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Resting-state microstate dynamics predict behavioral and neural efficiency during working memory processing in healthy aging.

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ABSTRACT

Working memory performance declines with age, yet substantial variability exists among cognitively healthy older adults. Although task-evoked electrophysiological responses such as the P300b are sensitive to working memory load, it remains unclear how they relate to stable characteristics of intrinsic brain organization and individual differences in working memory performance. We combined resting-state and task-based electroencephalography (EEG) to examine relationships among working memory performance, P300b amplitude, and resting-state microstate dynamics in 33 cognitively healthy older adults. Participants completed an eyes-closed resting-state recording followed by a visual 0-, 1-, and 2-back task. Behavioral performance (d'), target-evoked P300b amplitude, and canonical resting-state microstate parameters were quantified using linear mixed-effects models.

Increasing working memory load produced significant reductions in both discrimination performance and P300b amplitude, confirming expected behavioral and neural modulation. However, load-related changes in P300b amplitude were not associated with behavioral modulation, and neither was absolute P300b amplitude. Resting-state microstate dynamics selectively predicted task outcomes. In particular, longer duration of microstate D was associated with poorer behavioral discrimination performance. Longer duration of microstate C, in contrast was not associated with task outcomes but with larger P300b amplitude.

These findings suggest that although behavioral performance and P300b amplitude reflect common neural responses to increasing working memory demands, stable characteristics of intrinsic brain organization account for meaningful individual differences in neural responsiveness and cognitive performance. Resting-state EEG microstates may therefore provide complementary, perhaps highly sensitive biomarkers of cognitive functioning beyond conventional task-evoked measures in healthy aging.

INTRODUCTION

Successful everyday functioning depends on the flexible allocation of cognitive resources in response to changing task demands. Central to this process is working memory (WM), a limited-capacity system responsible for temporary maintenance and manipulation of information supporting goal-directed cognition and behavior (Baddeley, 2012). Efficient WM functioning remains important in older adulthood but may be particularly challenged as age-related declines in processing efficiency and executive control increase demands placed on the cognitive control system (Conway et al., 2005; Harada et al., 2013; Miyake, 2001). Age-related declines in WM have been well documented (Cabeza et al., 2018), but substantial inter-individual differences have been observed both across the adult lifespan and among healthy older adults (Matysiak et al., 2019; Zuber et al., 2025). Understanding this variability will provide important insights into the mechanisms underlying successful cognitive aging, as inter-individual differences in WM functioning likely reflect variation in efficiency and organization of underlying neural processes (Nagel et al., 2011; Osaka et al., 2003).

This variability becomes particularly apparent under conditions of increasing cognitive demand, where limited-capacity WM systems have to flexibly adapt to varying processing requirements. Experimental paradigms that systematically manipulate WM load therefore provide an important framework for investigating inter-individual variability in WM functioning. One well-established paradigm that robustly induces WM load is the *N*-back task (Kirchner, 1958; Owen et al., 2005). In the *N*-back task, participants monitor a sequence of stimuli and indicate whether the current item matches an item presented *n* trials before. As *n* increases, the task places progressively greater demands on WM processing capacity. Successful performance requires continuous updating of WM representations, while irrelevant information must be suppressed (Palomäki et al., 2012; Pesonen et al., 2007; Rac-Lubashevsky & Kessler, 2016; Scharinger et al., 2017).

Behavioral performance during the *N*-back task is often quantified using signal detection theory metrics (Swets et al., 1961) such as *d*-prime (*d'*), which indexes discrimination ability of targets from non-targets by calculating the relative proportion of hits minus false alarms (Macmillan & Creelman, 1990). A decrease in *d'* indexes reduced discrimination performance, which has been observed as a result of increasing WM load (Jaeggi et al., 2010).

Neural responses elicited by the *N*-back task have been studied in electrophysiological research (Palomäki et al., 2012; Pesonen et al., 2007; Scharinger et al., 2015). Specifically, time-locked

neural responses derived from electroencephalographic (EEG) recordings, also known as event-related potential (ERPs), are especially suitable to investigate cognitive mechanisms because they often temporally precede behavioral responses and can provide better discrimination across sub-types of memory engagement (Huang et al., 2025). A prominent ERP component that has emerged in the context of WM performance is the P300, defined by a positive amplitude deflection between 250 and 500 ms, observable at centro-parietal electrodes post-stimulus onset (Scharinger et al., 2017; Soltani & Knight, 2000). The P300 amplitude reflects processes of attention allocation and information updating (Gray et al., 2004; Picton, 1992; Sutton et al., 1965). In fact, two subcomponents of the P300 with partially distinct roles have been proposed: an earlier fronto-central P300a, associated with stimulus-driven attentional orienting and novelty detection; and a later centroparietal P300b, more closely linked to task-relevant processing, including attentional resource allocation and context updating (Polich, 2007). There are several studies demonstrating a robust modulation reflected in P300 amplitude decrease as a function of increased WM demands (McEvoy et al., 2001; Scharinger et al., 2015; Watter et al., 2001), leading to a general assumption that the decreasing P300 amplitude might reflect the internal distribution of processing resources in order to manage working memory demands (Duncan et al., 2009). This sensitivity to task difficulty, together with its established functional role, makes the P300 a particularly suitable neural correlate for assessing inter-individual differences in neural responsiveness during WM.

