PART B
STUDY DESCRIPTION

TITLE OF PROTOCOL	**Seizure-like Hippocampal Activity in Alzheimer's Disease Neurodegeneration**		
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B1. PURPOSE OF PROTOCOL

To test the hypothesis that increased hippocampal perfusion on arterial spin labeling MRI in patients with Alzheimer's Disease (AD) is partially driven by focal epileptiform activity, and that this abnormal activity contributes to memory dysfunction.

Here we propose applying an imaging technique, arterial spin labeling MRI (ASL-MRI), ideally suited for testing this hypothesis. We will utilize a pharmacological probe, levetiracetam, to test whether sub-clinical epileptiform activity is driving increases in hippocampal perfusion and contributing to the declarative memory impairment in AD. Levetiracetam is an antiepileptic drug (AED) that inhibits epileptiform activity. Levetiracetam inhibits burst firing without affecting normal neuronal excitability. We will study patients with mild AD in three sessions, counterbalanced for order, in a crossover design. In each session, participants will undergo a baseline electroencephalogram (EEG). They will then be given either a placebo or intravenous levetiracetam low dose (2.5 mg/kg) or high dose (7.5 mg/kg). After drug administration, repeat EEG, ASL-MRI perfusion imaging, and behavioral testing will be conducted. Participants will return at 1-2 week intervals for the second and third sessions in the other conditions (levetiracetam low dose / levetiracetam high dose / placebo). This study will test whether increased hippocampal perfusion in AD is caused by epileptiform activity, whether this activity is contributing to memory impairment, and whether antiepileptic medications might be a new avenue of treatment in AD. If validated, ASL-MRI would be an ideal tool to non-invasively determine whether antiepileptic medications are effective in decreasing aberrant excitatory activity in the hippocampus in AD.

Aim 1: To determine whether increased hippocampal perfusion in AD is caused by epileptiform activity.

> **Hypothesis 1**: Hippocampal perfusion in AD will be decreased after the administration of an antiepileptic medication, levetiracetam, but not after placebo.

Aim 2: To determine whether inhibition of burst firing can improve cognitive performance and neural function in cortical regions beyond the hippocampus.

> **Hypothesis 1**: Decreases in hippocampal perfusion will correlate with improvements in

measures of declarative memory and spatial memory.

Hypothesis 2: Decreases in hippocampal perfusion will correlate with increases in cortical perfusion, particularly in lateral temporal and parietal association cortices.

B2. SIGNIFICANCE AND BACKGROUND FOR THE STUDY

Seizures and Alzheimer's Disease

Alzheimer's disease (AD) is associated with an increased risk of unprovoked seizures and accounts for approximately 2% of all epilepsy [1]. Clinical seizures develop in 1.5 -17% of long-term AD survivors, most commonly in those with early age at disease onset and with familial AD [2-4]. In familial AD due to mutations in presenilin, epilepsy is much more prevalent, approaching 80% of affected members [5-7]. Seizures may be more common in sporadic AD, however, than previously reported [8]. Seizures may be under-recognized in patients with dementia. Subjective seizure auras such as déjà vu, olfactory hallucinations, and other auras are uncommon in AD [3]. Clinical manifestations of temporal lobe seizures are often subtle. For example, focal seizures restricted to the hippocampus may produce only mild confusion or amnesia without other outward signs [9]. Fluctuations in memory or wandering in AD can be due to unrecognized complex partial seizures [10]. Such seizures might easily be missed in patients with baseline memory and cognitive deficits. Seizures in AD are usually considered to be rare and late byproducts of a diffuse neurodegenerative process, but recent evidence from animal studies has raised the intriguing possibility that epilepsy plays a more central role in the pathophysiology of AD.

The exact mechanism of epileptogenesis (development of spontaneous seizures) in AD is not known, but new data from transgenic mice expressing human amyloid precursor protein (APP) in neurons suggest that high levels of beta amyloid (Aβ), the amyloid protein tightly linked to AD, are sufficient to elicit epileptiform activity and seizures [11]. The excitatory connections within the perforant pathway of the hippocampus also promote focal epileptic activity. Inhibitory connections outside the hippocampus and entorhinal cortex can prevent the propagation of seizures beyond the medial temporal lobes. Epileptic activity directly damages the hippocampus via hypoxia and ischemia [12]. Ischemia in turn increases amyloid deposition in a potential feedback loop [13] (see Figure 1).

Human amyloid precursor protein (hAPPJ20) mice have high levels of Aβ peptides in the brain without significant neuronal loss, and develop cognitive deficits comparable to those seen in human AD. Continuous video-EEG monitoring with intracranial electrodes in hAPP mice demonstrates frequent epileptiform activity including spikes, sharp waves, and spontaneous nonconvulsive seizures in both hippocampus and multiple neocortical regions. These seizures have no outward clinical manifestations. Epileptic activity leads to hypoxia-ischemia, which can then lead to increased amyloid deposition [13]. Animal studies show that amyloid acts as an excitatory epileptogenic stimulus in the hippocampus, leading to compensatory upregulation of inhibitory, GABAergic interneurons [11]. Network changes in hAPP mice include both aberrant network excitability and compensatory inhibitory mechanisms, such as GABAergic sprouting, enhanced synaptic inhibition, and synaptic plasticity deficits in the dentate gyrus. The interplay of excitatory and inhibitory changes prevents seizure generalization, but inhibits proper hippocampal function. Similar epileptiform activity has been identified in independent transgenic mouse models of AD [14-17], further highlighting the importance of APP and Aβ for the genesis of epileptiform activity.

