Age-related differences in Autonomic/Central Coupling during a Daytime Nap

Pin-Chun Chen[1,2], Katharine C. Simon[1], Negin Sattari[1], Lauren N. Whitehurst[3] & Sara C. Mednick[1*]

[1]Department of Cognitive Science, University of California, Irvine USA

[2]Department of Statistics, University of California, Irvine USA

[3]Department of Psychiatry, University of California, San Francisco, CA, USA

Corresponding author:

Sara C. Mednick, PhD

Department of Cognitive Science University of California, Irvine

Irvine, CA 92697

Email: mednicks@uci.edu Telephone: 949-824-4607

Abstract

Age-dependent functional changes are mirrored by declines in both the central nervous system (CNS) and in the autonomic nervous system (ANS) and have been related to pathological aging. Prior studies have demonstrated inter-dependence between central and autonomic events that contribute to cognition. Moreover, our group recently identified a temporal coupling of Autonomic and Central Events (ACEs) during sleep using electrocardiogram (ECG) to measure heart rate and electroencephalography (EEG) to measure sleeping brain rhythms [40]. We showed that heart rate bursts (HRBs) temporally coincided with increased slow wave activity (SWA, 0.5-1Hz) and sigma activity (12-15Hz), followed by parasympathetic surge (RR_{HF}) during non-rapid eye movement (NREM) sleep. Given that there are paralleling age-related declines in both the ANS and CNS, the current study investigated how these declining systems impact ACE coupling during daytime naps in older and younger adults. Despite, lower overall EEG activity during ACE windows in older adults, both younger and older adults showed HRB-modulated increases in SWA and sigma during wake and N2. However, older adults did not show the same pattern during N3. Furthermore, while younger adults demonstrated a RR_{HF} increase only after HRBs, older adults showed an earlier rise and maintenance of the RR_{HF}. Taken together, our results demonstrated that ACE activity remains generally intact with age. Given that age-related deterioration in autonomic and central nervous system activity is implicated in pathological decline, the general maintenance of alignment between the two systems is intriguing and may facilitate novel insights to aging.

Keywords: Autonomic-Central Events, ACE, Aging, Sleep, Nap, Temporal Coupling

Statement of Significance

Interactions between the CNS and ANS and have emerged as one of the key markers for health and cognition. Given that both declines in ANS and CNS have been independently implicated with pathological aging and mortality, it's pressing to understand how the CNS-ANS interactions change with age. Here, we examined the temporal coupling during wake and sleep among young and older adults during a daytime nap. Similar coupling was demonstrated during wake and N2, with older adults showed less coupling in N2 compared to younger adults. Furthermore, older adults showed no coupling during N3. The current study identifying declines CNS-ANS coupling may facilitate novel insights and provide new targets to combat neurodegenerative disease.

1. Introduction

Sleep is associated with enhanced cognition, sustained attention, restorative physiological processes, and improved immune functioning. Yet, with increasing age, sleep's ability to support these functions declines [2,34,35]. These age-dependent changes parallel dramatic alterations in sleeping brain rhythms [34] between middle and late adulthood that are thought to reflect diminishing underlying neural integrity [33,34]. In older compared to younger adults, slow wave activity (0.5-1Hz, [SWA]), a signature brain rhythm of deep non-rapid eye movement (NREM) sleep and product of synchronous neuronal firing, shows reduced density (number of events/time) and amplitude in the prefrontal cortex [7]. In conjunction, sleep spindles, which are bursts of activation in the sigma range (12-15 Hz) and a hallmark of NREM N2, show similar

declines in density, length of burst, amplitude, and topography [15,18,31,37,39]. Declining sleep EEG physiology and general sleep characteristics, such as changes in sleep rhythms that include increased napping, nighttime arousal, and fragmented sleep, can prognostically predict future cognitive decline and are related to increased pathology and mortality [16,29,42]. Critically, these sleep EEG features are evidence of declining central nervous system activity (CNS) and can be accompanied by age-related declines in the autonomic nervous system (ANS) [26]. How these systems interact during sleep as a function of age is unknown and could be critical for prognostic evaluation and understanding worsening cognition and health in later life.

The ANS's primary role is to quickly adapt and regulate our involuntary body functions in the presence of changing physiological and psychological demands. Efferent and afferent sympathetic and parasympathetic projections from the brain and spinal cord coordinate this effort to alter internal processes including heart rate, blood pressure, breathing and digestion [36]. Cardiac vagal activity, as measured by high frequency heart rate variability (HF HRV: 0.14 - 0.4Hz), is a traditional measure of parasympathetic activity [30]. Greater HF HRV during wake is related to better physical health and emotion regulation, and recent evidence links HF HRV during sleep to enhanced memory [1,9,10,52]. In young, healthy people, a balance exists between activity in the sympathetic and parasympathetic branches, moving from a sympathetic-dominant state during wakefulness to parasympathetic dominant state during NREM sleep [5,8,9,20,51]. Due to a range of factors, age-related autonomic imbalance can develop, with greater sympathetic activity and lower parasympathetic activity, which has been associated with pathological aging, cardiovascular disease, and early mortality [19,45, 48].

Emergent literature has focused on the interaction between ANS and CNS in sleep characterized by specific ANS profiles during each sleep stage [4,17]. For example, de Zambotti

and colleagues (2016) found brief temporal increases in heart rate with a k-complex during N2. Similarly, fluctuations in HF-HRV activity positively correlate with SWA [47]. Using a high temporal precision method to determine ANS-CNS coupling, our group recently evaluated changes in EEG and ECG signals that occurred during brief bursts in heart rate during daytime sleep [40,41]. We indentified short increased in heart rate that occurred at a rate of 1 per minute during N2 and N3 sleep, termed heartrate bursts [HRBs]. Prior to the peak of the HRB, we found increases in SWA and sigma [ACE-SWA and ACE-sigma], followed by vagal rebound [RR_{HF}] after the peak, and named these coupling events collectively Autonomic-Central Events [ACEs]. Importantly, ACE events were positively associated with post-sleep improvement in long-term [40] and working memory [10]. In fact, regression models showed a greater proportion of the sleep-dependent memory improvement was explained by the ACE activity than by the typical EEG sleep events alone.

Given the independent lines of research showing age-related changes in CNS and ANS during sleep, the lack of research examining the interaction between ANS and CNS is surprising. As such, the current study investigated whether aging impacts ACE activity in sleep. 50 young and 51 older adults took a daytime nap in the lab with EEG and ECG monitoring. We predicted a reduction in ACEs for older compared to younger adults, such that the ACE-SWA and ACE-sigma activity and RR_{HF} would be reduced in older compared to younger adults. These results will inform how multiple systems change across the life span and identify novel areas for age-based interventions that may enhance functioning.

