**INTRODUCTION TO REVISED APPLICATION:** I thank the reviewers whose input has strengthened this proposal. The reviewers were enthusiastic about the initial proposal, speaking to the "strong publication record" of the applicant (R2), "exceptionally strong" (R1) and "highly committed" (R3) mentorship team, "stellar research program" (R1), "strong training plan with benchmarks that are attainable" (R2), and an institution that "offers a strong environment for the applicant's training project, especially for PET/MRI techniques" (R3). R1 also spoke to the "novelty" of the proposed research. Modifications addressing Reviewers' concerns are summarized below.

**Improved Training Plan*: Emphasis on training in aging and Alzheimer's disease relevant research:*** *"If training by Dr. Price is critical … she might have a more central role" (R1). "Many of the activities appear to suggest that the applicant will simply learn on the job" (R1).* The revised Training Plan now centers on concrete training goals in cognitive aging and Alzheimer's disease (Training Goal #1), including didactic training through the "Alzheimer's Fast Track" short course (Brightfocus Foundation), involvement with the Alzheimer's Association's ISTAART organization, frequent collaboration with Dr. Julie Price (see LOS), and presentations at the Alzheimer's Assoc. International Conference, Human Amyloid Imaging, and Dallas Aging & Cog. Conference.
***Reframing of MRI/PET training goals:*** *"The need for additional training in PET and MRI research is unclear" (R1) "The applicant has limited experience in PET imaging" (R3). "Unclear…training needs in regards to moment-to-moment variability in brain activity using fMRI" (R2).* We apologize for the lack of clarity in the MR/PET training goals, which may account for somewhat conflicting reviewer comments. The applicant has foundational experience with PET kinetic modeling (undergraduate training) and fMRI (graduate training), without which this multisession combined MRI/PET study would be considered too ambitious. We now emphasize training in the successful *integration* of this multimodal information acquired from simultaneous MR/PET scans (Training Goal #2). Sponsor Dr. Jacob Hooker is a leading expert in this area and the incorporation of the pharmacologic manipulation to measure simultaneous alteration in dopamine function (PET) and BOLD activity (fMRI) takes unique advantage of this promising technology. Consultant Dr. Garrett will provide essential expertise on the nuanced field of neural variability and the interactions between the dopamine system and fMRI signal. Moment-to-moment variability research has benefitted greatly from rigorous (and rapid) methods development, and has been shown to be a sensitive individual differences measure relevant to aging and disease. Careful interpretation of results across lab and methodological approaches requires training.

**Improved Research Strategy:** ***Improved clarity on research timelines and work accomplished:*** *"it appears as though much of the research is already done…The results in figure 5 imply that the study has already been completed" (R1)* The caption in Figure 5 has been updated to more clearly explain that it presents data from an entirely separate study conducted at UC Berkeley by Co-Sponsor Dr. Anne Berry using a static measure of dopamine synthesis. These data provide initial proof-of-concept support that dopamine moderates associations between tau and cognition in aging, and suggests our new study is adequately powered to detect these interaction effects. The proposed project critically expands upon this previous work by examining dynamic *endogenous dopamine release* with [$^{11}$C]-Raclopride and second-generation tau-PET radiotracer (MK6240).
***Clarification of research hypotheses:*** *"The hypothesis in Aim 1 that increased levels of tau will be associated with poorer memory has been demonstrated" (R3). "No alternative approaches if the hypotheses need to be modified" (R3).* Aim 1 has been updated to focus on only the novel hypotheses. We have expanded our discussion of approaches (e.g. analysis of baseline D2/3 receptor occupancy in participants who do not qualify to receive methylphenidate, and alternative metrics of dynamic shifts in functional connectivity)
***Analysis of ptau-181:*** *"Plasma measures of amyloid are almost certainly less helpful than plasma measures of phosphorylated tau" (R1)*. Will also collect ptau181 in addition to beta-amyloid 40/42.

**Additional Changes:** ***Addition of biostatistician:*** *"There is no integration of a neuroimaging statistician" (R1, 3). "The likelihood that the interaction effect between dopamine and tauopathy on memory is unlikely to be statistically powered." (R1)* Dr. Nate Mercaldo, a biostatistician in the MGH Department of Radiology and Instructor of Statistics at Harvard Medical School, will join the mentoring team as a collaborator (see LOS). Interaction power analyses are included.
***Additional safety information included:*** *"Participants will receive methylphenidate 20mg but precautions including exclusion criteria have not considered potential for adverse effects for the age of the sample" (R2).*
*"Plans for identifying and ensuring validity of chemical resources is not provided" (R2).* Now included.
***Efforts to recruit a diverse participant cohort:*** *"It may be helpful… to over-sample individuals from non-white groups to facilitate subgroup analysis" (R1).* We have partnered with a marketing firm, BuildClincial, to recruit participants with Black, Hispanic/Latino, and Indigenous racial/ethnic backgrounds.
***Primary sponsor's funding:*** *"The primary sponsor's current NIH R01 grant focuses on Huntington's disease" (R3).* Dr. Anne Berry (Co-Sponsor) is the PI on two ongoing projects that will fund the proposed data collection (R00 AG058748; R01 AG074330). Dr. Hooker (Sponsor) is a Co-I on these ongoing projects.

**APPLICANT'S BACKGROUND AND GOALS FOR FELLOWSHIP TRAINING**

**A. DOCTORAL DISSERTATION AND RESEARCH EXPERIENCE**

My scientific goal is to understand how neurochemistry modulates age-related changes in cognition and brain activity. I am particularly interested in the potential protective role neuromodulatory systems may play in the earliest stages of Alzheimer's disease. My proposed research combines multimodal neuroimaging with advanced computational methods to track the cognitive and neural markers of AD in a longitudinal cohort of healthy older adults. I am a newcomer to the field of cognitive aging and Alzheimer's disease, and while I have a lot to learn, I also believe that I have a lot to offer. I have a strong quantitative background with a Bachelor's degree in Computer Science and a PhD in Computational Neuroscience. During graduate school, I gained extensive experience using functional magnetic resonance imaging (fMRI) to study memory and learning, publishing two first-authored and one co-authored manuscripts. During my postdoctoral fellowship, I will apply my previous quantitative and neuroscientific training to investigate the neurochemical factors that may preserve cognitive function in the face of AD pathology. Together with Dr. Jacob Hooker (Sponsor) and Dr. Anne Berry (Co-Sponsor), **I have developed a training plan to gain expertise in cognitive aging and AD-relevant research (Goal #1), learn innovative techniques for integrating MRI with PET imaging (Goal #2), and receive training in leading collaborative, interdisciplinary scientific teams (Goals #3)**.

**Undergraduate Research Assistant (March 2015 – May 2017)**

*Martinos Center for Biomedical Imaging, Mass. General Hospital, Charlestown, MA*

*Mentors: Jacob Hooker, PhD; Hsiao-Ying Wey, PhD*

My undergraduate coursework spurred my interest in studying the human brain. I was first exposed to studies of memory and learning in my Cognitive Psychology class, where I was fascinated by the way functional neuroimaging offered a "window" into the mind. This interest prompted me to join the lab of Dr. Jacob Hooker at the MGH Martinos Center as an undergraduate intern. My primary project was to develop a user-friendly simulation tool for computational modeling of PET radiotracer pharmacodynamics. Through the development of this tool, I learned to code in MATLAB and gained a deeper understanding of what PET signal is measuring. This project resulted in a graphical tool that chemists in the lab can use to model pharmacodynamics of potential new radiotracers without the need to write any code themselves. The tool is posted to GitHub so that other PET researchers can take advantage of it. Work on this project contributed to a co-authored review paper on PET neuroimaging (Placzek et al. 2015). I was also able to assist other lab members with their pharmacokinetic analyses, resulting in two additional co-authored publications (Gilbert et al., 2019; Strebl et al., 2017).

During the final year of my undergraduate degree from Tufts University, I conducted a senior honors thesis project in Dr Hooker's lab. The focus of the project was to optimize a new *functional* neuroimaging technology using FDG-PET. Fluorodeoxyglucose (FDG) is a radiotracer used by neuroscientists to measure regional differences in cerebral glucose metabolism. In traditional PET imaging, FDG-PET gives researchers a static summary image of the average glucose metabolism in the brain. Through the development of functional PET with FDG (fPET-FDG), we aimed to track *dynamic* task-related changes in cerebral glucose metabolism. My thesis compared the effect of different radiotracer delivery methods (constant infusion vs. bolus-plus-infusion) on fPET-FDG signal. Culminating in a poster, written thesis, and oral defense, results from this project demonstrated that the bolus-plus-infusion method strengthened the signal of fPET-FDG in occipital cortex when subjects viewed a visual flashing checkerboard stimulus. This project taught me basic skills in PET image analysis that I will expand upon during my postdoctoral research. Additionally, this project sparked my interest in dynamic neuroimaging analyses which I continued during my dissertation and will expand upon through proposed neural variability analyses in my postdoc (see Aim #2).

