Title
A study of alpha desynchronization, heart rate, and MRI during stroop testing unmasks pre-symptomatic Alzheimer’s disease

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Alpha desynchronization during Stroop testing and its link to heart rate unmask pre-symptomatic AD

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Abstract

Objective: Determine the alpha desynchronization changes during cognitive challenge in pre-symptomatic AD individuals and their linkage to heart rate to better predict early AD risk.

Methods: We used quantitative electroencephalography (qEEG) to investigate brain activities during resting state and Stroop interference testing at low load (congruent trials) and high load conditions (incongruent trials) in cognitively healthy (CH) elderly participants. Participants fit one of two subgroups from cerebrospinal fluid (CSF) proteins: with normal amyloid/tau ratio (CH-NAT, n = 20) or pathological amyloid/tau ratio (CH-PAT, n = 21). Alpha event-related desynchronization (ERD) and alpha spectral entropy (SE) by quantitative EEG were compared. We explored the relations between alpha ERD with heart rate and estimated cognitive reserve.

Results: Alpha power during resting state did not change between groups . Compared to CH-NATs, CH-PATs have more negative occipital alpha power, and higher frontal and occipital alpha SE during congruent trials, both indicating hyperactivity. CH-PATs have less frontal SE changes from congruent to incongruent trials, indicating they have insufficient cognitive resource in response to the higher interference load. Correlations of alpha ERD with heart rate and cognitive reserve were significant in CH-PATs, but not CH-NATs.

Interpretation: Our study suggests Stroop challenge reveals compensatory hyper-excitability and insufficient brain-heart resources in pre-symptomatic AD.

Abbreviations:

AD: Alzheimer’s disease; CH-NATs: cognitively healthy with normal amyloid/tau ratio; CH-PATs: cognitively healthy with pathological amyloid/tau ratio; EEG: electroencephalography; ERD: event-related desynchronization; SE: spectral entropy.

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Introduction

Neuropathology and biomarkers of Alzheimer's disease (AD) have demonstrated that pathology (amyloid/tau) precedes cognitive impairment by decades[1, 2]. With the lack of effective AD treatment, current efforts to recognize early pathology and predict clinical onset in late onset AD are an area of active research[3]. The research framework from the recent US National Institute on Aging–Alzheimer's Association (NIA-AA) focuses on early AD diagnosis, and encourages investigating the interaction among pathology (amyloid/tau biomarkers and genotype) and cognitive symptoms[4]. Subtle systemic dysfunctions provide an alternative opportunity for biomarker (amyloid/tau) detection in pre-symptomatic AD. Therefore, non-invasive, easy applicable, and inexpensive approaches to classify pre-symptomatic AD are the focus of our study.

Electrophysiological approaches have potential to understand how neurodegeneration affects the pre-symptomatic stage of AD. Electroencephalography (EEG) has revealed altered occipital alpha frequency in progressive MCI or AD[5, 6]. Using quantitative EEG (qEEG), alpha event-related desynchronization (ERD) reflects brain activation in response to stimuli [7] and relates to "neural efficiency"[8]. Alpha spectral entropy (SE) quantifies signal complexity of power density in the alpha band[9, 10]. Pre-symptomatic AD individuals demonstrated compromised alpha ERD and alpha SE during working memory testing, indicating hyper-excitability[11]. Since working memory is related to Stroop interference processing[12], we predict that altered alpha ERD and SE during Stroop interference will reveal individuals with pre-symptomatic AD pathology.

Previous studies by us and others demonstrated decreased inhibitory control in AD, using Stroop interference testing[13-15]. Pre-symptomatic AD individuals have impaired behavioral performance during the Stroop task, but how their interference processing change

remains unknown. Our goal is to test whether alpha ERD and SE during an Stroop interference challenge can detect pre-symptomatic AD individuals[1, 2].

We expect that systemic physiological measures such as heart rate, will be affected by underlying neurodegeneration[16]. Heart rate reflects the balance between acceleratory sympathetic and inhibitory parasympathetic nerves activities, and is regulated by the neurovisceral network, eg. amygdala and hypothalamus that are inhibited by the prefrontal cortex (PFC)[17-20]. Resting heart rate is related to cognitive decline in a large cohort study[21] and heart rate increases on cognitive challenge, reflecting brain-heart responses to cognitive load[22]. Since pre-symptomatic AD individuals show mildly impaired executive functions[11, 14], we predict that their heart rate will be dysregulated by pathology of the PFC-amygdala network and correlate with changes in frontal alpha ERD.

This study uses cognitive challenge with objective measures of both central (qEEG) and peripheral (heart rate) nervous systems to understand systemic cognitive dysfunction in pre-symptomatic AD. Though these pioneering studies need to be replicated and extended to other and larger populations and with longitudinal assessment, these results provide a template strategy and objective measures to detect pre-symptomatic AD that correlate with invasive biomarkers (CSF amyloid/tau), and to assess potential preventive treatments for cognitively unimpaired individuals: treatment that improves the alpha ERD, SE, and heart rate will predict benefit, while no effect will predict treatment failure.