Although behavioral performance and P300 amplitude are both sensitive to increasing WM load, they may reflect partially distinct aspects of WM functioning, and their relationship remains incompletely characterized (Christie et al., 2013; Lenartowicz et al., 2021; Voegtle et al., 2022). These findings suggest that inter-individual variability in WM may not be fully explained by either behavioral performance or task-evoked responses alone but may additionally depend on intrinsic properties of the underlying neural system organization. Resting-state EEG provides access to such intrinsic brain dynamics and reflects the ongoing organization of large-scale brain activity thought to index the functional organization of neural systems (Cabral et al., 2014; Foster et al., 2016; Mitra & Raichle, 2016).

Resting-state neural activity reflects spontaneous and coordinated activity of large-scale brain networks that support cognitive functioning (Bressler & Kelso, 2016; Varela et al., 2001; Zanesco et al., 2020). Rather than representing passive or random activity, intrinsic neural dynamics are increasingly understood as part of the functional architecture and organization of neural systems, potentially reflecting stable inter-individual differences that contribute to

variability in cognitive functioning (Campbell & Schacter, 2017; Dubois & Adolphs, 2016). Contemporary models of brain function further emphasize that intrinsic brain activity is inherently dynamic, with large-scale neural networks continuously transitioning between coordinated functional configurations over time (Bressler & Tognoli, 2006; Preti et al., 2017).

A promising approach for characterizing the fast spatiotemporal organization of intrinsic brain activity is the analysis of topographic voltage patterns in scalp-recorded EEG data (Michel & Koenig, 2018a). Resting-state EEG activity is characterized by brief periods during which the spatial configuration of scalp-recorded electrical activity remains relatively stable for approximately 40 to 120 milliseconds before rapidly transitioning into distinct configurations over time. These transient periods of stable scalp topographies, also known as *microstates* (Lehmann et al., 1987), are thought to reflect coordinated large-scale neural activity and have been proposed as markers of the temporal dynamics underlying global brain network organization (Michel & Koenig, 2018a). As such, they provide a complementary perspective to spectral features, which primarily characterize the strength of neural activity, by capturing the temporal organization of intrinsic neural activity.

The literature identifies four canonical data-driven clusters (microstate A-D) with remarkable consistency across individuals, accounting for a large proportion of the observed variance in EEG time series during rest (Michel et al., 2024; Michel & Koenig, 2018a; Tarailis et al., 2024). By quantifying how long individual microstate episodes persist before transitioning to another state (duration), how frequently these states occur (occurrence), and the proportion of total recording time spent in each state (coverage), microstate analysis provides a detailed characterization of the temporal organization of large-scale neural activity. Current work supports large consistency regarding these temporal parameters. At the same time, understanding about stability or change in resting-state microstates with age is limited (Michel & Koenig, 2018a; Tomescu et al., 2018; Zanesco et al., 2020). Considering intrinsic neural organization at rest in the context of cognitive aging might therefore provide a valuable component to better understand inter-individual differences in healthy older adults.

The precise functional role of canonical microstates remains an active topic of debate, but converging evidence from source localization, EEG-fMRI, and task-based studies suggest partially dissociable functional roles across microstate classes. Microstate A and B have most consistently been linked to sensory and perceptual processing, particularly involving auditory and visual networks, respectively (Bréchet et al., 2019; Britz et al., 2010; Custo et al., 2017).

In contrast, microstate C and D appear more strongly associated with higher-order cognitive processing and large-scale associative networks. Specifically, microstate C has been linked to activity in regions of the default mode and salience networks and has been associated with internally directed cognition and task processing (Bréchet et al., 2019; Britz et al., 2010). Microstate D, in turn, has been associated with frontoparietal and dorsal attention networks and has repeatedly been linked to attentional control (Custo et al., 2017; Murphy et al., 2018).

Given the proposed association of microstate C and D with higher-order cognitive and attentional networks, longer microstate duration may indicate a greater tendency of the brain to maintain functionally coordinated network states over time, potentially supporting more efficient allocation of attentional and working memory resources during task performance. Consequently, resting-state microstate dynamics may be associated not only with absolute behavioral and neural task responses, but also with the degree to which these responses adapt to increasing cognitive demand.

Despite evidence that both behavioral performance and P300 amplitude are sensitive to increasing WM demands, the relationship between these task-evoked measures remains poorly understood. Moreover, although resting-state microstates have been proposed as markers of intrinsic neural system organization, relatively little is known about how microstate dynamics relate to inter-individual differences in neural and behavioral responses during WM processing in older adults. Understanding these relationships may provide important insights into whether variability in WM reflects differences in task-evoked processing, intrinsic neural organization, or the interaction between both.