Could a similar pathophysiological chain be occurring in patients with AD and, if so, could these events contribute to the pathological changes in the hippocampus and connected regions (figure 1)? In hAPP mice, the degree of network changes is correlated with deficits in learning and memory [11, 18]. More importantly, many of the Aβ-induced neuronal alterations as well as learning and memory deficits could be prevented by blocking excitotoxin-induced overexcitation [19]. These findings raise the possibility that hippocampal epileptiform and seizure activity may not be solely an epiphenomenon of progressive neurodegeneration, but may play a causal role in the cognitive deficits in hAPP mice and possibly also humans with AD. Confirmation of a relationship between excitatory neuronal activity and cognitive deficits in humans with AD would provide new insights into the pathogenesis of AD as well as open new potential avenues for therapy.

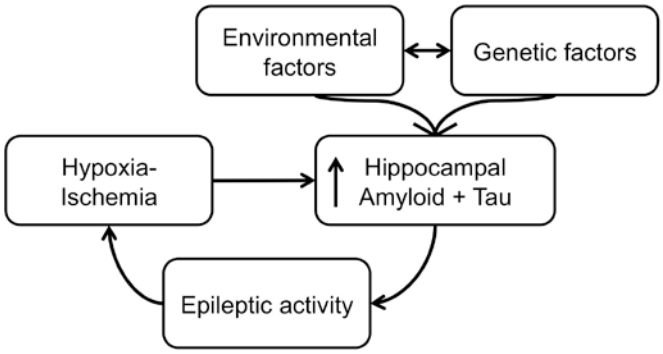

Figure 1. Possible pathway linking AD with aberrant epileptic activity. Amyloid deposition leads to epileptic activity.

Arterial spin-labelling MRI to detect hippocampal blood flow changes

Demonstrating whether similar epileptiform activity and seizures occurs in the hippocampus of humans with AD is challenging. Surface EEG, dense array EEG, and magnetoencephalography are extremely limited in their ability to detect hippocampal epileptic activity [20-22]. The hippocampus can be recorded with intracranial depth electrodes, but these are invasive and carry a risk of hemorrhage and infection. For this reason, indirect measures of hyperexcitability, such as increases in cerebral blood flow by functional MRI or SPECT, are commonly used to aid in localization of deep epileptic foci. The mechanism of hyperperfusion due to seizure activity is not completely understood, but may be related to transient loss of autoregulatory function in surrounding vasculature or to the release of excitatory neurotransmitters such as glutamate in areas of increased neuronal firing.

In this project, we hypothesize that aberrant epileptiform and seizure activity occurs in the hippocampus in patients with AD, and that suppression of epileptiform activity will improve memory deficits. We will study hippocampal activity using a proxy for epileptiform activity, Arterial Spin Labeling MRI (ASL-MRI), as well as EEG. We have completed a study of brain perfusion in mild-AD using Arterial Spin Labeling MRI (ASL-MRI), a non- invasive technique to quantifiably measure cerebral blood flow [23]. We found decreases in cerebral perfusion in lateral temporal and parietal cortex, in agreement with previous PET studies. In addition, we found increased perfusion in the hippocampus, particularly when the perfusion measure was corrected for atrophy [23], Figure 2. These unexpected findings were recently corroborated [24, 25]. A similar pattern of increased hippocampal activity has been seen with functional MRI, where patients with mild cognitive impairment show increased activation, a finding recently extended to presymptomatic patients with familial AD [26, 27]. Hippocampal hyperperfusion can also be seen in

mild cognitive impairment and even in healthy elderly patients with apoE4 allele, which predisposes to development of AD.

