

Research Articles: Systems/Circuits

The hierarchy of coupled sleep oscillations reverses with aging in humans

<https://doi.org/10.1523/JNEUROSCI.0586-23.2023>

Cite as: J. Neurosci 2023; 10.1523/JNEUROSCI.0586-23.2023

Received: 29 March 2023

Revised: 11 July 2023

Accepted: 31 July 2023

This Early Release article has been peer-reviewed and accepted, but has not been through the composition and copyediting processes. The final version may differ slightly in style or formatting and will contain links to any extended data.

Alerts: Sign up at www.jneurosci.org/alerts to receive customized email alerts when the fully formatted version of this article is published.

Copyright © 2023 Züst et al.

This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license, which permits unrestricted use, distribution and reproduction in any medium provided that the original work is properly attributed.

Abbreviated title: Coupled sleep oscillations across the human lifespan

1 The hierarchy of coupled sleep oscillations reverses with aging in
 2 humans
 3

4 Marc Alain Züst^{1*}, Christian Mikutta^{2,3,4}, Ximena Omlin², Tatjana DeStefani², Marina Wunderlin¹,
 5 Céline Jacqueline Zeller¹, Kristoffer Daniel Fehér^{2,5}, Elisabeth Hertenstein², Carlotta L. Schneider²,
 6 Charlotte Elisabeth Teunissen⁶, Leila Tarokh^{2,7}, Stefan Klöppel¹, Bernd Feige⁸, Dieter Riemann⁸,
 7 Christoph Nissen^{2,5}

8
 9 ¹ University Hospital of Old Age Psychiatry and Psychotherapy, University of Bern, Bern, Switzerland

10 ² University Hospital of Psychiatry and Psychotherapy, University of Bern, Bern, Switzerland

11 ³ Private Clinic Meiringen, Meiringen, Switzerland

12 ⁴ Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, United Kingdom

13 ⁵ Division of Psychiatric Specialties, Geneva University Hospitals (HUG), Geneva, Switzerland

14 ⁶ Neurochemistry Laboratory, Department of Clinical Chemistry, Amsterdam Neuroscience, Neurodegeneration,
 15 Vrije Universiteit Amsterdam, Amsterdam UMC, Amsterdam, Netherlands

16 ⁷ University Hospital of Child and Adolescent Psychiatry and Psychotherapy University of Bern, Switzerland

17 ⁸ Department of Psychiatry & Psychotherapy, University of Freiburg Medical Center, Freiburg, Germany

18

19

20

21 Number of pages: 34

22 Number of figures: 4 Number of tables: 1

23 Number of words: 245 (Abstract) 954 (Introduction) 1727 (Discussion)

24 **Conflicts of interest**

25 CN has served on advisory boards of Idorsia, Lundbeck and Janssen. The other authors have no
 26 conflict of interest to declare.

27 **Acknowledgements**

28 This work was supported by the Dementia Research: Synapsis Foundation Switzerland, in
 29 collaboration with the Peter Bockhoff Foundation, the Heidi Seiler Foundation, and the Kurt Fries
 30 Foundation [grants No. 2018-PI02 to SK, CN, MZ and MW, and 2021-CDA03 to MZ]. The funding
 31 agencies had no role in conceptualization, design or analysis plan of this research.

32 * Please address correspondence to:

33 Marc Alain Züst, PhD

34 University Hospital of Old Age Psychiatry and Psychotherapy

35 Bolligenstrasse 111, 3000 Bern 60, Switzerland

36 Tel.: +41 (0)31 930 89 03

37 e-mail: marc.zuest@upd.unibe.ch

38

Abbreviated title: Coupled sleep oscillations across the human lifespan

39 **Abstract**

40 A well-orchestrated coupling hierarchy of slow waves and spindles during slow wave sleep supports
41 memory consolidation. In old age, duration of slow wave sleep and number of coupling events
42 decreases. The coupling hierarchy deteriorates, predicting memory loss and brain atrophy. Here, we
43 investigate the dynamics of this physiological change in slow wave-spindle coupling in a frontocentral
44 electroencephalography position in a large sample (N=340, 237 female, 103 male) spanning most of
45 the human lifespan (ages 15-83). We find that, instead of changing abruptly, spindles gradually shift
46 from being driven by-, to driving slow waves with age, reversing the coupling hierarchy typically seen
47 in younger brains. Reversal was stronger the lower the slow wave frequency, and starts around
48 midlife (~age 40-48), with an established reversed hierarchy at age 56-83. Notably, coupling strength
49 remains unaffected by age. In older adults, deteriorating slow wave-spindle coupling, measured using
50 phase slope index (PSI) and number of coupling events, is associated with blood plasma glial fibrillary
51 acidic protein (GFAP) levels, a marker for astrocyte activation. Data-driven models suggest
52 decreased sleep time and higher age lead to fewer coupling events, paralleled by increased astrocyte
53 activation. Counterintuitively, astrocyte activation is associated with a back-shift of the coupling
54 hierarchy (PSI) towards a “younger” status along with increased coupling occurrence and strength,
55 potentially suggesting compensatory processes. As the changes in coupling hierarchy occur gradually
56 starting at midlife, we suggest there exists a sizable window of opportunity for early interventions to
57 counteract undesirable trajectories associated with neurodegeneration.

58 *Keywords:* Slow wave sleep, sleep spindles, phase-amplitude coupling, aging, astrocyte activation,
59 biomarkers, neurodegeneration, human life-span

60

Abbreviated title: Coupled sleep oscillations across the human lifespan

61 **Significance Statement**

62 Evidence accumulates that sleep disturbances and cognitive decline are bi-directionally and causally
63 linked forming a vicious cycle. Improving sleep quality could break this cycle. One marker for sleep
64 quality is a clear hierarchical structure of sleep oscillations. Previous studies showed that sleep
65 oscillations decouple in old age. Here, we show that, rather, the hierarchical structure gradually shifts
66 across the human lifespan and reverses in old age, while coupling strength remains unchanged. This
67 shift is associated with markers for astrocyte activation in old age. The shifting hierarchy resembles
68 brain maturation, plateau, and wear processes. This study furthers our comprehension of this
69 important neurophysiological process and its dynamic evolution across the human lifespan.

Abbreviated title: Coupled sleep oscillations across the human lifespan

70 **1. Introduction**

71 Sleep is of central importance for the brain, promoting vital functions like memory consolidation,
72 synaptic renormalization, and clearance of metabolic waste-products like amyloid beta ($A\beta$), a
73 hallmark for Alzheimer's disease (Mander et al., 2016; Rasch & Born, 2013; Tononi & Cirelli, 2020;
74 Xie et al., 2013). Coupled oscillations, especially during slow wave sleep (SWS), have been identified
75 as a cornerstone of the function of sleep for the brain. Neocortical slow waves (SW, <1.25 Hz),
76 thalamo-cortical spindles (12-16 Hz) and hippocampal sharp-wave ripples (80-100 Hz) are
77 hierarchically orchestrated to allow for optimized, synchronized information processing that enables
78 memory consolidation (Rasch & Born, 2013; Staresina et al., 2015). For optimal functionality, the
79 layers of this hierarchy are organized in a relationship of phase-amplitude coupling, where the faster
80 spindles are nested into the depolarizing up-phase of the slower SW. This allows for synchronized,
81 widespread communication during periods of high responsiveness and therefore efficient top-down
82 control of processes like memory consolidation (Bastian et al., 2022; Helfrich et al., 2018; Mikutta et
83 al., 2019; Rasch & Born, 2013; Tort et al., 2010).

84 With age, sleep quality and quantity declines, leading to a loss of SWS (Mander et al., 2017). This
85 loss inevitably entails less opportunity for sleep's important functions. While part of normal aging
86 (Carrier et al., 2011; Hertenstein et al., 2018), this loss is more severe in neurodegenerative
87 disorders, like Alzheimer's disease (Rauchs et al., 2008; Westerberg et al., 2012; Zhang et al., 2022).
88 As neurodegeneration progresses, sleep quality declines, which in turn robs the brain of crucial
89 recuperative functions, worsening neurodegeneration (Mander et al., 2016). With lacking SWS, $A\beta$ is
90 not cleared from the brain as effectively, and the residual $A\beta$ in turn disrupts sleep (Eide et al., 2021;
91 Fultz et al., 2019; Ju et al., 2017; Kang et al., 2009; Mander et al., 2015; Roh et al., 2012; Varga et al.,
92 2016; Winer et al., 2019, 2020), leading to a vicious cycle (Mander et al., 2016; Wunderlin et al.,
93 2020; Zeller et al., 2023).

94 The orchestrated coupling of spindles and SW follows along with these age-related sleep changes.
95 Recent studies posit that spindles become uncoupled from SW in the aging brain, and this change is
96 associated with degrading memory and medial frontal brain atrophy (Helfrich et al., 2018; Muehlroth
97 et al., 2019). In younger individuals, SW drive spindles, signifying that SW inhabit a higher position in

Abbreviated title: Coupled sleep oscillations across the human lifespan

98 the hierarchy of coupled oscillations. In older individuals, however, this clear cross-frequency
99 directionality deteriorates (Helfrich et al., 2018).

100 Importantly, it is known that older individuals with higher structural brain integrity in areas like the
101 medial prefrontal cortex and hippocampus exhibit a SW-spindle coupling physiology reminiscent of a
102 younger brain (Muehlroth et al., 2019). Moreover, enhancing SW-spindle coupling using transcranial
103 electric stimulation has been shown to improve post-sleep declarative memory retrieval in older adults
104 with mild cognitive impairment (Ladenbauer et al., 2017), suggesting the unfavorable age-associated
105 deterioration of SW-spindle coupling can potentially be compensated to prevent cognitive decline.

106 While currently available research paints quite a stark contrast between younger and older adults, it is
107 not clear how and when these changes emerge. Are changes in SW-spindle coupling gradually
108 appearing across the adult human lifespan, or suddenly at a specific age? At what age does the
109 process become apparent? Here, we address these open questions by examining SW-spindle
110 coupling in an extensive sample (N=340) spanning a large portion of the human lifespan (age 15-83).
111 Instead of focusing on group differences between younger and older individuals including all
112 associated cross-generational inhomogeneity, we investigate SW-spindle coupling as a continuum
113 across the human lifespan.

114 When aiming to prevent cognitive decline, early detection of unfavorable trajectories is key. Recently,
115 blood-based biomarker assessments have become an affordable, minimally invasive approach for the
116 early prediction of cognitive decline (Beyer et al., 2022; Thijssen et al., 2021; Verberk et al., 2020).
117 The most promising prognostic blood-based biomarkers currently discussed are A β 42/40 ratio and
118 glial fibrillary acidic protein (GFAP) levels. A lower blood A β 42/40 ratio is thought to be a marker for
119 impaired clearance of A β from the brain. Increased levels of GFAP is a marker for astrocyte
120 activation, with a potential role in neuroinflammation due to neuronal damage or degeneration
121 (Thijssen et al., 2021; Verberk et al., 2020). Experimentally induced sleep deprivation is linked with
122 astrocyte activation and neuroinflammation as indicated by increased cytokine and GFAP levels in
123 rodents (Manchanda et al., 2018; Xiao et al., 2022). High GFAP levels are associated with a steeper
124 rate of decline in memory, executive functioning and attention, and had a high prognostic value for
125 incident dementia in humans (Verberk et al., 2021). Using a combination of amyloid misfolding status

Abbreviated title: Coupled sleep oscillations across the human lifespan

126 and GFAP levels, the incidence of Alzheimer's diagnosis could be accurately predicted 17 years in
127 advance with receiver-operating characteristic area under the curve of .83 (Beyer et al., 2022), paving
128 the way for minimally invasive early detection of cognitive decline.

129 In addition to investigating the dynamics of the shift in SW-spindle coupling across the human
130 lifespan, we examine if changes in the hierarchical coupling structure of brain oscillations during slow
131 wave sleep are reflective of neuronal degradation as measured by blood-based biomarkers. For this
132 purpose, we analyze associations of SW-spindle coupling with readily accessible blood-based
133 biomarkers for dementia and astrocyte activation ($A\beta_{42/40}$ ratios and GFAP levels) in a subgroup of
134 older individuals.

135 A continuous investigation of brain physiology from adolescence to senescence allows for a deeper
136 understanding of the dynamic processes the brain undergoes throughout our lifetime. It can put
137 individual neurophysiological characteristics into context, allows us to better identify pathological
138 trajectories, and separate pathological from healthy trajectories. This knowledge can accelerate the
139 development of tailored treatment- and prevention methods for cognitive decline, especially as more
140 early warning signs are identified every year.