My work as an undergraduate in Dr. Hooker's lab taught me important skills in taking a project from start to finish. In my postdoc, I will expand my PET neuroimaging skills beyond pharmacokinetic modeling, learning to also collect and reconstruct data, build custom preprocessing pipelines, and learn to work with new radiotracers for measuring dopamine receptor density and tau burden. Moreover, the proposed project will integrate MRI and PET imaging in an innovative new way to investigate the relationships between brain chemistry and brain activity simultaneously during a memory task in healthy older adults.

**PhD Research Rotation (December 2017 – April 2018)**

*Department of Psychological and Brain Science, Boston University*

*Mentor: David Somers, PhD*

During my first year of graduate school, I completed a research rotation in the Attention and Perception Neuroimaging Lab, directed by Dr. David Somers. During this research rotation, I worked with an existing fMRI

dataset collected by a former graduate student to study the functional brain networks that support memory-guided attention (Rosen et al., 2016; Rosen et al., 2017). We confirmed that the frontoparietal control network was activated during tasks that required participants to incorporate information from long-term memory into guiding their perceptual attention. These results complemented existing literature that has implicated the frontoparietal control network in the integration of information from opposing attention and memory systems. Results from this research rotation greatly informed my dissertation, providing me with another perspective on the function of the frontoparietal control network. Additionally, I learned several methodologies from members of Dr. Somers's lab including preprocessing techniques for task-based and resting state fMRI data using FreeSurfer, statistical methods for multiple comparison correction, and use of Boston University's Shared Computing Cluster for efficiently running analysis pipelines in parallel. Through this rotation, I made a strong connection with Dr. Somers who has served informally as a collaborator and mentor, and formally as a member of my dissertation advisory committee.

When a new graduate student joined the lab in the fall of 2020, we didn't have much new data for her to work with because of the pandemic. I was able to share what I had worked on from this rotation project, and mentored the new student as she conducted a follow-up analysis. This formed the basis for a new co-authored manuscript was recently published (Isenburg et al., 2023).

**PhD Dissertation Research (August 2017 – May 2022)**
*Graduate Program for Neuroscience, Boston University, Boston, MA*
*Mentor: Chantal Stern, DPhil*

As a doctoral student at Boston University, I used functional magnetic resonance imaging (fMRI) to study how the brain's functional network architecture dynamically reconfigures to support reasoning, learning, and memory. My dissertation research resulted in two first author papers (Morin et al., 2021; Morin et al., 2022), and ten poster presentations at local, national, and international conferences (e.g. Society for Neuroscience, Organization for Human Brain Mapping, Cognitive Neuroscience Society).

My first project in Dr. Stern's lab was to develop a simplified version of the Raven's Progressive Matrices task – an abstract reasoning test originally designed to probe general fluid intelligence – that was suitable for testing in the fMRI scanner. After thorough behavioral piloting, we settled on a task design and successfully recruited and scanned a full cohort of healthy young adult participants (n=27). Using a dynamic network analysis, I showed that abstract reasoning is associated with flexible reconfiguration of the frontoparietal control network, and high stability within default and somatomotor networks. These findings were presented in several posters at the Society for Neuroscience and Cognitive Neuroscience Society meetings, and recently published as a first-author manuscript (Morin et al., 2022).

In another set of experiments, I studied the dynamic brain network characteristics associated with successful rule learning. In this study, naïve participants completed an associative learning task, attempting to learn a set of context-dependent rules through trial and error during fMRI scanning. I completed a dynamic functional connectivity analysis of the fMRI data comparing successful learners with unsuccessful learners. The results demonstrated that successful rule learning is associated with stable attention networks and a flexible frontoparietal control network. I presented these results in posters at the Organization for Human Brain Mapping and Society for Neuroscience conferences, and published a first-author manuscript (Morin et al., 2021).

Results from these projects contributed to the scientific understanding of how the brain's temporal dynamics (e.g., functional network flexibility and stability) support learning and memory. As part of the Graduate Program for Neuroscience (GPN) I completed coursework in graph theory, network science, advanced statistics, and computational neuroscience that have inspired my computational approaches to fMRI analysis. In my final year of graduate school, I was awarded the Russek Student Achievement Award, a prize given to one student in GPN each year for their scientific and community-building achievements. Throughout graduate school, I gained a deep interest in how the brain is able to learn and remember information. I was able to build a new research arm in Dr. Stern's lab by incorporating my computational and network-science training into the existing work happening in the lab. During my postdoctoral fellowship, I will extend my expertise in fMRI and integrate it with PET neuroimaging to study the neurochemical factors lead to deficits in (or the conservation of) memory and cognition in aging and Alzheimer's disease.

**Postdoctoral Fellowship & Visiting Research Scientist (July 2022 – Present)**
*Postdoctoral Research Fellow, Martinos Center for Biomedical Imaging, Mass. General Hospital, Boston, MA*
*Mentor: Jacob Hooker, PhD*
*Visiting Research Scientist, Neurochemistry and Cognition Lab, Brandeis University, Waltham, MA*

*Mentor: Anne Berry, PhD*

My previous research experience has established my interests in brain chemistry, cognition, and temporal brain dynamics. During my postdoctoral training, I will combine these interests to investigate how age-related changes in dopamine and tau impact dynamic brain activity and memory. My postdoctoral training, which began in July 2022, involves a unique cross-institutional collaborative mentorship between Dr. Jacob Hooker at the MGH Martinos Center for Biomedical Imaging and Dr. Anne Berry at Brandeis University. I am appointed as a Postdoctoral Research Fellow in the department of Radiology at Mass. General Hospital and I have also secured a Visiting Research Scholar appointment at Brandeis University. This unique mentoring arrangement offers several advantages. Because I have previously worked in Dr. Hooker's lab as an undergraduate research assistant, I sought a co-mentorship with Dr. Berry so that I could also experience a new research environment at Brandeis University. The collaborative mentorship facilitates training in both MR/PET neuroimaging (through both mentors) and cognitive aging (primarily through Dr. Berry). As an undergraduate research assistant in Dr. Hooker's lab I was primarily working on pharmacokinetic modeling of PET radiotracers. In my new role as a postdoc, with added training from Dr. Berry, I will transition from the theoretical world of mathematical models to applied neuroimaging research in cognitive aging. The proposed project will combine MRI and PET imaging to enhance our understanding of the interactions between neurochemistry and brain activity in aging. Moreover, my dissertation research focused entirely on basic cognitive processes using fMRI in healthy young adults. During my postdoc, I will apply advanced fMRI and PET imaging analyses to answer questions about how cognition changes with age. Finally, with a co-mentorship across MGH and Brandeis University, I get to experience working in both a large research hospital setting, and a small liberal arts college setting. I enjoy teaching and mentoring, so in the future, I plan to apply for faculty positions that include a teaching role. Gaining experience at Brandeis University in addition to Mass. General Hospital will help me prepare for such roles.

Dr. Hooker and Dr. Berry are ideal co-mentors. Dr. Hooker is a Co-Investigator on three grants that were recently awarded to the Berry lab (F31 AG079515, R01 AG074330, R00 AG058748). I am excited to join and learn from this interdisciplinary and cross-institutional collaborative team. During the first year of my postdoctoral training, I have worked hard to integrate myself into the cognitive aging and Alzheimer's research community. I joined the Alzheimer's Association's International Society to Advance Alzheimer's Research and Treatment (ISTAART), attending several of the workshops and seminars offered by the Neuromodulatory Subcortical Systems (NSS) and Neuroimaging Professional Interest Areas (PIA)s. In collaboration with researchers in Dr. Berry's lab, I worked to develop a preprocessing pipeline for the MR/PET data being collected for the proposed research project. In October 2022, I presented an invited talk on my research to the Cognitive Aging & Memory Lab, directed by Prof. Ayanna Thomas at Tufts University. In January 2023, I was invited to present at ISTAART's Neuromodulatory Subcortical Systems Professional Interest Area (NSS PIA)'s annual "year in review" webinar. In February 2023, I attended the Dallas Aging and Cognition Conference. I am a co-author on a manuscript (under review) with members from Dr. Berry's lab that investigates genetic polymorphisms related to BDNF and amyloid-beta using data from ADNI. Through the proposed research project and training plan, I look forward to establishing myself as an expert in multimodal neuroimaging, developing expertise in cognitive aging and AD, and gaining experience in collaborative, interdisciplinary science.