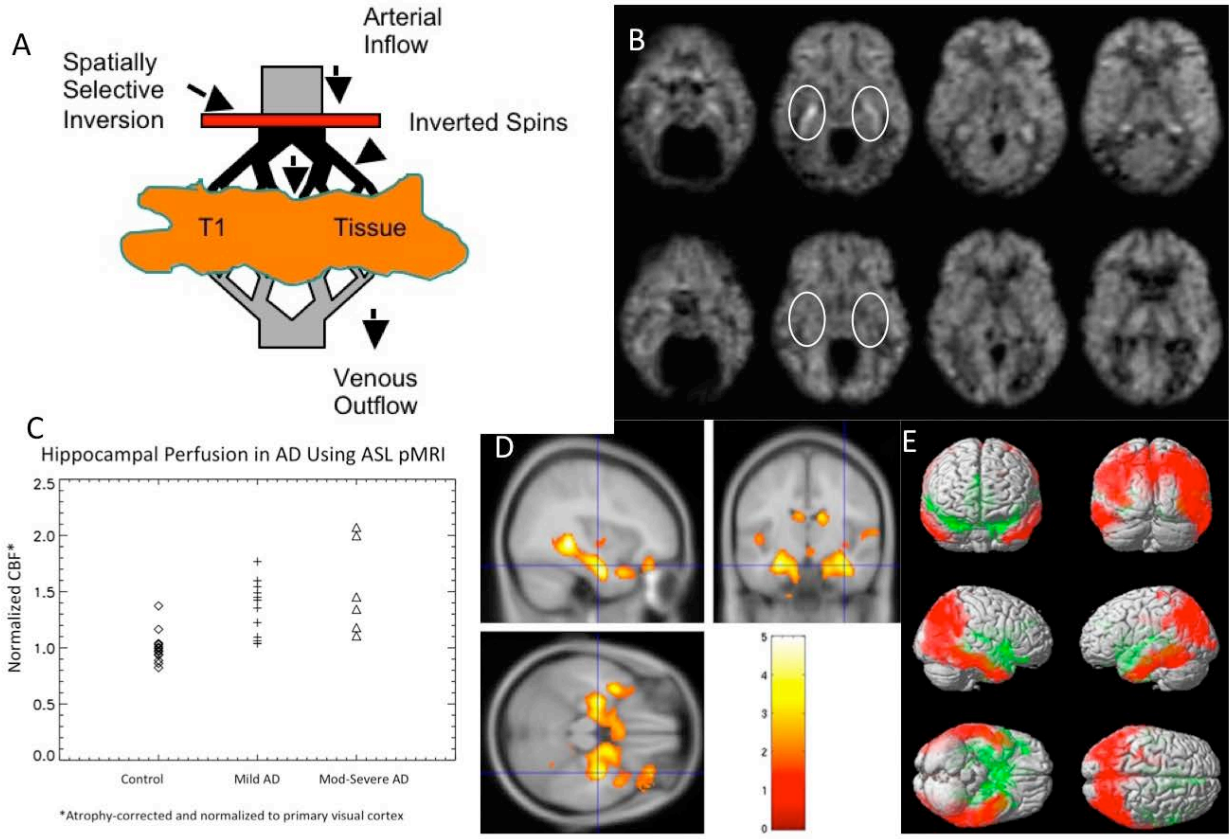

Figure 2. Arterial spin labeling is a non-invasive technique to measure cerebral perfusion by applying a magnetic "tag" to arterial blood without requiring administration of a contrast agent (panel A). We have previously found increased hippocampal perfusion in AD participants that was visible at the individual level (top of panel B is AD participant, bottom is age-matched control). The perfusion change was prominent at the group level (panel C shows individual data points, panel D shows statistical maps of group data and panel E shows areas of both decreased perfusion in temporal-parietal cortex (in red) and increased perfusion in medial temporal structures (in green).

In our previous study of 22 AD participants (mean age=75.6, MMSE 22.2) and 16 demographically matched controls (mean age=72.6, MMSE 27.9), we measured hippocampal, amygdala, and parahippocampus CBF as a % of visual cortex. In addition, we corrected for atrophy on a voxel-by-voxel basis by measuring gray matter volume in each voxel using T1 maps that were acquired in the same sequence as the perfusion maps. A region of interest analysis was performed using an *a priori* template, with left and right regions averaged together. The table below shows the results of the atrophy-corrected perfusion measurements as a percentage of visual cortex perfusion.

	Hippocampus	**Amygdala**	**Parahippocampus**
Mean AD (SD)	0.96 (0.29)	0.61 (0.19)	0.74 (0.20)

Mean CN (SD)	0.77 (0.17)	0.52 (0.11)	0.62 (0.14)
Prob of difference	0.008	0.043	0.017

Choice of levetiracetam as a pharmacologic probe

In this pilot study, we aim to determine whether the hippocampal hyperperfusion seen on ASL-MRI reflects abnormal epileptiform activity by using levetiracetam as a pharmacological probe. Even intermittent epileptic discharges, in the absence of clinically obvious seizures, may lead to memory impairment. If so, a reduction of hippocampal epileptiform activity could improve declarative memory and potentially alter progression of AD.

Levetiracetam, a newer antiepileptic drug, decreases the frequency and limits propagation of epileptiform activity without affecting normal neuronal excitability [28, 29]. The mechanism of action of levetiracetam is not fully understood, but it binds specifically to synaptic vesicle protein SV2A and likely modulates its function [30]. Levetiracetam may also have an indirect effect on GABA-A inhibitory neurotransmission and on high-voltage activated calcium channels. Levetiracetam decreases cortical excitability measured by transcranial magnetic stimulation [31]. In a study of levetiracetam in photosensitive epilepsy, 250mg oral doses decreased epileptiform activity induced by photic stimulation within 1 hour, and 750mg oral doses completely abolished epileptiform activity [32]. Acute dosing of levetiracetam 500mg caused significant reduction in the frequency of interictal epileptiform activity in 8/10 patients with epilepsy [33]. Because of its rapid onset of action and effect on interictal epileptiform activity as well as seizures, levetiracetam is an excellent candidate AED to examine changes in perfusion on ASL-MRI.

Levetiracetam is FDA-approved for the treatment of partial epilepsy in adults and children > age 4 years. Levetiracetam has a serum half-life of 10-11 hours in healthy elderly subjects [34]. Excretion is 100% renal; the half-life is prolonged in patients with renal impairment. There is no significant plasma protein binding. Because levetiracetam is not metabolized by hepatic microsomal P450 enzymes, it has no known drug interactions and kinetics are linear [35]. Levetiracetam intravenous infusion is bioequivalent to oral tablets [36].

Cognitive effects of acute administration of levetiracetam have been studied in healthy volunteers, ages 20-50 years [37]. There was no difference between levetiracetam and placebo in the summary score for the cognitive neurophysiologic test (CNT), which combines electroencephalography (EEG), evoked potential (EP), and cognitive performance measures.