141

142 **2. Methods**

143 **2.1. Sample**

144 The total sample consisted of 340 whole-night baseline sleep recordings of healthy participants (237
145 female, 103 male, age: 15-83, $M\pm SD$: 43.4 \pm 17.8, see Table 1) participating in various studies at the
146 Department of Psychiatry and Psychotherapy, University of Freiburg Medical Center (UFMC) between
147 2008 and 2018 and University Hospital for Old Age Psychiatry and Psychotherapy Bern (UPD)
148 between 2019 and 2021. Of the total sample, 310 participants were measured at UFMC (213 female,
149 age: 15-83, $M\pm SD$: 40.9 \pm 16.5) and 30 participants were measured at UPD (24 female, age: 61-80,
150 $M\pm SD$: 69.5 \pm 4.3). All participants underwent extensive screening procedures to confirm suitability as
151 healthy study participants, which was the first inclusion criterion. The second inclusion criterion was
152 availability of polysomnographic (PSG) recordings of an entire night under baseline measurement

Abbreviated title: Coupled sleep oscillations across the human lifespan

153 conditions after an adaptation night – i.e., natural sleep with no intervention. Exclusion criteria were
154 current or recent (over the last 6 months) psychiatric or physical illness, especially if impacting sleep
155 (e.g., insomnia, hypersomnia, sleep apnea syndrome, or restless legs syndrome), irregular sleep
156 patterns, substance abuse, use of prescription medication acting on the central nervous system, and
157 pregnancy. Studies were conducted in accordance with the Declaration of Helsinki as approved by
158 local ethics committees. All individuals (and their parents if underage) gave written informed consent.

159 **2.2. Procedures**

160 All participants completed one night of PSG. At UFMC, sleep was recorded on a 24-channel EEG
161 PSG device with a sampling rate of 200 or 256 Hz. Recorded EEG channels included C3, C4, Fz,
162 Fpz, and Oz. During recording, channels C3 and C4 were referenced against contralateral mastoids,
163 the other channels were referenced against pooled mastoids or Cz. More information about UFMC
164 data, infrastructure and standard procedures can be found elsewhere (Hertenstein et al., 2018). At
165 UPD, sleep was recorded using a high-density EEG system (128-channel MicroCel Geodesic Sensor
166 Net, 16-channel Physio16 input box, 400 Series Geodesic EEG System™) by Magstim EGI (Eugene,
167 OR, USA), with a sampling rate of 500 Hz, referenced against Cz. Polysomnographic scoring of sleep
168 stages was performed according to the criteria of the American Academy of Sleep Medicine (Iber et
169 al., 2007) by experienced somnologists for all 340 datasets.

170 For 28 of the 30 participants in the UPD sample (22 female, age: 61-80, $M \pm SD$: 69.5 \pm 3.9), blood
171 samples were taken in the morning (~1 hour after waking) and immediately centrifuged and stored at -
172 80°C. The resulting plasma samples were analyzed in the Neurochemistry Lab, Amsterdam University
173 Medical Center, Amsterdam, NL. Plasma A β 1-42 and 1-40, as well as GFAP levels were quantified
174 using Simoa immunoassays (IA-N4PE)(Thijssen et al., 2021), and A β 42/40 ratios were calculated. All
175 measurements were above the limits of detection and the functional lower limits of quantification as
176 per the manufacturer's specifications. High GFAP levels and low A β 42/40 ratios constitute risk factors
177 for neurodegenerative disease and are strongly associated with amyloid-positivity as assessed with
178 positron-emission tomography (Graff-Radford et al., 2007; Verberk et al., 2020, 2021).

Abbreviated title: Coupled sleep oscillations across the human lifespan

179 **2.3. Sleep parameters**

180 We determined the following sleep parameters individually, then averaged for the whole sample
181 (N=340) as well as for age quartiles: Sleep period time (SPT), defined as the time from sleep onset to
182 the final awakening (Hertenstein et al., 2018); total sleep time (TST, i.e. SPT minus intermittent
183 wakefulness), sleep (onset) latency (SL, i.e. the time until first occurrence of non-rapid eye movement
184 sleep stage 1), sleep efficiency (SE) as percentage of sleep during bedtime, spindle density (SD,
185 measured as spindle events per minute of N2/N3 sleep), slow wave amplitude (SW amp, in μV ,
186 negative-to-positive peak of detected SW events), as well as standard AASM sleep architectural
187 stages, i.e., wakefulness, non-rapid eye movement sleep stages 1 through 3 (N1-N3), and rapid eye
188 movement (REM) sleep. SPT, TST and SL are measured in hours, sleep architectural stages in
189 percent of SPT. For all sleep parameters, we tested association with age using Pearson's
190 determination coefficients. A more in-depth evaluation of sleep parameters of UFMC data is reported
191 elsewhere (Hertenstein et al., 2018).

192 **2.4. EEG processing**

193 EEG processing, as well as calculation and statistical analysis of SW-spindle coupling was achieved
194 in MATLAB R2019a (Natick, Massachusetts: The MathWorks Inc.) using EEGLAB (Delorme &
195 Makeig, 2004), the CircStat toolbox (Berens, 2009), the fieldtrip toolbox (Oostenveld et al., 2010) and
196 the phase-amplitude coupling analysis framework by Jiang et al. (2015). For the UFMC dataset, 30-
197 second segments of data containing artifacts were manually labelled and excluded from analysis. For
198 the UPD dataset, EEG data was preprocessed using the PREP pipeline for EEGLAB (Bigdely-Shamlo
199 et al., 2015) and the automatic artifact rejection pipeline as implemented in the fieldtrip toolbox
200 (Oostenveld et al., 2010). All analyses were conducted on artifact-free N2 or N3 sleep data on
201 channel Fz, referenced against pooled mastoids, resampled to 200 Hz if necessary.

202 **2.5. Slow wave-, spindle- and coupling event classification**

203 SW- and spindle events were detected using previously established methods (Helfrich et al., 2018;
204 Mölle et al., 2009; Staresina et al., 2015). For slow oscillations, we filtered data between 0.16 and
205 1.25 Hz and marked zero crossings. SW events were then defined as negative peaks between two
206 consecutive positive-to-negative zero crossings based on duration (0.8-2 seconds) and amplitude

Abbreviated title: Coupled sleep oscillations across the human lifespan

207 (individual 75th percentile) criteria (Helfrich et al., 2018; Mölle et al., 2009). For sleep spindles, we
208 filtered data between 12 and 16 Hz and extracted the amplitude of the Hilbert transform. Spindle
209 events were defined as peaks of the smoothed (200 ms moving average) Hilbert-amplitude curve in
210 regions that exceeded the individual 75th amplitude percentile for 0.5 to 3 seconds (Staresina et al.,
211 2015). Spindle events that were within 2.5 seconds of a SW-event were marked as SW-coupled
212 spindles and constitute coupling events. We then extracted the coupling phases, i.e., the
213 instantaneous SW-phase angles of SW-coupled spindles using the angle of the Hilbert transform in
214 SW-filtered (0.16-2 Hz) data. To counteract reduced SW power with age, we z-standardized data
215 within participants prior to analyses of SW-spindle coupling.

216 **2.6. Quantifying slow wave-spindle coupling**

217 The number of coupling events yields a first measure of SW-spindle coupling and can vary with
218 quantity (i.e., the time spent asleep) and/or quality (i.e. the exact electrophysiological synchronization
219 of SW and spindles) of SWS. In addition to the number of coupling events, we calculated three
220 principal SWS-quantity-independent measures of SW-spindle coupling:

221 1) The resultant vector angle (rvec angle), or mean circular direction (CircStat::circ_mean) of coupling
222 phases yields a measure of the preferred coupling phase of spindles within SW. An rvec angle of 0° is
223 equivalent to the positive peak, ±180° to the negative peak, negative values up to -90° are before a
224 positive peak, and positive values up to 90° are after a positive peak. As rvec angle is a circular
225 measure, its utility is limited to circular statistics, and it cannot be included in linear models.

226 2) The modulation index (MI) (Jiang et al., 2015; Tort et al., 2010) as a measure of cross-frequency
227 coupling was calculated between the phase of a lower frequency (SW, 0.39-1.95 Hz in 0.39 Hz steps)
228 and the amplitude of a higher frequency (spindles, 12-16 Hz in 1 Hz steps). The MI is a measure of
229 circular spread and indicates how far an empirical distribution deviates from uniformity using the
230 Kullback-Leibler divergence. The higher the MI, the more closely all coupling phases are grouped
231 around the preferred phase, i.e., the stronger the coupling.

232 3) The phase slope index (PSI) (Jiang et al., 2015) as a measure of cross-frequency directionality was
233 calculated between the phase of a lower frequency (SW, 0.5-2 Hz in 0.5 Hz steps) and the amplitude
234 of a higher frequency (spindles, 12-16 Hz in 1 Hz steps). The PSI robustly measures the consistency

Abbreviated title: Coupled sleep oscillations across the human lifespan

235 of phase lag or lead between the two frequencies, and a value significantly different from 0 is
236 suggestive of causal influence of the leading over the lagging frequency. A positive PSI indicates SW
237 drive spindles, a negative PSI indicates spindles drive SW. The PSI can be used instead of rvec angle
238 in linear models.

239 MI and PSI were calculated on 5-second data segments centered on the negative peak of detected
240 slow waves. We defined a sliding window of 2 seconds length with 1 second steps, using 5 cycles to
241 estimate frequency power. We then averaged the resulting MI and PSI values for all possible
242 frequency sub-band pairs to yield a single estimate for MI and PSI between the SW and spindle
243 bands per subject.

244 As the number of coupling events diminishes with age ($R^2=0.48$, $p<.001$), lower numbers of coupling
245 events might bias the estimation of SW-spindle coupling and its association with age. To counteract
246 this, we implemented a per-subject bootstrapping procedure where we repeated calculation of rvec
247 angles and MI with q randomly selected coupling events, where q equals the smallest number of
248 coupling events across all participants ($q = 120$ in a subject aged 77). This random draw was
249 repeated for 1000 iterations per subject (or, if not possible, for the maximum number of unique
250 draws), and an average coupling measure was then calculated from the mean of the bootstrapping
251 distribution. We used a leave-one-out jackknifing procedure to test stability of the estimation of the
252 PSI. If estimation of the PSI exhibited low stability ($|z(\text{jackknifing error})| > 2$), the subject was excluded
253 from PSI analyses, which was the case in 9/340 participants. These unstable estimates contained
254 three outliers ($|z(\text{PSI})| > 3$), and no outliers remained after exclusion of unstable estimates.

255 **2.7. Testing for association of slow wave-spindle coupling and age**

256 We tested for associations of measures of SW-spindle coupling (number of coupling events, rvec
257 angle, MI and PSI) with age. For linear coupling measures (number of coupling events, MI and PSI)
258 we calculated the Pearson correlation coefficient with age. For preferred coupling phase (rvec angle),
259 we used CircStat::circ_corrcl for a circular-linear correlation between rvec angle and age. Importantly,
260 an rvec angle can technically be calculated even in almost uniformly distributed data, but would not
261 produce a sensible estimate of preferred phase in that case. Therefore, we repeated the circular-
262 linear correlation between rvec angle and age, as well as the linear correlation between PSI and age,

Abbreviated title: Coupled sleep oscillations across the human lifespan

263 in a subset of individuals exhibiting high coupling strength as measured by MI to minimize bias due to
264 invalid estimations of preferred phase. As MI was right-skewed, we defined high MI as $z(\ln(MI)) > 0$,
265 which was the case in 177 participants. This logarithmic transformation normalizes the distribution of
266 MI and allows z-transformed values above 0 to represent the upper half of MI data. For effect sizes,
267 we calculated explained variance through determination coefficients (R^2).

268 For significant associations of SW-spindle coupling with age (rvec angle and PSI), we further
269 subdivided the sample into age quartiles and tested the quartiles separately against zero. Preferred
270 coupling phase (rvec angle) was tested against zero using a one sample test for mean angle
271 (CircStat::circ_mtest). Zero was chosen as test value because it marks the highest point on a positive
272 SW peak; thus allowing to test if spindles prefer to nest significantly before or after a SW peak. PSI
273 was tested against zero using one-sample t-tests. Zero was chosen as test value because it marks
274 the reversal-point of cross-frequency directionality, i.e., a reversal of which frequency drives the other.
275 We additionally calculated the x-zero-crossing of the best-fit models of the association between SW-
276 spindle coupling measures and age to estimate the age at which a reversal happens. For MI, we
277 tested age quartiles against each other in an ANOVA to test for potential non-linear shifts in coupling
278 strength between age quartiles.

