Supplementary Online Content


**eMethods.** Participants, Assessments, DLPFC Localization, Electroencephalography, PAS Administration, and DLPFC Plasticity

This supplementary material has been provided by the authors to give readers additional information about their work.
eMethods. Participants, Assessments, DLPFC Localization, Electroencephalography, PAS Administration, and DLPFC Plasticity

The study was conducted at the Centre for Addiction and Mental Health (CAMH), a university teaching hospital that provides psychiatric care to a large urban catchment area of Toronto and is a tertiary referral center in Ontario, Canada. The study was approved by CAMH Research Ethics Board which is in accordance with the declaration of Helsinki.

Participants: Alzheimer’s disease (AD) participants were recruited from CAMH and other collaborating hospitals in Toronto or in response to advertisements. AD participants were included if they met criteria for probable AD following the National Institute of Neurological and Communicative Disorders and Stroke, and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) core criteria\(^1\), and Diagnostic and Statistical Manual of Mental Disorders, 4th Edition- Text Revision (DSM IV-TR) criteria for dementia due to probable AD\(^2\), and scoring 17 or above on the Mini-Mental State Examination (MMSE)\(^3\). Other inclusion criteria were: age 65 or above, either not taking or on a stable dose of a cognitive enhancer (i.e., donepezil, galantamine, memantine, or rivastigmine) for at least three months, willingness and ability to speak English, willingness and ability to provide informed consent, corrected visual ability sufficient for reading of newspaper headlines and corrected hearing capacity adequate to respond to a raised conversational voice. Participant’s capacity to provide informed consent was determined by administering MacArthur Competence Assessment Tool for Clinical Research\(^4\) and only the participants who passed this assessment were included. Age matched, right handed healthy control (HC) participants were recruited via advertisements and from a database. HC participants were included in the study if they met the following inclusion criteria: age 65 or above, willingness and ability to speak English, willingness and ability to provide informed consent.
consent, and corrected visual ability sufficient for reading of newspaper headlines and corrected hearing capacity adequate to respond to a raised conversational voice. Participants with AD were excluded if they met criteria for a DSM IV-TR\(^2\) axis I diagnosis other than AD within the past 12 months or met diagnostic criteria for current alcohol or other drug dependence within the past six months, had electroconvulsive therapy within the past six months, were left handed, or had any contraindication for transcranial magnetic stimulation (TMS) such as history of more than one spontaneous seizure (other than febrile seizure of childhood), presence of a cardiac pacemaker or a defibrillator, and presence of any metal in head or neck region that may interfere with TMS. HC participants were excluded if they met lifetime criteria for a DSM IV-TR\(^2\) axis I diagnosis other than a simple phobia or adjustment disorder, had any neurological disorder affecting central nervous system, were on any psychotropic medication except for sedative/hypnotic at a stable dose for at least four weeks, were left handed, or had any contraindication for TMS. All participants provided their informed written consent. Sample size calculation was based on previous study of PAS done at our center\(^5\), based on which a sample of 32 AD and 16 HC participants would provide 80% power at an alpha = 0.05 to detect a significant difference in DLPFC plasticity between the two groups.

**Baseline Assessments:** Participants were assessed using the NINCDS-ADRDA criteria\(^1\), the Structured Clinical Interview for DSM-IV-TR\(^6\), Cornell Scale for Depression in Dementia\(^7\), and the MMSE\(^3\). They also underwent a thorough clinical assessment by a study psychiatrist. Participants were also assessed for cognitive status by the Repeatable Battery for the Assessment of Neuropsychological Status\(^8\) and the Executive Interview\(^9\).

**Working Memory Assessment:** Working memory performance was assessed with the \(n\)-back task\(^10,11\). The \(n\)-back task was performed right before PAS and on the same day. The \(n\)-back task
is a working memory task that requires participants to decide whether a letter presented on a monitor is the same as, or different from a letter presented \( n \) trials back. When the letter is the same, the trial is a Target trial. When the letter is different, the trial is a Non-Target trial. In our study, \( n \) was either 1 or 2 because AD participants could not generate meaningful data with 3-back condition. The \( n \)-back task also includes a 0-back condition during which participants respond to every letter presented, assessing pure attention to the task. The \( n \)-back letters are black capital letters presented for 250 ms followed by a delay period of 3000 ms during which the participant is required to respond. Then, a plus sign is presented to indicate the end of each trial. In the 1- and 2-back conditions, letters are presented continuously for 15 min. The proportion of Target trials in 1- and 2-back conditions was 0.23 and 0.16, respectively.

For calculating working memory performance accuracy scores, we used A Prime (\( A' \)) as the composite outcome measure that takes into account hits and false alarms using the following formula\(^{12,13}\):

\[
A' = 0.5 + \left[ \text{Sign} \left( H - F \right) \frac{(H - F)^2 + |H - F|}{4 \text{Max}(H, F) - 4HF} \right]
\]

\( H = \) Hit rate (percent target correct responses) and \( F = \) False alarm rate (percent non-target non-correct responses). In the equation, \( \text{sign} \left( H - F \right) \) equals +1 if \( H - F > 0 \) (i.e., if \( H > F \)), 0 if \( H = F \), and -1 otherwise. \( \text{Max} \left( H, F \right) \) equals either \( H \) or \( F \), whichever is greater\(^ {14}\). \( A' \) has advantages over other signal detection measures such as \( d' \) as it is free of assumptions of normality for signal and noise.
**DLPFC Localization:** DLPFC site of stimulation was localized through neuronavigation techniques using the MRICro/reg software, a T1-weight MRI scan obtained with seven fiducial markers in place, and the MINIBIRD system (Ascension Technologies, USA), as previously described. This site lies at the junction of the middle one-third and anterior one-third of the middle frontal gyrus (Talairach coordinates (x, y, z) = -50, 30, 36), which corresponds with junction of Brodmann areas 9 and 46. This site was chosen based on previous research showing the relevance of this DLPFC site for functional neurophysiological abnormalities in relation to working memory impairment and has been successfully used in several studies.