In the current study we recorded resting-state EEG and task EEG data during the *N*-back task in a sample of cognitively healthy older adults to address three aims: (1) Within this dataset and as a foundation to the other aims, we first validated the *N*-back paradigm by testing whether WM modulated task-evoked behavioral and neural measures. We hypothesized that *d'*, indexing discrimination accuracy (Hypothesis 1a), and P300b amplitude, indexing attentional orientation (Hypothesis 1b), would significantly decrease with increasing *N*-back task load. (2) Next, we evaluated the associations between behavioral and neural task-evoked measures. We hypothesized that modulation in *d'* would be positively associated with modulation in the P300b amplitude, reflecting task-evoked modulation coupling (Hypothesis 2a). Additionally, we hypothesized that the absolute *d'* values would be positively associated with absolute P300b amplitude (Hypothesis 2b). (3) We finally tested the associations between resting-state EEG

microstates and task-evoked measures (d' prime and P300b amplitude). Our primary analysis focused on microstates C and D, given their more consistent link to higher-order cognitive processing, and mean duration of these configurations, given its reflection of temporal stability. We expected that these parameters would have the strongest potential for effects. Specifically, we hypothesized that longer mean duration of microstate C (Hypothesis 3a) and D (Hypothesis 3b) would be positively associated with behavioral- and neural task-evoked measures.

METHODS AND MATERIALS

Sample

As part of a larger longitudinal study investigating the effects of language training on cognitive performance, neural signatures, and resting-state brain dynamics, participants were recruited at two different sites: the University of Florida (USA), and the University of Granada (Spain). The study was approved by the respective local university ethics committees, and all participants provided written informed consent and received financial compensation for their participation. The present study focused exclusively on pre-training assessment.

Participants at both sites were recruited from the local community through advertisements and at the University of Florida also through IRB-approved participant registries. All participants were screened for general health and eligibility for EEG recording. All participants were right-handed, neurologically healthy, and had no recent history of stroke, concussion, or signs of neurodegenerative cognitive decline.

Across the two sites, the final analysis sample comprised 33 older adults aged 60 to 83 years ($M = 68.74$, $SD = 6.31$; 61.8% female). Cognitive status was assessed using the Montreal Cognitive Assessment (MoCA; Nasreddine et al., 2005: $M = 26.35$, $SD = 2.07$, Range = 22 – 30). Participants recruited in Gainesville ($N = 17$; $M_{Age} = 70.47$, $SD_{Age} = 7.68$; $M_{MoCA} = 26.88$, $SD_{MoCA} = 1.72$) and Granada ($N = 16$; $M_{Age} = 66.80$, $SD_{Age} = 4.16$; $M_{MoCA} = 25.56$, $SD_{MoCA} = 2.18$) did not differ significantly in age ($p = .104$) or MoCA performance ($p = .065$).

A sensitivity analysis was conducted using G*Power (version 3.1) to determine the smallest detectable effect sizes given the available sample size. For correlation-based analysis (two-tailed, $\alpha = .05$, power = .80, $N = 33$), the study was sensitive to effects of approximately $r = .45$. For regression models testing individual predictors ($\alpha = .05$, power = .80, $N = 33$), the minimum detectable effect size was $f^2 = .25$. These values indicate that the study was adequately powered to detect medium-to-large effects.

Procedure and Measures

At both sites, the session started with informed written consent, followed by the MoCA to capture cognitive capacity. Additionally, participants completed several baseline tasks not further considered here (including a semantic verbal fluency task, a personal social network survey, and a story-telling task to collect spontaneous speech). After behavioral assessment, participants were fitted with an EEG cap and placed in a shielded soundproof booth. Participants first completed the resting-state EEG sequence during which they were asked to rest (5 minutes with eyes open; 5 minutes with eyes closed; order counterbalanced across participants), followed by an *N*-back task to assess WM.

As shown in Figure 1, this version of the *N*-back task used visual stimuli consisting of letters presented centrally on a white screen (font Consolas, 38pt). The task included a 0-back control condition, and two levels of WM load (1-back, and 2-back). Participants started the task with detailed instructions and practice blocks with 20 trials for each condition. Participants were told that they would see a single letter on the computer screen, one at a time, and that the letter would be either a target (to which they were to respond via the keyboard) or a non-target. In the 0-back condition, the letter Z was the target, and all other letters were non-targets. In the 1-back condition, the target was the letter that came one before the one currently on-screen (independent of what letter it was); and in the 2-back condition, the target was the letter that came two before the one currently on-screen (again independent of what it was).

The three load condition blocks were presented in a sequential order (0-Back, 1-Back, 2-Back), and instruction reminders were presented again before the beginning of each block. Each block consisted of 36 items (12 target letters, 6 distractor letters, and 18 novel letters). Each experimental trial began with a pre-clean screen of 100 ms. Next, the letter was presented for 500 ms, followed by an interstimulus interval of 2000 ms consisting of a fixation cross in the center of the screen. The task was implemented using Eprime3.0 software (Psychology Software Tools, Pittsburgh, PA).