Levetiracetam is a well-tolerated and safe AED in elderly patients. In 166 elderly patients with cognitive disorders (post-stroke or post-traumatic cognitive disorder or memory disorders), asthenia (16.9%), somnolence (7.8%), headache (7.2%), and dizziness (7.2%) were the most common adverse effects [35]. In an open label phase IV study of patients > 65 years with epilepsy, approximately 40% of patients reported one or more adverse events, usually mild or moderate in severity, with 19.2% discontinuing levetiracetam because of side effects [38]. Somnolence (16.7%) and dizziness (9%) were the most commonly reported adverse effects. In 24 cognitively impaired elderly patients with epilepsy treated with levetiracetam for 12 weeks, significant overall improvements were observed for the Folstein's Mini-Mental State Examination and the Alzheimer's Disease Assessment Scale-Cognitive [39]. A study comparing AED retention rates in patients > age 55 found levetiracetam to have the second-highest one-year retention and seizure-free rate (73%), at a median daily dose of 1500mg [40]. In an open label study in

patients with AD and epilepsy, levetiracetam was effective and well tolerated [41]. Of 25 patients with severe AD treated with levetiracetam 1000–1500 mg daily, 72% were seizure-free for at least one year, 16% discontinued for adverse effects, 8% were unresponsive, and 4% were lost to follow-up.

Data on acute use of intravenous levetiracetam in elderly patients is limited. Levetiracetam is well tolerated in healthy young subjects (< 65 years) after 15 minute (2000-4000mg) and 5 minute (1500-2500mg) infusions [42]. One retrospective observational study included 14 older patients (mean age 73.9 years) with a series of complex partial seizures or convulsive or non-convulsive status epilepticus needing emergent intravenous (IV) treatment [43]. Seizure control was achieved in 11/14 patients (78.6%) at a mean dosage of 1,643 mg/day (range 500-4,000). The only adverse effect was sedation.

Levetiracetam was recently tested in animal models of AD and in adults with mild cognitive impairment. Levetiracetam 5 mg/kg reduced epileptiform discharges and improved memory performance in hAPP mice, while sodium channel blocking AEDs resulted in worsened memory performance. In a recent study in patients with mild cognitive impairment, levetiracetam 250mg twice daily for two weeks normalized hippocampal hyperperfusion, as measured by blood oxygen level dependent (BOLD) MRI. For this study, we chose two doses of levetiracetam (low 2.5 mg/kg and high 7.5 mg/kg) based on the information above.

B3. DESCRIPTION OF RESEARCH PROTOCOL

A. Study Design – Overview, Methods, Procedures

1. Study Overview

This double-blind, within-subject crossover pilot study will test whether increased hippocampal perfusion on ASL-MRI in AD is caused by epileptiform activity. If epileptic activity is occurring and impairing hippocampal function, then levetiracetam should lead to decreased rCBF in the hippocampus and improved declarative memory.

Twenty subjects with mild AD (MMSE≥20) will participate in this study in the Clinical Research Center at Beth Israel Deaconess Medical Center. Subjects will be screened to ensure they meet inclusion and exclusion criteria and will provide written informed consent before any study procedures are performed. Each eligible subject will undergo three sessions, held 1-2 weeks apart, in a double blind, within-subject crossover design, counterbalanced for order (placebo / levetiracetam 2.5 mg/kg / levetiracetam 7.5 mg/kg), Figure 3. During each session, placebo or levetiracetam will be administered intravenously over 20 minutes. Drug administration will occur during EEG recording so that neurophysiological recordings can be acquired before, during, and after administration. Changes in EEG background frequency and level of synchrony will be analyzed [44-46]. After drug administration and EEG recording, hippocampal perfusion and functional connectivity will be measured with ASL-MRI and resting BOLD fMRI. Finally, participants will undergo a cognitive battery with memory tests particularly sensitive to hippocampal function (declarative memory, attention, working memory and executive function).