## B. TRAINING GOALS AND OBJECTIVES

My long-term goal is to become an independent researcher leading a positive, cross-disciplinary training group that uses multimodal neuroimaging technologies and advanced computational analyses to study cognitive aging and age-related disease. I enjoy teaching and mentoring and would like to have a faculty position at a research university or medical school where I could also have a small teaching role. Through the research and training goals described in this proposal, I will apply my computational background to exciting new questions in the field of cognitive aging. I will gain expertise in the cognitive neuroscience of aging and Alzheimer's disease, learn to integrate information from MRI and PET imaging, and build cross-institutional, interdisciplinary collaborations.

### 1. Training in Cognitive Aging and Alzheimer's-Relevant Research

**Develop expertise in cognitive aging, particularly in relation to the neuromodulatory systems that impact aging, Alzheimer's disease, and other dementias.**

During my postdoctoral research, I will gain training and first-hand experience working with older adults through collaborations with Dr. Anne Berry's (Co-Sponsor) lab. In Dr. Berry's lab, I will learn to run neuropsychological and MR/PET neuroimaging experiments with healthy older adult participants. Additionally, through regular meetings and joint-lab meetings with Dr. Julie Price and Dr. Bill Jagust (*Collaborators, see LoS*), I will have training in the analysis and interpretation of MR/PET neuroimages acquired with older adult populations. By

focusing on the impacts of dopamine and tau on memory in older adults, the proposed research will bridge two subfields of cognitive aging. Recent research places an increasing focus on the hedonic and affective changes that arise in AD, which have been posited to involve altered catecholamine function. Intriguingly, the locus coeruleus, a noradrenergic nucleus in the brainstem, is one of the first brain regions to show tau aggregation in aging. With this evidence in mind, understanding the combined influence of catecholamine neuromodulators and tau on cognitive aging is essential to making progress in the field of Alzheimer's and related diseases. Following my postdoctoral training, I will be uniquely positioned to address the neurobiological mechanisms underlying alterations in reward sensitivity in Alzheimer's Disease. Most previous PET neuroimaging research focuses on a single neurochemical factor such as dopamine in isolation. Through the proposed research, I will examine the joint effects of dopamine and tau on memory in older adults. Dr. Berry (Co-Sponsor) and Dr. Jagust (Collaborator) have experience considering multiple neurochemical factors in a single study, such as the joint effects of norepinephrine and tau on memory in older adults (Ciampa et al., 2022). Bridging the gap between these two subfields of cognitive aging will set me apart as an investigator and help me begin to build independence as a researcher.

During the first year of my postdoctoral research, I will enroll in a short course offered by the Brighfocus Foundation: "Alzheimer's Fast Track". This is a crash-course for researchers entering the field of Alzheimer's disease research with talks from experts and workshops to develop aging grants. Additionally, I will attend Dr. Berry's seminar course offered at Brandeis University: PSY 121b "Alzheimer's disease resilience and risk factors." I also plan to join the Executive Committee for the Neuromodulatory Subcortical Systems Professional Interest Group (NSS PIA) through the Alzheimer's Association International Society to Advance Alzheimer's Research and Treatment (ISTAART). In January, I delivered an invited talk for the NSS PIA's Year-in-Review Webinar where they discuss recent research advances in the field. As a member of the executive board, I will help plan additional webinars and journal clubs related to the neuromodulatory subsystems that are altered in aging and ADRD. To further expand my knowledge of cognitive aging and become a part of the research community, I will attend the Neurology Grand Rounds at MGH (monthly), and annual meetings including the Dallas Aging & Cognition Conference, Alzheimer's Association International Conference (AAIC) and Human Amyloid Imaging annual meetings.

**2. <u>Research Training in Integration of MRI and PET</u>**

**Develop innovative analysis methods to integrate MRI and PET imaging data.**

MRI and PET neuroimaging provide complementary information about brain activity and brain chemistry respectively. Together, information from the two modalities offer a unique opportunity to investigate how neuromodulators influence brain activity and vice-versa. In the proposed research, I will explicitly examine whether endogenous dopamine release (measured with [$^{11}$C]-Raclopride) is related to variability in BOLD signal (measured with fMRI). The study benefits from having both of these measures acquired simultaneously while subjects are also engaged in a reward-memory encoding task. Previous work suggests that dopamine may play a role in toning behavioral and neural flexibility. In our study, we can explicitly examine whether endogenous dopamine levels are related to changes in BOLD signal, before and after the administration of a dopamine-enhancing drug (methylphenidate). I will consult with Dr. Doug Garrett (collaborator) for assistance in analyzing and interpreting the variability of BOLD signal. Dr. Garret's group has shown that changes in neural variability are associated with age, working memory load, and dopamine availability (Garrett et al, 2015; 2022; Grady & Garrett 2014; Guitart-Masip et al., 2015). Consultation with Dr. Garrett will play a key role in interpreting neural variability data in the context of the nuanced literature on variability in aging. Dr. Garrett has agreed to regular (monthly) meetings via Zoom, and for me to visit his lab in Germany to learn more from his group. I will also consult with Dr. Nate Mercaldo (see LOS), a statistician at MGH, to ensure that we are using robust statistical methods that are appropriate for our hypotheses and sample size.

This proposal builds off of my extensive training with fMRI and limited previous experience with PET. Training in PET image analysis will occur primarily through Dr. Jacob Hooker (sponsor) who has extensive expertise in chemical neuroimaging. I will also participate in Dr. Julie Price's (collaborator) annual Pharmacokinetic Modeling workshop at the MGH Martinos Center. Through additional (monthly) meetings with Dr. Price and collaboration with. Members of her lab, I will learn to analyze the PET images collected for this project. Dr. Price's lab is easily accessible to me at the MGH Martinos Center and she has invited me to attend her regular lab meetings. Additionally, Dr. William Jagust (*Collaborator, see Letter of Support*) is an expert in tau and amyloid PET measures in cognitively normal older adults and will provide guidance on interpreting our dopamine findings in the context of preclinical AD. Dr. Jagust has agreed to regular (monthly) meetings and has invited me to present my research at one of his future lab meetings and visit his lab at UC Berkeley. Under the

mentorship of Dr. Berry, I will also play an active role in collecting MR/PET neuroimaging data from participants in the ongoing longitudinal Brandeis Aging Brain Study. These participants undergo neuropsychological testing, structural and functional MRI, as well as PET scans to measure endogenous dopamine release and tau pathology. At the MGH Martinos Center, I will have access to two simultaneous MR/PET scanners which I can use to acquire the data proposed in Aims 1-3. I will present my neuroimaging findings locally during the Martinos Center's annual Summer Symposium and Clinical Neuroscience Day, and at conferences including the Organization for Human Brain Mapping Conference.

**3. Training in Team-based, Collaborative Science**

**Learn effective mentoring strategies, understand team-based management techniques, and engage in collaborative cross-institutional research for training the next generation of scientists.**

The field of neuroscience is facing increasingly complex questions that require cross-institutional and interdisciplinary collaborations. During my postdoctoral fellowship, I will gain experience working across institutions with affiliations at both the Massachusetts General Hospital and Brandeis University. This unique collaborative mentoring opportunity will provide me with experience working in research settings at both a large research hospital and a small liberal arts university. The two research environments provide complementary benefits: the MGH Martinos Center is one of the largest neuroimaging centers in the world, and provides access to some of the world's leading experts in neuroimaging. Brandeis University has its own thriving neuroscience research environment, as well as a talented population of graduate and undergraduate students who I will mentor in the lab. Dr. Hooker (Sponsor) has extensive experience leading multi-institutional scientific teams. My weekly meetings and daily interactions with Dr. Hooker will support not only my scientific goals, but also my career aspirations to lead collaborative, interdisciplinary research projects. Additionally, I will gain experience working with mentors from complementary fields. Dr. Berry's background is primarily in cognitive neuroscience and Dr. Hooker's background is primarily in chemistry. My mentoring team will augment my quantitative background and prepare me to lead an interdisciplinary group of my own in the future. Both Dr. Hooker and Dr. Berry have agreed to provide training in the more "administrative" aspects of their jobs as professors. I will join regular meetings with lab managers and clinical research staff to learn the ins-and-outs of lab administration. This will prepare me to run my own lab in the future. Dr. Hooker (Sponsor) and Dr. Berry (Co-Sponsor) have assured me that I can take any data, analyses, and results that I collect as part of this proposal with me when I start my own lab.