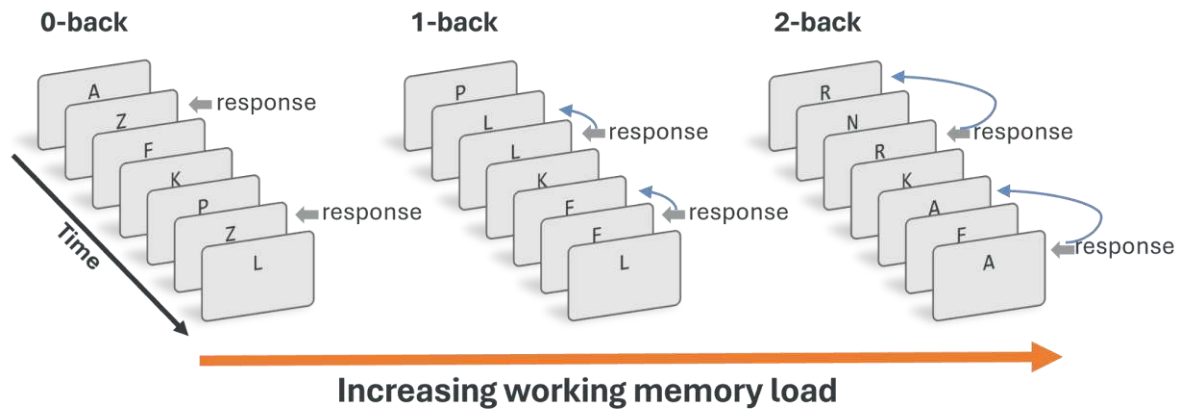


Figure 1. Schematic illustration of the visual N-back working memory task. Participants completed three working memory conditions with increasing cognitive demand: 0-back, 1-back, and 2-back. In the 0-back condition, participants responded whenever the pre-specified target letter ("X") was presented. In the 1-back condition, participants responded when the current letter matched the immediately preceding letter. In the 2-back condition, participants responded when the current letter matched the letter presented two trials earlier. Stimuli consisted of sequentially presented uppercase letters, and task difficulty increased with increasing working memory load. The figure illustrates example stimulus sequences and target trials.

Working memory performance

Accuracy on the *N*-back task was quantified using d' from signal detection theory (Swets et al., 1961), which captures the ability to discriminate targets from non-targets independent of response bias. For each participant and load condition (0-Back, 1-Back, 2-Back), trials were classified as target (signal) or non-target (noise, including novel and distractor trials). Hits were defined as correct responses to target trials, whereas false alarms were defined as responses to non-target trials. Hit and false alarm rates were calculated separately for each load condition. To account for extreme values (i.e., hit or false alarm rates of 0 or 1), rates were adjusted using the Hautus correction, whereby proportions were computed as $(\text{hits} + 0.5) / (\text{number of target trials} + 1)$ and $(\text{false alarms} + 0.5) / (\text{number of non-target trials} + 1)$. d' was then calculated as the difference between the z-transformed hit rate and false alarm rate. Higher d' values indicated better discrimination performance (higher WM functioning).

EEG recording and pre-processing

Data acquisition at testing sites

Continuous EEG was recorded using comparable acquisition parameters at both sites but slightly different electrode configurations. At the University of Florida, continuous EEG was recorded using an ActiChamp Brain Products system (Brain Products GmbH, Germany) with an array of 32 Ag/AgCl scalp active electrodes (BrainVision Analyzer Software, Version 2.2.2, 2021, Gilching, Germany: Brain Products GmbH., n.d.), arranged according to the 10–20 system. For online referencing and later re-referencing, two electrodes were placed on the right and left mastoids, respectively. Impedance was maintained below 10 k Ω . Additionally, two sets of bipolar electrooculogram (EOG) electrodes were placed above and below the left eye and on the right and left canthi to measure vertical and horizontal eye movements, respectively. The signal was amplified using a BrainVision actiCHamp amplifier with 24-bit analog-to-digital conversion (Brain Products, München, Germany) and continuously recorded at a sampling rate of 1000 Hz without online filtering.

At the University of Granada, continuous EEG signal was recorded using a Neuroscan system (Compumedics Neuroscan, Charlotte, NC, USA) and recorded with Curry 7 with a 40-channel Ag/AgCl electrode cap (Quick-Cap, Neuroscan Inc.), arranged according to the international 10–20 system including mastoid electrodes (A1, A2) and additional posterior channels (e.g., PO1, PO2). The signal was amplified and digitized using a Scan NuAmps system (Compumedics Neuroscan Ltd., VIC, Australia). The sampling rate was 1000 Hz, and an online 0.01–100 Hz band-pass filter was applied during acquisition. EEG activity was referenced online to the mastoids, and horizontal and vertical EOG activity was recorded using electrodes placed at the outer canthi of the eyes and above and below the left eye to monitor eye movements. Throughout the recording, electrode impedances were kept below 10 k Ω .

Because electrode layouts differed slightly between sites, preprocessing and subsequent analyses were restricted to electrodes common to both systems (Figure 2). This ensured comparable spatial sampling across datasets. In addition, subsequent analyses included study site as a covariate.