Participants and Methods

Participants

Fifty cognitively healthy elderly participants were initially recruited from advertisements placed in local newspapers and newsletters, from the Pasadena Huntington Hospital Senior Health Network, the Pasadena Senior Center, and from meetings with local physicians where we presented this research. Participants were then further divided depending on individual CSF Aβ/tau ratios compared to a cutoff value derived from a logistic regression model that correctly diagnosed >85% of clinically probable AD participants. We applied this regression to cognitively healthy (CH) seniors, which separated two groups with either normal CSF Aβ/tau ratio (CH-NATs) or pathological Aβ/tau ratio (CH-PATs)[14]. We have shown that CH-PATs are at higher risk for cognitive decline and are pre-symptomatic AD[23]. Twenty CH-NATs and twenty-one CH-PATs were included to match the education level. All participants signed consent in an Institutional Review Board (IRB) approved protocol (HMRI # 33797). Assessments included collection of demographic data, physical exam, fasting blood studies, disease severity and disability scales, and CSF amyloid/tau measurements[14]. Participants with any cognitive impairment, i.e., global clinical dementia rating scale (CDR) scores > 0.0, were excluded. Only participants who had a Uniform Data Set (UDS-3) format examination with no classifiable psychiatric or neurological disorder were diagnosed as CH and enrolled in this study.

Procedures

Study participants were seated in a quiet room and were first asked, during resting state baseline measures, to “sit still” and “empty their minds” for 5 minutes with eyes open (eyes fixed at the DELL sign on the bottom of the dark screen), and then for 5 minutes with eyes closed.

We administered the Stroop interference test using E-prime software (Psychology Software Tools, Inc., Sharpsburg PA) on a Dell Precision T5610 with a 20” screen. Participants were comfortably seated before the computer screen. One of the three colored words ‘Red’, ‘Blue,’ or ‘Green’ presented on the screen one at a time in three different colors (red color, blue color, or green color). The ink color and word denote can be congruent (“C”, eg. ‘Red’ in red ink), or incongruent (“I”, e. ‘Red’ in blue ink). Participants were instructed to respond to the color of the ink and ignore the word: press ‘1’ for ‘red’, ‘2’ for ‘blue’, and ‘3’ for ‘green’. Participants took the Stroop testing after a practice run of 2-3 minutes. Each test includes 3 blocks of 110 trials and the entire task took about 20 minutes to complete, depending on each participant’s performance.

EEG recordings

Online EEG data were collected during resting state or during the Stroop challenge as previously described[24]. Briefly, a 21-head-sensor, dry electrode system (Quasar Wearable Sensing, DSI-24, San Diego, CA) was used to collect EEG signals, and sensor configuration following the international 10–20 system and were placed approximately at the locations (Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, O2, M1, and M2. The EEG signals were sampled at 300 Hz, and bandpass filtered between 0.003–150Hz. To help signal processing, electrooculographic (EOG), electrocardiographic (ECG), and electromyography (EMG) on the right forearm activities were recorded by three auxiliary sensors. A trigger channel encoded the time of color-word stimuli onset, the participants’ responses, and the type of test (C or I) for further analysis.

Behavioral and EEG Data Processing

A researcher performed all behavioral and EEG data collection and processing without knowledge of CH-NAT/CH-PAT status. The behavioral performances were described and

compared by accuracy (ACC) and response time (RT): we define ACC as the percentage of correctly responded trials out of the total trials; RT as the duration from stimulus onset to participant's response.

We analyzed all dataset in EEGLAB version eeglab14_1_0b[25] running in MATLAB R2016b (The MathWorks, USA) and custom codes developed in-house. The continuous EEG recordings were segmented into epochs of 1500 ms duration during eyes closed for resting state, or using the stimulus onset as a reference during Stroop (500 ms before and 1000 ms after). Preprocessing and time-frequency analyses were performed as previously described[24]. Briefly, epochs were filtered between 2 and 30 Hz, and independent component analysis (ICA)[25] was used to remove eye blinks and cardiac and other muscle artifacts. Large artifact activity greater than three standard deviations (SDs) from the mean of a specific sensor, were rejected. For time-frequency analysis, epoched EEG data were decomposed with logarithmic scaling between 2 and 30 Hz by fast Fourier transform and Morlet wavelet [$e^{i2\pi tf}e^{-t^2/2\sigma^2}$] convolution in the frequency domain, followed by the inverse fast Fourier transform[26, 27]. Power values were normalized by decibels to the baseline power from -400 to -100 ms pre-stimulus at each frequency band [

$dB\,power = 10 * \log 10\,(\frac{power}{baseline})$]. Based on the TF plots and published data[28-30], alpha ERD (range 200-500 ms, 8-15 Hz) were then extracted for comparison across sensors, participants, and groups. This was done separately for each sensor, condition, and participant. In order to exclude individual processing speed differences, percentage of alpha ERD change from C to I trials were also compared.

Besides alpha ERD, other frequency bands, such as delta, theta, and beta bands, are also reported to be important for Stroop interference processing [31]. Therefore, we compared

delta (2-4), theta (4–8 Hz), alpha (8–15 Hz), and beta (15–30 Hz) bands at the early [0 to 500] ms window (except alpha at [200 to 500] ms) or late [500 to 1000] ms window between CH-NAT and CH-PAT participants.

Spectral entropy (SE) analysis

We calculated alpha SE for baseline EEG using the [-400 to -100] ms time window during Stroop test trials, and alpha SE for active (ERD) EEG using the [200-500] ms time window. The SE was calculated at each time point in the respective temporal windows of each EEG channel using the following formula:

$SE=\frac{1}{\ln(N)}\sum_{f_i=f_1}^{f_2} P_n(f_i)\ln\left(\frac{1}{P_n(f_i)}\right)$ [32], where N is the number of frequency components in the [f1 f2] range, with f1 and f2 being the lower (8 Hz) and upper (15 Hz) limit of the alpha frequency band respectively, and $P_n(f_i)$ is the normalized power spectrum.