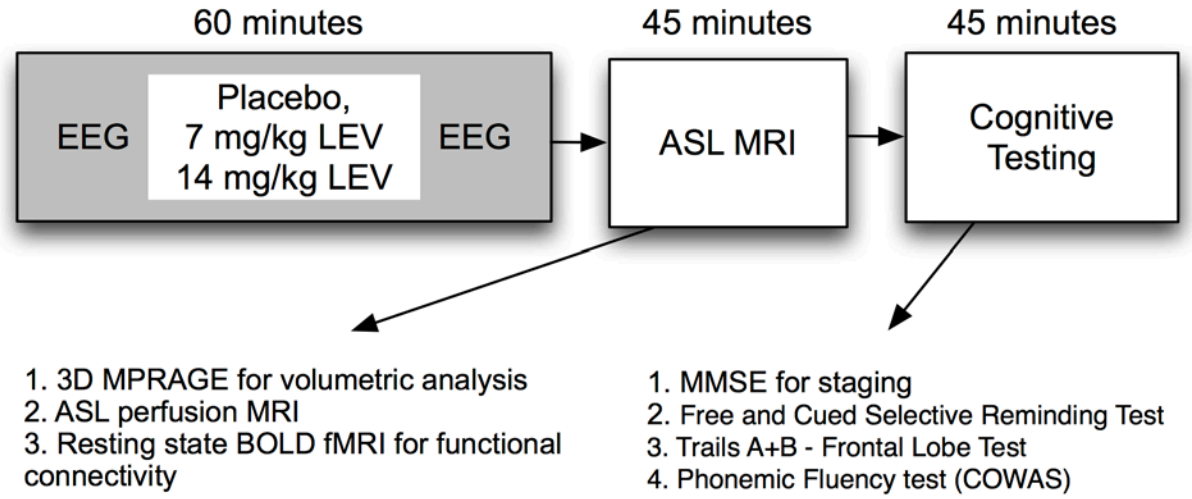

Figure 3. Overall paradigm. Participants will undergo testing in 3 sessions counterbalanced for order, with placebo, low dose LEV or higher dose LEV. Drug will be administered intravenously during EEG recording. Imaging will be acquired with anatomical, perfusion and resting state bold MRI. Cognitive testing will be performed with a battery including a memory test particularly sensitive to hippocampal function as well as other tests of frontal function.

2. Study Rationale

This protocol has three primary goals: to test the hypothesis that increased hippocampal perfusion in AD is at least partially driven by epileptic activity, that lowering epileptic activity may improve cognitive performance, and that perfusion MRI can be used as a non-invasive assay of the effects of medications that decrease epileptic activity in AD.

3. Subject Recruitment and Screening

We plan on testing twenty AD participants (> age 50 years) meeting the NINCDS-ADRDA criteria for probable AD from the Cognitive Neurology Unit at BIDMC. All participants will have mild AD by the Mini-Mental Status Examination (MMSE≥20). The P.I., who specializes in neurodegenerative disorders, maintains an active clinic and treats over 100 patients with AD. In addition, the Cognitive Neurology Unit has over 13,000 patient visits per year. Exclusion criteria include a history of seizures prior to the onset of AD, current use of an antiepileptic medication, current use of a medication known to lower seizure threshold (e.g. bupropion or a neuroleptic), presence of parkinsonism, significant cerebrovascular disease, other CNS disease or major depression. Subjects will be screened for depression with the Geriatric Depression Scale and excluded if they score greater than 9 [47]. Participants receiving standard treatments for dementia including cholinesterase inhibitors, memantine and serotonergic selective reuptake inhibitors may participate.

4. Crossover Design, Randomization, and Blinding

This is a within-subject, crossover study. Each subject will receive one of 3 study drug regimens at each session:

a. Placebo
b. Low dose levetiracetam (2.5 mg/kg)

c. High dose levetiracetam (7.5mg/kg)

A computer-generated, blocked randomization list will be generated that will randomly assign subjects to one of 6 groups. The order of drug administration will be counterbalanced to avoid pitfalls of the crossover repeated measures design.

Group 1: A, B, C
Group 2: A, C, B
Group 3: B, A, C
Group 4: B, C, A
Group 5: C, A, B
Group 5: C, B, A

The BIDMC research pharmacy will provide for randomization and blinding. Patients and investigators will be blinded to study group.

5. Study Procedures

Details and timing of the study procedures are listed below in the section "Study Plan".

a. Demographics and baseline characteristics
b. Medical history
c. Neurologic history, including date of onset and progression of AD, history of prior seizures, and history of other neurological disorders.
d. Recent and concomitant medications
e. Assessment of renal function (blood urea nitrogen and creatinine)
f. Vital signs
g. At screening and at each visit: Systolic and diastolic blood pressure, heart rate, and respiratory rate; weight
h. 20 minutes before each dose of study drug, and at 10, 20, and 30 minutes after start of intravenous infusion of study drug: Systolic and diastolic blood pressure, heart rate, and respiratory rate
i. Physical exam: appearance, cardiovascular, lung/chest, abdomen, extremities, and musculoskeletal.
j. Neurological exam: mental status, cranial nerves, motor system, sensory system, reflexes, coordination, stance, gait
k. Mini-Mental Status Examination
l. Electroencephalogram
   A 21-electrode array (Fp1, Fp2, F3, F4, C3, C4, P3, P4, O1, O2, F7, F8, T3, T4, T5, T6, Fz, Cz, Pz, reference, ground) of gold disc electrodes will be placed using paste technique, with electrode positions determined by the 10-20 International System. Additional electrodes will be placed to monitor eye movements (superior and inferior to outer canthus of each eye) and electrocardiogram. EEG will be sampled at 250 Hz and band-pass filtered from 0.1-70Hz. EEG will be recorded for 20 minutes before, 20 minutes during, and 20 minutes after administration of study drug. During each 20 minute session, EEG will be recorded for 5 minutes with eyes open and 15 minutes resting with eyes closed. EEG will be analyzed using both visual and quantitative techniques [44-46]. EEG will be visually inspected to determine 1) whether baseline epileptiform activity is present and is altered by study drug, and 2) whether study drug has other effects on EEG (such as diffuse slowing), which might affect the results of cognitive testing. Automated artifact detection and artifact decontamination filters will be used to minimize contributions of eye movements and other artifact sources. Data

segments containing possible artifacts will be excluded from analysis. For each session, EEG measures will include alpha peak power, beta band power, and delta/theta band power. will be determined for each session.

m. Study drug administration
For each session, the BIDMC research pharmacy will prepare study drug (intravenous levetiracetam 2.5mg/kg, levetiracetam 7.5mg/kg, or identical-appearing placebo) according to the subject's assigned group. Commercially-available levetiracetam will be purchased using study funds. All IV study drug will be prepared, labeled, and dispensed by the research pharmacy in accordance with clinical GMP guidelines. All study drug will be stored in a secure location. The study drug will be administered over 20 minutes by a research nurse in the BIDMC General Clinical Research Center. Study drug will be infused at room temperature.

n. Imaging paradigm
All participants will undergo brain imaging with a structural MRI, a resting brain perfusion MRI using arterial spin labeling, and a resting BOLD fMRI for functional connectivity. Imaging will be acquired on a 3.0T research-dedicated General Electric scanner.