2. Methods

2.1 Participants

Fifty healthy young adults (18-23yo, Mean =20.8, SD = 6.453) and fifty-one older adults (60-85yo, Mean = 69.99, SD = 3.074) with no personal history of neurological, psychological, or other chronic illness provided informed consent, which was approved by the University of California, Riverside Human Research Review Board. Participants included in the study had a regular sleep wake schedule (reporting a habitual time in bed of about 7–9 h for young adults and 6-8 h for older adults per night; National Sleep Foundation, 2015). Demographics and prior self-reported sleep habits were reported in Table 1.

The personal health histories were measured twice. First, during a pre-screening questionnaire in which a general health condition report was collected over the online survey and the eligibility was determined. Second, eligible subjects were invited to participate in an orientation in which they were given details about the study and interviewed by a trained graduate student. Participants who met any of the following exclusion criteria were excluded from the study: a) extreme morning or evening-type tendencies (measured with the Morningness Eveningness Questionnaire; Horne & Östberg, 1976); b) excessive daytime sleepiness (reported by Epworth Sleepiness Scale; Johns, 1991; subjects' rating > 13 were excluded); c) a sleep disorder (assessed by questionnaires); d) any personal or immediate family (i.e., first degree relative) history of diagnosed significant psychopathology; e) personal history of head injury with loss of consciousness greater than 2 minutes or seizures; f) history of substance dependence; g) current use of any psychotropic medications; h) any cardiac, respiratory or other medical condition which may affect cerebral metabolism; i) non-correctable vision and auditory impairments.

Participants who did not met any of the exclusion criteria above and also met all the following inclusion criteria were enrolled in the study: a) aged 18-39/ 60-85 years old; b)

healthy, non-smoking adult without major medical problems; c) completed at least 12 years of education; d) a regular sleep-wake schedule, defined as obtaining 7–9 h (young adults) or 6-8 h (older adults) of sleep per night, with a habitual bedtime between 9pm and 2am (young adults) or 8pm and 1am (older adults) and a habitual wake time between 6am and 10am (young adults) or 5am and 9am (older adults). Enrolled participants were asked to maintain their schedule for one week prior to their visit, which was monitored with sleep diaries. In addition, participants were asked to wear an actigraph (Actiwatch Spectrum, Respironics) for one night prior to their visit. Subjects were rescheduled if they reported poor sleep quality in their sleep diary, such as having more than 2 nights of less than 6 h of sleep or more than 9 h of sleep during the week prior to their visit, or if subjects' actigraphy data showed less than 6 h of sleep or more than 9 h of sleep the night before the experimental visit. Rescheduled subjects were given another week to fill out a new sleep diary and maintain a regular sleep wake schedule prior to their visit.

During the orientation, participants were screened for cognitive impairment using Digit Span Backwards, which contains a multi variate length string of digits; subjects were asked to repeat the string of digits backwards after it was read to them. Older participants were screened for dementia using the Telephone Screening for Dementia questionnaires (referred to as TELE), which consists of variety of questions to identify dementia-like symptoms. For older subjects, the STOP-BANG questionnaire was used to screen for obstructive sleep apnea and those with a medium to high risk were excluded. Additionally, participants were instructed to abstain from caffeine and alcohol starting at noon the day prior to the study (detected on the sleep diary). Participants who consumed alcoholic beverages > 10 cans a week or caffeinated products > 3 cups a day were excluded prior to the study (reported in Table 1).

2.2 Procedures

Participants were asked to maintain a regular sleep-wake schedule for one week prior to their visit, which was monitored with sleep diaries and was based on a 2-hour bedtime/ waketime window assigned at the orientation. The 2-hour bedtime/wake time was chosen based on subjects' regular bedtime/wake time. In addition, participants were asked to wear an actigraphy (Actiwatch Spectrum, Respironics) for one night prior to their visit. Participants were rescheduled if they reported poor sleep quality in their sleep diary, such as having more than 2 nights of less than 6 h of sleep during the week prior to their visit, or if subjects' actigraphy data was showing less than 6 h of sleep the night before the experimental visit. Rescheduled subjects were given another week to fill out a new sleep diary and maintain a regular sleep wake schedule prior to their visit.

On the study day, participants had a 2-hour nap opportunity beginning at 1:30 PM while monitored with polysomnography (PSG), including electroencephalography (EEG), electrocardiogram (ECG), electromyogram (EMG), and electrooculogram (EOG). Sleep was monitored online by a trained sleep technician. Among our participants, 6 of older adults were not able to fall asleep, so they were excluded from further analyses. Participants received monetary compensation for participating in the study.

2.3. Data acquisition and pre-processing

2.3.1 Sleep recording and scoring

Electroencephalographic (EEG) data were acquired using a 32-channel cap (EASYCAP GmbH) with Ag/AgCI electrodes placed according to the international 10-20 System (Jasper, 1958). Electrodes included 24 scalp, two electrocardiography (ECG), two electromyography (EMG), two electrooculography (EOG), 1 ground, and 1 on-line common reference channel. The

EEG was recorded with a 1000 Hz sampling rate and was re-referenced to the contralateral mastoid (A1 & A2) post-recording. High pass filters were set at 0.3 Hz and low pass filters at 35 Hz for EEG and EOG. Only eight scalp electrodes (F3, F4, C3, C4, P3, P4, O1, O2), the EMG and EOG were used in the scoring of the nighttime sleep data. Sleep scoring were performed by two trained sleep technicians. Prior to scoring these data, technicians were required to reach 90% reliability with each other and one other rater on two independent data sets. Raw data were visually scored in 30-sec epochs into Wake, Stage 1 Sleep (N1), Stage 2 Sleep (N2), Slow Wave Sleep (N3) and rapid eye movement sleep (REM) according to the American Academy of Sleep Medicine (AASM) rules for sleep staging using HUME, a custom MATLAB toolbox. Prior to sleep scoring, data were pre-processed using BrainVision Analyzer 2.0 (BrainProducts, Munich Germany) and all epochs with artifacts and arousals were identified by visual inspection and rejected. Minutes in each sleep stage were calculated and sleep onset latency (SOL) were calculated as the number of minutes from lights out until the initial epoch of sleep, N2, N3 and REM. Additionally, wake after sleep onset (WASO) was calculated as total minutes awake after the initial epoch of sleep and sleep efficiency (SE) was computed as total time spent asleep after lights out divided by the total time spent in bed x 100.

2.3.3 Power spectral analysis

The EEG power spectrum was computed using the Welch method (4 sec Hanning windows with 50 % overlap). The frequency for sigma power was 12- 15Hz and for SWA was .5–1 Hz. For RR time-series, the power spectral estimation was performed by the autoregressive model and the model order was set at 16. Summary statistics for overall EEG power averaged across frontal and central areas (F3, F4, C3, and C4 channels) during each sleep stage were shown in Table 3.

