Study Protocol

Title: Heart rate and Breathing Effects on Attention and Memory (HeartBEAM)

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Research Background

As we age, the parasympathetic system declines as the sympathetic system becomes hyperactive. In our previous clinical trial (Nashiro et al., 2023; Yoo et al., 2023), we found that increasing parasympathetic activity through daily practice of slow-paced breathing significantly decreased plasma amyloid-β (Aβ) in healthy younger and older adults (Min et al., 2023). In healthy adults, higher plasma Aβ is associated with greater risk of Alzheimer's disease (AD). Our primary goal of the current trial is to extend our initial findings regarding effects of slow-paced breathing on Aβ. Our secondary objectives are to examine the effects of daily slow-paced breathing on brain structure and the rate of learning.

Research Objectives

The aim of the study is to examine how daily paced breathing affects plasma Aβ levels and the rate of learning in older adults. Healthy adults aged 50-70 who meet all eligibility criteria will be invited to this study. Participants will be randomly assigned to one of the two conditions: 1) Daily memory and attention training followed by a paced breathing protocol designed to increase relaxation or 2) Daily memory and attention training followed by a paced breathing protocol to increase alertness. Participants will be asked to complete pre and post intervention cognitive testing online, engage in 10 weeks of daily brain training (starting Week 2) and 9 weeks of paced breathing (starting Week 3) at home. They will also be asked to come in for lab visits on Weeks 2, 7 and 12 to provide blood and urine samples to assess amyloid beta levels and to complete magnetic resonance imaging scans to assess perivascular space volume.

Methods and Design

Overview of the study

Timeline	Lab Visit	Home Tasks
Week 1		online consent, home cognitive testing
Week 2	(Visit 1) EEG tasks, questionnaires (Visit 2) MRI, blood & urine samples, cognitive tasks, questionnaires, give heart rate monitoring device	(Training Week 1) cognitive training + resting pulse measures (~40 min/day)
Week 3		(Training Week 2) cognitive + breathing training (~60 min/day)
Week 4		(Training Week 3) cognitive + breathing training (~60 min/day)
Week 5		(Training Week 4) cognitive + breathing training (~60 min/day)
Week 6		(Training Week 5) cognitive + breathing training (~60 min/day)
Week 7	(Visit 3) MRI, blood & urine samples, questionnaires	(Training Week 6) cognitive + breathing training (~60 min/day)
Week 8		(Training Week 7) cognitive + breathing training (~60 min/day)
Week 9		(Training Week 8) cognitive + breathing training (~60 min/day)
Week 10		(Training Week 9) cognitive + breathing training (~60 min/day)
Week 11		(Training Week 10) cognitive + breathing training (~60 min/day)
Week 12	(Visit 4) EEG task, questionnaires (Visit 5) MRI, blood & urine samples, cognitive tasks, questionnaires, return heart rate monitoring device	home cognitive testing

Week 1 - Home Tasks:
After signing the consent form, participants will be asked to complete questionnaires and cognitive tasks online. We will email them a link to a website (custom programmed) that will administer a combination of the tasks listed below daily for a week. In the email, we will give them the following instructions: “Before you begin, please make sure all distractions are removed (i.e. TV, radio, phone and tablets are all turned off). While you complete the tasks, please do not eat, snack or drink; please do not leave your screen throughout the experiment (and if you have to, try to do it after you finish the task at hand); please wear glasses if needed.” Participants’ email will be their username and, on the first day, they will create a password for the website app that administers the tasks and questionnaires, which will take about 45 minutes each day. The information in parentheses below indicates domains of the tasks.

1) N-back task (memory)
2) Verbal learning memory task (memory)
3) Face-name recognition task (memory)
4) Pattern separation task (memory)
5) Task switching task (executive function)
6) Flanker task (executive function)
7) Stop-signal task (executive function)
8) Spatial orientation task (visuospatial function)
9) The Reading the Mind in the Eyes Test (emotion)
10) Profile of Mood States (emotion)
11) Verbal fluency task (language)
12) Daily Inventory of Stress Events (emotion)
13) Depression Anxiety Stress Scales – Short Form (emotion)
14) Positive and Negative Affect Schedule (emotion)
15) The Five Facet Mindfulness Questionnaire (emotion)
16) Physical activity ratings (physical activity; note that this questionnaire will be asked only at pre-intervention)
17) Epworth Sleepiness Scale (sleep)
18) Karolinska Sleepiness Scale (sleep)
19) Memory for emotional state in the past week and prediction of mood for next week (memory)
20) Demographic information
21) Emotional memory task (emotion and memory)

If participants are unable to complete the cognitive/questionnaire module for a particular day, they will be asked to pick up that module of the study where they left off the next day. They can complete that leftover module and start and complete a new module the same day. However, they will not be allowed to start more than one module per day. If participants miss more than one out of 6 days of cognitive testing, they will be asked to leave the study.