1. Structural MRI - A high-resolution MPRAGE sequence will be performed with 1x1x1mm isotropic voxels to allow for accurate measures of atrophy. This sequence requires 7 minutes for acquisition.

2. Perfusion MRI - Arterial spin labeling perfusion MRI will be performed using a custom sequence that employs several features to increase reliability for clinical studies: background suppression to reduce motion related noise and artifacts; fast spin echo imaging to eliminate distortion and susceptibility related signal attenuation; and a long post-labeling delay to insure labeled blood actually reaches brain tissue of interest (Dai et al., 2008). For quantification of the blood flow corrected for atrophy, a second, anatomical imaging acquisition with the identical imaging sequence as the perfusion sequence will be performed but with two inversion preparations replacing the ASL. We have successfully employed this sequence and method of atrophy correction in patients with Alzheimer's disease (Alsop et al., 2008). This sequence requires 10-15 minutes for acquisition.

3. Functional Connectivity MRI- A resting BOLD fMRI sequence will be applied a rapid-acquisition echoplanar sequence (TE 25msec, TR 2500ms, 3.75mm isotropic voxels) over a 10 minute epoch to measure correlated activity at low frequencies, a measure of the strength of functional connectivity.

o. Behavioral testing
Participants will undergo declarative memory testing with the Free and Cued Selective Reminding Test, a task that is particularly sensitive to hippocampal function [48]. In addition, a short battery will be administered to test executive function, naming, visuospatial ability and semantic function (Trail Making Test Parts A+B, Phonemic fluency Test, Boston Naming Test 15-item version, clock drawing, category fluency). The primary endpoint for memory testing will be the free recall score from the Free and Cued Selective Reminding Test. This battery is fairly brief (30 min) but sensitive to both hippocampal function and changes in other cognitive realms impaired in AD.

p. Adverse events observed by investigator or reported spontaneously by the subject

6. Study Plan

The following assessments and procedures will be done at each study visit:

a. Screening visit

1. Informed consent
2. Demographics and baseline characteristics
3. Medical history
4. Neurologic history
5. Recent and concomitant medications
6. Blood collection for blood urea nitrogen and creatinine)
7. Vital signs: Systolic and diastolic blood pressure, heart rate, and respiratory rate; weight
8. Physical exam
9. Neurological exam
10. Mini-Mental Status Examination

b. Visits #1-3 (identical procedures, visit length 5-7 hours)
   1. Vital signs: Systolic and diastolic blood pressure, heart rate, and respiratory rate; weight
   2. Physical exam
   3. Neurological exam
   4. IV catheter placement
   5. EEG (60 minute recording)
   6. Study drug infusion (20 minutes)
   7. Vital signs at 10, 20, 30 minutes after infusion
   8. Imaging paradigm (60 minutes)
   9. Behavioral testing (60-90 minutes)
   10. Adverse events

   There will be scheduled breaks between tests and scheduled lunch

c. Telephone follow-up
   1. Follow up for adverse effects

B. Statistical Considerations

Sample Size Justification:

We plan to study 20-25 subjects at BIDMC. We anticipate screening 25 subjects to enroll and randomize 20 eligible subjects. We have performed a power calculation based on our previous study finding hyperperfusion of the hippocampus in AD as compared to controls [23]. With a sample size of 20 AD participants, we will have a 72% probability of detecting a decrease in perfusion after levetiracetam, assuming administration of the drug lowers hippocampal CBF only to normal levels. This estimate is likely overly conservative for two reasons. First, the within subject design should markedly decrease variance. Second, if epileptiform discharges are contributing to hippocampal hyperperfusion and damage, then briefly ceasing the abnormal activity with drug should reveal underlying hypoperfusion like that typically seen in temporal lobe epilepsy.

Data Analysis:

The primary study outcomes are:

Differences in hippocampal perfusion on ASL-MRI
Differences in memory performance

The secondary study outcomes are:

Differences in whole brain regional cerebral blood flow on ASL-MRI
Differences in working memory and executive functions
Differences in EEG measures (alpha peak power, beta band power, delta/theta band power, coherence)

Behavioral Data
Measures of memory encoding, retrieval and storage will be analyzed using repeated measures ANOVA. We will test our a priori main hypothesis of improved memory performance after drug. In a secondary analysis, we will measure effects of drug on working memory and executive functions in a similar manner.