Heart rate

The researcher asked participants to maintain a relaxed sitting position in the chair, and measured their heart rate, systolic pressure, and diastolic pressure using a sphygmomanometer (MODEL: HEM-790IT, Omeron Healthcare, Inc) after the Stroop testing. Measurements also include resting heart rate at a different visit.

Cognitive reserve and neuropsychology tests

We calculated cognitive reserve from proxies of verbal IQ and education years. Both verbal IQ and education years were calculated for Z-scores for the recruited population, which were then averaged for cognitive reserve. *MMSE-7 and MoCA* were measured by standard questionnaire[14].

Statistical methods

Alpha power measurements were analyzed by averaging individual sensors within and across participants to derive statistics. We compared group differences on participant baseline characteristics using two-sided t-tests, or Fisher's exact test. We summarized alpha power and alpha SE statistics for 6 regions[24, 33]: frontal or F (Fz, F3, F4), central or C (Cz, C3, C4), parietal or P (Pz, P3, P4), left temporal or LT (F7, T3, T5), right temporal or RT (F8, T4, T6), and occipital or O (O1, O2). The strength of associations between EEG spectral power with other measurements from same individuals (heart rate, cognitive reserve, etc.) were assessed as slope factors, determined using linear regression methods and correlation coefficients, and p values were computed. Receiver operating characteristic (ROC) curves were performed to determine if brain and heart parameters can classify CH-NAT and CH-PAT participants. Analyses were done using PRISM v6.07 (GraphPad), excel (Office 2013), or MATLAB 2016b. Since this was an exploratory study, a significance level of 0.05 was used for all tests.

Results

Study participant demographics

Cognitively healthy participants with normal Aβ/tau ratios (CH-NAT, n=20), and abnormal Aβ/tau ratios (CH-PAT, n=21), were matched by age, gender, education, and handedness (Table 1),

Behavioral Performance (ACC and RT)

For the congruent and incongruent trials, neither ACC nor RT were significantly different between the CH-NAT and CH-PAT participants (Table 2). RT during incongruent trials was longer than that during congruent trials for both groups ($p<0.001$). Accuracy during incongruent groups was lower than that during congruent trials for both CH-NATs and CH-PATs ($p<0.01$).

Time-Frequency Plots

Figure 1 shows a general fronto-posterior distribution of alpha ERD during the Stroop test, in the comparison (CH-NAT vs. CH-PAT) in time-frequency plots of mean power of EEG in the sagittal plane at the frontal, central, parietal, and occipital regions of the brain. Despite “normal” behavioral performance measures (Table 2), total power of alpha ERD, in the white box in the occipital region (congruent trials), was significantly greater (more negative, blue color) (200~500 ms, 8-15 Hz) in the CH-PAT group compared to age-matched CH-NATs, indicated by the arrows in Figure 1.

Alpha baseline values

We compared CH-NAT and CH-PAT participants on time-frequency plots for mean alpha power during resting, as well as during congruent and incongruent trial baselines. Our

238 analysis suggests that alpha power was comparable between CH-NATs and CH-PATs,
239 during resting, and baseline of congruent and incongruent trials (data not shown).

240

241 ***Alpha ERD differences during cognitive challenge***

242 During congruent trials, alpha ERD was lower in CH-PAT than CH-NAT in the occipital
243 region (p=0.024) and marginally lower in CH-PATs in the right temporal region (p=0.059,
244 Fig 2, Table 3a). During incongruent trials, no different alpha ERD was seen between
245 groups (Fig 2 and Table 3b).

246 ***Alpha SE differences during cognitive challenge***

247 Congruent trials revealed a higher alpha SE (active ERD windows) in the CH-PATs
248 compared to CH-NATs in the frontal and occipital regions (p=0.042 and 0.039,
249 respectively); incongruent trials revealed no differences between CH-NAT and CH-PAT
250 groups (Fig 3 and Table 4). Comparing the congruent to incongruent trials, alpha SE in CH-
251 NATs was higher than in CH-PATs at the frontal region (p=0.012) (Fig 3, right column;
252 Table 4c).

253 ***Alpha ERD during cognitive challenge and heart rate by groups***

254 Alpha ERD correlated with heart rate during congruent trials at frontal, central, left
255 temporal, right temporal, and occipital region for CH-PATs (p=0.001~0.029,
256 r=0.48~0.66), but this was minimal for CH-NATs at the right temporal region
257 (p=0.047, r=-0.49). Correlations during incongruent trials for CH-PATs were more
258 significant (p<0.0005~0.007, r=0.57~0.78), but minimal for CH-NATs at central and
259 left temporal regions (p=0.021~0.031, r=-0.55~-0.52) (Fig 4).

Alpha ERD during cognitive challenge and cognitive reserve correlation by groups

Alpha ERD correlated with cognitive reserve during incongruent trials at F, LT, RT, and occipital regions for CH-PATs ($p=0.001$, $r=0.66$ for F), but not for CH-NATs. Neither groups show correlations during congruent trials (Fig 5). Cognitive reserve correlated with alpha SE during incongruent trials at F, C, LT, and occipital regions for CH-PATs ($p=0.007$, $r=-0.57$ for F), but not CH-NATs. Neither groups show correlations during congruent trials (Fig 5E-H).

ROC curves

We examined how well combined brain and heart parameters classify CH-PATs from CH-NATs. Alpha ERD at the occipital region during congruent trials is a significant classifier of two groups ($p<0.05$, $AUC=0.69$). Although not significant by themselves, adding cognitive reserve, MoCA, and heart rate improves the classification accuracy of alpha ERD ($p=0.0048$, $AUC=0.76$) (Fig 6A-B).