279 To account for potential sources of bias, we calculated a linear regression of age on PSI
280 (Matlab::lmfit) and let a data-driven stepwise procedure (Matlab::step) optimize this model by testing
281 change in model fit by the inclusion and exclusion of terms. Each step, the term yielding the highest
282 gain in R^2 is added, provided R^2 would increase by at least 0.1. Gender, age, linear coupling
283 measures, SW up- and down-phase duration, number of coupling events, sleep parameters (TST, SL,
284 SPT in hours; stages N1-3 & REM, as well as intermittent wakefulness in % SPT, SD, SE, and SW
285 amplitude) and interactions of existing terms may be added as factors if not already present. If no
286 term can be added this way, the term resulting in the least loss of R^2 is removed provided R^2 would
287 decrease by no more than 0.05. If neither threshold is met through further changes in the model, the
288 procedure ends. Final models were F -tested against intercept-only models. To prevent overfitting,
289 model R^2 was adjusted for the number of included terms. To explicitly test for an influence of up- and
290 down-phase duration on coupling, an additional model was calculated to include up- and down-phase
291 duration a-priori. To explicitly test for gender differences, an additional model was calculated to

Abbreviated title: Coupled sleep oscillations across the human lifespan

292 include gender a-priori. For these models, the ΔR^2 threshold for excluding terms was set more
293 liberally at 0.02 to allow control for gender and SW duration effects even if they are small. All model
294 optimizations finished within four steps.

295 **2.8. Blood biomarker analysis**

296 For 28 participants in the UPD sample, blood-based biomarkers were analyzed for associations with
297 SW-spindle coupling while controlling for potential confounders such as gender, age and sleep
298 parameters. Initially, a-priori baseline models were defined explaining blood biomarkers (A β 42/40
299 ratios and GFAP levels) by linear coupling measures (number of coupling events, MI, and PSI) and
300 age, and explaining linear coupling measures by blood biomarkers and age. Stepwise optimization
301 allowed for the inclusion and exclusion of gender, age, blood biomarkers, linear coupling measures,
302 sleep parameters and interactions as described above (section 2.7). All model optimizations finished
303 within three steps.

304 **2.9. Data availability**

305 Data will be deposited on an open repository (e.g., <https://boris-portal.unibe.ch/>) upon article
306 acceptance.

307 **3. Results**

308 **3.1. Trends for sleep parameters across the human lifespan replicate earlier findings**

309 Consistent with earlier studies (Carrier et al., 2011; Hertenstein et al., 2018), the structure of sleep
310 changes with age (table 1). We found significant decreases in sleep period time (SPT, $R^2=.14$), total
311 sleep time (TST, $R^2=.43$), proportional non-rapid eye movement sleep (N) stages N3 ($R^2=.38$) and
312 rapid eye movement sleep (REM, $R^2=.18$) sleep, as well as spindle density ($R^2=0.41$), SW amplitude
313 ($R^2=0.25$), and the number of coupling events ($R^2=0.48$) with age ($p<.001$). Conversely, N1 sleep
314 ($R^2=.31$) and periods of intermittent wakefulness ($R^2=.37$) were increased ($p<.001$) with age. Sleep
315 onset latency (SL) and stage N2 did not change with age ($R^2<.01$, n.s.).

316

Abbreviated title: Coupled sleep oscillations across the human lifespan

317

TABLE 1 ABOUT HERE

318

3.2. Spindle density, age, and total sleep time determine number of slow wave-spindle

319

coupling events

320

With age the number of coupling events is strongly reduced ($R^2=0.48$, $p<.001$, see table 1). A

321

stepwise optimized linear regression model ($F(338)=1910$, $R^2_{adj}=.85$, $p<.001$) indicated that number of

322

coupling events is best explained by spindle density ($t=43.69$, $p<.001$), an association so strong no

323

other factors were being considered. If spindle density is removed from the pool of available

324

regressors, an optimized model ($F(337)=282$, $R^2_{adj}=.62$, $p<.001$) indicated that number of coupling

325

events is best explained by age ($t=-8.24$, $p<.001$) and TST ($t=11.51$, $p<.001$). It is therefore difficult to

326

isolate the effect of age on SW-spindle coupling measures (rvec angle, MI, and PSI) from reduced

327

numbers of coupling events due to reduced spindle density. To counteract this, we implemented

328

bootstrapping and jackknifing procedures (see Methods – Quantifying slow wave-spindle coupling) to

329

test robustness of age effects on SW-spindle coupling measures against variance in number of

330

coupling events. This allows the evaluation of age-related effects on SW-spindle coupling while

331

number of coupling events is held constant. Notably, all results regarding age effects in rvec angle,

332

MI, and PSI are unchanged whether these procedures are implemented or not.

333

3.3. Spindles shift from lagging to leading slow waves without loss of coupling strength

334

with age

335

Spindles prefer to nest into the positive half-wave of the SW for almost all participants across all ages,

336

as 338/340 (>99%) of individual rvec angles lay within SW-phase angles of -90° and $+90^\circ$. However,

337

with age, the average preferred coupling angle shifts from after to before the peak of the SW. This is

338

indicated by a significant circular-linear correlation of rvec angle and age ($r=.57$, $p<.001$), with age

339

explaining 33% of variance in rvec angle. For the youngest age-quartile (Q1), the average preferred

340

coupling phase occurs significantly after peak ($M=24.8^\circ$, $CI_{95}=[15.9^\circ, 33.7^\circ]$, $p<.001$), while for the

341

oldest age-quartile (Q4), the average preferred coupling phase occurs significantly before the peak

342

($M=-22.0^\circ$, $CI_{95}=[-35.7^\circ, -8.3^\circ]$, $p<.01$). Age quartiles Q2 and Q3 did not exhibit significant deviations

343

of rvec angle from 0° . The best-fit model suggests a reversal of preferred spindle coupling from after-

344

to before the SW peak at age 43.9 (fig. 1C).

Abbreviated title: Coupled sleep oscillations across the human lifespan

345 The age-dependent forward-shifting of preferred spindle-coupling phase within SW becomes even
346 more pronounced if participants exhibiting weak coupling are excluded. We repeated the circular-
347 linear correlation of rvec angle and age only in participants exhibiting a high MI between SW and
348 spindle frequencies. High MI was defined as $z(\ln(MI)) > 0$ (data above the red dotted line in fig. 1B),
349 yielding a subgroup of $N_{\text{highMI}}=177$. The resulting correlation was highly significant ($r=.65$, $p<.001$) with
350 age explaining 42% of variance in rvec angle, which is significantly higher than using the entire
351 sample (Pearson & Filon's z : -12.58 , $p<.001$). In this high MI subgroup, age quartiles Q1 ($M=20.6^\circ$,
352 $CI_{95}=[7.9^\circ, 33.3^\circ]$, $p<.001$), Q3 ($M=-6.9^\circ$, $CI_{95}=[-12.2^\circ, -1.6^\circ]$, $p<.05$) and Q4 ($M=-24.3^\circ$, $CI_{95}=[-43.3^\circ, -$
353 $5.2^\circ]$, $p<.001$) show significant deviations of preferred coupling from the peak of the SW (0°), with a
354 best-fit model suggested reversal at age 40.4 (fig. 1D).

355 The observed forward-shift of preferred coupling phase with age manifested in a reversal of cross-
356 frequency directionality. While in younger adults, SW drive spindles, in older adults, spindles drive
357 SW. This is illustrated by a significant correlation of PSI and age ($r=-.19$, $p<.001$). However, this
358 measure allowing stronger claims exhibits higher variance compared to preferred coupling phase
359 using rvec angles, with age explaining only 3% of the variance in PSI. Notably, there was a shift in
360 SW peak frequency across age, manifesting in a slightly increased duration of up-phase ($R^2=0.01$,
361 $p=.012$), and a markedly increased duration of down-phase ($R^2=0.26$, $p<.001$) of SW events in line
362 with previous reports (Carrier et al., 2011). Up- ($r=.29$, $p<.001$), but not down-phase ($r=-.05$, $p=.408$)
363 duration was correlated with PSI. PSI is inherently capable of addressing shifting frequency peaks
364 (Jiang et al., 2015), especially since we chose a wider window for lower frequency between 0.5 and
365 2.0 Hz to allow for individual drifts. Still, this age-related shift in SW frequency may confound
366 calculation of age-related trends in coupling. To account for this, we ran a stepwise optimized
367 regression analysis initially including both up- and down-phase duration of SW events as regressors.
368 During optimization, down-phase duration was removed as regressor, yielding a final model that
369 revealed an improved effect of age on PSI ($F(328)=21.7$, $R^2_{\text{adj}}=.11$, $p<.001$, age $R^2_{\text{partial}}=.05$),
370 indicating varying SW frequency with age partially masked the effect of age on PSI.

371 Some studies suggest gender to be an interacting factor in age-associated changes in SWS (Ohayon
372 et al., 2004; Redline et al., 2004) and therefore, gender could influence age-associated changes in
373 SW-spindle coupling. Our stepwise regression optimization procedures were allowed to control for

Abbreviated title: Coupled sleep oscillations across the human lifespan

374 gender, but never included gender as a factor because it did not explain enough variance to pass the
375 entry threshold. Still, we wanted to explicitly test for effects of gender using gender as a-priori
376 regressor in a model explaining PSI by age and optimized this model stepwise using a more liberal
377 threshold to keep terms (see Methods, section 2.7). This model still showed a significant effect of age
378 on PSI ($F(328)=6.3$, $R^2_{\text{adj}}=.031$, $p=.002$, age $R^2_{\text{partial}}=.032$) with gender having almost no influence
379 ($p=.62$). Stepwise optimization reverted to the model above, removing gender but including SW up-
380 phase duration.

381 Similarly, the COVID-19 pandemic may have influenced sleep quality of participants enrolled in years
382 2020/21. We repeated the procedure described above for gender, substituting gender for a regressor
383 coding whether study participation occurred during the pandemic (true for 24 participants). This
384 analysis yielded similar results, showing no effect of pandemic ($p=.83$) on the significant age-related
385 change in PSI ($F(328)=6.2$, $R^2_{\text{adj}}=.031$, $p=.002$, age $R^2_{\text{partial}}=.029$). Again, stepwise optimization
386 reverted to the model only including age and SW up-phase duration.

387 Importantly, age quartiles Q2 ($M=0.0011$, $CI_{95}=[0.0004, 0.0017]$, $t(87)=3.29$, $p=.001$) and Q4 ($M=-$
388 0.0011 , $CI_{95}=[-0.0017, -0.0005]$, $t(88)=-3.44$, $p<.001$) exhibit significant deviations of PSI from 0,
389 including a sign-flip, indicating a reversal of which frequency drives the other at best-fit model
390 suggested age 43.2 (fig. 1E).

391 Interestingly, the youngest age quartile did not exhibit a significant PSI, indicating no clear cross-
392 frequency directionality in the age group 15-26. To investigate a potential rising and falling
393 relationship between age and PSI, a stepwise linear model was allowed to fit higher-order polynomial
394 age terms. To prevent overfitting in data-sparse regions, four data at age >73 were removed from this
395 model. Including a cubic polynomial peaking at age 29.2 resulted in the best model fit, increasing
396 explained variance (ΔAIC vs. linear: -11.95 , $F(323)=9.32$, $R^2_{\text{adj}}=.07$, $p<.001$). This model suggests a
397 reversal of which frequency drives the other at age 47.7 (fig. 1E, blue curve).

398 As with rvec angle, the age-dependent shift of PSI, including a sign-flip, becomes more pronounced if
399 only participants exhibiting strong coupling (high MI) are included (fig. 1F). As with rvec angle, high MI
400 was defined as $z(\ln(\text{MI}))>0$, yielding a subgroup of $N_{\text{highMI}}=172$. In this subgroup, the association of
401 age and PSI became stronger, about doubling explained variance. A linear correlation yielded $R^2=.07$

Abbreviated title: Coupled sleep oscillations across the human lifespan

402 ($p < .001$), which is significantly stronger than including the entire sample (Pearson & Filon's z : 33.14,
 403 $p < .001$). This association was again improved by including SW up-phase duration as predictor in a
 404 linear regression ($F(169)=19.9$, $R^2_{adj}=.18$, $p < .001$, age $R^2_{partial}=.09$), and showed an even stronger
 405 cubic relationship (ΔAIC vs. linear: -13.93 , $F(168)=10.50$, $R^2_{adj}=.15$, $p < .001$). A suggested reversal of
 406 which frequency drives the other was between ages 44.5 (linear) and 48.1 (cubic). This was again
 407 paralleled by age quartiles Q2 ($M=0.0019$, $CI_{95}=[0.0009, 0.0030]$, $t(44)=3.74$, $p < .001$) and Q4 ($M=-$
 408 0.0022 , $CI_{95}=[-0.0033, -0.0012]$, $t(42)=-4.35$, $p < .001$) exhibiting significant and opposite deviations of
 409 PSI from 0.

