modifications to the protocol/research plan.

8 STATISTICAL CONSIDERATIONS

8.1 STATISTICAL ANALYSIS PLAN

Preliminary data analysis: One-way ANOVA will be used to compare demographic and baseline clinical characteristics across groups. **no missing data expected, SAS, p-value

Aim 1 Analysis: For the between-group analysis of functional connectivity, general linear model multivariate analysis of variance will be used with FC values between pairs of LSBN parcellations as dependent variables and group (DLB, AD, and CN) as a factor variable. Bonferroni post hoc test will be used to correct for multiple comparisons. The primary pairs of interest (see **Table 4**) based on available literature (see background and **Table 2**) will be analyzed first. Other combinations of these seven parcellations will be analyzed secondarily. If additional, unexpected anomalies are present between groups, we will test them in an exploratory fashion to advance this novel field of study.

Aim 2 Analysis: Pearson correlation analyses will be performed between functional connectivity values and neuropsychological test scores to evaluate the relationship between connectivity strengths and the clinical phenotype of DLB. Similar to Aim 1, we will focus on pre-specified pairs of interest, but will explore others if they present themselves in the functional connectivity analysis.

Table 4: Data Variables
Population Descriptors: age, sex, race, ethnicity, education, measures of baseline cognition (e.g. MoCA)
Aim 1: Evaluate the role of rs-fMRI and connectomic analysis as a biomarker of disease in DLB using data from an ADNI-2/3 population of MCI and mild AD subjects and a clinic population of DLB subjects
Group variable: DLB, AD, or CN

Dependent variables: Functional connectivity (FC) scores between pairs of LSBN parcellations
• Primary pairs of interest (see background for justification of these choices):
  ○ Locus coeruleus v. Basal nucleus of Meynert
  ○ Locus coeruleus v. Intralaminar nucleus of thalamus
  ○ Basal nucleus of Meynert v. Intralaminar nucleus of thalamus
  ○ PH v. FST
  ○ Substantia nigra v. Caudate nucleus
• Exploratory pairs: Other 15 combinations of the pre-specified parcellations, other pairs of parcellations found to be anomalies by Infinitome program

Aim 2: Correlate cross-sectional dysfunction between LSBN parcellations with specific neuropsychological abnormalities in DLB Patients
Functional connectivity (FC) scores between pairs of LSBN parcellations (same primary pairs of interest as Aim 1).
Scores on neuropsychological tests: WMS-R Logical Memory Story A, Boston Naming Test Short, Category Fluency, WMS-R Digit Span Forward and Backward, ANART, RAVLT, Trails A/B, WMS-R Digit Symbol, Clock Drawing, NPI-Q, FAQ, ADAS-Cog 13

8.2 POWER ANALYSIS OR STATEMENT OF PRECISION

In a one-way ANOVA, sample sizes of 19 DLB, 38 AD, and 38 CN achieve 80% power to detect differences among the means versus the alternative of equal means using an F test with a 0.01 significance level and assuming a common standard deviation within a group of 0.05. This is true for any set of group means with a standard deviation greater than or equal to 0.02, which we anticipate being able to achieve based on previous research. All significant brain connection couples in Franciotti's work had standard deviations of the group means greater than 0.06.

For post-hoc tests, group sample sizes of 19 DLB, 38 AD, and 38 CN patients achieve 81% power to reject the null hypothesis of equal means when the population mean difference is 0.05 with a standard deviation for both groups of 0.05 and with a significance level (alpha) of 0.010 using a two-sided two-sample equal-variance t-test.

9 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

9.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

9.1.1 INFORMED CONSENT PROCESS

ADNI: We are requesting a waiver of written informed consent for all ADNI data for the following reasons: 1) The data is retrospective and will be downloaded from a database, 2) There is no additional data collection, and 3) It would be impractical to consent patients (due to large numbers) if written informed consent of patients were required. A data use agreement with ADNI will be completed prior to downloading the data for analysis from the ADNI database.