Explorations of other low frequency spectral power by groups

Delta, theta, alpha, and beta power at early (before 500ms) and late (500-1000ms) windows during Stroop testing were compared. Details are shown in supplementary table (Tables S1a&b).

Spectral power correlates with behavioral responses (RT and ACC) by groups

The correlation between participants' spectral power and RT and ACC for different trials sre shown in supplementary tables (Tables S2a&b).

Spectral power correlates with heart rate, CSF amyloid, Tau, and MMSE-7

The correlations between participants' EEG spectral power and heart rate, CSF amyloid, total Tau, and MMSE-7 for congruent and incongruent trials are shown in supplementary tables (Tables S3a&b).

Discussion

Our study demonstrates that Stroop challenge reveals subtle changes in pre-symptomatic AD and supports our two predictions: First, despite similar behavioral responses, alpha ERD was more negative at the occipital region in CH-PATs during a low interference load, indicating hyperactivities; alpha SE changes from low to high interference load at the frontal region are lower in CH-PATs, indicating they have insufficient cognitive resource in response to the higher interference load. The capability of qEEG to differentiate the pre-symptomatic AD individuals highlights the importance of the qEEG data in revealing the underlying susceptibility that we report for participants with the AD biomarkers of abnormal CSF amyloid/tau. We emphasize the veracity of our qEEG in this paradigm, since the RTs (600~1000 ms) of our particpants are consistent with the reported range for computerized Stroop testing[34], and both groups exhibited the expected Stroop effects with longer RT during incongruent than congruent trials. Second, there are significant correlations between alpha ERD with heart rate in CH-PATs compared to CH-NATs. Thus, we report that quantitative measures of brain functions during a simple cognitive "interference" challenge linked to peripheral physiology (heart functions) and cognitive reserve, which reflect systemic physiological changes related to pre-symptomatic AD pathology.

Yield of interference challenge

In comparison to published approaches, our study is novel in both using an early stage of AD pathology recognized only by CSF biomarkers, as well as by using cognitive challenge (interference) to bring out alterations in dynamic neural responses detected by both qEEG and by its link to heart rate and cognitive reserve. A few published studies have examined pathophysiology in cognitively healthy individuals with known AD biomarkers, and those

that have followed healthy people longitudinally have analyzed the predictive power of the more established CSF and brain imaging biomarkers[1, 2]. We discuss how our results add to the existing knowledge and why they are important and discuss the limitations and the main conclusions of our study. In addition, after finding this new systemic evidence including central (qEEG) and peripheral (heart rate) involvement of AD in cognitively healthy individuals, we undertook initial studies of their potential interactions with other biomarkers, including MMSE, MoCA, and CSF amyloid/tau; we present preliminary findings in the Supplementary Tables to stimulate future analyses between these new qEEG and heart rate findings and the more widespread interoceptive pathophysiology of AD.

Context of Previous Work

In the symptomatic stages of AD, studies of MCI and AD patients have reported a preponderance of changes in occipital alpha: Bajo et al. demonstrated higher parieto-occipital synchronization in alpha and beta bands for progressive MCI compared to stable MCI[5]; Dr. Babiloni's group reported alpha and delta/alpha differences at the occipital region between AD and healthy elderly participants[6]; Occipital hypo-synchronous alpha was shown in AD by resting magnetoencephalography (MEG)[35]; and early AD individuals demonstrate frontal hyperactivity[36]. We interprete our finding of more negative alpha ERD in the occipital region with higher alpha SE over the frontal and occipital regions during congruent trials as demonstrating compensatory hyperactivity in CH-PATs. This result corroborates our previous findings of higher frontal alpha SE during working memory testing during low load[11], as working memory capacity can predict the Stroop interference level[34, 37]. Our data during low interference load challenge confirmed our hypothesis that more negative alpha ERD would be revealed in the occipital region and higher alpha SE observed over the frontal and occipital regions in CH-PATs. This frontal and occipital hyperactivity seems to be transitory for lower long-distant connections in AD

participants[38]. Additionally, we interpret the lower alpha SE from congruent to incongruent trials at the frontal region in CH-PATs to indicate they have insufficient cognitive recruitment in response to the challenge with a higher interference load.

Heart rate and alpha ERD are correlated in CH-PATs

Heart rate and resting alpha power are tightly linked to BOLD signals[39]. Heart rate was positively related to cognitive decline in a large scale population study, with odds ratio about 1.01-1.08[21]. In our study, alpha ERD correlated positively with heart rate only in the CH-PAT group, reflecting tighter connections between cognitive and systemic efforts in response to interference challenges. This tighter connection in CH-PATs compared to CH-NATs might reflect the reduced influence of PFC on the neurovisceral network[20] from pathology. That is also consistent with the frontal dysfunction reported in pre-symptomatic AD[11]. The decreased prefrontal cortex influence on CH-PATs may be related to autonomic strain, or to compromised connections between prefrontal cortex and amygdala or hypothalamus[17,18,20,19]. However, the direct link between frontal cortex and heart rate regulation in pre-symptomatic AD needs further investigation.

Furthermore, those CH-PAT individuals with more negative alpha ERD show lower heart rate, reflecting an unmatched oxygen supply with increasing demand. This could reflect an energy conflict between oxygen demand versus supply in CH-PATs, which could further burden neural efficiency, with more negative alpha ERD reflecting an insufficient neurovisceral system in pre-symptomatic AD.