Week 2 - Lab Visit 1 (around 2 hours):
Participants will be asked to complete the following cognitive tests and questionnaires at the Emotion & Cognition Lab:

- Trail Making Test (TMT)
- Animal fluency test
- Multilingual Naming Test (MINT)
- Functional Activities Questionnaire (FAQ)
- Montreal Cognitive Assessment (MoCA)
- Sleep questionnaire
- EEG tasks

Week 2 - Lab Visit 2 (around 2.5 hours):
The second visit includes the MRI assessments, which will take place at the USC Dana and David Dornsife Cognitive Neuroimaging Center (DNI). In the MRI session, the participant will be asked to simply rest (i.e., do nothing without falling asleep) during different types of scans. During the entire MRI session, their heart rate will be measured by a pulse oximeter. The total scan time will be an hour.

The rest of the visit will take place at the Emotion & Cognition Lab and the Psychology Department (SGM 906), which will take about an hour and half. Phlebotomists will collect blood samples up to 30 mL via venipuncture. Note that blood draw volume for blood donation in the US is 1 pint (~450 mL); thus, 30 mL should be safe for participants. Fasting is not required for the blood draw. Participants will also be asked to provide a urine sample (~20mL). Blood pressure will be measured by a blood pressure cuff. In addition, participants will be asked to complete the Profile of Mood States, the Daily Inventory of Stress Events, and the sleep questionnaire.

At the end of this visit, participants will receive their devices. The devices include a laptop with a customized paced breathing app, USB-module and a sensor that clips onto the earlobe and measures heart rate.

Week 2-12 (10-week intervention at home):
After the second lab visit, participants will be asked to start an intervention, which involves doing 15-30 minutes of cognitive training and 30 minutes of paced breathing practice each day. In the first week of intervention, participants will be asked to do cognitive training only followed by measuring their resting heart rate. From the second week, they will be asked to do both cognitive training and paced breathing practice (with heart rate monitoring via an ear clip) each day. Participants will be randomly assigned to one of two conditions.

Condition 1: Slow-paced breathing condition (a paced breathing protocol designed to increase relaxation)
Each day, participants will be guided to do slow paced breathing at paces between 10-15 seconds per breath. Participants will receive continuous biofeedback in the form of a

score that is higher the **more** that their heart rate shows strong oscillatory activity at a slow breathing frequency. This is described as the 'relaxation' condition to participants.

Condition 2: Random-paced breathing condition (a paced breathing protocol to increase alertness)
Each day, participants will be guided to do random-paced breathing at paces between 4-6 seconds per breath. Participants will receive continuous biofeedback in the form of a score that is higher the **less** that their heart rate shows strong oscillatory activity at a slow breathing frequency. This is described as the 'alertness' condition to participants.

Participants in both conditions will also play Lumosity cognitive training games daily. Lumosity games are commercial cognitive training games designed to improve various core cognitive skills including memory, attention, processing speed, spatial orientation, mental flexibility, reasoning, and problem-solving skills (https://www.lumosity.com/en/brain-games/).

Lumosity provides a platform for researchers who aim to better understand human cognition. They agreed to provide services to our research team. Our liaison at Lumosity, Nicole Ng, has consulted with us on some study design decisions but will not be involved in the collection or analysis of the data. In addition, Lumosity will not have access to identifiable data and will make the data collected on their platform available to us, as described below:
Data requested from the Lumosity database is available in a securely de-identified format. There is sufficient data meeting the criteria provided by the investigator to complete a thorough and robust analysis appropriate for the research question.

The app will assign each participant a Lumosity email. This Lumosity email will be stored in our app database along with the study participant ID.

Participants will play a list of games shown below and we will be getting final scores for each game each time they played it via email. Participants will have free access to the Lumosity games for the duration of the study. At the end of the study when they return the devices, their access to Lumosity will terminate. If they wish to continue with Lumosity after the completion of the study, they would need to sign up for an account and pay for the service.