410 While SW-spindle coupling clearly shifts across the human lifespan, reversing the coupling hierarchy
 411 around age 40-48, coupling strength remains unaffected. This is illustrated by the absence of an
 412 association of MI and age ($R^2 < 10^{-4}$, $p = .84$, fig. 1B). An ANOVA on MI age quartiles did not yield a
 413 significant effect ($F(3,336)=1.32$, $p = .267$). Pairwise comparisons showed a trend of Q2>Q4
 414 ($t(84)=1.82$, $p = .070$), which is reminiscent of previous findings (Helfrich et al., 2018), but this result is
 415 not robust and should be treated as a negative finding. In addition, the age groups where the effect
 416 seems to occur are not directly comparable (Helfrich et al.'s younger group's age was 20.4 ± 2.0 years,
 417 $M \pm SD$, while our Q2's age was 38.2 ± 6.3 years). Alternatively, the right-skewed nature of MI,
 418 combined with higher variance in the middle age quartiles compared to Q1/4 (Bartlett's $\chi^2=34.93$,
 419 $p < .001$) may cause the appearance of changing means.

420 FIGURE 1 ABOUT HERE

421

422 **3.4. The lower the slow wave frequency, the stronger slow wave-spindle coupling and** 423 **reversal of information flow**

424 To more closely investigate the exact nature of SW-spindle coupling and the observed reversal of
 425 information flow (from SW leading spindles to vice versa) with age, we re-ran the regression analyses
 426 of age on PSI for SW subbands (0.5, 1.0, 1.5, and 2.0 Hz) separately. A 4×4 repeated-measures
 427 ANOVA with the factors "SW subband" and "age quartile" (Q1-Q4) revealed a significant main effect
 428 for SW subband ($F(3,981)=3.32$, $p = .033$, Greenhouse-Geisser corrected) and a significant interaction
 429 with age quartile ($F(9,981)=7.37$, $p < .001$). The significant interaction is due to lower SW subbands

Abbreviated title: Coupled sleep oscillations across the human lifespan

430 showing a stronger age-dependent reversal effect than higher subbands. Additional FDR-corrected t-
 431 tests of single subbands against 0 indicated that significant sign-flips from positive to negative PSI
 432 (i.e., reversal of information flow) occurred for subbands 0.5 and 1.0 Hz only (cf. asterisks for age
 433 quartiles in fig. 2).

434 Next, we investigated age-trends for the four PSI SW subbands. For each SW subband, two models
 435 were calculated, paralleling the final models from the analysis of age on PSI in section 3.3 and figure
 436 1E: 1) a linear model including SW up-phase duration as covariate; 2) a model including up to cubic
 437 terms of age. The strongest age-related PSI shift occurred for the lowest SW subband, 0.5 Hz (linear:
 438 $F(328)=28.4$, $R^2_{\text{adj}}=.14$, $p<.001$, age $R^2_{\text{partial}}=.11$; cubic: $F(323)=17.2$, $R^2_{\text{adj}}=.13$, $p<.001$; fig. 2, left-most
 439 panel). With increasing SW subband frequency, this relationship got progressively less pronounced
 440 (1.0 Hz linear: $F(328)=24.0$, $R^2_{\text{adj}}=.12$, $p<.001$, age $R^2_{\text{partial}}=.03$; 1.0 Hz cubic: $F(323)=9.0$, $R^2_{\text{adj}}=.07$,
 441 $p<.001$; 1.5 Hz linear: $F(328)=9.1$, $R^2_{\text{adj}}=.05$, $p<.001$, age $R^2_{\text{partial}}=.01$; 1.5 Hz cubic: $F(323)=4.0$,
 442 $R^2_{\text{adj}}=.03$, $p=.008$; fig. 2, middle panels). The highest SW subband, at 2.0 Hz, was no longer
 443 significantly associated with age (linear: $F(328)=2.0$, $R^2_{\text{adj}}=.01$, $p=.132$, age $R^2_{\text{partial}}=.004$; cubic:
 444 $F(323)=1.2$, $R^2_{\text{adj}}=.001$, $p=.31$; fig 2., right-most panel). PSI exhibits markedly reduced variance in the
 445 highest SW subband (2.0 Hz), hovering around 0 (fig. 2, right most panel). This illustrates the
 446 transition away from slow wave frequencies into the upper delta range. Counterintuitively, the first two
 447 age-quartiles in the 2.0 Hz subband even exhibit significantly negative PSI, but due to the very low
 448 absolute values and variance, we treat this as a false positive finding.

449 FIGURE 2 ABOUT HERE

450

451 **3.5. Plasma GFAP, but not plasma amyloid β 42/40, is associated with slow wave-spindle** 452 **coupling in older individuals**

453 Plasma GFAP levels after waking were strongly associated with SW-spindle coupling in 28 older
 454 individuals with biomarker measurements in an optimized linear regression model ($F(25)=6.45$,
 455 $R^2_{\text{adj}}=.29$, $p=.006$). Number of coupling events ($t=-2.17$, $p=.039$) and PSI ($t=2.77$, $p=.010$) were
 456 significant predictors of GFAP levels (fig. 3). The negative association between number of coupling
 457 events and GFAP levels indicate that individuals with lower overall SWS quality and/or quantity show

Abbreviated title: Coupled sleep oscillations across the human lifespan

458 increased signs of astrocyte activation. Somewhat counterintuitively, the positive association between
459 PSI and GFAP indicates that older individuals exhibiting a more positive cross-frequency directionality
460 typical for younger individuals (i.e., SW driving spindles rather than spindles driving SW) showed
461 increased signs of astrocyte activation. Age and MI were dropped from the a-priori baseline model
462 and no other terms (e.g., sleep parameters) were added during stepwise model optimization. Notably,
463 as neither proportion of N2/N3 sleep, nor TST were considered predictors of plasma GFAP levels, the
464 association of the number of coupling events with GFAP seems to be independent of the absolute
465 available time (quantity) in SWS for coupling to occur, and more dependent on the quality of SWS
466 determining whether coupling occurs or not.

467 The number of coupling events was best explained using an optimized model ($F(22)=5.42$, $R^2_{adj}=.45$,
468 $p=.002$) including all a-priori terms (MI, PSI, age, and GFAP), as well as TST as strong predictor.
469 While MI ($t=2.20$, $p=.039$) and TST ($t=3.09$, $p=.005$) significantly predicted the number of coupling
470 events, PSI ($t=1.63$, $p=.118$), age ($t=-1.74$, $p=.096$), and GFAP levels ($t=-1.69$, $p=.106$) explained
471 enough variance to remain in the model. Unsurprisingly, the number of coupling events increases with
472 total sleep time (TST), illustrating its dependency upon sleep quantity. The positive association of MI
473 and coupling events, on the other hand, explains how qualitative aspects of SWS as indicated by
474 coupling strength are associated with an increased occurrence of coupling events.

475 MI was best explained using an optimized model ($F(23)=4.00$, $R^2_{adj}=.31$, $p=.013$) including the number
476 of coupling events ($t=2.27$, $p=.033$), PSI ($t=-2.68$, $p=.013$), GFAP ($t=0.48$, $p=.638$) and the interaction
477 of PSI*GFAP ($t=2.22$, $p=.036$). Age was removed from the model during stepwise model optimization,
478 paralleling the result of the whole-sample analysis (N=340, fig. 1B).

479 PSI was best explained using an optimized model ($F(24)=3.62$, $R^2_{adj}=.23$, $p=.028$) including the
480 number of coupling events ($t=1.36$, $p=.19$), MI ($t=-1.62$, $p=.118$), and GFAP ($t=2.54$, $p=.018$),
481 excluding age. Although number of coupling events and MI explained enough variance to stay in the
482 model, GFAP was the only term significantly explaining variance in PSI.

483 Plasma A β 42/40 ratios were not explained by any of the available measures ($F(26)=1.40$, $R^2_{adj}=.01$,
484 $p=.248$). Number of coupling phases, PSI, and age were dropped from the a-priori baseline model,

Abbreviated title: Coupled sleep oscillations across the human lifespan

485 and MI remained as a non-significant predictor in the optimized model ($p=.248$), indicating that
486 amyloid clearance is seemingly not related to SW-spindle coupling in healthy older adults.

487 In summary, these optimized models suggest a link between SW-spindle coupling and astrocyte
488 activation: falling sleep quality- and quantity-related reduction in SW-spindle coupling events was
489 associated with increased signs of astrocyte activation as measured by plasma GFAP levels (figs.
490 2&3). Increased GFAP levels in turn were paralleled by a shift in PSI resembling the physiology of
491 younger participants. Age is not directly associated with this process, suggesting this older age
492 subgroup to be of homogeneous age. We deliberate on potential explanations (e.g., compensatory
493 increase in PSI in response to deteriorating neurophysiology and neural integrity) in the discussion
494 section and fig. 4.

495

496 FIGURE 3 ABOUT HERE

497 FIGURE 4 ABOUT HERE

498

Abbreviated title: Coupled sleep oscillations across the human lifespan

499 **4. Discussion**

500 We show that SW-spindle phase-amplitude coupling gradually shifts across the human lifespan
501 without losing coupling strength. Corroborating previous reports (Mikutta et al., 2019; Muehlroth et al.,
502 2019; Winer et al., 2019), SW drive spindles in younger individuals, representing the canonical
503 hierarchy of top-down neocortical control of information flow (Rasch & Born, 2013; Staresina et al.,
504 2015). However, while others report that this hierarchical structure dissipates with age (Helfrich et al.,
505 2018), we found that the hierarchy reverses, settling into a configuration of spindles driving SW in old
506 age. The extent of this reversal of coupling hierarchy was associated with markers for astrocyte
507 activation.

508 Importantly, we demonstrate a gradual, not sudden, forward-shift of SW-spindle coupling across the
509 adult human life, paralleling another large-sample study (McConnell et al., 2021). This gradual nature
510 notwithstanding, this shift results in a fundamental structural change – a reversal of the order of
511 events and thereby the hierarchical structure observed in younger adults – starting around age 40-48.
512 In old age, spindles shift from being driven by- to driving SW. This effect was stronger, the lower the
513 SW-subband analyzed: 0.5 Hz exhibited the strongest shift & coupling (PSI) with spindles, 1.0–1.5 Hz
514 exhibited gradually reduced shift & coupling, and 2.0 Hz exhibited no shift/coupling, marking the
515 transition away from SW and into the upper delta frequency band, which no longer seems to
516 orchestrate sleep oscillations. What exactly the downstream effects of this shift regarding information
517 flow inside the brain networks are must be speculated on, but others suggest that a precise hierarchy
518 of SW driving spindles is not a necessity for information processing, but helps making it more efficient
519 (Muehlroth et al., 2019). These authors find that in old age, a coupling hierarchy reminiscent of a
520 younger brain is associated with higher structural integrity in key brain regions for memory processing
521 (e.g. hippocampus and medial prefrontal cortex). This hints towards the existence of mechanisms for
522 the preservation of a younger brain's physiology, or potentially for compensation of loss thereof. This
523 dovetails with findings that lifelong learning and cognitively stimulating environments contribute to
524 cognitive fitness and neuronal integrity, aid the clearance of amyloid beta, and may counteract
525 cognitive decline (Brown et al., 2003; Fischer et al., 2007; Flexman, 2021; Lazarov et al., 2005).

Abbreviated title: Coupled sleep oscillations across the human lifespan

526 Our subgroup analysis relating astrocyte activation, and therefore, potential neuroinflammatory
527 processes to SW-spindle coupling provides a result that dovetails into such a model of maintenance
528 and/or compensation. We find that age-related loss of sleep leads to reduced coupling. Reduced
529 coupling is associated with an increase in plasma GFAP, a biomarker for astrocyte activation and a
530 warning sign for potential impending cognitive decline and Alzheimer's disease. Interestingly,
531 increased astrocyte activation is accompanied with a back-shift of SW-spindle coupling towards a
532 younger brain's physiology. This back-shift, in turn, is associated with an increase in coupling strength
533 and indirectly may lead to more coupling overall. This could be an indication that the aging brain
534 attempts to compensate for loss of sleep and structural integrity by shifting the coupling hierarchy
535 back into a more optimal configuration. An alternative explanation would be that the age-associated
536 reversal of coupling hierarchy is a normal, healthy process, and a failure to do so is a sign of a
537 suboptimal development, paralleled by astrocyte activation. However, as other studies strongly
538 indicate that the age-related forward-shift in SW-spindle coupling physiology is a detrimental
539 development associated with memory loss and brain atrophy (Chylinski et al., 2022; Helfrich et al.,
540 2018; Ladenbauer et al., 2017; Muehlroth et al., 2019), we find this alternative explanation to be
541 unlikely.