During my dissertation work in Dr. Stern's lab I mentored several undergraduate students, teaching them to analyze fMRI data with AFNI, and working with them to develop independent research projects. Undergraduates I have mentored have presented their research at conferences like the Society for Neuroscience, been included as co-authors on my manuscripts, and secured their own undergraduate research funding. As a postdoctoral fellow, I will continue to gain mentoring experience through the supervision of graduate and undergraduate students in Dr. Berry and Dr. Hooker's labs. I will also continue to participate as a volunteer mentor in programs that facilitate the recruitment and professional development of new graduate students including the Application Statement Feedback Program which helps grad school applicants craft a compelling personal statement (10/hours per year) and the MIT IMPACT program (6 weeks, 2hours/week) which helps graduate students and postdoctoral fellows create compelling scientific presentations to a variety of audiences. To gain training in project management, I will also enroll in courses at Harvard Business School, such as "Leadership Principles" and "Management Essentials" (6-8 weeks, 4-7 hours/week). Although the proposed research is not a clinical trial, I will also participate in the "Clinical Project Management Fundamentals Certification Program" (30 hours, Barnett International), to learn the mechanics of team-led clinical trials.

## C. ACTIVITIES PLANNED UNDER THIS AWARD

**RESEARCH**

- **Year 1 (Aim 1: Dopamine PET/MR Analysis; Tau PET Data Collection):** Dopamine PET and fMRI data will be collected from all participants prior to the start of the award. Tau PET data collection is currently underway and is expected to continue throughout Year 1 and the beginning of Year 2. Analyses in Year 1 will focus on pharmacokinetic modeling of dopamine PET (for which I will attend Dr. Price's annual PK modeling course) and preprocessing fMRI data. I will submit my findings from these analyses in the form of abstracts to the annual meetings of the Alzheimer's Association International Conference (AAIC) and the Organization for Human Brain Mapping (OHBM). I will also present these findings at the annual MGH Clinical Neuroscience Day. The year will conclude by preparing a manuscript summarizing results from the dopamine-PET and fMRI analyses.

- **Year 2 (Aim 1: Analysis of Dopamine/Tau Interaction on Memory; Aim 2: Neural Variability Analysis):** Tau PET data collection will be completed in Year 2. Analyses for Aim 1 will be completed, including regression analysis to determine whether dopamine receptor density modulates the expected association between tau burden and memory performance. Analyses for Aim 2 will commence, including visiting Dr. Garrett's lab and attendance at the "Biomarkers in Neurodegenerative Diseases" course, to learn how to analyze and interpret neural variability data. I will submit results from these analyses as abstracts to AAIC and the Dallas Aging and Cognition Conference (DACC). Finally, I will submit a K-award application to continue on my path towards independence.
- **Year 3 (Aim 3):** Analyses for Aim 3 will focus on data-driven approaches (e.g. partial least squares correlation, see Aim 3) to determine the factors that best predict the relationship between tau burden and memory performance. Consultations with Dr. Nate Mercaldo, a biostatistician will assist in utilizing the appropriate methods for this analysis. I will again attend several conferences (AAIC, DACC, OHBM) to continue building my network. I will convert previous abstracts from Aims 1 and 2 into manuscripts, and resubmit my K-award. These steps will position me well to go on the job market for independent faculty positions.

**WRITING**

- **Grants:** By the end of Year 2, I will use preliminary data from this project to apply for an NIH K-award. I will participate in the MGH workshop on writing K-grants (8 weeks online). This is an important step on the path towards securing independence as an investigator.
- **Abstracts:** I will submit abstracts to at least two national/international conferences each year. I will also present my work at local symposia, poster sessions, and invited talks.
- **Manuscripts:** I will aim to publish at least one first-authored manuscript for each year of the fellowship. I will also co-author manuscripts with other members of Dr. Hooker and Dr. Berry's labs.

**CAREER DEVELOPMENT**

- **Seminars (2-3 hrs/week):** I will attend relevant seminars including the weekly BrainMap and "Martinnovate" Seminars the MGH Martinos Center, and weekly Brandeis Neuroscience Brown Bag talks.
- **Grand Rounds (1hr/month):** I will attend MGH neurology grand rounds once per month to gain a stronger clinical perspective of Alzheimer's disease and related disorders.
- **Hooker Lab Meetings (1hr/week):** I will attend the weekly Hooker lab meetings, as well as our group's monthly section meeting on human-neuroimaging. I will present in lab meeting at least twice per year.
- **Berry Lab Meetings (1 hr/week):** I will also attend the weekly Berry Lab meetings at Brandeis University and present on my research once per year. I will become familiar with the Berry Lab's work on cognitive aging.
- **Price Lab Meetings & Training (1 hr/month):** I will attend the Price Lab meetings at the MGH Martinos Center once per month to stay updated on the latest pharmacokinetic modeling methods for dopamine and tau PET tracers. I will also attend Dr. Price's annual course (1 week) on Pharmacokinetic Modeling of PET Radiotracers.
- **Training at Garrett Lab (2 weeks):** I will spend two weeks training at the Garrett Lab at Max Planck Institute in Germany where I will learn fMRI neural-variability methodologies and interpretation.
- **Training at Jagust Lab (2 weeks):** I will spend two weeks training at the Jagust Lab where I will learn about the tau-PET and fMRI methods previously employed to study dopamine and tau during cognitive aging.
- **Consults with Dr. Mercaldo (1 hr/month):** I will regularly meet with Dr. Mercaldo to discuss the statistical approaches to the proposed work. This will include sampling considerations, appropriate modeling approaches, and hypothesis testing-strategies.
- **Conferences:** Locally, I will present annually at MGH Clinical Neuroscience Day and Martinos Summer Symposium. I will attend national/international meetings focused on cognitive aging and Alzheimer's disease including the AAIC, Human Amyloid Imaging meeting, and the Dallas Aging and Cognition Conference.
- **Workshops & Coursework:** During Year 1, I will participate in the Brightfocus Foundation's "Alzheimer's Fast Track" short course (1 week), and attend Dr. Berry's "Alzheimer's disease resilience and risk factors" seminar course at Brandeis. In Year 2, I will attend Dr. Price's course of Pharmacokinetic Modeling for PET (1 week). In pursuit of Training Goal 3, I will participate in a Clinical Project Management Certification Program (Fall Year 2; 1hr/week) and a Research Management course offered through Harvard Business School (Fall Year 3; 2hrs/week) to gain expertise in science leadership and in running a lab.
- **Individual Development Plan (IDP):** To ensure that this training plan is achieved, I will generate a formal IDP to be discussed with the mentoring team at least once per year. With the IDP, we will evaluate overall progress, address any unanticipated training issues, and evaluate career goals and progress toward those goals.

**Specific Aims**

Normal aging is associated with both dopamine system dysfunction and the aggregation of hyperphosphorylated tau[1,2]. These alterations in neural systems are broadly considered to arise through independent processes, and are thus studied independently. Dopamine-centric fields propose that age-related declines in memory are mediated through reductions in dopamine (the "correlative triad" hypothesis[3,4]), while advancements in positron emission tomography (PET) imaging have inspired Alzheimer's disease (AD)-centric fields to propose medial temporal lobe tau pathology is the major driver of reduced memory performance, even in cognitively unimpaired individuals[5]. Evidence from our group suggests there are interactions between age-related neuromodulatory changes and neuropathological changes in predicting memory performance[6,7]. While individuals with a "double hit," meaning reduced dopamine receptors and higher tau, will likely show poorest aging trajectories, we find that maintaining or restoring dopamine can produce better-than-expected memory performance given neural losses[7].

Understanding the many factors that influence age-related cognitive decline is essential for predicting who will benefit most from future therapies that may target the dopamine system and/or tau aggregates. The overall objective of this proposal is to study the joint effects of dopamine and tau on episodic memory in cognitive aging. In this proposal, we outline a study using simultaneous MR/PET neuroimaging to investigate the relationship between dopamine availability, tau burden, and memory in aging. We will use [$^{11}$C]raclopride to measure dopamine D2/3 receptor density and simultaneous fMRI to measure brain activity during a reward-memory task: first after receiving a placebo, and then after receiving a dopamine-enhancing drug (methylphenidate) to measure endogenous dopamine release. Participants will return for a [$^{18}$F]MK-6240 scan to measure tau burden. Our proposal will sample from an existing cohort of subjects participating in a longitudinal study of cognitive aging at Brandeis University. Our central hypothesis is that increased levels of dopamine are protective in aging, and can stabilize memory performance in the face of tau burden.