Game Name	Category	Type	Description (taken from lumosity.com/en/brain-games/)

Familiar Faces	Memory	Episodic memory (long-term memory for face-name pairs)	Play the role of a waiter and remember your customers' names and orders in order to earn higher tips and job promotions. Face-Name Recall: Face-name recall involves being able to pay attention to and remember names and physical characteristics.
Tidal treasures	Memory	Episodic memory (binding action to item across rounds)	Keep choosing unique ocean treasures from a tide pool as more and more items wash up. Objects will be more difficult to tell apart as they accumulate in the tide pool -- so you'll need to remember more details about each item on your growing list of treasures as you spend more time at the shore. Working Memory: Working memory is used for temporarily storing and manipulating information.
Memory Match	Memory	2-back working memory	In Memory Match, you must quickly determine whether a flashcard symbol matches the one presented 2 times previously. Quickly memorizing and comparing the symbols challenges your working memory. This game also makes you continually update your working memory with new information. Working Memory: Working memory is used for temporarily storing and manipulating information.

Word Bubble rising	Language	Verbal fluency	In Word Bubbles, you write words that start with the same starting letters. This task challenges verbal fluency — your ability to rapidly retrieve words from your mental vocabulary. More specifically, Word Bubbles challenges phonemic fluency, or fluency with words that begin with a certain letter or sound. Verbal Fluency: Verbal fluency is the ability to rapidly retrieve words from your mental vocabulary.
Raindrops	Math	Arithmetic	Raindrops uses arithmetic to improve problem-solving skills. Play Raindrops to become better at mental calculations. You might even find yourself volunteering the next time someone needs to calculate a tip! Numerical Calculation: Numerical calculation is the ability to perform simple arithmetic operations including addition, subtraction, multiplication, and division.
Penguin pursuit	Attention	Spatial orientation + speed	Penguin Pursuit makes you use your spatial orientation, your ability to adjust your perspective in a mental map, to guide a penguin through a maze. When the maze rotates, you must rotate your mental map of the maze and recalibrate the directions to get to the fish. Spatial Orientation: Spatial orientation, also known as sense of direction, involves being aware of the surrounding environment.

Lost in Migration	Attention	Flanker task	Lost in Migration exercises your attention abilities. Games like Lost in Migration can boost your focus, helping you avoid distraction and improve productivity at work. Selective Attention: The ability to focus on relevant information while ignoring irrelevant distractions
Splitting Seeds	Attention	Subitizing (visual estimation)	In this game, you evenly divide a pile of seeds. To do this quickly, you'll need your subitization skills. Subitization is your ability to quickly discern the number of items in a small group without having to count. Information Processing: Information processing is the initial identification and analysis of incoming sensory input.
Pirate passage	Reasoning	Planning	Exercise your planning skills by finding the route to buried treasure. Planning: Planning involves thinking ahead, evaluating options, and choosing the best course of action.
Brain Shift	Flexibility	Task Switching	In this game, you will switch between two rules depending on whether the letter-number pair shows up on the top or bottom card. The location of the letter-number pair will be unpredictable, so you will exercise your mental flexibility to answer as quickly as possible. Task Switching: Task switching is the process of adapting to changing

			circumstances, switching from one goal to another.
Ebb and Flow	Flexibility	Task Switching	In this game, you shift your focus between two details: where the leaves point and how they move. This challenges your ability to switch between 2 cognitive processes: one interprets shape, the other movement. When you switch tasks, your brain suppresses one of these processes while activating the other — and this complex process often leads to mistakes. Task Switching: Task switching is the process of adapting to changing circumstances, switching from one goal to another.
Color match 2	Flexibility	Stroop[SL34]	Color Match is a response-inhibition game that enhances your flexibility skills. Improving these abilities makes it easier to resist temptation, control impulses, and stay focused on your goals. Response Inhibition: Response inhibition is the ability to suppress inappropriate responses that interfere with goal-directed actions.

The app is intended to use for collecting data, providing feedback on their performance and sending notifications (i.e., we will send a reminder to practice). We will use the data collected by the app as part of our research study. These data mainly involve amount/time of practice, the sequence of intervals between heartbeats during the practice sessions, and the strategies they were asked to try during each session. We will need these data for reporting the success of our intervention and comparison condition in our study. These data will not be shared with the FDA.