542 A notable contrast to previous findings (Helfrich et al., 2018) is that here, coupling strength (MI) did
543 not change as a function of age. Our results indicate that MI does not exhibit age related changes in
544 the mean, but rather in variance, resembling an inverted-U shaped curve, with middle age quartiles
545 exhibiting larger variance in MI than extreme age quartiles (fig. 1B). This may lead to spurious
546 changes in the mean: MI is right-skewed and cannot become negative. The lower variance in Q1 and
547 Q4 thus leads to an asymmetrical absence of high (but not low) MI values that causes a lowered
548 mean. The analysis in the original report by Helfrich et al. (2018) may have captured this effect, but its
549 larger dynamics across the lifespan remained hidden from those authors as they only had access to
550 distinct age groups.

551 The change in coupling phase with concomitant stability of coupling strength might explain why
552 declarative memory is generally more severely impacted in aging and neurodegeneration compared
553 to procedural memory (Tromp et al., 2015), as declarative memory has been associated with coupling
554 phase, while procedural memory has been associated with coupling strength (Mikutta et al., 2019).

Abbreviated title: Coupled sleep oscillations across the human lifespan

555 We found that the typical coupling hierarchy (as measured using PSI) of younger adults does not yet
556 exist in our youngest age quartile, even though the overall event order typical for younger adults
557 (spindles after SW peak, measured using rvec angle) is already established. This hints towards a
558 dissociation between mere order of events versus the leading event exerting influence over the
559 lagging event. This quartile spanned ages 15-26, with adolescents under the age of 18 featuring
560 prominently. Only in the second quartile ranging ages 27-46, the canonical young adult coupling
561 hierarchy (PSI>0) is established. Our data-driven model suggested that a non-linear relationship
562 exists between coupling hierarchy and age, with an early “adolescent-young adult” and a later “adult
563 lifespan” component. During the early component, the canonical hierarchy is established, peaking at
564 age 29.2, and subsequently shifts gradually into the reported reversed hierarchy during the later
565 component. This early component tracks with brain maturation, especially of white matter, which
566 continues well into young adulthood of the early 20’s (Konrad et al., 2013). Paralleling our finding, a
567 recent study found that SW-spindle coupling strength increases during childhood into adolescence
568 and is associated with enhanced memory formation (Hahn et al., 2020). We find a similar inverted U-
569 shaped dynamic in changing variance in coupling strength (MI) with age. Taken together, the non-
570 linear waxing and waning of the SW-spindle coupling hierarchy and, arguably, coupling strength
571 across the human lifespan may reflect different biological processes: maturation, plateau, and wear.

572

573 **4.1. Limitations**

574 Our study has several limitations. Although we were able to investigate a large sample of baseline
575 sleep recordings, we were limited to a single frontal EEG derivation, and there were no behavioral
576 tasks available to associate memory, executive functions or other cognitive domains to SW-spindle
577 coupling. However, among other studies that did measure memory, there is a strong consensus that a
578 “younger” SW-spindle coupling physiology is optimal for memory consolidation, and age-related
579 changes in coupling are associated with reduced memory performance (Bastian et al., 2022; Chylinski
580 et al., 2022; Helfrich et al., 2018; Ladenbauer et al., 2017; Mikutta et al., 2019; Muehlroth et al., 2019).

581 We could only assess the association of SW-spindle coupling with blood-based biomarkers in a
582 subset of 28 older individuals. The lower statistical power of this comparatively small subset may

Abbreviated title: Coupled sleep oscillations across the human lifespan

583 explain why we were not able to find an association with plasma amyloid levels. However, this lack of
584 association between coupling and amyloid is consistent with other studies (Winer et al., 2019, 2020),
585 even though one report finds that a forward-shift of spindles was associated with A β burden in the
586 medial prefrontal cortex and memory decline (Chylinski et al., 2022). Arguably, aberrant amyloid
587 dynamics, although predictive of cognitive decline years in advance (Beyer et al., 2022) may not be
588 prominent enough in healthy older adults (yet) to associate with sleep-microstructural dynamics like
589 SW-spindle coupling (Winer et al., 2020).

590 Based on an ample body of literature, we speculate that increased GFAP levels may be indicative of
591 neuroinflammation (Beyer et al., 2022; Manchanda et al., 2018; Verberk et al., 2021; Xiao et al.,
592 2022). However, GFAP levels are also associated with general and benign astrocyte activation
593 (Verkhatsky & Nedergaard, 2018). Finally, our stepwise regression method is rather exploratory in
594 nature. The suggested mechanistic pathway model attempting to explain the association of astrocyte
595 activation/neuroinflammation with a “younger” coupling physiology is hypothetical, with directions of
596 causality not resolved. We interpreted the regressor structure in a way that made sense in context of
597 other studies. However, more research is needed to confirm or refute this model, including human
598 intracranial recording studies for more direct physiological measurements, or animal studies directly
599 manipulating cellular processes and assessing biomarker responses (Katsuki et al., 2022).

600 **4.2. Conclusions and future directions**

601 Our results generally agree with previous studies. However, the specific finding that SW-spindle
602 coupling shifts across the human lifespan without losing coupling strength, with a reversal of the
603 typical hierarchical coupling structure at midlife, is a novel finding in slight contrast with previous
604 reports (Helfrich et al., 2018). It has generally been the assumption that the tight SW-spindle coupling
605 typically seen in younger individuals becomes fuzzier in old age, but we do not see a decrease in
606 coupling strength or a dissolution of a clear hierarchical structure of cross-frequency directionality in
607 our data. On the contrary, we see that in the oldest age quartile, a hierarchical structure of cross-
608 frequency directionality re-emerges, but in reversed form, with spindles driving SW. Zooming into this
609 older age group, we find that deteriorating sleep, coupling physiology, and astrocyte activation go

Abbreviated title: Coupled sleep oscillations across the human lifespan

610 hand in hand. Astrocyte activation is associated with a hierarchical back-shift of cross-frequency
611 directionality to a younger status, potentially indicating compensation.

612 This assumption of compensation is exploratory and should be followed-up on with more systematic,
613 prospective studies. However, if the model holds, it may hint towards SW-spindle coupling during
614 sleep as a potential target for intervention against- or prevention of cognitive decline. As the process
615 needing to be reversed (i.e., the shifting coupling hierarchy) starts to gradually, not suddenly, shift into
616 a qualitatively different configuration at midlife, and as the GFAP/amyloid biomarker profile can be
617 used to predict neurodegeneration up to 17 years before onset (Beyer et al., 2022), there remains
618 ample time to intervene. This potentially enables early, low threshold, “soft” lifestyle adjustments to
619 serve as a sufficient push in the right direction to avoid pathological trajectories, saving resources and
620 preserving quality of life for otherwise afflicted individuals. What these adjustments might be should
621 be investigated further, but as our model suggests a connection between total sleep time and
622 coupling physiology, a focus on good sleep hygiene throughout one’s life would be a good starting
623 point.

624

Abbreviated title: Coupled sleep oscillations across the human lifespan

625 **References**

- 626 Bastian, L., Samanta, A., Ribeiro de Paula, D., Weber, F. D., Schoenfeld, R., Dresler, M., & Genzel, L. (2022).
 627 Spindle-slow oscillation coupling correlates with memory performance and connectivity changes in a
 628 hippocampal network after sleep. *Human Brain Mapping*, *43*(13), 3923–3943.
 629 <https://doi.org/10.1002/hbm.25893>
- 630 Berens, P. (2009). CircStat: A MATLAB Toolbox for Circular Statistics. *Journal of Statistical Software*, *31*, 1–21.
 631 <https://doi.org/10.18637/jss.v031.i10>
- 632 Beyer, L., Stocker, H., Rujescu, D., Holleczeck, B., Stockmann, J., Nabers, A., Brenner, H., & Gerwert, K. (2022).
 633 Amyloid-beta misfolding and GFAP predict risk of clinical Alzheimer's disease diagnosis within 17 years.
 634 *Alzheimer's & Dementia: The Journal of the Alzheimer's Association*. <https://doi.org/10.1002/alz.12745>
- 635 Bigdely-Shamlo, N., Mullen, T., Kothe, C., Su, K.-M., & Robbins, K. A. (2015). The PREP pipeline: Standardized
 636 preprocessing for large-scale EEG analysis. *Frontiers in Neuroinformatics*, *9*, 16.
 637 <https://doi.org/10.3389/fninf.2015.00016>
- 638 Brown, J., Cooper-Kuhn, C. M., Kempermann, G., Van Praag, H., Winkler, J., Gage, F. H., & Kuhn, H. G. (2003).
 639 Enriched environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis.
 640 *European Journal of Neuroscience*, *17*(10), 2042–2046. [https://doi.org/10.1046/j.1460-](https://doi.org/10.1046/j.1460-9568.2003.02647.x)
 641 [9568.2003.02647.x](https://doi.org/10.1046/j.1460-9568.2003.02647.x)
- 642 Carrier, J., Viens, I., Poirier, G., Robillard, R., Lafortune, M., Vandewalle, G., Martin, N., Barakat, M., Paquet, J.,
 643 & Filipini, D. (2011). Sleep slow wave changes during the middle years of life. *Eur J Neurosci*, *33*(4),
 644 758–766. <https://doi.org/10.1111/j.1460-9568.2010.07543.x>
- 645 Chylinski, D., Van Egroo, M., Narbutas, J., Muto, V., Bahri, M. A., Berthomier, C., Salmon, E., Bastin, C., Phillips,
 646 C., Collette, F., Maquet, P., Carrier, J., Lina, J.-M., & Vandewalle, G. (2022). Timely coupling of sleep
 647 spindles and slow waves linked to early amyloid- β burden and predicts memory decline. *ELife*, *11*,
 648 e78191. <https://doi.org/10.7554/eLife.78191>
- 649 Delorme, A., & Makeig, S. (2004). EEGLAB: An open source toolbox for analysis of single-trial EEG dynamics
 650 including independent component analysis. *Journal of Neuroscience Methods*, *134*(1), 9–21.
- 651 Eide, P. K., Vinje, V., Pripp, A. H., Mardal, K.-A., & Ringstad, G. (2021). Sleep deprivation impairs molecular
 652 clearance from the human brain. *Brain*, *144*(3), 863–874. <https://doi.org/10.1093/brain/awaa443>
- 653 Fischer, A., Sananbenesi, F., Wang, X., Dobbin, M., & Tsai, L.-H. (2007). Recovery of learning and memory is
 654 associated with chromatin remodelling. *Nature*, *447*(7141), Article 7141.
 655 <https://doi.org/10.1038/nature05772>