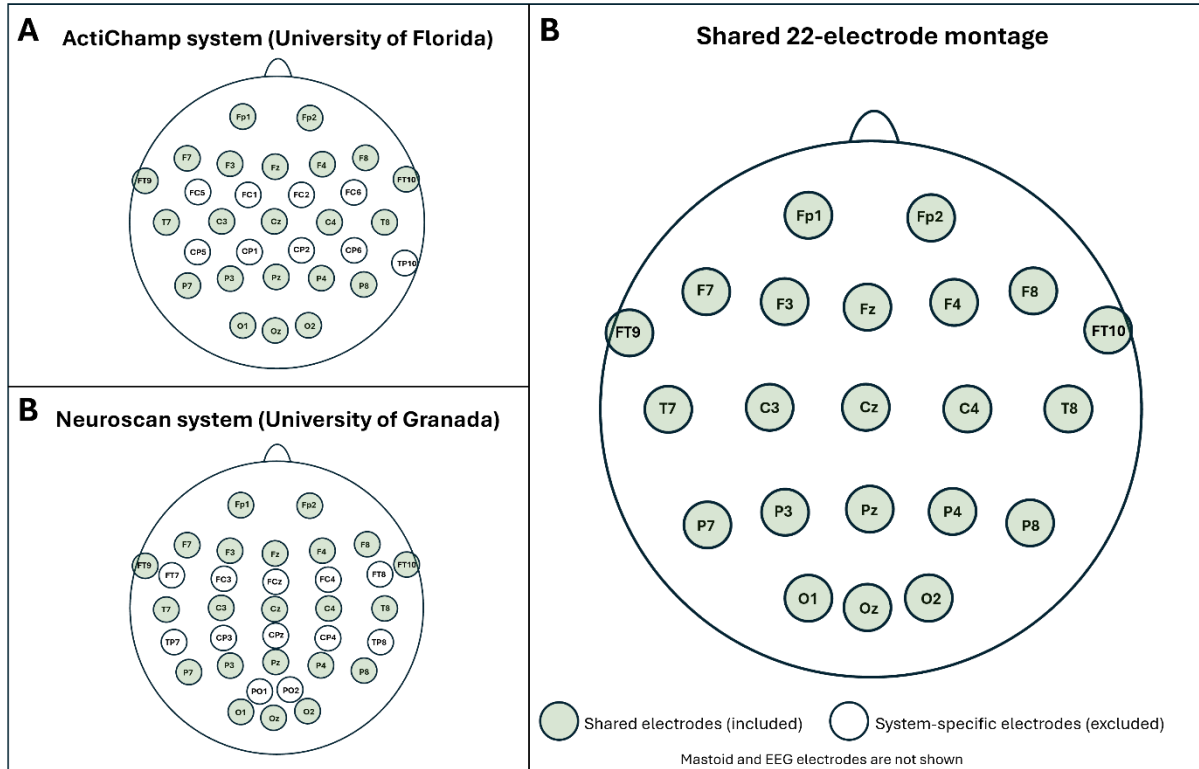


Figure 2. Electrode layouts and shared electrode montage across study sites. Scalp electrode configurations used with the ActiChamp system at the University of Florida (A) and the Neuroscan system at the University of Granada (B). Green electrodes indicate the 22 channels present in both configurations and retained for common-average re-referencing and subsequent analyses; white electrodes indicate system-specific channels that were excluded to ensure comparable spatial sampling across sites. The resulting shared 22-electrode montage is shown in (C). Mastoid reference and electrooculogram electrodes are not displayed.

Preprocessing for resting-state microstates EEG

Raw resting-state EEG for both the eyes open and the eyes closed condition were preprocessed offline using Brain Vision Analyzer (BVA) version 2.3 (Brain Products GmbH, Gilching, Germany). The raw data recorded with the ActiChamp Brain Products system was natively imported in Brain Vision Analyzer. However, the raw EEG recordings acquired with the Neuroscan system and recorded with Curry 7 were exported from the native curry file structure and converted into BVA-compatible file formats using the MNE-Python package (Gramfort et al., 2013) and the BrainVision functionality implemented in MNE. After all the data was imported into BVA, continuous data was visually inspected to ensure overall data quality prior to further processing. Channels exhibiting persistent noise were identified and interpolated using standard spherical interpolation methods.

After visual inspection, data was band-pass filtered to remove slow drifts and high-frequency noise (0.1–40 Hz; zero-phase Butterworth filter, order 4–8 depending on dataset). Independent Component Analysis (ICA) was performed on the entire dataset using 512 steps and a restricted infomax (Gradient) algorithm to identify eye movements and other non-neural artifacts based on their temporal and spatial characteristics and removed following visual inspection. To avoid violating the assumption of independence and to maximize accuracy in eye artifact detection, bipolar eye channels were excluded from ICA, even though the signal from the bipolar eye channels were utilized to maximize accuracy in recognizing ocular artifacts components. The continuous EEG signal was then re-referenced using a common average including electrodes that were identical in both datasets (i.e., 22 electrodes: Fp1, Fp2, F7, F3, Fz, F4, F8, T7, C3, Cz, C4, T8, P7, P3, Pz, P4, P8, O1, Oz, O2, FT9, and FT10; see López-Rojas et al., 2022 for a similar approach). Re-referenced data was exported in a binary and vectorized format and imported into MATLAB (R2025b, MathWorks) for further analysis.

Preprocessing for N-Back EEG and ERP analysis

All raw *N*-Back EEG data were preprocessed offline using BVA version 2.3. The raw data recorded with the ActiChamp Brain Products system was natively imported in Brain Vision Analyzer. The raw EEG data from the Curry system was transformed to be read in by Brain Vision Software using the MNE-Python package (Gramfort et al., 2013) and BrainVision functionality implemented in MNE. In BVA, data was visually inspected to ensure overall data quality (e.g., flat channels, extreme noise) prior to further processing.