Clinic: All research study staff will maintain certification in human subject's protection. All study investigators and staff will take an active role in developing procedures to protect against or minimize potential risks to the safety and well-being of enrolled participants. Potential research subjects will be informed that participation in this study is voluntary and will not be discriminated against if they choose not to participate. Written informed or electronic consent and assent will be obtained from participants, and family member/caregivers or legally authorized representatives (LAR). Participants will be asked to describe in their own words the study's expectations. Subjects will be informed that they can withdraw from the study at any time and will be given a copy of the consent form. Subjects will have written assurance that while de-identified individual subject data may be available to other researchers for research purposes, or used to improve the software program, only a summary of the results will ever be published or otherwise publicly released. Subjects will be assured that participation in the study will be strictly confidential, that any identifying information will be available to the study staff only, and that no identifying information concerning the data and results will be made known.

Potential research subjects will be informed that participation in this study is voluntary and that their decision to participate will not reflect upon their relationships with the Center for Memory and Aging, Regions Hospital, or HealthPartners. Subjects will be informed that they can withdraw from the study at any time and will be given a copy of the consent form.

With the electronic consent via REDCap the patient/caregiver providing consent will be able to review the consent form themselves and sign electronically with a stylus, touch screen, or cursor using a signature field in REDCap. After the individual has received the link and can view the consent form, the research staff member will go through the consent form with the individual as would be typical in person. Following the consent conversation, the staff member will sign and e-mail the consent and HIPPA electronically to the patient. The patient will electronically sign, certify, and submit the consent and HIPPA in REDCap. A fully executed PDF copy of the consent and HIPPA will be provided electronically to the patient for their records as well as saved via the auto-archiver function in REDCap.

9.1.2 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, the safety and oversight monitor(s), and the sponsor(s). This confidentiality is extended to the data being collected as part of this study. Data that could be used to identify a specific study participant will be held

in strict confidence within the research team. No personally-identifiable information from the study will be released to any unauthorized third party without prior written approval of the sponsor/funding agency.

All research activities will be conducted in as private a setting as possible.

All study regulatory binders will be stored in a locked file cabinet within a secure office. The internal study monitor, representatives of the IRB, or regulatory agencies, may inspect all documents and records required to be maintained by the investigator, for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor/funding agency requirements.

A unique study ID code unrelated to the medical record number or other study subject-specific information will be assigned to each patient and used to link data from various sources and needed for analysis. The study number will be used on the RedCap database and Infinitome Program. All imaging data uploaded from the NSC research site will be de-identified and uploaded to the Infinitome cloud-based server.

Infinitome program uses an industrial grade cyber security that is superior to what is offered at the leading medical device companies. No personal health information is stored on servers and only de-identified scans are stored on a dedicated edge node on encrypted MinIO instances. Patient data is only accessed through authenticated calls form Kubernetes pods contained in the VPC. The web service requires user authentication. Only the data related to a specific facility can be retrieved through authenticated calls and are restricted to their use

The PI will ensure all mechanisms used to share data will include proper plans and safeguards for the protection of privacy, confidentiality, and security for data dissemination and reuse (e.g., all data will be thoroughly de-identified and will not be traceable to a specific study participant). Plans for archiving and long-term preservation of the data will be implemented, as appropriate.

9.1.3 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator
Michael Rosenbloom, MD
HealthPartners Neuroscience Center
295 Phalen Blvd. St. Paul, MN 55130

9.1.4 SAFETY OVERSIGHT

There is no Data Safety Monitoring Board for this study, as there is no intervention and has minimal risks

9.1.5 CLINICAL MONITORING

N/A, refer to next section.

9.1.6 QUALITY ASSURANCE AND QUALITY CONTROL

Study staff will perform internal quality management of study conduct, data collection, documentation and completion.

Quality control (QC) procedures will be implemented as follows:

Informed consent --- Study staff will review both the documentation of the consenting process and 10% of the completed consent documents. Feedback will be provided to study staff to ensure proper consenting procedures are followed.

Protocol Deviations – The study team will review documented protocol deviations on an ongoing basis and will implement corrective actions when the quantity or nature of deviations are deemed to be at a level of concern.

Should independent monitoring become necessary, the PI will provide direct access to all study related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor/funding agency, and inspection by local and regulatory authorities.

9.1.7 DATA HANDLING AND RECORD KEEPING

9.1.7.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection will be the responsibility of the research study staff under the supervision of the PI. The PI will be responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Data collection/reporting tools will be developed internally (i.e. CRFs or eCRFs (RedCap Database) and source documents). Data collected and stored electronically will remain confidential and secure (e.g. secured server, encrypted data, password protected file).