Abbreviated title: Coupled sleep oscillations across the human lifespan

- 656 Flexman, R. (2021). Lifelong Learning: *Delaware Journal of Public Health*, 7(4), 124–127.
 657 <https://doi.org/10.32481/djph.2021.09.015>
- 658 Fultz, N. E., Bonmassar, G., Setsompop, K., Stickgold, R. A., Rosen, B. R., Polimeni, J. R., & Lewis, L. D. (2019).
 659 Coupled electrophysiological, hemodynamic, and cerebrospinal fluid oscillations in human sleep.
 660 *Science*, 366(6465), 628–631. <https://doi.org/10.1126/science.aax5440>
- 661 Graff-Radford, N. R., Crook, J. E., Lucas, J., Boeve, B. F., Knopman, D. S., Ivnik, R. J., Smith, G. E., Younkin, L.
 662 H., Petersen, R. C., & Younkin, S. G. (2007). Association of Low Plasma A β 42/A β 40 Ratios With
 663 Increased Imminent Risk for Mild Cognitive Impairment and Alzheimer Disease. *Archives of Neurology*,
 664 64(3), 354–362. <https://doi.org/10.1001/archneur.64.3.354>
- 665 Hahn, M. A., Heib, D., Schabus, M., Hoedlmoser, K., & Helfrich, R. F. (2020). Slow oscillation-spindle coupling
 666 predicts enhanced memory formation from childhood to adolescence. *ELife*, 9, e53730.
 667 <https://doi.org/10.7554/eLife.53730>
- 668 Helfrich, R. F., Mander, B. A., Jagust, W. J., Knight, R. T., & Walker, M. P. (2018). Old Brains Come Uncoupled
 669 in Sleep: Slow Wave-Spindle Synchrony, Brain Atrophy, and Forgetting. *Neuron*, 97(1), 221-230.e4.
 670 <https://doi.org/10.1016/j.neuron.2017.11.020>
- 671 Hertenstein, E., Gabryelska, A., Spiegelhalder, K., Nissen, C., Johann, A. F., Umarova, R., Riemann, D.,
 672 Baglioni, C., & Feige, B. (2018). Reference Data for Polysomnography-Measured and Subjective Sleep
 673 in Healthy Adults. *Journal of Clinical Sleep Medicine*, 14(04), 523–532.
 674 <https://doi.org/10.5664/jcsm.7036>
- 675 Iber, C., Ancoli-Israel, S., Chesson, A., & Quan, S. F. (2007). *The AASM Manual for the Scoring of Sleep and*
 676 *Associated Events: Rules, Terminology and Technical Specifications*. American Academy of Sleep
 677 Medicine. <http://www.aasmnet.org/scoringmanual/>
- 678 Jiang, H., Bahramisharif, A., van Gerven, M. A. J., & Jensen, O. (2015). Measuring directionality between
 679 neuronal oscillations of different frequencies. *NeuroImage*, 118, 359–367.
 680 <https://doi.org/10.1016/j.neuroimage.2015.05.044>
- 681 Ju, Y.-E. S., Ooms, S. J., Sutphen, C., Macauley, S. L., Zangrilli, M. A., Jerome, G., Fagan, A. M., Mignot, E.,
 682 Zempel, J. M., Claassen, J. A. H. R., & Holtzman, D. M. (2017). Slow wave sleep disruption increases
 683 cerebrospinal fluid amyloid- β levels. *Brain*, 140(8), 2104–2111. <https://doi.org/10.1093/brain/awx148>
- 684 Kang, J. E., Lim, M. M., Bateman, R. J., Lee, J. J., Smyth, L. P., Cirrito, J. R., Fujiki, N., Nishino, S., & Holtzman,
 685 D. M. (2009). Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. *Science*,
 686 326(5955), 1005–1007. <https://doi.org/10.1126/science.1180962>

Abbreviated title: Coupled sleep oscillations across the human lifespan

- 687 Katsuki, F., Gerashchenko, D., & Brown, R. E. (2022). Alterations of sleep oscillations in Alzheimer's disease: A
 688 potential role for GABAergic neurons in the cortex, hippocampus, and thalamus. *Brain Research*
 689 *Bulletin*, 187, 181–198. <https://doi.org/10.1016/j.brainresbull.2022.07.002>
- 690 Konrad, K., Firk, C., & Uhlhaas, P. J. (2013). Brain Development During Adolescence. *Deutsches Ärzteblatt*
 691 *International*, 110(25), 425–431. <https://doi.org/10.3238/arztebl.2013.0425>
- 692 Ladenbauer, J., Ladenbauer, J., Külzow, N., de Boor, R., Avramova, E., Grittner, U., & Flöel, A. (2017).
 693 Promoting Sleep Oscillations and Their Functional Coupling by Transcranial Stimulation Enhances
 694 Memory Consolidation in Mild Cognitive Impairment. *The Journal of Neuroscience: The Official Journal*
 695 *of the Society for Neuroscience*, 37(30), 7111–7124. <https://doi.org/10.1523/JNEUROSCI.0260-17.2017>
- 696 Lazarov, O., Robinson, J., Tang, Y.-P., Hairston, I. S., Korade-Mirnics, Z., Lee, V. M.-Y., Hersh, L. B., Sapolsky,
 697 R. M., Mirnics, K., & Sisodia, S. S. (2005). Environmental Enrichment Reduces A β Levels and Amyloid
 698 Deposition in Transgenic Mice. *Cell*, 120(5), 701–713. <https://doi.org/10.1016/j.cell.2005.01.015>
- 699 Manchanda, S., Singh, H., Kaur, T., & Kaur, G. (2018). Low-grade neuroinflammation due to chronic sleep
 700 deprivation results in anxiety and learning and memory impairments. *Molecular and Cellular*
 701 *Biochemistry*, 449(1), 63–72. <https://doi.org/10.1007/s11010-018-3343-7>
- 702 Mander, B. A., Marks, S. M., Vogel, J. W., Rao, V., Lu, B., Saletin, J. M., Ancoli-Israel, S., Jagust, W. J., &
 703 Walker, M. P. (2015). β -amyloid disrupts human NREM slow waves and related hippocampus-
 704 dependent memory consolidation. *Nature Neuroscience*, 18(7), 1051–1057.
 705 <https://doi.org/10.1038/nn.4035>
- 706 Mander, B. A., Winer, J. R., Jagust, W. J., & Walker, M. P. (2016). Sleep: A novel mechanistic pathway,
 707 biomarker, and treatment target in the pathology of Alzheimer's disease? *Trends in Neurosciences*,
 708 39(8), 552–566. <https://doi.org/10.1016/j.tins.2016.05.002>
- 709 Mander, B. A., Winer, J. R., & Walker, M. P. (2017). Sleep and Human Aging. *Neuron*, 94(1), 19–36.
 710 <https://doi.org/10.1016/j.neuron.2017.02.004>
- 711 McConnell, B. V., Kronberg, E., Teale, P. D., Sillau, S. H., Fishback, G. M., Kaplan, R. I., Fought, A. J.,
 712 Dhanasekaran, A. R., Berman, B. D., Ramos, A. R., McClure, R. L., & Bettcher, B. M. (2021). The aging
 713 slow wave: A shifting amalgam of distinct slow wave and spindle coupling subtypes define slow wave
 714 sleep across the human lifespan. *Sleep*, 44(10), zsab125. <https://doi.org/10.1093/sleep/zsab125>
- 715 Mikutta, C., Feige, B., Maier, J. G., Hertenstein, E., Holz, J., Riemann, D., & Nissen, C. (2019). Phase-amplitude
 716 coupling of sleep slow oscillatory and spindle activity correlates with overnight memory consolidation.
 717 *Journal of Sleep Research*, 28(6), e12835. <https://doi.org/10.1111/jsr.12835>

Abbreviated title: Coupled sleep oscillations across the human lifespan

- 718 Mölle, M., Eschenko, O., Gais, S., Sara, S. J., & Born, J. (2009). The influence of learning on sleep slow
 719 oscillations and associated spindles and ripples in humans and rats. *European Journal of Neuroscience*,
 720 29(5), 1071–1081. <https://doi.org/10.1111/j.1460-9568.2009.06654.x>
- 721 Muehlroth, B. E., Sander, M. C., Fandakova, Y., Grandy, T. H., Rasch, B., Shing, Y. L., & Werkle-Bergner, M.
 722 (2019). Precise Slow Oscillation–Spindle Coupling Promotes Memory Consolidation in Younger and
 723 Older Adults. *Scientific Reports*, 9(1), 1940. <https://doi.org/10.1038/s41598-018-36557-z>
- 724 Ohayon, M. M., Carskadon, M. A., Guilleminault, C., & Vitiello, M. V. (2004). Meta-analysis of quantitative sleep
 725 parameters from childhood to old age in healthy individuals: Developing normative sleep values across
 726 the human lifespan. *Sleep*, 27(7), 1255–1273. <https://doi.org/10.1093/sleep/27.7.1255>
- 727 Oostenveld, R., Fries, P., Maris, E., & Schoffelen, J.-M. (2010). FieldTrip: Open Source Software for Advanced
 728 Analysis of MEG, EEG, and Invasive Electrophysiological Data. *Computational Intelligence and*
 729 *Neuroscience*, 2011, e156869. <https://doi.org/10.1155/2011/156869>
- 730 Rasch, B., & Born, J. (2013). About Sleep's Role in Memory. *Physiological Reviews*, 93(2), 681–766.
 731 <https://doi.org/10.1152/physrev.00032.2012>
- 732 Rauchs, G., Schabus, M., Parapatics, S., Bertran, F., Clochon, P., Hot, P., Denise, P., Desgranges, B., Eustache,
 733 F., Gruber, G., & Anderer, P. (2008). Is there a link between sleep changes and memory in Alzheimer's
 734 disease? *Neuroreport*, 19(11), 1159–1162. <https://doi.org/10.1097/WNR.0b013e32830867c4>
- 735 Redline, S., Kirchner, H. L., Quan, S. F., Gottlieb, D. J., Kapur, V., & Newman, A. (2004). The effects of age, sex,
 736 ethnicity, and sleep-disordered breathing on sleep architecture. *Archives of Internal Medicine*, 164(4),
 737 406–418. <https://doi.org/10.1001/archinte.164.4.406>
- 738 Roh, J. H., Huang, Y., Bero, A. W., Kasten, T., Stewart, F. R., Bateman, R. J., & Holtzman, D. M. (2012).
 739 Disruption of the sleep-wake cycle and diurnal fluctuation of beta-amyloid in mice with Alzheimer's
 740 disease pathology. *Sci Transl Med*, 4(150), 150ra122. <https://doi.org/10.1126/scitranslmed.3004291>
- 741 Staresina, B. P., Bergmann, T. O., Bonnefond, M., van der Meij, R., Jensen, O., Deuker, L., Elger, C. E.,
 742 Axmacher, N., & Fell, J. (2015). Hierarchical nesting of slow oscillations, spindles and ripples in the
 743 human hippocampus during sleep. *Nature Neuroscience*, 18(11), 1679–1686.
 744 <https://doi.org/10.1038/nn.4119>
- 745 Thijssen, E. H., Verberk, I. M. W., Vanbrabant, J., Koelewijn, A., Heijst, H., Scheltens, P., van der Flier, W.,
 746 Vanderstichele, H., Stoops, E., & Teunissen, C. E. (2021). Highly specific and ultrasensitive plasma test
 747 detects Aβ(1-42) and Aβ(1-40) in Alzheimer's disease. *Scientific Reports*, 11(1), 9736.
 748 <https://doi.org/10.1038/s41598-021-89004-x>
- 749 Tononi, G., & Cirelli, C. (2020). Sleep and synaptic down-selection. *European Journal of Neuroscience*, 51(1),
 750 413–421. <https://doi.org/10.1111/ejn.14335>

Abbreviated title: Coupled sleep oscillations across the human lifespan

- 751 Tort, A. B. L., Komorowski, R., Eichenbaum, H., & Kopell, N. (2010). Measuring Phase-Amplitude Coupling
 752 Between Neuronal Oscillations of Different Frequencies. *Journal of Neurophysiology*, *104*(2), 1195–
 753 1210. <https://doi.org/10.1152/jn.00106.2010>
- 754 Tromp, D., Dufour, A., Lithfous, S., Pebayle, T., & Després, O. (2015). Episodic memory in normal aging and
 755 Alzheimer disease: Insights from imaging and behavioral studies. *Ageing Research Reviews*, *24*, 232–
 756 262. <https://doi.org/10.1016/j.arr.2015.08.006>
- 757 Varga, A. W., Wohlleber, M. E., Gimenez, S., Romero, S., Alonso, J. F., Ducca, E. L., Kam, K., Lewis, C., Tanzi,
 758 E. B., Tweardy, S., Kishi, A., Parekh, A., Fischer, E., Gumb, T., Alcolea, D., Fortea, J., Lleo, A.,
 759 Blennow, K., Zetterberg, H., ... Osorio, R. S. (2016). Reduced Slow-Wave Sleep Is Associated with High
 760 Cerebrospinal Fluid Abeta42 Levels in Cognitively Normal Elderly. *Sleep*, *39*(11), 2041–2048.
 761 <https://doi.org/10.5665/sleep.6240>
- 762 Verberk, I. M. W., Laarhuis, M. B., Bosch, K. A. van den, Ebenau, J. L., Leeuwenstijn, M. van, Prins, N. D.,
 763 Scheltens, P., Teunissen, C. E., & Flier, W. M. van der. (2021). Serum markers glial fibrillary acidic
 764 protein and neurofilament light for prognosis and monitoring in cognitively normal older people: A
 765 prospective memory clinic-based cohort study. *The Lancet Healthy Longevity*, *2*(2), e87–e95.
 766 [https://doi.org/10.1016/S2666-7568\(20\)30061-1](https://doi.org/10.1016/S2666-7568(20)30061-1)
- 767 Verberk, I. M. W., Thijssen, E., Koelewijn, J., Mauroo, K., Vanbrabant, J., de Wilde, A., Zwan, M. D., Verfaillie, S.,
 768 C. J., Ossenkoppele, R., Barkhof, F., van Berckel, B. N. M., Scheltens, P., van der Flier, W. M., Stoops,
 769 E., Vanderstichele, H. M., & Teunissen, C. E. (2020). Combination of plasma amyloid beta(1-42/1-40)
 770 and glial fibrillary acidic protein strongly associates with cerebral amyloid pathology. *Alzheimer's*
 771 *Research & Therapy*, *12*(1), 118. <https://doi.org/10.1186/s13195-020-00682-7>
- 772 Verkhratsky, A., & Nedergaard, M. (2018). Physiology of Astroglia. *Physiological Reviews*, *98*(1), 239–389.
 773 <https://doi.org/10.1152/physrev.00042.2016>
- 774 Westerberg, C. E., Mander, B. A., Florczak, S. M., Weintraub, S., Mesulam, M. M., Zee, P. C., & Paller, K. A.
 775 (2012). Concurrent impairments in sleep and memory in amnesic mild cognitive impairment. *J Int*
 776 *Neuropsychol Soc*, *18*(3), 490–500. <https://doi.org/10.1017/S135561771200001X>
- 777 Winer, J. R., Mander, B. A., Helfrich, R. F., Maass, A., Harrison, T. M., Baker, S. L., Knight, R. T., Jagust, W. J.,
 778 & Walker, M. P. (2019). Sleep as a Potential Biomarker of Tau and β -Amyloid Burden in the Human
 779 Brain. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *39*(32), 6315–
 780 6324. <https://doi.org/10.1523/JNEUROSCI.0503-19.2019>
- 781 Winer, J. R., Mander, B. A., Kumar, S., Reed, M., Baker, S. L., Jagust, W. J., & Walker, M. P. (2020). Sleep
 782 Disturbance Forecasts β -Amyloid Accumulation across Subsequent Years. *Current Biology*,
 783 S0960982220311714. <https://doi.org/10.1016/j.cub.2020.08.017>