Continuous EEG data were band-pass filtered to remove slow drifts and high-frequency noise (0.1–30 Hz; zero-phase IIR Butterworth filter, order 4–8 depending on dataset to better correct for line noise, if present). Horizontal and vertical eye movements were detected using the automatic ICA implemented in BVA. ICA was performed on the entire dataset using 512 steps and a restricted infomax (Gradient) algorithm. To avoid violating the assumption of independence and to maximize accuracy in eye artifact detection, bipolar eye channels were excluded from ICA, but they were utilized to maximize accuracy in recognizing ocular artifacts (Zhang et al., 2024). The EEG signal was then re-referenced using a common average including electrodes that were identical in both datasets (same approach as above). Re-referenced data was segmented into epochs time-locked to the presentation of each stimulus (-200 ms to 800 ms). The signal was baseline-corrected using the -200 ms pre-stimulus interval. Remaining

artifacts were semi-automatically identified using BVA's algorithm, and rejection was confirmed manually. After artifact rejection, the overall mean rejection rate was under 1 %.

For the ERP analysis all trials in each condition ($n = 24$) were included in the average, as including only correct trials would have significantly reduced the signal-to-noise ratio, especially in the 1-back and 2-back conditions. Epochs were averaged per condition and participants across all trial types; 0-Back Targets, 1-Back Targets, 2-Back Targets. ERP analyses focused on the P300b (300ms to 600ms) component at electrode Pz, given its well-established association with WM updating and its characteristic centroparietal topography.

To ensure robustness and to evaluate potential topographical variations, additional ERP measures for P300b were extracted at electrodes Cz and Fz. Furthermore, additional components, namely N200 and P300a at Pz, Cz and Fz, were explored and results from these analyses are reported in the supplementary material.

EEG microstate analysis

Dataset preparation

Resting-state EEG microstate analysis was conducted in MATLAB using EEGLAB 2025.1.0 and the MicrostateLab Toolbox implemented in EEGLAB, following established procedures (Michel & Koenig, 2018a). The analysis was restricted to the eyes-closed resting-state condition. This decision was made because eyes-closed data provides the most established reference condition in canonical microstate research, is less affected by visual processing, and yielded more stable and canonical topographies than eyes-open recordings during preliminary inspection.

To ensure valid spatial comparisons across the two study sites, all datasets were restricted to the intersection of channels available in both datasets (as described above). Posterior electrodes PO1 and PO2 were excluded due to inconsistent availability across the two datasets. For each participant, all non-common channels were removed, and the remaining channels were reordered into an identical sequence. The same channel list and order were used consistently across template estimation, labeling, validation, and backfitting. This harmonization was essential because microstate clustering and backfitting rely on spatial correlations of scalp topographies, which require identical channel configurations across datasets.

Template estimation

Microstate templates were estimated from the pooled eyes-closed dataset across participants from both sites. For each individual recording, Global Field Power (GFP) was computed across electrodes at every sampling point as the standard deviation of the scalp potential distribution. Microstate clustering was restricted to local maxima of the GFP time series, such that only maps corresponding to GFP peaks were submitted to clustering. This procedure ensured that clustering was performed on time points with maximal global signal strength, thereby increasing signal-to-noise ratio and reducing temporal redundancy in the input data.

The extracted GFP-peak maps were pooled across all participants and submitted to modified k-means clustering as implemented in MicrostateLab (function `pop_FindMSMaps`). Clustering was performed using a spatial correlation-based distance metric with polarity ignored (i.e., absolute spatial correlation). All maps were normalized prior to clustering. The clustering algorithm was configured to evaluate solutions between four and seven classes without limiting the number of input maps. To improve stability and reduce the likelihood of local minimum, the clustering procedure was repeated across 20 random iterations. The Sample:atomize-and-agglomerate hierarchical clustering option was not used, such that clustering relied exclusively on the modified k-means procedure.

Solutions from four to seven classes were estimated. Although global explained variance increased with higher numbers of classes, improvement beyond four classes was limited and interpretability decreased. The final solution was therefore fixed at four classes, corresponding to the canonical A-D microstate solution, providing the best balance between explained variance, interpretability, and comparability with prior work.

The pooled mean solution was generated by combining individual microstate maps across all included eyes-closed recordings using polarity-invariant averaging. The resulting four-class solution (Figure 3) was sorted and labeled using the canonical microstate templates described by Koenig et al. (2002) via spatial correlation matching (function `pop_SortMSMaps`). The pooled eyes-closed templates showed high correspondence with the canonical maps ($A = .94$, $B = .91$, $C = .96$, $D = .96$), supporting validity of the final template solution.

Cross-site validation

As a quality control step, microstate templates were estimated separately for the two recording sites using identical clustering and labeling procedure. The resulting site-specific templates

were matched to the Koenig and colleague's (2002) canonical maps and compared with each other using polarity-invariant spatial correlations of their common channels. Correspondence between site-specific templates was high for microstates A and B and moderate for microstate C ($A = .89$, $B = .90$, $C = .61$). Microstate D showed low cross-site correspondence ($r = .10$), indicating reduced stability of this state across the two datasets. This pattern suggests that while the overall microstate structure is largely preserved across sites, individual states, particularly microstate D, may be more sensitive to site-specific factors or noise. Considering these findings, a pooled template approach was retained to ensure a common reference space across participants across sites and to maximize the stability of the overall clustering solution. To account for potential residual site-related variance, study site was included as a covariate in all subsequent statistical analyses.