**Aim 1: Define the independent and interactive effects of dopamine and tau pathology on memory in aging.** *Hypothesis 1:* Tau will moderate the relationship between dopamine and memory. From preliminary data, we predict that individuals with lower levels of tau will show a significant positive relationship between dopamine D2/3 receptor density and memory, but that this relationship will be disrupted in the context of high tau.

**Aim 2: Establish the effects of dopamine and tau on hippocampal activity in aging.** *Hypothesis 2a*: Hippocampal neural variability during memory encoding will be increased by enhancing dopamine via methylphenidate. *Hypothesis 2b*: Individuals with the greatest endogenous dopamine release (defined as the difference in [$^{11}$C]raclopride signal on methylphenidate vs. placebo) will have the highest levels of hippocampal neural variability during memory encoding, when adjusting for individual differences in tau burden.

**Aim 3: Define the factors that best predict which individuals will show memory benefits following dopamine enhancement.** The Brandeis Aging Brain Study provides us with additional cognitive, behavioral, and biological measures for the participants who will be scanned in our study. We will conduct a partial least squares correlation analysis to determine which brain measures best predict memory benefits following dopamine enhancement. *Hypothesis 3*: We predict that older adults with lower D2/3 receptor density will show the greatest potential for memory improvements following pharmaceutically-induced dopamine enhancement, despite levels of tau. Brain measures will include D2/3 receptor density, endogenous dopamine release, regional tau pathology, regional activation, and regional neural variability. Secondary analyses will also consider plasma amyloid, plasma tau, neuropsychological measures (e.g., executive function), years of education, and trait anxiety.

Based on preliminary data, we expect to find that increased levels of dopamine will lead to increased neural variability in the hippocampus and better reward memory in older adults, but that these effects will be attenuated by tau aggregation. Previously, the influences of dopamine and tau on cognitive aging have mostly been studied independently. This work will begin to bridge these subfields by studying the joint influences tau and dopamine on memory together in the same individuals. These experiments will contribute to an improved understanding of how neurochemistry and protein pathology interact to affect memory in older adults, and may suggest a mechanism by which cognitive function is preserved in the pre-clinical stages of age-related disease. The work capitalizes on a unique collaborative training opportunity between interdisciplinary researchers at the MGH Martinos Center for Biomedical Imaging and Brandeis University. I will gain expertise in cognitive aging and advanced fMRI/PET methodologies, setting me on a path toward independence and significant scientific impact.

## Research Strategy: SIGNIFICANCE

A recent position paper from a working group of Alzheimer's researchers expressed the urgent need for research investigating the role of neuromodulators (e.g. dopamine) in Alzheimer's disease (AD). They cited the unique opportunity neuromodulatory systems present as strategic prospects for disease-modifying therapies[8]. Previously, most research on the dopaminergic system in older adults has not considered AD biomarkers such as neural and plasma markers of tau and amyloid burden. In this proposal, I will explicitly examine the relationships among dopamine, AD-pathology, and memory in aging and preclinical AD, years before any significant cognitive deficits are apparent and before tau and amyloid pathology become widespread.

**Tau and Dopamine in Cognitive Aging:** The deposition of hyperphosphorylated tau is an inevitable part of aging that occurs years before the onset of disabling AD symptoms[1,2]. In clinically normal older adults, medial temporal lobe tau burden is associated with memory performance[5,9,10]. Normal aging also involves dopamine system decline (striatal dopamine innervation decreases at a rate of 6-10% per decade)[11]. Disruption to the dopamine system is associated with age-related deficits in memory and executive functioning[3,4,12]. Pharmacologically enhancing dopamine in older adults has been shown to improve memory performance and strengthen associated MRI brain measures[13,14]. Although older adults experience both the accumulation of tau and dopamine-system-disruption, some individuals are resilient to cognitive decline. Previous work from our group suggests that the dopamine system is one avenue through which some older adults may maintain intact memory performance, even in the face of tau pathology[6,15].

**Dopamine, Age, and Neural Variability:** As a neuromodulator, dopamine tunes activity in target brain regions. In humans, these modulatory effects can be measured through analyses of moment-to-moment fluctuations in brain activity. Dr. Doug Garrett (*Collaborator, see LoS*) has demonstrated that dopamine and age modulate the temporal variation in BOLD signal detected by fMRI (neural variability)[16]. Specifically, Garrett's group and others have demonstrated that older adults typically exhibit decreased neural variability compared to younger adults[17–19]. Recent evidence suggests that age-related loss of dopamine receptors could be one factor driving the reduction in neural variability[20]. Computational modeling suggests that restoring variability to neuronal models of aging/dopamine loss improves neuronal stimulus detection[21]. Furthermore, pharmaceutically enhancing dopamine via amphetamine administration can induce youth-like levels of neural variability and improved working-memory performance in older adults[17]. To our knowledge, no previous work has examined the relationship between tau burden and neural variability, nor potential interactions of dopamine on this relationship.

**Conceptual model and key hypotheses:** Based on a proof-of-concept analysis of data from a study at UC Berkeley (see **Fig. 1**), we predict that dopamine release is positively associated with memory performance in older adults, but that this relationship is disrupted in the presence of elevated tau. We predict that the dopaminergic system improves memory by modulating hippocampal activation, which we can measure through neural variability. That is, we predict that increased dopamine release improves memory by increasing neural variability in the hippocampus during encoding. We predict that tau aggregation dampens this effect by disrupting dopamine's ability to act on hippocampal memory circuits. Successful completion of this research will improve our understanding of who might benefit from future therapies that target the dopamine system in cognitive aging. Additionally, this research may improve our understanding of how the dopamine system is associated with cognitive reserve in healthy older adults.

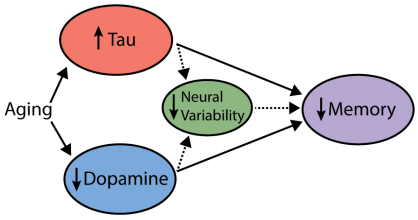



**Fig. 1:** Aims 1-3 will test our conceptual model that tau burden and dopamine dysfunction are associated with poorer memory in aging. Aim 1 will test the joint effects of tau and dopamine on memory. Aim 2 will test the influence of tau and dopamine on hippocampal neural variability. Aim 3 will determine the factors that best predict memory benefits following pharmaceutically-induced

**Strengths and weaknesses in the rigor of prior research:** Previously, research has considered dopamine and tau largely in isolation of each other. By considering how protein pathology and neuromodulatory systems interact in the aging brain, we can build a multifactorial explanation of how memory is affected in aging. We will use simultaneous MR/PET and pharmacological administration to measure both between-subjects and within-subjects differences in dopamine and memory performance.

## APPROACH

We will use simultaneous [$^{11}$C]raclopride PET/MRI and administration of methylphenidate to measure dopamine release and hippocampal activity during a reward-memory task in older adults. [$^{18}$F]MK-6240 PET will be completed in a separate session for assessment of medial temporal lobe tau. Aims 1-3 will use the same dataset.

***Experimental Design and Methods:***

*Participants:* For Aims 1-3, 45 participants (ages 60-80; 50% female) will be recruited from an existing cohort of cognitively normal older adults who are participating in the ongoing longitudinal Brandeis Aging Brain Study (BABS). Dr. Berry (Co-Sponsor) began recruiting participants for the BABS study in 2019. BABS participants complete a comprehensive neuropsychological battery (described below) upon joining the study and every two-years thereafter. BABS participants also agree, in principle, to participate in follow-up neuroimaging studies. Select eligible subjects will be asked to participate in simultaneous MR/PET neuroimaging studies to measure dopamine (using [$^{11}$C]raclopride) and tau (using [$^{18}$F]MK-6240) at the MGH Martinos Center. All IRB approvals for this study are complete, and **data collection is underway (n=40 subjects are currently enrolled and have completed the [$^{11}$C]raclopride MR/PET scans. Five of these subjects also completed [$^{18}$F]MK-6240 PET).**