9.1.7.2 STUDY RECORDS RETENTION

Investigator records will be retained in accordance with regulatory, organizational and sponsor or grantor requirements. All records will be maintained securely with limited access. Disposal of investigator records will be done in such a manner that no identifying information can be linked to research data.

9.1.8 PROTOCOL DEVIATIONS

This protocol defines a protocol deviation as any noncompliance with the study protocol. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions will be developed by the site and implemented promptly.

9.1.9 PUBLICATION AND DATA SHARING POLICY

This study will be registered at ClinicalTrials.gov, and results information from this study will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals.

Data from the de-identifed images may be utilized by Omniscent for improvement of Infinitome program and potential future imaging research studies.

9.1.10 CONFLICT OF INTEREST POLICY

The study leadership in conjunction with HealthPartners Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

9.2 ADDITIONAL CONSIDERATIONS

N/A

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11 APPENDIX

11.1 SCHEDULE OF VISITS

Procedure	Screen/Consent	Cognitive	Imaging	Results Review
	Visit 1	Visit 2	Visit 3 (Within 3 weeks of visit 2)	Optional (After Visit 3)
	Telephone/Video	At NSC	At NSC	At NSC or Virtual
E-consent	X			
Medical History	X			
Demographics	X			
MRI Contraindications	X		X	
Inclusion/Exclusion Criteria	X	X	X	
MMSE	X			
Cognitive Tests		X		
MRI Imaging			X	
Brain Imaging Results				X

Updated *A Priori* SAP
06 FEB 2024

Aim 1

Our primary aim was to evaluate the role of rs-fMRI and connectomic analysis as a diagnostic imaging biomarker of disease for LSBN deterioration in DLB by comparing a clinical population of DLB patients with the ADNI 2/3 cohort of CN and AD/CI subjects. Specifically, we compared FC scores of prespecified parcellation pairs between the three groups using a one-way ANOVA. Eight parcellation pairs were chosen as the primary pairs, and twenty-two as the exploratory pairs.

Aim 2

Our secondary aim (aim 2) was to correlate cross-sectional dysfunction between LSBN parcellations with specific neuropsychological abnormalities in DLB patients. To accomplish this, we performed a two-stage analysis. First, we compared cognitive test scores between groups to understand which successfully distinguished between the cohorts. When possible, test scores were normed, i.e., converted to a Z- or T-score adjusted for age, sex, and years of education.

Primary Comparisons/A Priori Hypotheses:

1) ADAS Number Cancellation Targets will be lower in DLB patients

2) Trails B time will be higher in DLB patients

3) Clock Drawing Tests will be lower in DLB patients

4) ADAS construction praxis score will be lower in DLB patients

5) Category Fluency Animals will be lower in AD patients

6) RAVLT recognition will be lower in AD patients

The remaining comparisons were exploratory: Trails A time, RAVLT immediate recall, RAVLT delayed recall, Functional Activities Questionnaire (FAQ), Logical Memory Immediate, and Logical Memory Delayed. Digit Span Forward, Digit Span Backward, and Boston Naming Test were either missing or highly incomplete in ADNI controls; therefore, they were described in the DLB cohort only.

In the second part of the Aim 2 analysis, we correlated test scores with FC scores from the primary parcellation group in Aim 1, within diagnosis (DLB, CN, or AD/CI) and compared these correlations across diagnosis group. As above, we prespecified 4 hypotheses to test and consider the remaining correlations to be exploratory. Because of the high volume of statistical tests performed in the exploratory analysis, we used the Holm correction to adjust the p-values in the exploratory correlations only.

Primary/ A Priori Hypotheses:

1) Caudate R / Substantia Nigra R and the Clock Drawing Test will be differently correlated for DLB patients vs other groups.

2) Caudate R/ Substantia Nigra R and the ADAS Number Cancellation Targets will be differently correlated for DLB patients vs other groups.

3) FST R/PH Rand the ADAS construction praxis will be differently correlated for DLB patients vs other groups.

4) FST R / PH R and the Clock Drawing Test will be differently correlated for DLB patients vs other groups.

With respect to the exploratory correlations, we used a significance threshold of $p < 0.15$, which is an accepted approach for detecting effects in emerging research areas where it's preferable to err on the side of identifying novel effects versus failing to reject a null hypothesis when it is false (Type II error).