Backfitting procedure

The final pooled and labeled microstate templates were subsequently applied to each participant's continuous EEG recording using a spatial correlation-based backfitting procedure implemented in MicrostateLab (function `pop_FitMSMaps`). At each time-point (i.e., GFP), the scalp voltage map was compared to all four template maps. Each time point was assigned to the microstate class with which it showed the highest spatial correlation. GFP peak restriction during backfitting was disabled, ensuring full temporal segmentation of the EEG signal. To reduce spurious rapid state transitions and enforce physiologically plausible segment durations, temporal smoothing was applied using a sliding window of 30 ms and a non-smoothness penalty parameter of 5. This smoothing procedure suppresses very short segments and reduces microstate fragmentation, resulting in more stable and interpretable temporal segmentation.

Temporal parameter extraction and quality control

For each participant and microstate, temporal parameters were extracted from the MicrostateLab output (function `EEG.msinfo.MSSStats`). Extracted parameters included coverage, mean occurrence, mean duration, individual explained variance, total explained variance, and total recording time. Coverage was converted from percent to proportion, occurrence was expressed in segments per second, and duration was expressed in milliseconds.

Quality control procedures included verification that coverage values summed to 1 within each participant, inspection of parameter distributions for physiologically plausible ranges, and identification of extreme outliers. No participant was excluded based on these criteria. Final parameter values fell within expected ranges, with mean durations approximately 90–110 ms,

occurrence rates approximately 2–3 Hz, and coverage values approximately .15–.40 across microstates.

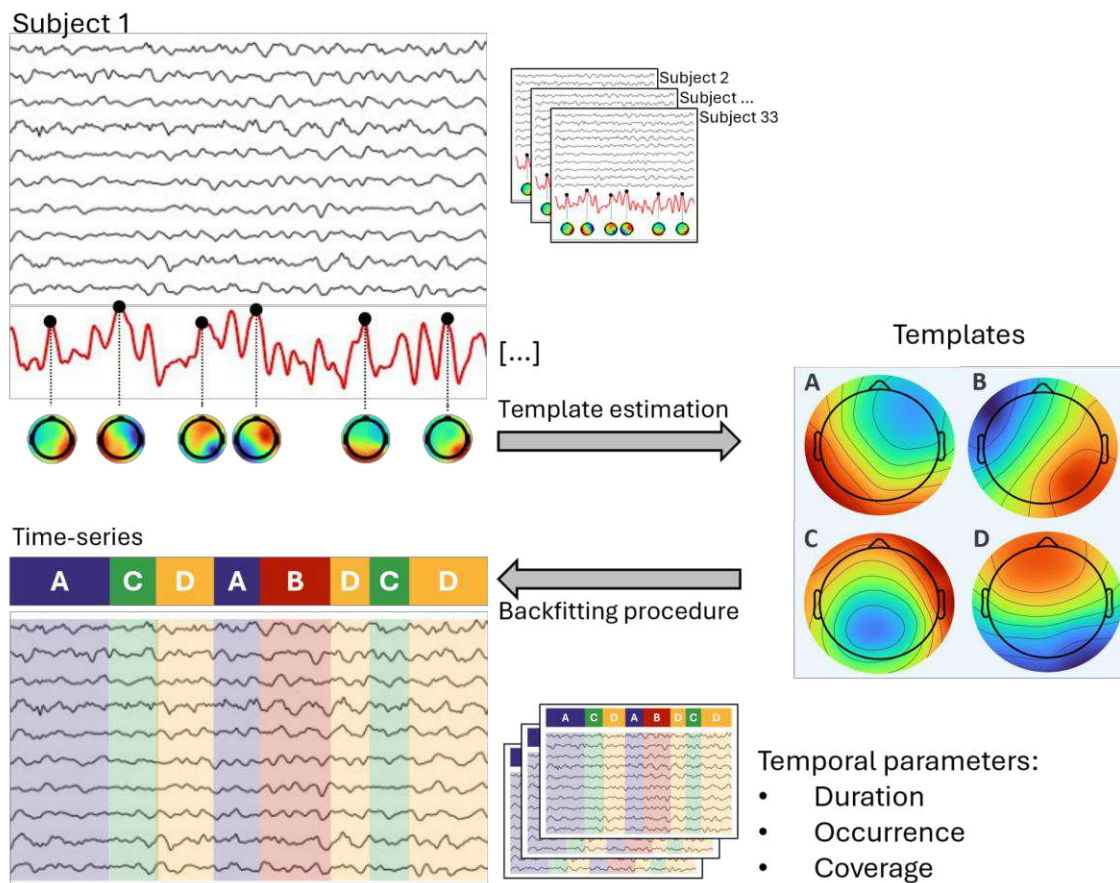


Figure 3. Schematic overview of the resting-state EEG microstate analysis pipeline. Continuous eyes-closed EEG recordings were first reduced to scalp topographies corresponding to local maxima of the Global Field Power (GFP) time series, representing moments of maximal global signal strength (top left). GFP-peak maps from all participants were pooled and submitted to modified *k*-means clustering, yielding four canonical microstate templates (A–D) identified by spatial correlation with the canonical solution described by Koenig et al. (2002) (right panel). The resulting template maps were subsequently backfitted to each participant's continuous EEG recording by assigning each time point to the template exhibiting the highest polarity-invariant spatial correlation, followed by temporal smoothing to reduce spurious rapid state transitions (bottom left). This procedure produced a complete temporal segmentation of the resting-state EEG into successive microstate classes (A–D), for which temporal parameters including duration, occurrence and coverage were extracted for subsequent statistical analyses.