2.4 Autonomic/Central Event (ACE) Detection

For ACE analyses, consecutive artifact-free 3-min windows of undisturbed sleep (free from stage transitions, arousal, or movements) were selected across the whole nap. Epochs of N1 were not analyzed as only 2 older adults and 4 young adults had stable 3-min bins. For some subjects, we were unable to detect stable 3-min bins during other sleep stages, so they were excluded. In summary, we included 30 young and 42 older adults in analyses for Wake, 49 young and 42 older adults in analyses for N2, 38 young and 23 older adults in analyses for N3, as well as 19 young and 5 older adults in analyses for REM sleep. Since too few older subjects had REM sleep, we dropped REM from all the analyses.

2.4.1 Heart-beat detection

Electrocardiogram (ECG) data were acquired at a 1000-Hz sampling rate using a modified Lead II Einthoven configuration. We analyzed HRV of the R-waves series using Kubios HRV Analysis Software 2.2 (Biosignal Analysis and Medical Imaging Group, University of Kuopio, Finland), according to the Task Force guidelines (Electrophysiology Task Force of the European Society of Cardiology the North American Society of Pacing, 1996). RR peaks were automatically detected by the Kubios software and visually examined by trained technicians. Incorrectly detected R-peaks were manually edited. Next, the data was passed through a lab tool based in MATLAB to perform further analyses. The ECG signals were filtered with a passband of 0.5-100 Hz by Butterworth filter. R waves were identified in the ECG using the Pan-Tompkins method, and confirmed with visual inspection. In order to extract continuous RR tachograms, the RR intervals were resampled (at 4 Hz for power spectrum estimation) and

interpolated by piecewise cubic spline. Zero-phase Butterworth filters were applied to the interpolated RR time-series to extract RR_{HF}.

2.4.2 HR burst detection

Within each continuative and undisturbed 3-min bin, the mean and standard deviation of RR were calculated, and HR bursts were identified as the RR intervals shorter than 1.25 standard deviations below the mean. We investigated EEG changes during 20-sec windows around the HR bursts because EEG fluctuation typically returned to baseline level within 20 seconds in the previous study from our group [40]. No other burst occurred in the 20 second windows around the HR bursts. Summary for HR bursts Density were shown in Table 4.

2.5 Event-based Analysis for Autonomic/Central Event

2.5.1 Time-locked analysis

In order to calculate changes in SWA and sigma power around the HR burst, the Hilbert transform was applied on filtered EEG signals in bands of interest (0.5–1 Hz for SWA and 12–15 Hz for sigma activity). To assess the HF amplitude fluctuation around the HR burst (RR_{HF}), the Hilbert transform was applied on RR_{HF} (0.15–0.4 Hz). See Naji et al 2019a for detailed methods.

2.5.2 ACE Change scores

We investigated ACE coupling during wake and sleep stages by tracking fluctuations in the EEG in a 20-sec window from 10 second before to 10 second after the HR burst peak. As we were specifically interested in sleep EEG activity previously demonstrated to correlate with cognitive

enhancement, we focused on SWA and sigma activity. In addition, we examined RR_{HF} in the ECG channel, about which we did not have a specific hypothesis.

EEG/ECG data were binned into 5-sec intervals within the 20-sec windows around the HR burst, named -10, -5, +5, +10 window. For ACE activity, the average RR_{HF}, SWA, and sigma activity were calculated in each of the four 5-sec windows. For non-ACE brain activity, we calculate average RR_{HF}, SWA, and sigma activity in periods with no HR burst (including 20 s windows around them). We computed ACE change scores for each 5-sec interval as follows: (ACE activity in each 5 s interval – non-ACE activity)/ (ACE activity in each 5 s interval + non-ACE activity). Figure 1 showed an example of ACE time-locked analysis. Summary for ACE change scores were shown in Table 5 and Figure 2.

2.6 Statistics Analyses

Statistical analyses were conducted using R version 3.4.3, and alpha level was set at <.05. In order to investigate within-subject profiles of ACE activity across sleep stages, channels, and windows, we used a linear-mixed effect model (LME), which does not depend on limited assumptions about variance-covariance matrix assumptions (sphericity). Additionally, LME models eliminates the need for averaging epochs across sleep stages and allows inclusion of an unbalanced number of observations per subject in the analyses. Moreover, LME models take into account the influence of factors whose levels are extracted randomly from a population (i.e. participants), thus yielding more generalizable results. For RR intervals and HR bursts Density, we built an LME model using participant as crossed random effects, with a between-subject factor (two age groups) and a within-subject factor (sleep stages: N2, N3, Wake). For ACE-SWA and ACE-sigma change scores, we built an LME model using participant as crossed random

effect, with a between-subject factor (two age groups) and two within-subject factor (windows: -10, -5, +5, +10; sleep stages: N2, N3, Wake) as within-subject fixed effects. ACE-SWA and ACE-sigma change scores were averaged across frontal and central areas (F3, F4, C3, and C4 channels) in the statistical models given our preliminary analyses showing no significant topographical differences in ACE change scores. For ACE-RR_{HF} change scores, we built an LME model using participant as crossed random effect, with a between-subject factor (two age groups) and two within-subject factor (windows: -10, -5, +5, +10; sleep stages: N2, N3, Wake) as within-subject fixed effects. Post hoc comparisons were corrected using the Bonferroni method.

To examine the relationship between overall EEG power and modulation of EEG around HR burst, Pearson's correlations were used to test the bivariate correlations between overall sigma power and ACE-sigma change scores, as well as overall SWA power and ACE-SWA change scores.

3. Results

3.1 Sleep Architecture

Older adults demonstrated shorter total sleep time, longer N1 sleep duration, shorter N3 sleep duration, shorter REM sleep duration, longer wake after sleep onset, and lower sleep. Efficiency. Descriptive statistics for sleep architecture were shown in Table 2.

3.2 Age Differences in RR intervals and HR burst Density (per Minute)

We first examined age differences in RR intervals (see Table 4), and found a main effect of Sleep stages ($F_{(2, 113)} = 27.628$, p=<.0001), with heart rate slower during N2 and N3 compared

to Wake (all ps < .0001); and an interaction between Sleep stages and Age ($F_{(2, 113)}$ = 5.366, p=.0059) on RR intervals. No significant main effect of Age was found ($F_{(1, 101)}$ = 3.777, p=.0547). Post hoc comparisons revealed that the young adults, compared to the older adults, showed a significant slower heart rate during N2 (p = .0164) and N3 (p= .0202) but not during Wake (p = .4288). Within each age group, the young adults showed significantly faster heart rate during Wake, compared to all other sleep stages (all ps < 0.0001), while the older adults showed no differences across sleep stages (all ps > 0.1022).