Abbreviated title: Coupled sleep oscillations across the human lifespan

- 784 Wunderlin, M., Züst, M. A., Fehér, K. D., Klöppel, S., & Nissen, C. (2020). The role of slow wave sleep in the
 785 development of dementia and its potential for preventative interventions. *Psychiatry Research: Neuroimaging*, 111178. <https://doi.org/10.1016/j.psychresns.2020.111178>
 786
 787 Xiao, S.-Y., Liu, Y.-J., Lu, W., Sha, Z.-W., Xu, C., Yu, Z.-H., & Lee, S.-D. (2022). Possible Neuropathology of
 788 Sleep Disturbance Linking to Alzheimer's Disease: Astrocytic and Microglial Roles. *Frontiers in Cellular
 789 Neuroscience*, 16. <https://www.frontiersin.org/articles/10.3389/fncel.2022.875138>
 790 Xie, L., Kang, H., Xu, Q., Chen, M. J., Liao, Y., Thiyagarajan, M., O'Donnell, J., Christensen, D. J., Nicholson, C.,
 791 Iliff, J. J., Takano, T., Deane, R., & Nedergaard, M. (2013). Sleep Drives Metabolite Clearance from the
 792 Adult Brain. *Science*, 342(6156), 373–377. <https://doi.org/10.1126/science.1241224>
 793 Zeller, C. J., Züst, M. A., Wunderlin, M., Nissen, C., & Klöppel, S. (2023). The promise of portable remote
 794 auditory stimulation tools to enhance slow-wave sleep and prevent cognitive decline. *Journal of Sleep
 795 Research*, e13818. <https://doi.org/10.1111/jsr.13818>
 796 Zhang, Y., Ren, R., Yang, L., Zhang, H., Shi, Y., Okhravi, H. R., Vitiello, M. V., Sanford, L. D., & Tang, X. (2022).
 797 Sleep in Alzheimer's disease: A systematic review and meta-analysis of polysomnographic findings.
 798 *Translational Psychiatry*, 12, 136. <https://doi.org/10.1038/s41398-022-01897-y>
 799

800 Figure Legends

801 **Figure 1:** Slow-wave-spindle phase-amplitude coupling across the human life span. **A** Illustration of
 802 measurement of slow waves (SW), spindles, and their coupling. In brief, SW and spindles are
 803 detected using established duration and relative amplitude criteria (red and crimson). SW events are
 804 centered on their negative peak, and each spindle event is classified as SW-coupled if it lies within
 805 2.5 seconds of a SW event (green). Coupling (blue) is measured based on the SW-phase occurring at
 806 the spindle peak (resultant vector angle, rvec angle; the average circular direction of spindle-SW
 807 coupling events), coupling strength (modulation index, MI) and cross-frequency directionality (phase
 808 slope index, PSI; the consistency of phase lag or lead between two signals). A PSI significantly
 809 different from 0 suggests the leading signal drives the lagging signal. A positive PSI indicates SW
 810 drive spindles, and vice versa for negative PSI. **B** MI is not associated with age across the entire
 811 sample (N_{total}), indicating that coupling strength does not change with age. Consequently, the high-MI
 812 subgroup analyses in panels D and F are not biased by age. The red dotted line separates high- from
 813 low-MI subsets at $z(\ln(\text{MI}))=0$. Inset bar graphs show the means of MI in age quartiles Q1-Q4. There

Abbreviated title: Coupled sleep oscillations across the human lifespan

814 was no overall group difference among age quartiles ($p=.27$), but pairwise comparisons revealed a
 815 trend for Q2>Q4 ($p=.07$). **C** Circular-linear correlation of rvec angle with age across the entire sample
 816 ($N_{\text{total}}=340$). 0° represents the peak of the SW, $\pm 180^\circ$ the valley. While >99% of preferred coupling
 817 phases across all ages lie within the positive half-wave of the SW (i.e., between -90° to $+90^\circ$), there
 818 is a strong correlation of age and preferred coupling phase ($R^2=.33$). For younger individuals, spindles
 819 couple after the peak of the SW, while for older individuals, spindles couple before the peak of the
 820 SW. Inset phase histograms show the distribution of preferred coupling phases (dark bars) and all
 821 coupling events (light bars with blue outline) in age quartiles Q1-Q4. For the youngest quartile (Q1),
 822 the average preferred coupling phase (red indicator) occurs significantly after peak, while for the
 823 oldest quartile (Q4), the average preferred coupling phase occurs significantly before the peak (small
 824 red arrows and vectors). The best-fit model suggests a reversal of coupling from after- to before the
 825 SW peak at age 43.9 (green arrow). **D** Same as C, but only in individuals exhibiting strong phase
 826 preference as measured by the modulation index (MI) between spindles and SW ($N_{\text{highMI}}=177$; High MI
 827 = $z(\ln(\text{MI}))>0$). In this sample, the relationship of preferred coupling phase and age is even more
 828 pronounced ($R^2=.42$). The best-fit model suggests a reversal at age 40.4 (green arrow), and Q3
 829 already exhibits a significant shift of average preferred coupling phase to before the SW peak. **E** PSI
 830 between slow waves and spindles as a function of age. Nine data were excluded due to unstable
 831 estimates ($N_{\text{PSI}}=331$). A significant linear regression reveals a gradual reversal from SW leading
 832 spindles in younger individuals to spindles leading SW in older individuals ($R^2=.03$). When controlling
 833 for an age-related change in up-phase duration, this relationship becomes more pronounced
 834 ($R^2_{\text{partial}}=.05$). The best-fit model suggests a reversal at age 43.2. The inset bar graph shows age
 835 quartile means, t-tested against 0. Notably, the youngest quartile (Q1) does not show clear cross-
 836 frequency directionality, but Q2 and the oldest quartile (Q4) do in line with the findings in C & D. A
 837 stepwise linear model fitting higher-order polynomials resulted in a best fit using a cubic relationship
 838 ($R^2_{\text{adj}}=.07$, blue curve), suggesting a rising- and falling PSI across age (peaking at age 29.2),
 839 potentially reflecting brain maturation processes in adolescents. **F** Same as C, but only in individuals
 840 exhibiting strong phase preference as measured by the modulation index (MI) between spindles and
 841 SW. In this sample, the relationship of PSI and age is more pronounced (linear: $R^2=.07$; linear,
 842 controlling for up-phase duration: $R^2_{\text{partial}}=.09$; cubic: $R^2_{\text{adj}}=.14$). The best-fit model suggests a reversal

Abbreviated title: Coupled sleep oscillations across the human lifespan

843 between age 44.5 (linear, green arrow) and 48.1 (cubic, blue arrow). Note: for cubic relationships in E
844 and F, data above age 73 were excluded to prevent overfitting. * $p < .05$, ** $p < .01$, *** $p < .001$.

845

846 **Figure 2:** Cross-frequency directionality as measured using phase slope index (PSI) between slow
847 wave (SW) subbands (0.5, 1.0, 1.5, 2.0 Hz) and spindles (12-16 Hz) as a function of age. Nine data
848 were excluded due to unstable PSI estimates (N=331). Coupling between SW and spindles is
849 strongest for the lowest SW-frequency subband and gradually diminishes with increasing subband
850 frequency, until it no longer is present for 2.0 Hz, marking the transition away from SW into delta
851 frequency. Significant linear regressions, controlling for SW up-phase duration, show the reversal
852 from SW leading spindles in younger individuals to spindles leading SW in older individuals for
853 subbands 0.5, 1.0, and 1.5 Hz, but not 2.0 Hz (black lines). Cubic relationships follow the same trend
854 (blue curves), suggesting a rising- and falling PSI between SW and spindles across age for SW
855 frequencies 1.5 Hz and lower. The lower the SW subband, the stronger the association. There is no
856 linear or cubic association between age and PSI for the highest subband (2.0 Hz), which marks the
857 transition away from SW into delta frequency range. Boxplots show age quartiles (Q1-4), t-tested
858 against 0 (FDR-corrected). * $p < .05$, ** $p < .01$, *** $p < .001$, ns $p > .1$.

859 **Figure 3:** Association of number of slow wave (SW)-spindle coupling events (N coupling events, blue)
860 and phase slope index (PSI, red) with plasma glial fibrillary acidic protein (GFAP) levels in older
861 subgroup with biomarker measurements (N=28, age 61-80). N coupling events and PSI significantly
862 predict GFAP levels in an optimized linear regression (model: $F(25)=6.45$, $R^2_{adj}=.29$, $p=.006$, see t-
863 values for regressors in plot). No other terms were included during stepwise model optimization – i.e.,
864 age, gender, sleep parameters and MI do not contribute to explaining GFAP levels. * $p < .05$

865

866 **Figure 4:** Regressor structure of optimized linear models in older subgroup with biomarker
867 measurements (N=28, age 61-80). Models were calculated for linear slow wave (SW)-spindle
868 coupling measures (phase slope index, PSI; modulation index, MI; number of SW-spindle coupling
869 events, N coupling events; in blue) and plasma glial fibrillary acidic protein levels (GFAP, a biomarker
870 for astrocyte activation; in red). Additional variables are total sleep time (TST) and age, in gray.
871 Pointer lines indicate regressors for pointees. Arrows are positive associations, T-ends are negative

Abbreviated title: Coupled sleep oscillations across the human lifespan

872 associations. Black lines are significant regressors ($p < .05$), the solid gray line is a trend ($p = .096$), and
873 gray dotted lines are non-significant regressors explaining enough variance to remain in models (i.e.,
874 model R^2 would drop by $> .05$ if removed). The converging pointer from GFAP and PSI to MI indicates
875 the significant interaction GFAP*PSI on MI. We hypothesize the following model to explain this
876 regressor structure: ① With age, sleep becomes fragmented, reducing the available time for SW-
877 spindle coupling to occur. ② The reduction in N coupling events is associated with a decrease in MI
878 and ③ an increase in plasma GFAP, suggesting decreased coupling quality and increased astrocyte
879 activation, potentially due to deteriorating neural integrity. ④ Notably, an increase of plasma GFAP is
880 associated with an increase in PSI, suggesting a astrocyte activation-associated shift of coupling
881 phase towards the physiology of a younger brain. ⑤ This shift, in interaction with the increase in
882 GFAP, is in turn associated with an increase in coupling strength (MI), ⑥ which is positively
883 associated with N coupling events. The positive association between PSI and GFAP ④ is unexpected
884 and may be explained in two ways: A) Coupling phase (PSI) is back-shifted towards a “younger” state
885 to compensate for the suboptimal development of sleep quality, coupling physiology and astrocyte
886 activation. This back-shift improves coupling strength directly and may indirectly lead to more overall
887 coupling (N coupling events). B) Alternatively, the forward-shifted coupling phase observed across the
888 human lifespan (see fig. 1) is a normal physiological process, and a lack of this shift (as indexed by an
889 age-relative positive PSI) is suboptimal and associated with astrocyte activation. We favor the
890 compensatory explanation (A) because the age-associated forward-shift in coupling phase has been
891 shown to be associated with neurodegeneration (Helfrich et al., 2018), and an age-relative back-shift
892 has been associated with improved brain integrity and memory (Muehlroth et al., 2019).
893
894