Imaging Data
We will do two analyses, one focused specifically on hippocampal perfusion and a second analyzing regional CBF throughout the whole brain. First, we will use a repeated measures ANOVA with hippocampal perfusion and session (placebo, 2.5mg/kg and 7.5mg/kg) being the factors. Hippocampalm perfusion is measured as a percentage of visual cortex perfusion, using a template available within the Statistical Parametric Mapping package. Second, we will measure regional CBF throughout the whole brain using SPM5 and measure changes after drug vs. placebo using a random effects model and accounting for the repeated measures design. In addition to direct analysis of perfusion, we will examine cortical changes in CBF correlated with our seed region in the hippocampus. We predict that decreases in perfusion in the hippocampus after levetiracetam will correlate with increases in perfusion in association cortical regions. Lastly, we will test for correlations between behavioral measures of memory and regional changes in CBF in hippocampus and whole brain. We predict that decreases in hippocampal perfusion will paradoxically correlate with improved declarative memory performance.

EEG Data
EEG will be visually analyzed for presence of epileptiform activity. Peak alpha power, beta band power, delta/theta power, and coherence will be calculated using BESA (Brain Electrical Source Imaging) software and Persyst MagicMarker software. Measures will be analyzed using repeated measures ANOVA. We hypothesize that levetiracetam will decrease source coherence in temporal regions.

Analyses will be performed in the Cognitive Neurology Unit, Magnetic Resonance Imaging Center of the Department of Radiology, and EEG Laboratory at Beth Israel Deaconess Medical Center.

C. Subject Selection

Subjects will be recruited from the Cognitive Neurology Unit at BIDMC. The P.I., who specializes in neurodegenerative disorders, maintains an active clinic and treats over 100 patients with AD. In addition, the Cognitive Neurology Unit has over 13,000 patient visits per year.

Inclusion Criteria:

- Meets the NINCDS-ADRDA criteria for probable Alzheimer's disease
- Mild AD (MMSE≥20)
- Age >= 50 years
- English as first language

Exclusion Criteria:

- A history of seizures prior to the onset of AD
- Familial Alzheimer's Disease due to known genetic mutations
- Current use of an anti-epileptic medication
- Current use of a medication known to lower seizure threshold (e.g. bupropion or a neuroleptic)
- Presence of parkinsonism
- Significant cerebrovascular disease
- Other Central Nervous System disease (e.g. stroke, severe traumatic brain injury)
- Major depression or other psychiatric or behavioral disorders (psychosis, agitation)
- Medical contraindication to MRI (e.g. cardiac pacemaker, intraocular or intracranial metallic objects)
- Severe claustrophobia or inability to lie flat for MRI
- Known allergy to levetiracetam, or history of previous adverse reaction to levetiracetam.
- Serum creatinine >= 2

Participants receiving standard treatments for dementia including cholinesterase inhibitors, memantine and serotonergic selective reuptake inhibitors may participate.

B4. POSSIBLE BENEFITS

The present study is designed to enhance our understanding of the role of epileptiform activity in Alzheimer's disease. There is no direct benefit to the subject from participating in this study.

Confirmation of a relationship between excitatory neuronal activity and cognitive deficits in humans with AD would provide new insights into the pathogenesis of AD. If levetiracetam decreases this excitatory activity, antiepileptic drugs may be a new potential avenue for treatment or prevention of AD.

B5. POSSIBLE RISKS AND ANALYSIS OF RISK/BENEFIT RATIO

Several aspects of this trial have a potential for subject discomfort or injury. All study procedures are low- to moderate-risk procedures, as described below. These risks are balanced by the potential benefit that the knowledge gained from this research may help to determine new methods to treat or prevent Alzheimer's Disease.

Study drug: Levetiracetam has been approved by the FDA as safe and effective in the treatment of seizures and epilepsy and is generally well tolerated. No laboratory monitoring is recommended by the FDA for patients receiving levetiracetam for seizures. Levetiracetam may cause some, all, or none of the side-effects listed below. Subjects will receive only one dose of study medication at each visit, so side effects should not last longer than 12-24 hours.

> More Common: The most frequently reported side effects from levetiracetam are drowsiness, dizziness, nausea, and headache. Other side effects can include vomiting, tremor, irritability, or depression. More than 10% of subjects may experience these side effects when levetiracetam is given intravenously. The side effects are usually mild and temporary. The medication will be given slowly over 20 minutes to minimize these side effects.
>
> Less Common: Less common side effects (occurring in 1-10% of subjects) include anger, anxiety, decreased appetite, diarrhea, and infection (colds or viral infections).
>
> Rare: Rare side effects (occurring in less than 1% of subjects) include severe depression or the development of suicidal thoughts, effects on blood cells (low blood counts, low platelets or white blood cells), liver failure and weight loss.

Blood draws: The risks and discomforts of blood drawing from a vein include the possibility of pain or bruising at the site of the blood draw; occasional feeling of lightheadedness; and rarely, infection at the site of the blood draw.

Intravenous catheter placement and medication infusion: Levetiracetam and placebo will be given through an intravenous catheter. Possible risks of the catheter or infusion include: skin infection at the catheter site; redness, swelling, discomfort, pain, or bruising at the injection (IV) site; bruising of the skin caused by broken blood vessels leaking blood under the skin; or local thrombosis.

EEG: EEG is a commonly used procedure which has low risk. Some subjects experience mild discomfort during scalp preparation for electrode placement, or have itching or skin irritation at the electrode site. Some subjects find it difficult to lie still during the EEG, which will last approximately one hour.

MRI scans are safe and routinely used in medical diagnosis. Exposure to strong, static magnetic fields is accepted as safe. Subjects will be screened for MRI safety, including presence of metal in their body. For this protocol, all MRI studies will be performed on a 3T General Electric research magnet that has received FDA approval. There is no exposure to ionizing radiation. This technique is non-invasive and does not require administration of exogenous contrast agents. The most common adverse event associated with MRI is claustrophobia. Subjects will have voice contact with the technologist performing the study at

all times, and the MRI scan can be immediately stopped at the subject's request.