Next, we found a main effect of Sleep stages ($F_{(2, 141)}$ = 52.0966, p=<.0001) and Age ($F_{(1, 102)}$ = 8.1390, p=.0052), as well as an interaction between Sleep stages and Age ($F_{(2, 141)}$ = 15.4628, p=<.0001) on HR burst Density (see Table 4). Post hoc comparisons revealed that the young adults, compared to the older adults, showed significant more HR bursts during N2 (p<.0001) and N3 (p=.0002), but not during Wake (p=0.3120). Within each age group, the young adults showed significantly less HR bursts during Wake, compared to all other sleep stages (all ps < .0001), while the older adults significantly less HR bursts during Wake, compared to N2 (p=.0028) but not N3 (p=.2211). Summary for HR bursts Density were shown in Table 4.

In summary, age-related decrease in RR intervals and HR burst density were found during NREM sleep but not Wake.

3.3 ACE-SWA change score is modulated by ACE Window and Age

Next, we examined ACE-SWA change scores (see Figure 2) with a between-subject factor (two age groups) and two within-subject factors (windows: -10, -5, +5, +10; sleep stages: N2, N3, Wake). The analysis revealed a main effect of sleep stages ($F_{(2, 3389)}$ = 139.7949, p<.0001), a main effect of windows ($F_{(3, 3389)}$ = 186.9396, p<.0001), a two-way interaction

between sleep stages and age groups ($F_{(2, 3389)} = 5.7078$, p=.0034), a two-way interaction between sleep stages and windows ($F_{(6, 3389)} = 16.9731$, p<.0001), a two-way interaction between age groups and windows ($F_{(3, 3389)} = 6.0064$, p=.0004), a three-way interaction between Windows, Age, and Sleep stages ($F_{(6, 3389)} = 2.2528$, p=.0358).

The Bonferroni post-hoc comparisons revealed that during N2 and N3, young adults showed greater ACE-SWA change scores than the older adults during the -5 window (N2: p=.0068; N3: p=.0001) but no age differences between the rest of the windows (all ps > .2117). No age-related differences were found during Wake (all ps > .4379).

Within each age group, young adults showed significantly greater ACE-SWA change scores during the -5 window compared to the rest of the windows during N2 and N3 (all ps < .0001), while older adults showed significantly greater ACE-SWA change scores during the -5 window compared to the rest of the windows during N2 (all ps < .0001) but not during N3 (all ps > 0.3845). During Wake, both age groups showed significant pairwise differences between windows (all ps < .0001) except that no differences were found between the -10 and +5 windows (all ps > .2379).

Taken together, age-related decrease in peak ACE-SWA change scores were found during NREM sleep but not Wake. Older adults showed similar ACE modulation, with the peak EEG power during the -5 window, during N2 and Wake but they did not show significant ACE modulation during N3.

3.4 ACE-sigma change score is modulated by ACE Window and Age

Similarly, we examined ACE-sigma change scores (see Figure 2) with a between-subject factor (two age groups) and two within-subject factors (windows: -10, -5, +5, +10; sleep stages:

N2, N3, Wake). The analysis revealed a main effect of sleep stages ($F_{(2, 3389)}$ = 146.6889, p<.0001), a main effect of windows ($F_{(3, 3389)}$ = 131.9417, p<.0001), a two-way interaction between sleep stages and windows ($F_{(6, 3389)}$ = 6.7877, p<.0001), a two-way interaction between age groups and windows ($F_{(3, 3389)}$ = 26.8665, p<.0001), a three-way interaction between Windows, Age, and Sleep stages ($F_{(6, 3389)}$ = 2.6803, p=.0135).

The Bonferroni post-hoc comparisons revealed that during N2, young adults showed higher ACE-Sigma change scores during the -5 window than the older adults (p=.0055) but lower during the +5 (p=.0111) and +10windows (p<.0001). During N3, older adults showed higher ACE-Sigma change scores during the +5 window (p=.0492) than the young adults but not the rest of the windows (all ps>.1411). During Wake, older adults showed higher ACE-Sigma change scores during the +5 (p=.0128) and +10 window (p=.0101) than the young adults but not the rest of the windows (all ps>.1406).

Within each age group, during N3, Bonferroni post-hoc comparisons revealed significantly greater ACE-Sigma change scores during the -5 window compared to the rest of the windows (all ps < .0003) among the young adults, whereas the older adults showed significantly greater ACE-Sigma change scores during the +5 window compared to the -10 and +10 windows (all ps <.0383). During N2, young adults showed significantly greater ACE-Sigma change scores during the -5 window compared to the rest of the windows (all ps < .0001) and lower ACE-Sigma change scores during the +10 window compared to the -10 and +5 windows (all ps < .0001), whereas the older adults showed significantly greater ACE-Sigma change scores during the -5 window compared to the -10 and +10 windows (all ps <.0007). During Wake, young adults showed significantly greater ACE-Sigma change scores during the -5 window compared to the rest of the windows (all ps < .0001) and lower ACE-Sigma change scores during the +10

window compared to the -10 and +5 windows (all ps < .0001), and the older adults showed similar pattern, with significantly greater ACE-Sigma change scores during the -5 window compared to the rest of the windows (all ps < .0376) and greater ACE-Sigma change scores during the +5 window compared to the -10 and +10 windows (all ps < .0002).

Taken together, age-related decrease in peak ACE-Sigma change scores were found during N2 but not N3 or Wake. Older adults showed similar ACE modulation, with the peak EEG power during the -5 window, during N2 and Wake but a delay peak EEG power during the +5 window during N3. Similar to SWA, older adults showed no differences in ACE change scores during Wake, comparable ACE modulation with lower peak power than the young adults during N2 sleep, and a delay or blunt ACE EEG modulation during N3.

3.5 ACE-RR_{HF} change score is modulated by ACE Window and Age

In addition to EEG fluctuation, we examined ACE-RR_{HF} change scores (see Figure 2) with a between-subject factor (two age groups) and two within-subject factors (windows: -10, -5, +5, +10; sleep stages: N2, N3, Wake). The analysis revealed a main effect of sleep stages ($F_{(2, 984)}$ = 7.5304, p=.0006), a main effect of windows ($F_{(3, 984)}$ = 60.5159, p<.0001), a two-way interaction between sleep stages and age groups ($F_{(2, 984)}$ = 5.7119, p=.0034), a two-way interaction between age groups and windows ($F_{(3, 984)}$ = 10.8937, p<.0001). No significant three-way interaction between age groups, sleep stages, and windows was found ($F_{(6, 984)}$ = 0.7177, p=.6354).