Cognitive reserve and alpha ERD are correlated in CH-PATs

Cognitive reserve has been used to account for cognitive performance variabilities. To our knowledge, there are no other studies correlating cognitive reserve with alpha ERD. Cognitive reserve can be estimated by proxies of education years and verbal IQ[40]. Higher

cognitive reserve was associated with more effective strategies in the executive task[41] and with lower levels of cardiovascular disease (white matter hyperintensities), which may offer resilience to other pathologies[42]. We interpret the alpha ERD correlation with cognitive reserve that we find only in CH-PATs during incongruent trials to indicate a strain on cognitive reserve by the high load interference challenge. Interestingly, those CH-PATs with higher cognitive reserve have better alpha ERD values (less negative), indicating a better neural efficiency, similar to that for the CH-NAT group. Although spectral entropy is not different between the two groups during incongruent trials, the correlation with cognitive reserve is only significant (negatively) in CH-PATs: those CH-PATs with higher cognitive reserve used less alpha SE for high load incongruent trials, again indicating higher neural efficiency with less resources used for the task, results that are consistent with our interpretation of the alpha ERD results. This interpretation, therefore, suggests that cognitive reserve may improve neural function (higher ERD and lower SE) when CSF amyloid/tau levels are abnormal to protect the CH-PATs from the toxic effects and help maintain normal neural efficiency. This interpretation supports the widely reported protection effect from education[43]. We used the commonly used cognitive reserve calculation from verbal IQ and education years; adding factors such as occupational complexity, physical activity, leisure activities, etc., may further improve this type of analysis[40].

In sum, during resting or real life (when only behavioral responses were observed), the limited brain-heart resources are difficult to be identified in CH-PATs. However, like the physical treadmill test to diagnose coronary disease , the insufficient brain-heart resources in pre-symptomatic AD can be brought out at the early stage by increasing cognitive load: low interference load induces hyper-excitability (occipital or frontal), and high interference load reveals insufficient cognitive resource to support performance.

Conclusions

Stroop testing combined with qEEG in a cross-sectional study revealed that interference processing is compromised in cognitively healthy individuals with AD pathology defined by CSF amyloid/tau levels. Our results show that alpha ERD correlates with measurements of heart rate, cognitive reserve, and other AD related measures in individuals with pre-symptomatic AD pathology. We demonstrate for the first time that insufficient brain-heart resources in pre-symptomatic AD individuals are revealed by interference Stroop testing, and is likely a transitory stage to further deterioration. We propose that treatment strategies to improve cognitive reserve and cardiovascular health at the pre-symptomatic stage of AD may attenuate neurodegeneration[44].Cognitive challenge with Stroop testing combined with qEEG and systemic physiological measures have valuable screening potential to differentiate pre-symptomatic AD from normal aging and offer useful tools to monitor and guide new therapies.

Limitations, clinical implications, and future studies

There are limitations to this study. First, the investigation was exploratory, and our participants from the local Pasadena area were mainly Caucasian with higher socioeconomic status. Our work needs to be repeated in a larger and more mixed populationand from other locations. With longitudinal follow up, potential biomarkers can be calculated to set standards for clinical screening. In addition, Stroop interference does not include the common conflicts in real life (eg. left vs. right), but the computerized testing with EEG provide objective measures of the compromised oscillatory component behind interference processing. Future studies can incorporate different interference factors, such as the Simon effect[45]. Finally, more participants are female than male, both for CH-NATs and CH-PATs, which may reflect greater altruism or greater resilience in

females for aging. Our study can exclude age-related general slowing effects as a potential concern, since we have two groups with similar age range and can be further excluded by our findings of SE change from congruent to incongruent trials[46]. Given these limitations, our findings regarding alpha ERD and alpha SE during Stroop task performance provide new insight to early AD pathology and should encourage further research.

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Author Contributions

Conception and design of the study: XA MGH. Acquisition of the data: XA MGH. Analysis of the data: XA SH MK RK AF MGH. Wrote the paper, XA. Edited the paper: XA SH RK AF MGH. All authors contributed toward final manuscript.

Conflicts of Interest

Nothing to report.