Abbreviated title: Coupled sleep oscillations across the human lifespan

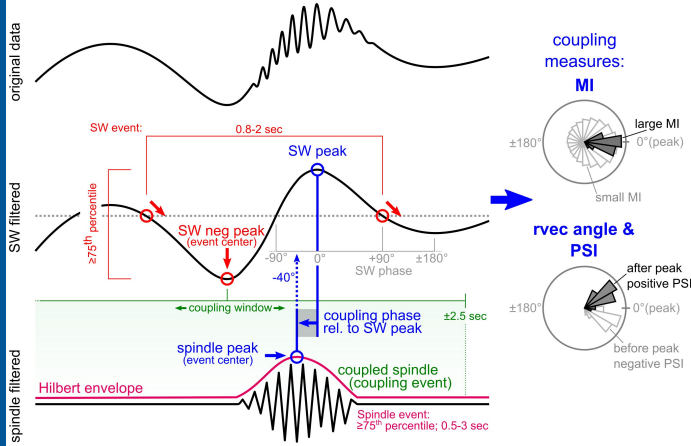
895 **Tables**

896 **Table 1:** Sleep parameters

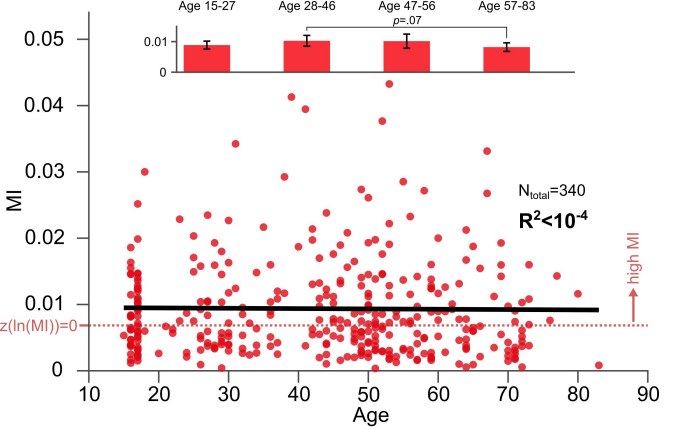
	ALL (N = 340)	Q1 (age 15-27, N = 84)	Q2 (age 28-46, N = 85)	Q3 (age 47-56, N = 85)	Q4 (age 57-83, R ² _{age} N = 86)	
SPT hrs	7.8 ± 0.8	8.4 ± 0.5	7.7 ± 0.3	7.6 ± 0.8	7.6 ± 1.0	.14↓
TST hrs	7.0 ± 1.0	7.9 ± 0.6	7.0 ± 0.6	6.8 ± 0.8	6.2 ± 1.1	.43↓
SL hrs	0.9 ± 0.8	0.6 ± 0.7	0.9 ± 0.8	1.0 ± 0.8	0.9 ± 1.0	<.01
Wake %	11.1 ± 8.3	5.2 ± 3.9	9.7 ± 5.7	11.1 ± 6.1	18.4 ± 10.0	.37↑
Stage N1 %	9.7 ± 6.7	5.6 ± 2.9	7.8 ± 3.7	10.5 ± 6.6	14.9 ± 8.0	.31↑
Stage N2 %	50.1 ± 9.5	47.4 ± 7.5	53.8 ± 6.7	53.3 ± 7.9	45.8 ± 12.3	<.01
Stage N3 %	10.5 ± 9.5	21.3 ± 8.8	8.5 ± 7.1	6.3 ± 6.4	5.8 ± 6.2	.38↓
Stage R %	18.6 ± 5.0	20.5 ± 4.3	20.2 ± 4.0	18.8 ± 4.9	15.1 ± 4.9	.18↓
SE %	88.9 ± 8.3	94.8 ± 3.9	90.3 ± 5.7	88.9 ± 6.1	81.6 ± 10.0	.37↓
SD	9.3 ± 1.6	10.4 ± 0.8	9.8 ± 1.2	9.2 ± 1.4	7.8 ± 1.4	.41↓
SW amp	60.6 ± 25.6	84.9 ± 22.1	58.1 ± 25.6	47.8 ± 15.8	51.9 ± 20.1	.25↓
NCE (×10 ³)	1.8 ± 0.6	2.3 ± 0.4	1.8 ± 0.4	1.7 ± 0.5	1.2 ± 0.5	.48↓

897 Note: Sleep period time (SPT), total sleep time (TST) and sleep latency (SL) in hours (hrs). Sleep
 898 stages (N1-3, R) and intermittent wakefulness (Wake) as a percentage of SPT, sleep efficiency (SE)
 899 as percentage of sleep during bedtime, spindle density (SD) during N2/N3 in spindle events per
 900 minute, slow wave amplitude (SW amp) in μ V, number of coupling events (NCE) in thousands, all
 901 $M \pm SD$. The first data column represents the whole sample (N=340), the columns "Q1-Q4" represent
 902 age quartiles Q1-Q4 of the whole sample. The last column indicates age trends and explained
 903 variance by age (R²_{age}, Pearson's determination coefficient). ↓ trending down, $p < .001$; ↑ trending up,
 904 $p < .001$.
 905

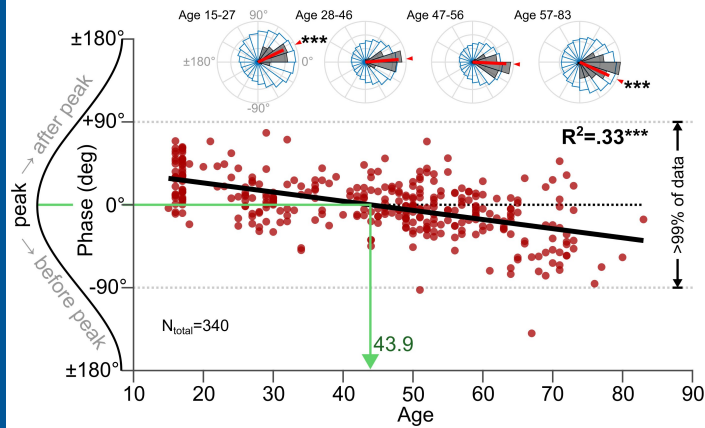
A Measurement of slow waves, spindles, and their coupling



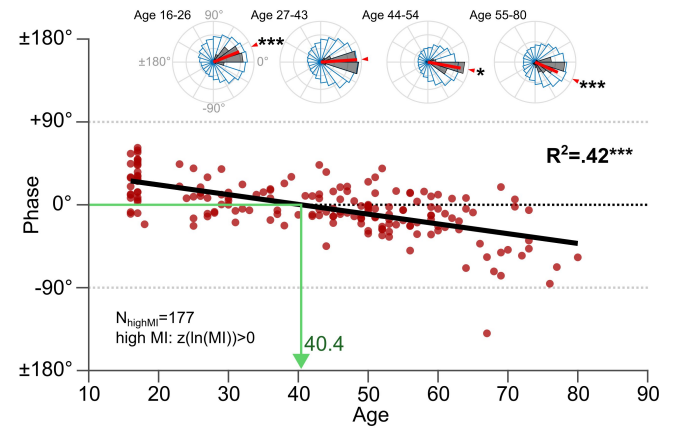
B Modulation index (MI) between spindles and SW by age



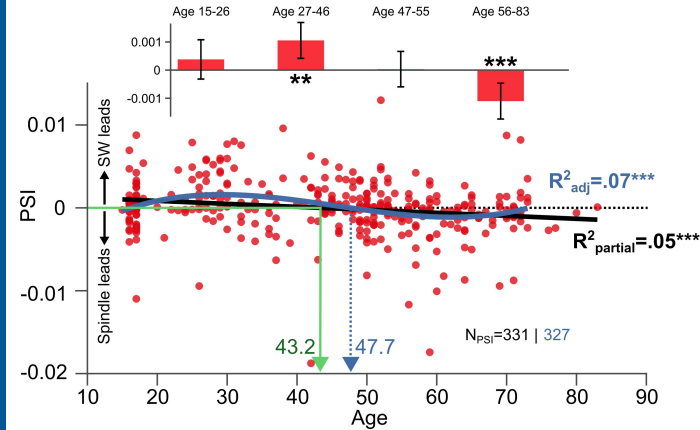
C Preferred phase of spindles in SW (rvec angle) by age



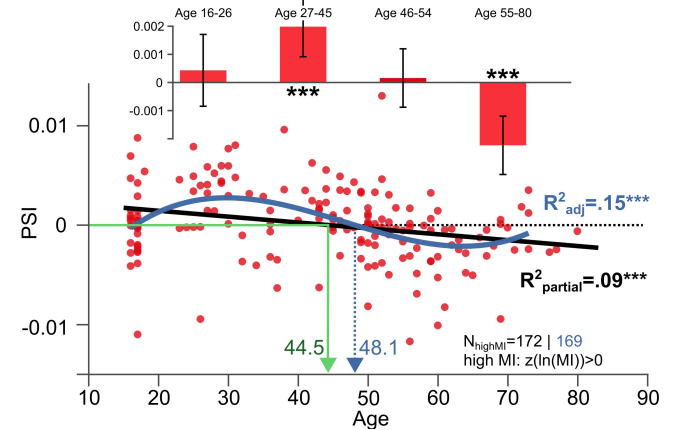
D Preferred phase (rvec angle) by age, high MI only



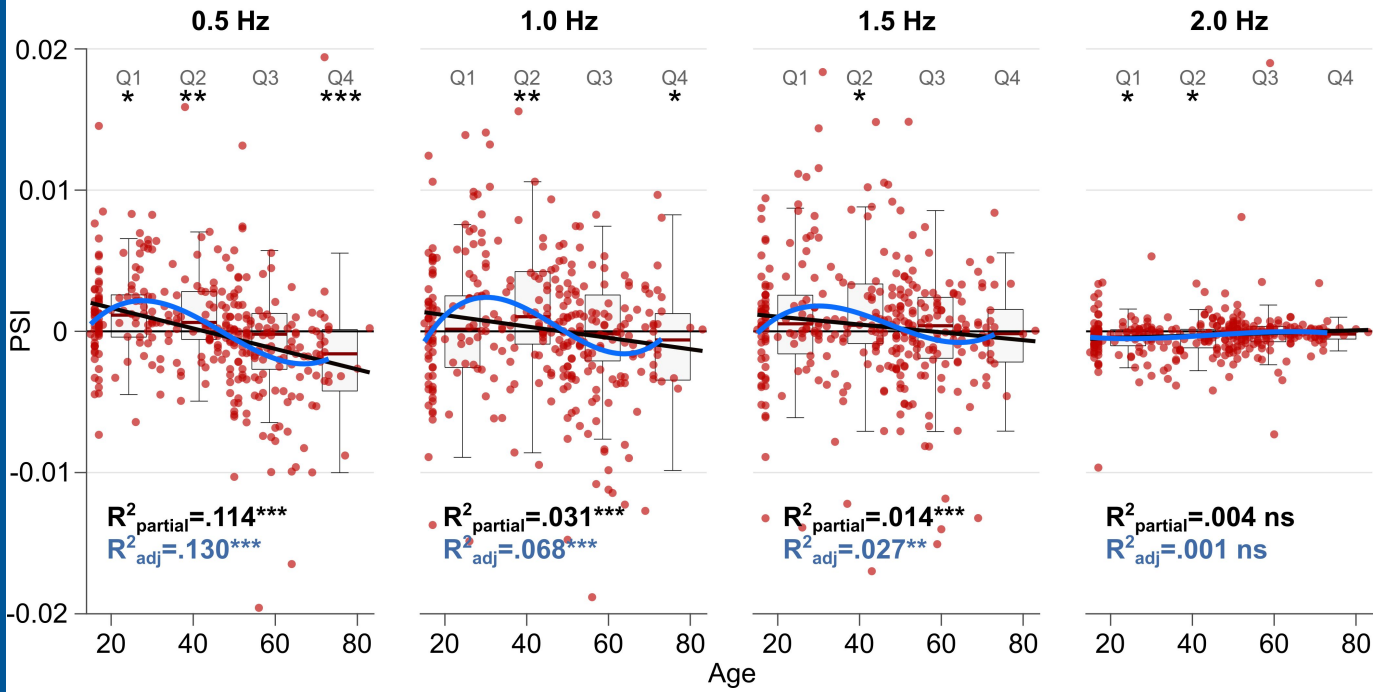
E Phase slope index (PSI) between spindles and SW by age



F PSI by age, high MI only



Phase slope index (PSI) between spindles and SW subbands by age



Number of coupling events and PSI by GFAP