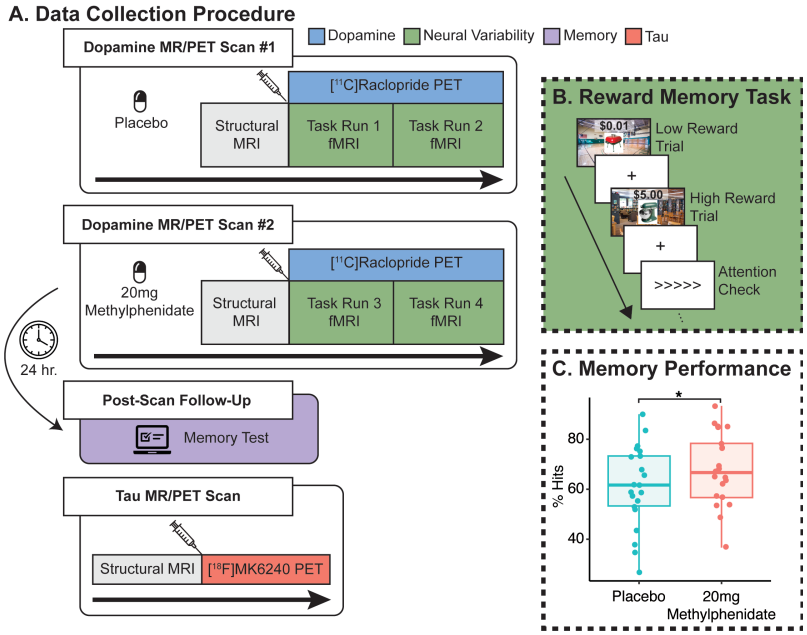



**Fig. 2: (A)** Participants will complete two dopamine-PET scans (placebo and 20mg methylphenidate), a memory test, and a tau-PET scan. **(B)** Reward memory fMRI task. **(C)** Preliminary data showing improved memory with methylphenidate ($T(20)=4.38$, $p<0.01$)

*Study Design:* Simultaneous MR/PET will be collected to measure brain activity and dopamine (with [$^{11}$C]raclopride) during a reward-memory task. Participants will complete a two-scan protocol on the same day: first with placebo, followed by a second scan after administration of methylphenidate (20 mg, oral), which increases synaptic dopamine concentrations by blocking dopamine re-uptake. The tau-PET scan (using [$^{18}$F]MK-6240) will be completed in a separate session. (**Fig. 2a**)

*Reward-Memory Task:* During each of the two simultaneous [$^{11}$C]raclopride PET/MRI scanning sessions (one with placebo, one with methylphenidate), participants will complete two runs of a reward-memory task (**Fig. 2a,b**). Each block consists of 44 trials. Participants will have the opportunity to practice the task prior to scanning. Unique stimuli will be presented in the practice version and in both scanning versions of the task. During the task, participants will view a series of items, and be instructed to remember as many as possible. Items will appear in one of two contexts: a high-reward context worth $5.00, in which an item is overlayed on a photo of a library; and a low-reward context worth 1¢, in which an item is overlayed on a photo of a gymnasium. 24-hours later during a follow-up session outside the scanner, participants complete a memory test. During the memory test, participants view stimuli and are asked if the item is novel or remembered (something they viewed previously during the scan). For remembered items, subjects are asked to select which context (library or gymnasium) the item appeared in. Memory will be scored for correct recognition (hits), false alarms, and correct context. We will compare memory scores within subjects across high and low-reward contexts and during the placebo and methylphenidate scans. Preliminary data (**Fig. 2c**) shows that methylphenidate administration is associated with improved correct recognition (% hits) for the stimuli across all contexts ($T(20)=4.38$, $p < 0.01$).

*MRI Acquisition:* During the first scanning session, we will acquire high-resolution structural (MPRAGE) and FLAIR scans to examine brain anatomy. Then, T2* weighted EPI (TR=2.4s, TE=37ms, flip angle=45°) BOLD images will be collected during the reward memory task. Dr. Jagust (*Collaborator, see LoS*) has agreed to review FLAIR scans for potential participant exclusion due to large white matter hyperintensities.

*[$^{11}$C]raclopride Acquisition:* [$^{11}$C]raclopride will be synthesized at the MGH Martinos Center as previously described[22]. Each participant will complete two 60-minute dynamic scans (following oral placebo and 20mg oral methylphenidate administration). **Methylphenidate is used only as a methodological tool for optimizing measurement of dopamine release. It is implemented solely for methodological and not clinical purposes. All eligible participants are administered methylphenidate and undergo the same PET scanning procedures.** For each scan, participants will receive a bolus injection of approximately 10mCi of [$^{11}$C]raclopride administered to the antecubital vein. To measure baseline D2/3 receptor occupancy, participants ingest a placebo pill approximately one hour before scan 1. The placebo scan will always be performed first. To

measure endogenous dopamine release, participants will ingest 20mg of methylphenidate one hour prior to scan 2. Methylphenidate blocks the dopamine transporter, enabling PET detection of intrinsic dopamine release[23]. This method has been successfully employed previously by Dr. Anne Berry (*Co-Sponsor)* and Dr. Bill Jagust (*Collaborator*) (see **Fig. 3**)[24]. Data-collection is currently underway at the MGH Martinos Center in collaboration with Dr. Jacob Hooker's lab (*Sponsor*). **Figure 4** shows preprocessed data from two currently enrolled subjects.

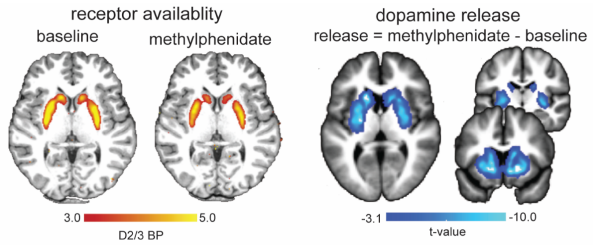



**Fig. 3:** Dopamine D2/3 Receptor availability is measured with [$^{11}$C]raclopride (left). Dopamine release is quantified by comparing placebo and methylphenidate scans (right) (Berry et al., 2018).

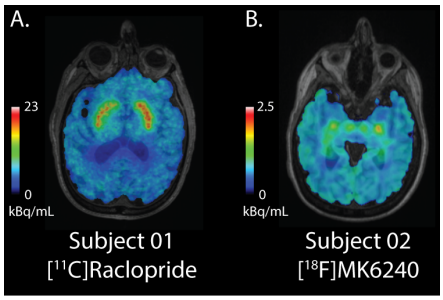



**Fig. 4:** Two healthy older adult participants from the proposed study. Preprocessed **(A)** [11C]raclopride showing dopamine D2/3 receptor density & **(B)** [18F]MK6240 tau PET.

*[$^{11}$C]Raclopride Analysis:* Endogenous dopamine release will be measured as the percent change in binding potential form scan 1 to scan 2 (placebo [$^{11}$C]raclopride - methylphenidate [$^{11}$C]raclopride) / placebo [$^{11}$C]raclopride. Data will be reconstructed and preprocessed using SPM and in-house scripts. Non-displaceable binding potential will be quantified using the simplified reference tissue model analysis (SRTM)[25]. Primary analyses will focus on partial volume-corrected dorsal caudate regions of interest as previously described[26,27]. Previous [$^{11}$C]raclopride studies specifically implicate caudate dopamine in episodic memory in aging[12]. **Fig 4a** shows a preprocessed [$^{11}$C]raclopride scan from one example subject during the placebo condition.

*[$^{18}$F]MK-6240 Acquisition & Analysis:* [$^{18}$F]MK-6240 will be used to measure tau in early Braak stage MTL regions (entorhinal cortex, hippocampus). Dr. Julie Price (*Collaborator*) and Dr. Hooker (*Sponsor*) have successfully used [$^{18}$F]MK-6240 at the MGH Martinos Center previously to measure tau in healthy older adults as well as older adults with Alzheimer's disease and related disorders (see **Fig. 4b**). Compared to [$^{18}$F]Flortaucipir, [$^{18}$F]MK-6240 shows increased dynamic range in Standardized Uptake Value Ratio (SUVR), reduced off-target binding in choroid plexus, and improved quantification of early tau (Braak I regions) in healthy older adults[28,29]. Analysis will follow POINTER neuroimaging ancillary protocols (SUVR 90-110 min.)[30]. Data will be partial volume corrected using the Rousset geometric transfer matrix method[31].