If subjects are injured as a direct result of participation in this study, BIDMC will provide the necessary care to treat the injury. The subject or his/her insurance company will be billed for medical care and/or hospitalization related to this injury. Subjects will be responsible for all copayments and deductibles required under their insurance. BIDMC will consider reimbursement of injury related expenses not covered by insurance on a case-by-case basis. There is no reimbursement for items such as lost wages or lost time from work.

B6. RECRUITMENT AND CONSENT PROCEDURES

Recruitment

Investigators will recruit from their own patient population as well as screen the neurology clinical research database and appointment books for patients who qualify for the study. The investigators will reach out to other neurology physicians to inform them about the study so that they may refer patients who are interested in participating to the investigators. Flyers describing the study will be placed in the Cognitive Neurology Unit and in the outpatient neurology clinic.

A neurologist in the Cognitive Neurology Unit at the Beth Israel Deaconess Medical Center will approach potential study participants. The Principal Investigator will then contact those patients who express an interest in participating in the study. The Principal Investigator will tell potential subjects about the ongoing study, including the specific aims of the study, outline of the study design, techniques to be used, and anticipated risks and benefits. If the potential subject expresses an interest in the study, Dr. Press will ensure that exclusion and inclusion criteria are met and that there are no contraindications for study participation by reviewing the patient's medical record and/or completing a full neurological history and physical examination of the patient. If the potential subject meets these criteria, he or she will be invited to participate in the study. Written informed consent will be obtained after all of the subject's questions have been completely answered.

Consent/Subject Protection

The Principal Investigator will obtain informed consent from all subjects up to one week prior to their participation in this study. The consent form describes the study and provides sufficient information for subjects to make an informed decision about their participation in this study. The principal investigator or his designee will discuss the study with the subject. Patients with mild AD (MMSE≥20) are able to understand a consent form and over 95% can supply true consent (Buckles et. al, Neurology 2003). Participants will be reminded at the start of each session that they may discontinue at any time and that this will not affect their care in any way. In addition, we will engage a health care proxy, spouse, or close family member, where available. The subject will be given time to read the consent form and to ask questions. Upon entry into the study, the subject or surrogate will receive a copy of the consent form, and a copy will be filed with their study binder.

B7. STUDY LOCATION

Privacy

The subjects' privacy will be protected by ensuring that the conditions under which information will be collected afford protections against interactions with participants being witnessed, overheard or inadvertently intercepted or viewed. Furthermore, the information being collected will be minimized to include only the data necessary to accomplish the research.

A unique identifier code will be assigned to each subject. All information, including EEG data files, will be stripped of personal identifying information. Patient confidentiality will be strictly maintained, and results of all testing will be available only to subjects and/or their health care providers, if requested by the subjects.

Physical Setting

Study sessions will in the Clinical Research Center on the East Campus of BIDMC. MRI scans will be conducted in the Research Radiology department in an adjoining building (Rabb Building). Drug administration will take place at the Clinical Research Center and the Research Pharmacy will be responsible for counterbalancing.

B8. DATA SECURITY

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization.

Subject binders will be kept in in a locked cabinet accessible only to the investigators. For data analysis, data will be entered into a password-protected computerized database behind the BIDMC firewall. The investigator will retain study essential documents for at least 2 years after completion of the study.

General Safety

All safety considerations were included as part of the design of this study.

Data Safety Monitoring Plan

Monitoring of Adverse Events: While levetiracetam is not used in treating Alzheimer's disease, it is widely used in the treatment of epilepsy, including epilepsy that develops in patients with Alzheimer's disease. We will be using a single dose, so the risk of side effects is low. Participants will complete the side effects questionnaire prior to and after every session in order to evaluate side effects.

The occurrence of any unexpected undesirable side effect will result in holding the trial until communication with the IRB, the CRC administrative staff, and the Research Subject Advocate

has taken place, and the risk for participants reassessed. Participation in the study for any given participant will stop in the event of any unexpected side effects or any cognitive deterioration as measured by the serial assessments described above.

Reporting of Adverse Events: All adverse events will be reported and reviewed every week according to laboratory policies. Information about all adverse events relating to this study will be collected and reported on the attached Report of Adverse Event Form. Any adverse event occurring in participants after they sign the consent form and until two weeks after the trial will be reported according to the following guidelines:

(1) All fatal, life-threatening or serious adverse events will be reported verbally (7-1764) and faxed (7-5953) to the CRC RSSO Director and the CCI within 24 hours.
(2) Unexpected serious adverse events will be reported verbally in real time to the CCI and the CRC RSSO Director.
(3) Unexpected moderate adverse events will be reported in writing to the CCI and the CRC RSSO Director within 10 days of occurrence or recognition.
(4) The PI will provide the CRC RSSO with any copies of adverse event reports that are sent to any entity such as the CCI,FDA, and NIH within the same time constraints.

The PI will also provide the CRC RSSO with an annual summary of adverse events (including anticipated, non-serious adverse events).

B9 Multi-Site Studies

Is the BIDMC the coordinating site or is the BIDMC PI the lead investigator of the multi-site study?
☐ Yes ☒ No

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