References

1. Nathan PJ, Lim YY, Abbott R, et al. Association between CSF biomarkers, hippocampal volume and cognitive function in patients with amnestic mild cognitive impairment (MCI). Neurobiol Aging. 2017 May;53:1-10.
2. Harari O, Cruchaga C, Kauwe JS, et al. Phosphorylated tau-Abeta42 ratio as a continuous trait for biomarker discovery for early-stage Alzheimer's disease in multiplex immunoassay panels of cerebrospinal fluid. Biol Psychiatry. 2014 May 1;75(9):723-31.
3. Cummings J, Lee G, Ritter A, Sabbagh M, Zhong K. Alzheimer's disease drug development pipeline: 2019. Alzheimers Dement (N Y). 2019;5:272-93.
4. Jack CR, Jr., Bennett DA, Blennow K, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimers Dement. 2018 Apr;14(4):535-62.
5. Bajo R, Castellanos NP, Cuesta P, et al. Differential patterns of connectivity in progressive mild cognitive impairment. Brain Connect. 2012;2(1):21-4.
6. Babiloni C, Triggiani AI, Lizio R, et al. Classification of Single Normal and Alzheimer's Disease Individuals from Cortical Sources of Resting State EEG Rhythms. Front Neurosci. 2016;10:47.
7. Klimesch W. alpha-band oscillations, attention, and controlled access to stored information. Trends Cogn Sci. 2012 Dec;16(12):606-17.
8. Grabner RH, Fink A, Stipacek A, Neuper C, Neubauer AC. Intelligence and working memory systems: evidence of neural efficiency in alpha band ERD. Brain Res Cogn Brain Res. 2004 Jul;20(2):212-25.
9. Inouye T, Shinosaki K, Sakamoto H, et al. Quantification of EEG irregularity by use of the entropy of the power spectrum. Electroencephalogr Clin Neurophysiol. 1991 Sep;79(3):204-10.
10. Nunes RR, Almeida MP, Sleigh JW. [Spectral entropy: a new method for anesthetic adequacy.]. Rev Bras Anestesiol. 2004 Jun;54(3):404-22.
11. Arakaki X, Lee R, King KS, Fonteh AN, Harrington MG. Alpha desynchronization during simple working memory unmasks pathological aging in cognitively healthy individuals. PLoS One. 2019;14(1):e0208517.
12. Jaiswal S, Tsai SY, Juan CH, Muggleton NG, Liang WK. Low delta and high alpha power are associated with better conflict control and working memory in high mindfulness, low anxiety individuals. Soc Cogn Affect Neurosci. 2019 Aug 7;14(6):645-55.
13. Tissier C, Linzarini A, Allaire-Duquette G, et al. Sulcal Polymorphisms of the IFC and ACC Contribute to Inhibitory Control Variability in Children and Adults. eNeuro. 2018 Jan-Feb;5(1).
14. Harrington MG, Chiang J, Pogoda JM, et al. Executive function changes before memory in preclinical Alzheimer's pathology: a prospective, cross-sectional, case control study. PLoS One. 2013;8(11):e79378.
15. van Veen V, Carter CS. Separating semantic conflict and response conflict in the Stroop task: a functional MRI study. Neuroimage. 2005 Sep;27(3):497-504.
16. Deater-Deckard K, Bell MA. Maternal executive function, heart rate, and EEG alpha reactivity interact in the prediction of harsh parenting. J Fam Psychol. 2017 Feb;31(1):41-50.
17. Thayer JF, Ahs F, Fredrikson M, Sollers JJ, 3rd, Wager TD. A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate

variability as a marker of stress and health. Neurosci Biobehav Rev. 2012 Feb;36(2):747-56.
18. Silvani A, Calandra-Buonaura G, Dampney RA, Cortelli P. Brain-heart interactions: physiology and clinical implications. Philos Trans A Math Phys Eng Sci. 2016 May 13;374(2067).
19. Kataoka N, Shima Y, Nakajima K, Nakamura K. A central master driver of psychosocial stress responses in the rat. Science. 2020 Mar 6;367(6482):1105-12.
20. Thayer JF, Hansen AL, Saus-Rose E, Johnsen BH. Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health. Ann Behav Med. 2009 Apr;37(2):141-53.
21. Leong; DP, O'Donnell; MJ, Teo; K, et al. Resting heart rate and decline in cognitive function: observations from the ONTARGET/TRANSCEND studies. European Heart Journal. 2013 01 August 2013;34(Issue suppl_1):499-500.
22. Kennedy DO, Scholey AB. Glucose administration, heart rate and cognitive performance: effects of increasing mental effort. Psychopharmacology (Berl). 2000 Mar;149(1):63-71.
23. Wilder C, Moncrieffe K, Nolty A, Arakaki X, Fonteh AN, Harrington MG. Boston Naming Test predicts deterioration of cerebrospinal fluid biomarkers in pre-symptomatic Alzheimer’s disease. FASEB Journal. 2018;accepted.
24. Arakaki X, Shoga M, Li L, et al. Alpha desynchronization/synchronization during working memory testing is compromised in acute mild traumatic brain injury (mTBI). PLoS One. 2018;13(2):e0188101.
25. Delorme A, Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods. 2004 Mar 15;134(1):9-21.
26. Cohen MX, Donner TH. Midfrontal conflict-related theta-band power reflects neural oscillations that predict behavior. J Neurophysiol. 2013 Dec;110(12):2752-63.
27. Cohen MX. Analyzing Neural Time Series Data: Theory and Practice. 2014.
28. Vazquez-Marrufo M, Galvao-Carmona A, Benitez Lugo ML, Ruiz-Pena JL, Borges Guerra M, Izquierdo Ayuso G. Retest reliability of individual alpha ERD topography assessed by human electroencephalography. PLoS One. 2017;12(10):e0187244.
29. Hu L, Peng W, Valentini E, Zhang Z, Hu Y. Functional features of nociceptive-induced suppression of alpha band electroencephalographic oscillations. J Pain. 2013 Jan;14(1):89-99.
30. Pagano S, Fait E, Monti A, Brignani D, Mazza V. Electrophysiological Correlates of Subitizing in Healthy Aging. PLoS One. 2015;10(6):e0131063.
31. Ergen M, Saban S, Kirmizi-Alsan E, Uslu A, Keskin-Ergen Y, Demiralp T. Time-frequency analysis of the event-related potentials associated with the Stroop test. Int J Psychophysiol. 2014 Dec;94(3):463-72.
32. Quandt F, Bonstrup M, Schulz R, et al. Spectral Variability in the Aged Brain during Fine Motor Control. Front Aging Neurosci. 2016;8:305.
33. Lianyang L, Arakaki X, Thao T, Harrington M, Padhye N, Zouridakis G. Brain activation profiles in mTBI: evidence from ERP activity of working memory response. Conf Proc IEEE Eng Med Biol Soc. 2016 Aug;2016:1862-5.
34. Tse CS, Balota DA, Yap MJ, Duchek JM, McCabe DP. Effects of healthy aging and early stage dementia of the Alzheimer's type on components of response time distributions in three attention tasks. Neuropsychology. 2010 May;24(3):300-15.