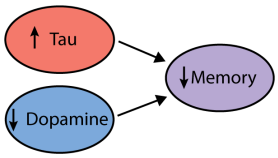
*BABS Neuropsychological Battery & Inclusion Criteria:* Participants are defined as cognitively normal based on their performance on the BABS neuropsychological battery, which was developed by Dr. Berry (Co-Sponsor) in consultation with clinical psychologists (for R01 AG074330). BABS participants are assessed with the Montreal Cognitive Assessment (MoCA; cutoff based on demographics[32]), the Quick Dementia Rating System (score 0)[33], and are screened for depression using the Geriatric Depression Scale (score 10 and lower)[34]. Participants will be within 1.5 SD of demographics-based normative data for memory (California Verbal Learning Test-II[35], Visual Reproduction[36]) and executive function measures (NIH toolbox[37,38]: Dimensional Change Card Sort, Flanker) and will not have subjective memory complaints. For inclusion in neuroimaging, participants will not have a severe general medical illness that affects cognition, or diagnosis of a neurological or psychological disorder. **To be eligible to receive oral methylphenidate, participants will have no history of arrhythmia or panic attacks and will be screened by a nurse practitioner with a physical exam and 12-lead EKG.**

*Blood plasma amyloid-β a ptau181:* To measure blood plasma amyloid-β and ptau181, blood samples are acquired from participants in the Brandeis Aging Brain Study. These samples are processed by the Alzheimer's Clinical & Translational Research Unit at Massachusetts General Hospital, and is supported by Dr. Anne Berry's R01AG074330 (Dr. Hooker, Co-PI). Aβ42/40 ratios will be calculated from Aβ42 and Aβ40 levels obtained from Euroimmun ELISA assays. Lower Aβ42/40 ratios have been associated with greater cortical amyloid as measured by PET, steeper cognitive decline, and increased risk of developing AD later on[39,40]. Recent work suggests that levels of ptau181 are also associated with cortical amyloid[41]. Aims 1-3 will account for Aβ42/40 status a ptau181 levels in secondary analyses.

We have adopted the following strategies to ensure a robust and unbiased approach – We will actively recruit a diverse sample and work to mitigate bias in our cognitive screening through the adoption of cognitive measures form the NIH toolbox. We propose well-powered tests of specific hypotheses supported by proof-of-concept data collected in cognitively normal older adults: the population we propose to study. Additional strategies are **highlighted** below.

**Aim 1: Define the independent and interactive effects of dopamine and tau on memory in aging.**

***Rationale:*** Normal aging is associated with dopamine-system dysfunction and the aggregation of phosphorylated tau in the medial temporal lobe. Most previous work examining the effects of tau and dopamine on cognitive aging have considered the two variables independently. The objective of this aim is to examine the interactive effects of tau and dopamine on reward-memory in aging. We will test the working hypothesis that low-tau individuals show a strong relationship between dopamine and memory, but that this relationship is disrupted in the context of high tau.



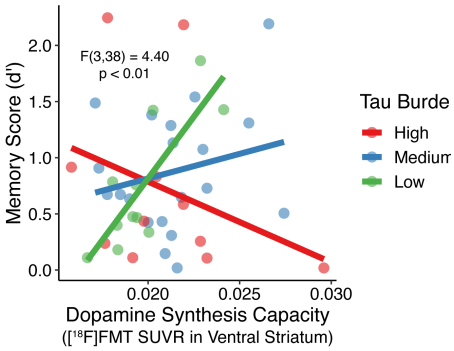




**Fig. 5:** Proof-of-concept data from a UC Berkeley study demonstrating that participants with low-tau (green) show a significant association between dopamine synthesis capacity and reward memory, but that this relationship is disrupted in high-tau individuals (red). (*Provided by Dr. Berry and Dr. Jagust*)

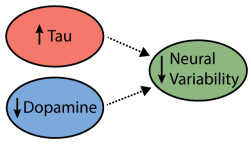
***Proof-of-Concept Data*:** We analyzed pre-existing data from a UC Berkeley study using [$^{18}$F]Fluoro-m-tyrosine and [$^{18}$F]Flourtaucipir PET datasets to examine the relationship between dopamine synthesis capacity and tau burden in participants from the Berkeley Aging Cohort Study (n=42) (*Data courtesy of Dr. Anne Berry: Co-Sponsor, and Dr. Bill Jagust: Collaborator*). These data demonstrate that low-tau individuals (green) show a positive relationship between dopamine-synthesis capacity and memory, but that this relationship is disrupted in individuals with higher levels of entorhinal tau (red) $F(3,38) = 4.40$, $p < 0.01$ (**Fig. 5**). Notably, these proof-of-concept data demonstrate that we can detect a statistically significant interaction in a sample of this size. **Our proposed study expands upon this result by measuring *endogenous dopamine release* (using [$^{11}$C]raclopride and methylphenidate) rather than dopamine synthesis-capacity. We will also use the second-generation radiotracer [$^{18}$F]MK-6240 to measure tau. This tracer boasts approximately twice the dynamic range in SUVRs compared to [$^{18}$F]Flortaucipir, which will allow us to more accurately detect early tau pathology (Braak I regions) in cognitively normal older adults**[28–30,42,43].

***Power Calculations:*** In the proof-of-concept analysis using data from a previous study conducted at UC Berkeley, the observed interaction between dopamine and tau had an effect size f=0.64. Assuming a Type I error of 0.05 and two covariates (dopamine and memory) analyzed across three groups (high, medium, and low tau), a minimum sample size of 42 is required for the proposed study. With this sample size, the model will detect significant interactions with $F \geq 3.25$ (G*Power v3.1). Our proposed sample size of n=45 exceeds the determined minimum sample size. (Power analysis conducted in consultation with Dr. Mercaldo, *Collaborator, see LoS.*)

***Research Design & Hypothesis Testing:*** Baseline dopamine D2/3 receptor density will be measured as [$^{11}$C]raclopride binding potential in the caudate during the placebo scan (scan 1)[12]. Tau burden will be measured as [$^{18}$F]MK6240 SUVR in entorhinal cortex and hippocampus. Memory will be measured as the difference between correct hits and false alarms (d') on the memory test. A generalized linear regression model will be constructed to quantify the association between memory scores and (1) baseline dopamine D2/3 receptor density, (2) entorhinal tau burden, and (3) the interaction between dopamine D2/3 receptor density and entorhinal tau burden. **An adjusted model will also be considered which accounts for age, sex, years of education, and plasma Aβ42/40 and ptau181**. Hypothesis 1 predicts a significant interaction effect whereby individuals with low tau burden will show a significant relationship between dopamine D2/3 receptor expression and memory scores, but that this relationship will be disrupted in high-tau individuals. Graphical summaries and residual analyses will be performed to assess modeling assumptions (e.g., linearity, normality of residuals). Model results will be submitted to statistical significance testing including correction for multiple comparisons.

***Alternative Approaches:*** Other health factors may obscure the predicted relationship with memory scores. **Secondary analysis will consider the influence of lifestyle factors** including sleep (PSQI)[44], physical activity (CHAMPS)[45], and cognitive reserve proxies (LEQ)[46], as these data are collected during BABS neuropsychological testing. Aim 3 will examine the relative influence of brain measures on memory scores in greater detail using a partial least squares correlation (PLSC) analysis. Finally, some participants may not qualify for the methylphenidate administration (i.e., EKG abnormality found prior to scanning). These participants will be included in a between-subjects analysis of baseline dopamine D2/3 receptor density, tau burden and memory.

**Aim 2: Establish the effect of dopamine and tau on hippocampal activity in aging.**




***Rationale:*** While most fMRI studies measure the *amplitude* of BOLD signal (neural activation), recent work has emphasized the importance of also considering the *temporal variation* of BOLD signal (neural variability)[16,47]. Neural variability is especially important to consider in the context of dopamine, which is purported to modulate the flexibility and stability of neural activation[48–50]. Dr. Garrett's (*Collaborator*) lab has demonstrated that neural variability is generally reduced in older adults, and that increased dopamine availability is associated with increased neural variability[17,20]. The *objective* of this aim is to define the relationship between endogenous dopamine release, entorhinal tau, and neural variability in the hippocampus during memory encoding in older adults. We will test the *hypotheses* that (*hypothesis 2a*) hippocampal neural variability during memory encoding will be increased by pharmaceutically-induced dopamine-enhancement, and that (*hypothesis 2b*) individuals with the greatest endogenous dopamine release will have the highest levels of hippocampal neural variability during memory encoding, when adjusting for individual differences in tau burden. Successful completion of this aim promises to offer mechanistic insight into how dopamine and tau influence the stability of hippocampal activation, leading to changes in memory performance.