**EEG Recording and Cleaning:** EEG was recorded during the PAS protocol (TMS–EEG) using a 64-channel Synamps 2 EEG system using Neuroscan software (Compumedics Ltd.) as per 10-20 system as previously described. Briefly, EEG 10-20 system is based on location of scalp electrodes and its relationship to underlying cerebral cortex where numbers 10-20 indicate that the distance between adjacent electrodes is either 10 or 20% of the total distance across sagittal or coronal axes of the skull. The distance across sagittal axis is measured between nasion (the point between the forehead and the nose just superior to the bridge of the nose) and inion (lowest and most prominent point of the skull from the back of the head). The distance across coronal axis is measured as the total distance over the skull between left and right pre auricular points. An EEG cap was used to record the cortical signals, and the impedance of all electrodes (Ag/AgCl ring electrodes) was lowered to $\leq 5$ kΩ. Electrodes were referenced to an electrode position immediately posterior to Cz electrode. EEG signals were recorded using DC and a low pass filter of 100 Hz at 20 kHz sampling rate as previously described.
**EEG Data Analysis:** EEG data were down-sampled to 1000 Hz and segmented from -1000 ms to 2000 ms relative to the onset of TMS pulse, and baseline corrected with respect to the pre-stimulus interval -500 ms to -110 ms. To avoid TMS artifacts, EEG data were re-segmented from 25 ms to 2000 ms. Thereafter, EEG data were digitally filtered by using second order, Butterworth, zero-phase shift 1-55 Hz band pass filter (24dB/Oct). EEG recordings from all EEG sessions (pre-PAS, and 0, 15, and 30 min post-PAS) were concatenated together. Initially, EEG data were visually inspected to eliminate trials and channels that were highly contaminated with noise (muscle activity, electrode artifacts). Then, an electrodes-by-trials matrix of ones was created and assigned a value of zero if an epoch had: (1) amplitude larger than +/- 150 μV; or (2) amplitude larger than mean +/- three times standard deviation of all trials or (3) power spectrum that violated 1/f power law. An electrode was rejected if its corresponding row had more than 60% of columns (trials) coded as zeros. An epoch was removed if its corresponding column had more than 20% of rows (electrodes) coded as zeros. Then an independent component analysis (ICA) (EEGLAB toolbox; Infomax algorithm) was performed to remove eyeblink traces, muscle artifacts, and other noise from the EEG data. Finally, data was re-referenced to the average for further analysis.

**PAS Administration and Assessment of DLPFC plasticity:** PAS was administered using previously established protocol. It involved electrical stimulation of the right median nerve at the wrist followed by TMS of the left DLPFC after a 25-ms delay. Electrical stimulation of the median nerve was delivered at 300% of the sensory threshold paired with TMS pulse delivered at
the stimulus intensity required to produce peak-to-peak motor evoked potential equivalent to one millivolt and frequency of 0.1 Hz for a 30-min period to deliver a total of 180 PAS pairs. Participants were instructed to focus their attention at the sensory stimuli to their right wrist and count the number of stimuli delivered during the 30-min period. Participants were not aware of the frequency or total number of the stimuli to be delivered. During the procedure participants were intermittently asked to report their current count of sensory stimuli which was recorded against the actual count. The absolute difference between the participant’s count and the actual count (Count Difference) was used as an index of attention during the PAS procedure given that attention is known to be critical for PAS-LTP\textsuperscript{5,19}. A Count Difference of 0 indicates “perfect” attention during PAS. Pre-PAS, cortical-evoked activity (CEA) of the DLPFC was indexed using a train of 100 monophasic TMS pulses at 0.1 Hz administered to the left DLPFC using a 7 cm figure-of-eight coil and a Bistim module (Magstim Company Ltd., UK). CEA from the EEG electrodes located over the left DLPFC was recorded using Neuroscan (Compumedics Ltd.). In order to measure the effect of PAS, first, CEA for each session was calculated by averaging the response over all epochs. Second, using Hilbert transform the area between 50-275 ms post TMS pulse was selected to calculate the overall CEA. The first interval (i.e., 50 ms) was chosen because it represents the earliest artefact-free data and the second interval (i.e., 275 ms) was chosen as it is thought to be important for gamma-aminobutyric acid receptor activity that is critical for paired pulse paradigms\textsuperscript{20}. We used rectified area under the curve for TMS evoked potential to calculate cortical evoked activity in line with previous publications on TMS-EEG\textsuperscript{15,21} and DLPFC plasticity\textsuperscript{5,22}. At 0, 17, and 34 min post-PAS CEA was indexed following the same procedures used for pre-PAS as described above. These time points were chosen based on previous research showing the maximum likelihood of potentiation during this interval both for
motor cortex and the DLPFC and the fact that the delivery of 100 pulses at 0.1 Hz takes approximately 16 minutes. DLPFC plasticity, i.e. PAS-LTP, was defined as potentiation of CEA and measured using the ratio of post-PAS to pre-PAS CEA. As the post-PAS timing of maximum potentiation of CEA could vary among participants, we selected the maximum CEA ratio for each participant post-PAS. This method has been used in several previous publications and has been shown to adequately correct for variability in baseline CEA in response to TMS.

**References**


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