The Bonferroni post-hoc comparisons revealed that older adults showed higher ACE-RR_{HF} change scores during the -5 window than the young adults (p=.0017) but not during the rest of the windows (p>.0795). Within each age groups, young adults showed significantly

greater ACE-RR_{HF} change scores during the +5 window than the rest of the windows (all ps<.0023), whereas the older counterparts showed significantly greater ACE-RR_{HF} change scores during both the -5 and +5 window than the rest two windows (all ps<.0001). In addition, regardless of windows, young adults showed greater ACE-RR_{HF} change scores during N2 and N3 compared to Wake (all ps<.0002), whereas the older adults showed no significant differences between sleep stages (all ps>.9307).

In summary, young adults demonstrated peak RR_{HF} during the +5 window, similar to Naji et al. (2019a). However, the older counterparts showed an earlier rise of RR_{HF} with the peak amplitude during both the -5 and +5 windows.

3.6 Relationship between Overall EEG Power and ACE EEG Change Scores

One possibility is that the overall EEG power loss in older adults contributed to the ACE declines. As such, we examined overall EEG power across the whole sleep period regardless of ACE windows in the SWA and sigma bands, and confirmed an age-related loss in EEG power, with older adults demonstrating lower SWA power during N2, N3 and Wake, as well as less sigma power during N3 sleep. Summary statistics for overall EEG power during each sleep stage were shown in Table 3.

Next, we used Pearson correlation coefficients to examine the bivariate relationship between overall EEG power and ACE EEG change scores. No significant associations between overall SWA power and ACE-SWA change score during N2 (Young: all ps > .3564; Older: all ps > .0956), N3 (Young: all ps > .1783; Older: all ps > .5319), or Wake (Young: all ps > .1540; Older: all ps > .2972). Similarly, no significant associations between overall Sigma power and ACE-Sigma change score during N2 (Young: all ps > .3239; Older: all ps > .3184), N3 (Young:

all ps > .1861; Older: all ps > .4239), or Wake (Young: all ps > .3575; Older: all ps > .0880). Taken together, these suggested that the age-related differences in EEG modulation around HR bursts cannot be explained by overall EEG loss in older adults.

3.5 Age Group is Associated with ACE Misalignment

Upon visual examination (Supplemental Figure S1-3), young adults demonstrated a uniform pattern with peak EEG occurring during the -5 window, whereas a less clear picture emerged in older adult group with some reaching the peak EEG during the +5 window. Similarly, for RR_{HF}, most of the young adults reached the peak RR_{HF} during the +5 window, whereas some older adults reached the peak EEG during the -5 window and decreased during the +5 window. Interestingly, older individuals who showed misalignment in one measure (e.g. ACE-SWA), also more likely to have misalignment in another measure (e.g. ACE-Sigma). We devised a measure of central/autonomic misalignment by identifying if the peak ACE-EEG occurred during the +5 window or if the peak RR_{HF} occurred during the -5 window on average during a sleep stage. We then statistically examined if the number of misalignments (see Table 6.1-6.2 for contingency tables; If a subject showed misalignment in ACE-Sigma and ACE-SWA during N2, this counted as misalignment = 2.) is associated with either age group by a Chi-squared independent test, and showed that being in the older adult group was associated with more misalignments (N2: chi-squared=29.73298, $p< 0.0001$; N3: chi-squared=13.80196; $p=0.0010$).

Next, using a logistic regression, we investigated if the number of misalignments can be predicted by napping habits among the older adults. We found that those who reported having napping habits have a lower likelihood of showing more than one measure of misalignment during N2 (β=-1.7918, $p=0.0434$), and that nap frequency was inversely related to the number of

misalignments during N2 (β=-0.4920, p=0.0549). Napping habit was not correlated with the number of misalignments in N3 sleep (all ps >. 0.3749). Furthermore, the number of misalignments during either N2 or N3 cannot be predicted by STOP-Bang score, BMI, ESS, or TELE score (all ps > 0.0824). Taken together, our results suggested that central/ autonomic misalignment increased with age, and can potentially be mitigated by frequent napping.

4. Discussion

We aimed to characterize the impact of age on autonomic-central couplings profiles across wake and sleep. To this end, we compared younger and older adults in Autonomic-Central Events (ACEs) during daytime naps. In the young adult population, our previous sleep findings were replicated in the present study: During wake, N2 and N3, a significant increase in SWA and sigma activity preceded the HRB peak. Similarly, older adults showed ACE modulation during wake and N2, with the peak EEG power during the -5 window. But, they did not show typical ACE modulation during N3, where they showed delayed or no ACE modulation. In addition, our older subjects demonstrated lower peak ACE-EEG power in both N2 and N3, but not wake. Replicating our original study, we also observed in younger adults a significant vagal response with increased RR_{HF} after the HRB peak. In contrast, for older adults, we observed an earlier-onset and sustained RR_{HF} rise before and after the HRB peak. Furthermore, we found that the change in age-related ACE activity was not driven by age-associated EEG decline. We also found that a subset of our older adults showed a misaligned ACE pattern, with a delayed increase in EEG activity after the HRB or an earlier rise in RR_{HF} before the HRB. Interestingly, this misaligned ACE pattern during N2 was found to be more prevalent in non-nappers. Altogether,

our results reflect that with age, subjects continue to show a temporal coupling between the autonomic and central nervous systems during wake and N2, but not in N3.

4.1 Age-dependent changes in sleep not implicated in ACE coupling

Aging is known to impact sleep, with dramatic changes in sleep quality, efficiency, and physiology [31,49,59]. In particular, beginning in middle age, adults experience increased difficulty initiating and maintaining sleep [37], as well as increased time spent in lighter compared to deeper stages of sleep, increased sleep fragmentation, greater sleep latency, and reduced total sleep time [6,37,42,54]. In evaluating the power spectra of aging sleep, a significant decline in SWA is observed between young and middle adulthood, with further age-associated declines in older age [37]. This decline is most apparent over the prefrontal cortex [37]. In part this is due to the decreasing amplitude of SOs and associated with typical age-dependent neural degeneration, including atrophy of the medial prefrontal cortex gray matter [37,39].