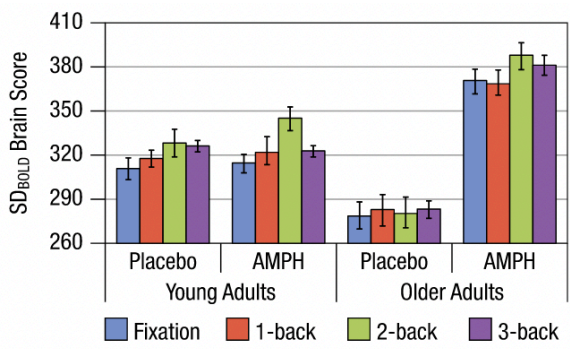



**Fig. 6:** Partial Least Squares model of relationship between Neural Variability ($SD_{BOLD}$), Age Group, Amphetamine (AMPH), and Task Condition. Higher $SD_{BOLD}$ brain scores reflect higher neural variability. Error bars represent bootstrapped 95% confidence intervals (1000x with replacement) (Garrett et al. 2015).

***Preliminary Data:*** Previous work from Garrett et al. demonstrates that healthy older adults tend to show reduced neural variability compared to better-performing healthy younger adults[16,51–53]. Relevant to our proposal, neural variability is increased in older adults upon administration of amphetamine, which stimulates dopamine release (see **Fig. 6**)[17]. **Aim 2 will expand upon previous work by 1) leveraging PET imaging to directly measure individual differences in dopamine release and 2) considering the joint influence of dopamine and tau on neural variability in the context of an episodic memory task**.

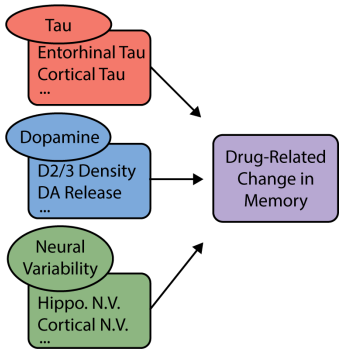
***Power Calculations:*** In the preliminary data analysis above, a partial least squares model found a latent variable that explained 60.86% of the variance in neural variability measures[17]. This translates to an effect size of $f^2 = 1.55$. Assuming a Type I error of 0.05 and a PLS model with seven predictors (drug, age group, task condition, and interactions), a minimum sample size of 23 is required (G*Power v3.1). Our proposed sample size of n=45 exceeds the determined minimum sample size. (Power analysis conducted in consultation with Dr. Mercaldo, *Collaborator, see LoS.*)

***Research Design & Hypothesis Testing:*** To facilitate neural variability analyses, the reward-memory task uses a blocked design, and expanded fMRI preprocessing steps including block normalization (as described in Garrett et al., 2015) will be employed. Neural variability ($SD_{BOLD}$) will be calculated as the standard deviation in BOLD signal across concatenated task blocks. A general linear regression model will be employed to quantify the association between (1) $SD_{BOLD}$ in the hippocampus, (2) endogenous dopamine release ([$^{11}$C]raclopride in caudate during methylphenidate – [$^{11}$C]raclopride in caudate during placebo) / [$^{11}$C]raclopride in caudate during placebo, and (3) Braak I tau ([$^{18}$F]MK-6240 SUVR). **An adjusted model will also be considered which accounts for age, sex, years of education. Secondary analyses will account for plasma Aβ42/40 and ptau181**. Graphical summaries and residual analyses will be performed to assess modeling assumptions (e.g., linearity, normality of residuals). Model results will be submitted to statistical significance testing.

***Alternative Approaches:*** The main motivation for studying neural variability is to examine how dopamine's modulation of the flexibility and stability of neural signals affects memory. Considering additional fMRI metrics, beyond neural variability may also prove useful. Previous work has identified changes in functional connectivity and graph theoretical metrics that are associated with interindividual differences in dopamine measures[12,48,54]. Specifically, we might expect to see changes in hippocampal-striatal and hippocampal-prefrontal functional connectivity that are associated with reward memory. During my dissertation, I gained expertise in the dynamic functional connectivity metrics associated with learning and executive function[55,56]. Alternative analyses will consider dynamic shifts in functional connectivity and brain network metrics that may be associated with memory.

**Aim 3: Define the factors that best predict which individuals will show memory benefits following dopamine enhancement.**

***Rationale:*** A robust literature suggests that not all individuals benefit equally from an increase in dopamine availability (e.g. inverted-U trends)[48–50]. Partial least squares correlation (PLSC) is a multivariate statistical approach that has been employed previously to identify a pattern of interindividual brain measures that optimally explains interindividual differences in cognitive measures[57–59]. The *objective* of this aim is to determine which factors best predict memory enhancement in our sample of older adults. Using PLSC, we will test the *working hypothesis* that lower baseline dopamine D2/3 receptor availability and lower tau pathology best predict pharmaceutically-induced improvements in memory performance.



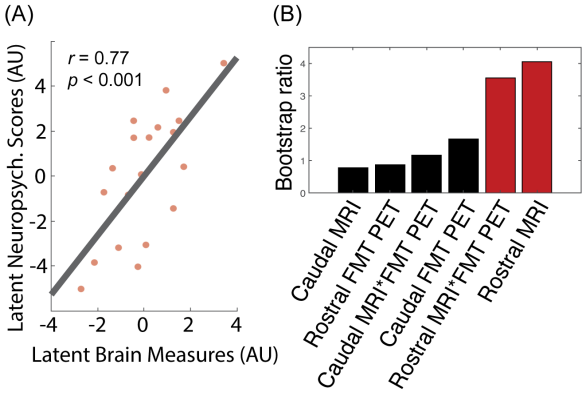




**Fig. 7: Example PLSC Analysis (A)** Scatter plot depicting the association between latent brain measures and latent neuropsychological scores. **(B)** Contribution of individual brain measures to the latent PLSC variable (bootstrap ratios > 1.96 in red are considered reliable).

***Preliminary Data:*** Dr. Berry's (Co-Sponsor) lab has employed PLSC models to examine the multivariate relationships between brain measures and cognition. **Fig. 7** shows one such PLSC analysis demonstrating correlation between one latent variable extracted from locus coeruleus brain measures and a second latent variable extracted from neuropsychological scores (**Fig. 7A**). To examine which specific brain measures best explained interindividual differences in neuropsychological scores, a bootstrap method was employed (**Fig. 7B**). Red bars indicate brain measures that were most predictive in this model, in this case MRI and PET measures from the rostral locus coeruleus.

***Power Calculations:*** In the preliminary PLSC analysis above, the observed correlation was r = 0.77. Assuming a Type I error of 0.05 and null hypothesis $H_0$: r=0, a minimum sample size of 15 is required. With this sample size, the model will detect significant correlations where -0.514 ≤ r ≥ 0.514 (G*Power v3.1). Our proposed sample size of n=45 exceeds the determined minimum sample size. (Power analysis conducted in consultation with Dr. Mercaldo, *Collaborator, see LoS.*)

***Research Design & Hypothesis Testing:*** We will employ a PLSC analysis to capture the multivariate association between improved memory performance following methylphenidate and neurocognitive measures. The PLSC analysis will estimate the brain measures that are maximally related to interindividual differences in memory performance. Brain measures to be included in the model include baseline D2/3 receptor density ([$^{11}$C]raclopride binding potential during placebo scan), endogenous dopamine release (the percent change in [$^{11}$C]raclopride binding potential during placebo scan vs. the methylphenidate scan), entorhinal and hippocampal tau burden ([$^{18}$F]MK-6240 SUVR), and regional neural variability on and off methylphenidate. To evaluate the statistical strength of the latent variables that are extracted by the PLSC method, a permutation test (n = 10,000) will be employed. Additionally, the reliability of vector weights to the latent variable will be determined using a bootstrapping procedure (n = 10,000). Vector weights with bootstrap ratios <-1.96 or >1.96 will be considered reliable. **Follow-up analyses will also consider age, sex, and years of education, and plasma measures of amyloid-*β***.

***Alternative Approaches:*** Our older adult sample of n=45 should reliably show interindividual differences in brain imaging and neuropsychological measures. However, extrapolating interindividual differences to the general population may be difficult with a sample of this size. **We can attempt to replicate our findings in similar datasets collected by Dr. Bill Jagust (Collaborator) and Dr. Doug Garrett (Collaborator)**. Follow-up analyses can also be conducted in large open datasets such as ADNI and the Human Connectome Lifespan dataset to validate the predictors we identify in our sample. While dopamine PET scanning may not be available in these large datasets, genetic data often is, and can provide predictions about dopamine D2/3 receptor expression. Successful completion of this research will improve our understanding of who might benefit from future therapies that target the dopamine system in cognitive aging. Additionally, this research may improve our understanding of how the dopamine system is associated with cognitive reserve in healthy older adults.