35. Ranasinghe KG, Cha J, Iaccarino L, et al. Neurophysiological signatures in Alzheimer's disease are distinctly associated with TAU, amyloid-beta accumulation, and cognitive decline. Sci Transl Med. 2020 Mar 11;12(534).
36. Nakamura A, Cuesta P, Fernandez A, et al. Electromagnetic signatures of the preclinical and prodromal stages of Alzheimer's disease. Brain. 2018 Mar 7.
37. Kane MJ, Engle RW. Working-memory capacity and the control of attention: the contributions of goal neglect, response competition, and task set to Stroop interference. J Exp Psychol Gen. 2003 Mar;132(1):47-70.
38. Stam CJ, de Haan W, Daffertshofer A, et al. Graph theoretical analysis of magnetoencephalographic functional connectivity in Alzheimer's disease. Brain. 2009 Jan;132(Pt 1):213-24.
39. Munck; Jd, Goncalves; S, Faes; TJ, et al. THE RELATION BETWEEN ALPHA BAND POWER, HEART RATE AND FMRI. IEEE international symposium on Biomed Imaging. 2007:4.
40. Narbutas J, Egroo MV, Chylinski D, et al. Cognitive efficiency in late midlife is linked to lifestyle characteristics and allostatic load. Aging (Albany NY). 2019 Sep 8;11(17):7169-86.
41. Frankenmolen NL, Fasotti L, Kessels RPC, Oosterman JM. The influence of cognitive reserve and age on the use of memory strategies. Exp Aging Res. 2018 Mar-Apr;44(2):117-34.
42. Pettigrew C, Soldan A, Zhu Y, et al. Cognitive reserve and rate of change in Alzheimer's and cerebrovascular disease biomarkers among cognitively normal individuals. Neurobiol Aging. 2020 Apr;88:33-41.
43. Gatz M. Educating the brain to avoid dementia: can mental exercise prevent Alzheimer disease? PLoS Med. 2005 Jan;2(1):e7.
44. Suffczynski P, Kalitzin S, Pfurtscheller G, Lopes da Silva FH. Computational model of thalamo-cortical networks: dynamical control of alpha rhythms in relation to focal attention. Int J Psychophysiol. 2001 Dec;43(1):25-40.
45. Ma Q, Shang Q. The influence of negative emotion on the Simon effect as reflected by p300. ScientificWorldJournal. 2013;2013:516906.
46. Bugg JM, DeLosh EL, Davalos DB, Davis HP. Age differences in Stroop interference: contributions of general slowing and task-specific deficits. Neuropsychol Dev Cogn B Aging Neuropsychol Cogn. 2007 Mar;14(2):155-67.

Figure legends

Fig 1. Time-frequency plots (F, C, P, and O regions) of mean Stroop test. 3D plot with time reference to stimulus onset (x axis in ms), frequency (y axis in Hz), and power (color scale in dB units). The rectangle indicates the representative alpha ERDs (200~500 ms, 8~15 Hz) that was compared between CH-NAT and CH-PAT groups in frontal, central, parietal, and occipital regions. Alpha ERDs were lower in CH-PAT (-2.11+/-1.32, N=21) vs. CH-NAT (-1.25+/-1.03, N=20) participants in occipital region, p=0.024. Column 1 shows power from F = frontal, column 2 C = central, column 3 P = parietal, and column 4 O = occipital, as indicated.

Fig 2. Topoplots of mean alpha ERD during different Stroop interference load (congruent trials and incongruent trials), by group. Alpha ERD is in dB units based on the colored scale bar on the right; p values are on the bottom row based on the pink scale bar on the right of each plot. Alpha ERD during congruent (C, left column) and incongruent (I, right column) were shown for CH-NAT (row 1), CH-PATs (row 2), and for p values of group comparisons (row 3).

Fig 3. Topoplots of mean alpha SE during active ERD window for C and I trials, and changes from C to I trials, by group. Alpha SE was shown in values based on the colored scale bar on the right (top two rows); p values were shown on the bottom row based on the pink scale bar. Alpha SE during C (left column) and I (middle column) and C to I percentage changes (right column) were shown for CH-NAT (top row), CH-PATs (middle row), and p values of group comparisons (bottom row).

Fig. 4. Correlation of alpha desynchronization with heart rate by groups. The correlation (p-values and correlation coefficient r values) of alpha ERD and

heart rate for CH-NATs and CH-PATs are shown in the topo map during congruent (A) and incongruent (B) trials. Correlation of frontal alpha ERD with heart rate for CH-NATs (green circles) and CH-PATs (red crosses) are shown during congruent (C) and incongruent (D) trials, corresponding p and r values, are shown when significant.

Fig 5. Correlation of alpha ERD with cognitive reserve by groups. The correlation of alpha ERD and cognitive reserve for CH-NATs and CH-PATs for all 6 brain regions are shown in color map during congruent (A) and incongruent (B) trials. Color indicates r values, *: $p<0.05$; **: $p<0.01$. Frontal alpha ERD correlation details are shown for CH-NATs (green circle) and CH-PATs (red cross) during congruent (C) and incongruent (D) trials. For significant correlation ($p<0.05$), p and r values are shown in corresponding colors ($p=0.001$, $r=0.66$ for CH-PATs during incongruent trials). The correlation of cognitive reserve and alpha SE by groups for brain regions are shown in color map during congruent (E) and incongruent (F) trials. Color indicate r values. *: $p<0.05$, **: $p<0.01$. Frontal alpha SE correlation details are shown for CH-NATs (green circle) and CH-PATs (red cross) during congruent (G) and incongruent (H) trials.