We, too, found a decline in older adults' overall EEG activity compared to younger adult subjects; however, the lack of a significant association between SWA and ACE activity suggests that this decline was not a driving factor in the age-associated loss of ACE during N3. Older adults also showed decreased overall peak EEG activity in the -5 window compared to younger adults. We further found that some older subjects showed ACE misalignment during N2 and N3, with delayed SWA and sigma rise after the HRB. This subset of older adults were also found to nap less frequently than older adults who did not show ACE misalignment. Within the sleep and aging literature, other studies have also discovered a weakening of EEG feature alignment in older, compared to younger, adults. For example, sleep dependent memory studies have shown

that independently, sleep spindles and slow oscillations are correlated with subsequent memory performance [55]. Temporally coincident spindles nested in the up-state of the slow oscillation further explain this improvement beyond individual EEG feature contributions [24,56,57]. Yet in older adults, this temporal slow oscillation-spindle relationship is misaligned, with decreased coupling of the two EEG features over the frontal lobes [13,24]. How this age-dependent change in EEG feature alignment is linked to global temporal coupling between the autonomic and central nervous systems is unclear; especially given that we only found a subset of older adults to show a change in ACE alignment. Despite the unknown mechanisms of ACE misalignment, the current study showed that overall EEG power and peak ACE-EEG activity in the -5 second window declines with age, and that older adults continue to show similar ACE modulation.

4.2 Age-dependent autonomic profiles

In line with the age-associated EEG changes, two significant timepoints signal a decline in parasympathetic activity, as measured by HF HRV during waking. The first time point occurs between adolescence and early adulthood, and the second at approximately 60 years, with continual decline until at least 80 [53]. This parasympathetic activity loss is also observed in older adults' sleep, as was discovered by studies investigating age-dependent changes in autonomic activity through the night and nap [3,11,14]. Further studies reported reduced cardiac modulated activity during NREM sleep, including dampened RR and RR_{HF} variability and HF_{nu}. Interestingly, in both our younger and older adults, we observed a significant cardiac modulated response as measured by increasing RR_{HF} after the HRB; however, older adults showed an earlier rise in RR_{HF} preceding the HRB. This earlier cardiac response could be the result of the shifting sympathovagal balance with age- or heart-related changes [32,38].

Heart-rate changes are a typical consequence of growing older. Possible influential factors include reduced SA node cell functionality, which results in greater time between heart beats; lower maximal aerobic exercise capacity (27, 45-47) caused by reduced physiological functioning; and reduced α and β adrenergic receptor sensitivity in the heart as a result of increased sympathetic activity [12,21-23,25,27,44,53]. Consistent with prior research, in our study we found that older adults had faster HR than younger adults during N2 and N3. However, older adults' rate of HRBs during sleep was half that observed in younger adults during NREM but not Wake, with young adults having approximately one HRB per minute while older adults had one approximately every two minutes. This loss of sleep-associated HRBs in older adults may be the result of age-associated heart rate changes or could reflect reduced vagal cardiac control. Typically the vagus nerve reduces the heart rate via a negative feedback loop, termed the vagal break, in which increased vagal activity reduces the heart rate on a beat-to-beat scale [58]. By observing the age-dependent loss of HRBs and earlier rise in RR_{HF} activity, we are likely seeing a less effective vagal break in our older adult subjects.

4.3 Coupling between the central and autonomic nervous systems in young adults

Prior literature has established an interaction between central and autonomic activity in young adults [10,40,41,50]. The interplay between the heart and brain has been identified during sleep at both broad and specific-feature levels. Broadly for example, each sleep stage is characterized by a specific EEG and ANS profile, with greater overall parasympathetic activity (HF-HRV) during NREM compared to waking or REM [5,8,9,11]. Brandenburger et al. (2001) also found that as slow waves dissipated with reducing homeostatic sleep pressure across the night, LF/HF HRV rose, suggesting an inverse relationship between the two. Furthermore, SWA in both N2 and N3 positively correlated with HF cardiopulmonary coupling (heart-respiration

coupling, [CPC]), with an increase in HF-CPC preceding an increase in SWA [47]. Altogether, these results support the broad intersection of the central and autonomic nervous systems during sleep.

In addition to macro-structure, at the feature level de Zambotti et al. (2016) found that both auditorily evoked and spontaneous k-complexes were quickly followed by brief increases and decreases in heart rate. Our initial contribution to the heart-brain interplay literature demonstrated a coupling of HRB-modulated EEG activity (ACEs), showing a temporally coincident increase in SWA and sigma preceding HRBs in N2 and N3. [40]. In this current study, we replicated our ACE observation in young adults across wake, N2, and N3 and found a similar pattern in older adults of ACE activity in wake and N2.

4.4 Central/Autonomic Coupling in older adults

While numerous studies have investigated the heart-brain interplay in young adults, research is sparse and results are mixed in studies with middle-aged and older populations. For example, Crasset et al. (2001) showed a loss in both HF HRV and SWA from young to middle age [28]. In contrast, Thomas et al. (2014) found that, during sleep, there was no change in the positive relationship between SWA and HF-CPC across the night in adults between the ages of 40 and 80 but a weakened relationship in adults 80 and older [47]. Similarly, Jurysta et al. (2005) found no age-dependent change in the association between HRV HFnu (normalized activity of HF/ HF + LF) and SWA when comparing adolescent males and middle-aged men [32]. The unique advantage in our study was our use of a high precision beat-to-beat analysis which showed similar ACE patterns to younger adults in wake and N2 but a loss of heart-brain coupling

during N3. These mixed age-associated coupling results may reflect differences in methodologies and analyses, and indicate the more research is needed.

4.5 Directionality of central-autonomic interaction

The driving force behind the age-related general maintenance of ACEs is unclear, and the ability to identify that force (or those forces) is further complicated by a lack of consensus regarding communication flow between heart and brain. Prior research has found evidence for cardiac changes preceding EEG increases. Jursyta and colleagues (2003, 2005) showed that shifts in HF HRV preceded delta activity changes by 12 minutes across a night of sleep [60,61]. In contrast, Rothenberger et al.'s (2015) research suggested that shifts in the EEG might drive autonomic fluctuations, pointing to the declining relationship between HF HRV and delta activity across successive NREM episodes [43]. This finding aligns with de Zambotti et al. (2016), who also showed that K-complexes preceded heart-rate changes, and our own findings that EEG activity precedes HRBs and subsequently drops afterwards [40].

The neurovisceral integration model may provide insight into the directionality of the heart-brain interplay pathways, at it has implicated several candidate brain structure networks in the communication and regulation of cognitive, emotional, and autonomic function [46]. Baroreceptors in the carotid sinus and aortic arch send messages to the vagal nerve which are relayed to the nucleus of solitary tract (NTS), a key component of the central autonomic network. The NTS further projects the message to higher-order cognitive areas such as hippocampus, amygdala, and prefrontal cortex, which can initiate a vagal break by top-down inhibitory mechanisms that initiate parasympathetic control of the heart and slow it down [62,63]. Increased vagal activity can subsequently activate the projecting NTS pathways to the

basal forebrain and locus coeruleus, which can result in an increased release of acetylcholine and norepinephrine leading to a reduction of slow oscillations [64]. Altogether, while the directionality of communication pathways between the heart and brain is not definitive, research points to the possibility that CNS changes may precede and drive alterations in the ANS. Overall age-related neural degeneration, primarily in frontal cortex, may cause impaired pathway communication, resulting in less successful control of ANS activity as well as poorer temporal convergence of the two nervous system branches.