The feedback will differ between the two conditions, in that the slow-paced breathing participants will be trained to try to maximize heart rate oscillations while the random-paced breathing participants will be trained to try to avoid heart rate oscillations. In each condition, they will receive a feedback score that is incremented during the session and has a final value at the end of the session. This score will reflect the opposing goals in the two conditions, with larger values for higher heart rate oscillations in the slow-paced breathing condition and larger values for less oscillatory power in the random-paced breathing condition.

Participants in the two conditions will be guided through which breathing paces to use to try to optimize their feedback scores each time they practice. During each 15-min practice session, participants will be guided through five 3-min segments of randomly selected paces from their set of paces. Over time, this set will get smaller, as the app gathers average performance of each pace for that participant and compares it to the other paces and discards those that are clearly worse than the numerically best pace because its 95% confidence interval no longer overlaps with the top ranked pace. Our intention is to find paces that work well for each individual to achieve their goal.

During the intervention, each participant's progress will be facilitated by the app that will send reminders when they fail to complete practices. Progress will be monitored by a group of researchers; if participants fall behind on practice, we will contact them by email, phone, or video calls (e.g., Zoom) for us to check if they have any problems with any aspects of the study, such as device and internet issues. There will be no opportunities to make up sessions and if participants only complete part of a session one day, they will start at the beginning of a session the next time they practice. We anticipate high compliance with practice, as in our prior 5-week HRV biofeedback study, older participants averaged **over 100%** of the practice we asked them to complete (and by week 3, this was 40 min/day of biofeedback). In that study, we did not ask them to do more than 40 min/day so this is an indicator that they enjoyed it or felt they were getting something worthwhile from it. Thus, we have some experience indicating that the HRV-biofeedback is something that participants can do every day. In addition, Lumosity is a highly engaging platform and we anticipate that older adults will enjoy the activity.

Week 7 - Lab Visit 3 (around 2 hours):
Participants will be asked to complete the same assessments as in Visit 2 (i.e., MRI, blood draw, urine collection, blood pressure measurement, questionnaires).

Week 12 - Lab Visit 4 (around 2 hours):

Participants will be asked to complete the same assessments as in Visit 1 except that we will not repeat the following cognitive tests: MINT, FAQ and MoCA.

Week 12 - Lab Visit 5 (around 2 hours)
Participants will be asked to complete the same assessments as in Visit 2. They will also be asked to return their devices.

A few days after Visit 2, 3, and 5, participants will be asked to complete the post blood draw questionnaire to report any adverse events associated with the blood draw, if any. We will report to the IRB if the responses to any of the questions were the most negative response option. For example, one of the questions is “do you notice any bleeding after your session (on your way home or at home)? The response options are 1) no bleeding, 2) a little (perhaps a few drops, 3) yes (needed a new bandage). We will report to the IRB when the response was #3.

Week 12 - Home Tasks:
After their last lab visit, participants will be asked to complete daily questionnaires and cognitive tasks as they did in the first week of the study. In addition, participants will be asked about their experience during this study. Pre and post cognitive data will be compared to see if there are any group differences in change.

References

Min, J., Rouanet, J., Martini, A. C., Nashiro, K., Yoo, H. J., Porat, S., Cho, C., Wan, J., Cole, S. W., Head, E., Nation, D. A., Thayer, J. F., & Mather, M. (2023). Modulating heart rate oscillation affects plasma amyloid beta and tau levels in younger and older adults. *Scientific Reports*, *13*(1), 3967.

Nashiro, K., Min, J., Yoo, H. J., Cho, C., Bachman, S. L., Dutt, S., Thayer, J. F., Lehrer, P. M., Feng, T., Mercer, N., Nasseri, P., Wang, D., Chang, C., Marmarelis, V. Z., Narayanan, S., Nation, D. A., & Mather, M. (2023). Increasing coordination and responsivity of emotion-related brain regions with a heart rate variability biofeedback randomized trial. *Cognitive, Affective & Behavioral Neuroscience*, *23*(1), 66–83.

Yoo, H. J., Nashiro, K., Min, J., Cho, C., Mercer, N., Bachman, S. L., Nasseri, P., Dutt, S., Porat, S., Choi, P., Zhang, Y., Grigoryan, V., Feng, T., Thayer, J. F., Lehrer, P., Chang, C., Stanley, J. A., Head, E., Rouanet, J., … Mather, M. (2023). Multimodal neuroimaging data from a 5-week heart rate variability biofeedback randomized clinical trial. *Scientific Data*, *10*(1), 503.