Fig. 6. ROC by brain and heart parameters. Plotting of overlap ROC curves for individual alpha desynchronization, cognitive reserve, MoCA, and heart rate (A), or ROC curve for combined alpha ERD, cognitive reserve, MoCA, and heart rate (B). CH-NAT: cognitively healthy with normal amyloid/tau ratio in cerebrospinal fluid (CSF); CH-PAT: cognitively healthy with pathological amyloid/tau ratio in cerebrospinal fluid (CSF); cognitive reserve: cognitive reserve; HR: heart rate; MoCA: Montreal Cognitive Assessment; p: Pearson’s correlation p-values; r: Pearson’s correlation coefficient r.

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Tables

Table 1. Baseline characteristics of participants.

		CH-NAT (n = 20)	CH-PAT (n = 21)	p-value
Mean Age (SD)	Mean (SD)	75.1 (7.5)	76.2 (8.4)	0.65[&]
Gender [n (%)]	Female	15 (75%)	16 (76.2%)	0.99[#]
	Male	5 (25%)	5 (23.8%)	
Mean Education (SD) (yrs)		15.7 (2.3)	16.3 (1.9)	0.33[&]
Handedness [n (%)]	R	19 (95%)	19 (90.5%)	0.99[#]
	L	1 (5%)	2 (9.5%)	

Abbreviations: R/L, right/left-handed; SD, standard deviation.

[&] Two-tailed t-test [#] Fisher's exact test.

Table 2. Mean (SD) response accuracy (ACC) and response time (RT) in the Stroop test

	CH-NAT	CH-PAT
Congruent trials		
N	20	21
ACC	0.94 (0.03)	0.94 (0.02)
RT (ms)	784.92 (125.16)	742.66 (98.90)
Incongruent trials		
N	20	21
ACC	0.90 (0.04)	0.90 (0.05)
RT (ms)	994.57 (186.64)	952.18 (143.23)

* p <0.05. Two-tail ttest

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Table 3a. Comparison of alpha ERD between CH-NAT and CH-PAT during congruent trials.

	CH-NAT (n=20)		CH-PAT (n=21)		P value
	Mean	SD	Mean	SD	
F	-1.20	0.68	-1.65	1.32	0.172
C	-1.26	0.78	-1.77	1.30	0.131
P	-1.23	1.00	-1.77	1.11	0.104
LT	-1.17	0.86	-1.74	1.28	0.106
RT	-1.10	0.74	-1.68	1.13	0.059
O	-1.25	1.03	-2.11	1.32	**0.024**

Table 3b. Comparison of alpha ERD between CH-NAT and CH-PAT during incongruent trials.

	CH-NAT (n=17)		CH-PAT (n=21)		P value
	Mean	SD	Mean	SD	
F	-1.39	0.91	-1.68	1.27	0.423
C	-1.50	0.96	-1.75	1.25	0.489
P	-1.42	1.15	-1.67	1.03	0.485
LT	-1.54	0.90	-1.79	1.26	0.473
RT	-1.19	0.75	-1.59	1.12	0.198
O	-1.41	1.13	-1.95	1.41	0.193

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Table 4a. Comparison of alpha SE between CH-NAT and CH-PAT during congruent trials.

	CH-NAT(n=17)		CH-PAT(n=21)		P value
	Mean	SD	Mean	SD	
F	0.94	0.03	0.96	0.03	**0.042**
C	0.94	0.03	0.95	0.04	0.377
P	0.93	0.04	0.95	0.04	0.254
LT	0.93	0.03	0.94	0.03	0.219
RT	0.94	0.03	0.95	0.03	0.144
O	0.93	0.03	0.96	0.03	**0.039**

Table 4b. Comparison of alpha SE between CH-NAT and CH-PAT during incongruent trials.

	CH-NAT(n=17)		CH-PAT(n=21)		P value
	Mean	SD	Mean	SD	
F	0.95	0.02	0.95	0.03	0.976
C	0.95	0.03	0.95	0.03	0.419
P	0.94	0.03	0.95	0.03	0.209
LT	0.95	0.03	0.95	0.02	0.589
RT	0.94	0.03	0.95	0.03	0.417
O	0.94	0.04	0.96	0.03	0.140

Table 4c. Comparison of alpha SE changes from congruent to incongruent trials ((I-C)/C) between CH-NAT and CH-PAT.

	CH-NAT(n=17)		CH-PAT(n=21)		P value
	Mean	SD	Mean	SD	
F	0.01	0.03	-0.01	0.02	**0.012**
C	0.01	0.03	0.01	0.03	0.887
P	0.01	0.05	0.01	0.04	0.879
LT	0.02	0.04	0.01	0.03	0.381
RT	0.00	0.05	0.00	0.03	0.509
O	0.01	0.02	0.00	0.02	0.548

Supplement Table 1. low frequency power comparison between CH-NATs and CH-PATs during congruent trials (a) and incongruent trials (b).

Supplement Table 2. Pearson's correlation between low frequency power during congruent trials (a) or incongruent trials (b) and behavioral performance (RT and ACC).

Supplement Table 3. Pearson's correlation between low frequency power during congruent trials (a) or incongruent trials (b) and other information (heart rate**, CSF amyloid, CSF tau, MMSE-7).**