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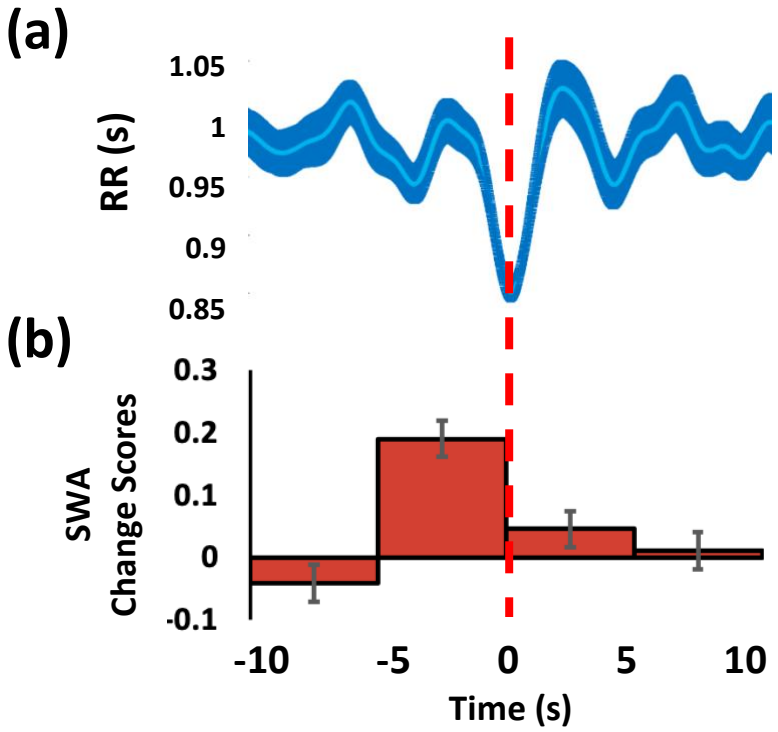
Figure Captions

Figure 1 *Illustration of time-locked analysis: Heart-rate-bursts and SWA change scores time-locked on HR bursts. (a) We first identified HR bursts and time-locked on the peak of HR burst to calculate EEG power spectrum within each 5-sec window. (b) Next, we averaged EEG power spectrum within individuals and calculated a change score for each individual as follows: (ACE activity in each 5 s interval – non-ACE activity)/ (ACE activity in each 5 s interval + non-ACE activity).*

Figure 2 *ACE change scores during the four windows across HR burst in younger (red) and older (blue) adults Error bars represent standard error of the mean. X axis represents the four 5-sec intervals within the 20-sec windows around the HR burst, named -10, -5, +5, +10 window. Y axis represents ACE change score during the four windows. Asterisks indicate three-way post-hoc comparisons corrected by Bonferroni method. Black asterisks indicate differences between age groups within the same windows. Red asterisks indicate differences between windows within the young adults. Red asterisks indicate differences between windows within the young adults. Blue asterisks indicate differences between windows within the older adults.*



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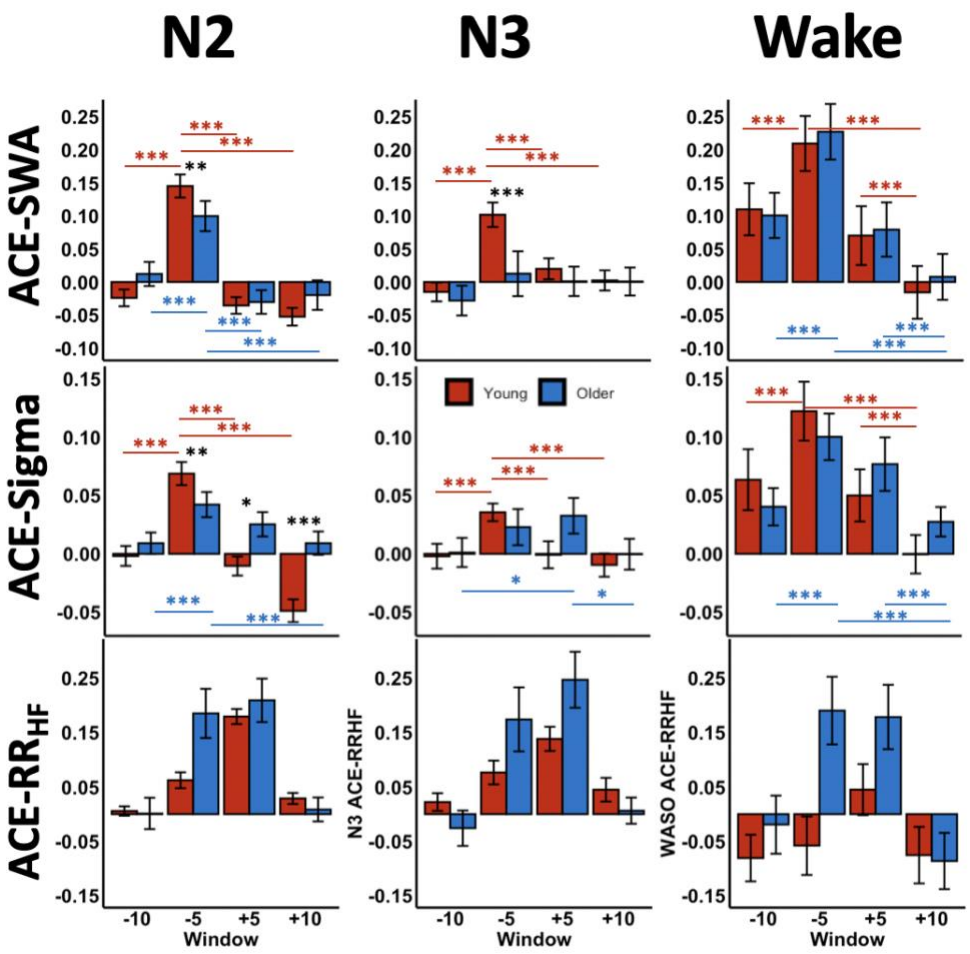

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Table 1. Descriptive statistics for Demographics

	Young Adults (N=50)	*Older Adults (N=51)*	
Age (years)	20.8 (6.453)	69.99 (3.074)	***
Male/female	27/23	24/27	
Caffeine/day	1.08 (1.554)	1.82 (1.629)	***
Alcohol/week	0.47 (3.137)	1.77 (3.311)	***
Nap/week	2.15 (2.635)	2.07 (2.762)	*n.s.*
Nap duration/ day	45.5 (52.386)	36.8 (54.926)	*n.s.*
ESS	7.39 (3.397)	6.56 (4.619)	*
BMI (kg/m^2)	24.5 (6.706)	27.7 (7.054)	***

Note: Data are reported as Mean (standard deviation) for quantitative variables and N for categorical variables; ESS: Epworth Sleepiness Scale; BMI: Body Mass Index;
*Asterisks indicate significant differences between age groups (n.s. p >0.05; *p < 0.05; ***p < 0.001).*

Table 2. Summary of Sleep Architecture

	Young	*Older*	
TIB (min)	78.069 (3.1)	80.763 (3.36)	*n.s.*
TST (min)	61.500 (2.94)	46.987 (3.92)	**
SOL (min)	7.664 (0.99)	9.118 (1.27)	*n.s.*
N1 (min)	4.586 (0.47)	7.934 (1.07)	**
N2 (min)	31.448 (1.94)	28.855 (2.86)	*n.s.*
N3 (min)	19.224 (1.96)	7.474 (1.58)	***
REM (min)	6.241 (1.09)	2.72 (0.98)	*
WASO (min)	6.560 (0.88)	19.01 (2.12)	***
SE (%)	77.588 (2.21)	56.52 (3.47)	***

*Note: Data are reported as Mean (standard error); TIB = Total Time in Bed; TST = Total Sleep Time; N3 = Slow Wave Sleep; REM = Rapid Eye Movement sleep; WASO = Wake After Sleep Onset (calculated as the minutes of wake after first epoch of sleep); SOL = Sleep Onset Latency (calculated as the time to first epoch of sleep); SE = Sleep Efficiency (calculated as 100*TST/TIB). Asterisks indicate significant differences between age groups (n.s. p >0.05; *p < 0.05;**p < 0.01; ***p < 0.001).*

Table 3. Summary of overall EEG Power Across Sleep Stages

Stage	N2			N3			Wake		
	Young	*Older*		*Young*	*Older*		*Young*	*Older*	
SWA (0.5-1Hz)	89.785 (10.328)	51.633 (5.658)	**	185.408 (14.037)	124.643 (19.396)	*	202.363 (24.726)	107.083 (18.606)	*
Sigma (12-15Hz)	5.30 (0.360)	4.26 (0.442)	*n.s.*	6.63 (0.724)	4.07 (0.603)	*	19.63 (3.387)	10.12 (7.732)	*n.s.*

Data are reported as Mean (standard error).
*Asterisks indicate significant differences between age groups (n.s. p >0.05; *p<0.05;**p<0.01;***p < 0.001).*

Table 4. Summary of HR Burst Density (per Minute) and RR intervals Across Sleep Stages

	N2			N3			Wake		
	Young	*Older*		*Young*	*Older*		*Young*	*Older*	
HR Burst Density	0.935 (0.0513)	0.616 (0.0522)	***	0.892 (0.0563)	0.557 (0.0673)	***	0.357 (0.0552)	0.433 (0.0496)	*n.s.*
RR intervals	991 (18.6)	920 (22.1)	*	988 (19.0)	917 (23.5)	*	920 (19.1)	896 (22.5)	*n.s.*

Data are reported as Mean (standard error).
*Asterisks indicate significant differences between age groups (n.s. p >0.05; *p<0.05;**p<0.01;***p < 0.001).*

Table 5.1: Change Scores for ACE variables during N2. Data were shown in mean (standard error of the mean).

Window		-10	-5	+5	+10
SWA	Young	-0.0239(0.0127)	0.1453(0.0174)	-0.0354(0.0126)	-0.0524(0.0133)
	Older	0.0122(0.0182)	0.0999(0.0227)	-0.0302(0.0179)	-0.0198(0.0223)
Sigma	Young	0.0634(0.0260)	0.1220(0.0252)	0.0500(0.0223)	-0.0004(0.0165)
	Older	0.0403(0.0160)	0.1001(0.0199)	0.0767(0.0228)	0.0274(0.0126)
RR_{HF}	Young	0.0058(0.0086)	0.0622(0.0145)	0.1794(0.0138)	0.0288(0.0101)
	Older	0.0034(0.0284)	0.187(0.0441)	0.2137(0.0391)	0.008(0.0217)

Table 5.2: Change Scores for ACE variables during N3. Data were shown in mean (standard error of the mean).

Window		-10	-5	+5	+10
SWA	Young	-0.0150(0.0141)	0.1019(0.0184)	0.0200(0.0159)	0.0024(0.0153)
	Older	-0.0280(0.0226)	0.0125(0.0339)	0.0011(0.0222)	0.0008(0.0212)
Sigma	Young	-0.0020(0.0107)	0.0355(0.0075)	-0.0008(0.0116)	-0.0096(0.0100)
	Older	0.0010(0.0125)	0.0228(0.0154)	0.0326(0.0152)	-0.0003(0.0132)
RR_{HF}	Young	0.0222(0.0162)	0.0764(0.0218)	0.1383(0.0222)	0.0449(0.0217)
	Older	-0.0261(0.0326)	0.1741(0.0588)	0.2470(0.0514)	0.0062(0.0243)

Table 5.3: Change Scores for ACE variables during Wake. Data were shown in mean (standard error of the mean).

Window		-10	-5	+5	+10
SWA	Young	0.1101(0.0394)	0.2095(0.0414)	0.0703(0.0444)	-0.0156(0.0399)
	Older	0.1009(0.0342)	0.2273(0.0419)	0.0795(0.0410)	0.0078(0.0347)
Sigma	Young	0.0634(0.0260)	0.1220(0.0252)	0.0500(0.0223)	-0.0004(0.0165)
	Older	0.0403(0.0160)	0.1001(0.0199)	0.0767(0.0228)	0.0274(0.0126)
RR_{HF}	Young	-0.0809(0.0428)	-0.0581(0.0538)	0.0450(0.0469)	-0.0757(0.0519)
	Older	-0.0207(0.0549)	0.1912(0.0628)	0.1817(0.0602)	-0.0680(0.0513)

Table 6.1 Contingency Table for Misalignment Count during N2 (chi-squared=29.73298; p< 0.0001)

	Misalignment = 0	Misalignment = 1	Misalignment > 1	Total
Older	15	14	13	42
Younger	44	4	1	49
Total	59	18	14	91

Table 6.2 Contingency Table for Misalignment Count during N3 (chi-squared=13.80196; p=0.0010)

	Misalignment = 0	Misalignment = 1	Misalignment > 1	Total
Older	7	6	10	23
Younger	24	12	2	38
Total	31	16	12	61