Statistical analysis

Statistical analyses were conducted in R Studio (Version 2021.09.2) using lme4 package (Bates et al., 2015) for linear mixed-effects models (LMMs). Based on the dependencies among data points and the robustness for unbalanced datasets, it was deemed appropriate to fit LMMs (see Frömer et al., 2018 for an overview of the advantages of LMMs in EEG research). Age and MoCA scores were mean centered prior to analysis, and site was defined as a categorical variable (Gainesville vs. Granada). All three variables were included as covariates across all models to ensure that potential effects were not driven by age, cognitive status, or site-specific differences.

Task-specific modulation (Hypotheses 1a & 1b)

To evaluate whether the *N*-back task elicited the expected load-dependent modulation in behavioral WM performance and ERP amplitude, LMMs were fitted. Behavioral WM performance (d') was modeled as a continuous outcome variable with fixed effects of load (0-Back, 1-Back, 2-Back), age, MoCA, and study site, as well as a by-subject random intercept to account for repeated measurements across load levels. ERP amplitude was analyzed analogously.

To further characterize inter-individual variability in load-related modulation, exploratory models were additionally fitted with load specified as a continuous predictor (0-Back, 1-Back, 2-Back) and a by-subject random slope for load. Variance components were inspected to evaluate the relative contribution of between-person differences in overall performance/amplitude levels (random intercepts) and load-related change (random slopes).

Associations between behavioral and neural modulation (Hypotheses 2a & 2b)

To examine whether load-dependent changes in neural responses were associated with changes in behavioral performance (Hypothesis 2a), two complementary approaches were used. First, change scores were computed as the difference between the 2-back and the 0-back conditions (resulting in $\Delta d'$ and $\Delta P300b$), reflecting load-dependent modulation across the full range of WM demands. The 2-back versus 0-back contrast was selected because it represents the maximal difference in task difficulty within the paradigm and therefore provides the most sensitive index of load-related change. As this transformation resulted in a single observation per participant and removed the within-subject structure of the data, associations between behavioral and neural modulation were assessed using multiple linear regressions. $\Delta d'$ was

entered as the dependent variable, with $\Delta P300b$ amplitude at Pz as the primary predictor, controlling for age, MoCA, and study site.

Second, to assess whether absolute neural response amplitude was associated with behavioral performance across load levels (Hypothesis 2b), LMMs were fitted. In this approach, d' was modeled as the outcome variable, with fixed effects of load and P300b amplitude (continuous), and a by-subject random intercept. This model retained the repeated-measures data structure and allowed for examination of overall associations between behavioral performance and neural responses.

Associations between resting-state microstate dynamics and task-related outcomes (Hypotheses 3a & 3b)

To examine whether resting-state microstate dynamics were associated with task-related behavioral and neural responses, LMMs were fitted using P300b amplitude at Pz or d' as dependent variables, respectively. In the primary models, both microstate C and D parameters were entered simultaneously as predictors to assess their unique contributions to task-related outcomes. Each model included a by-subject random intercept to account for repeated measurements across WM load levels.

To evaluate robustness and specificity of potential effects across alternative microstate metrics, parallel models were conducted using coverage and occurrence parameters, yielding a total of six models (see supplementary material for details). To control for multiple comparisons across these models, false discovery rate (FDR) correction (Benjamini-Hochberg procedure) was applied across the six tests. Duration-based models were considered hypotheses-driven, whereas coverage and occurrences analysis constituted exploratory extensions.

RESULTS

Task modulation of behavioral performance and ERP amplitude (Hypothesis 1)

As shown in Figure 4A, for behavioral performance, the main effect of load was significant ($F(2,64) = 73.95, p < .001$), in that d' decreased with increasing task load, confirming Hypothesis 1a. Specifically, performance was lower in the 1-back condition ($\beta = -0.94, SE = 0.18, t(64) = -5.33, p < .001$) and further reduced in the 2-back condition ($\beta = -2.13, SE = 0.18, t(64) = -12.13, p < .001$), relative to the 0-back condition. In this model, age was negatively associated with performance ($F(1,29) = 6.78, p = .014$), but MoCA and study site were not ($p > .329$).

Moreover, as shown in Figure 4B, P300b amplitude at Pz also showed a significant effect of load ($F(2, 64) = 5.42, p = .007$). Relative to the 0-back condition, P300b amplitude was reduced in both the 1-back ($\beta = -0.56, SE = 0.23, t(63) = -2.39, p = .020$) and the 2-back ($\beta = -0.73, SE = 0.23, t(63) = -3.15, p = .002$) condition, thus confirming Hypothesis 1b. In this model, age, MoCA, or study site were not significant ($p > .479$).

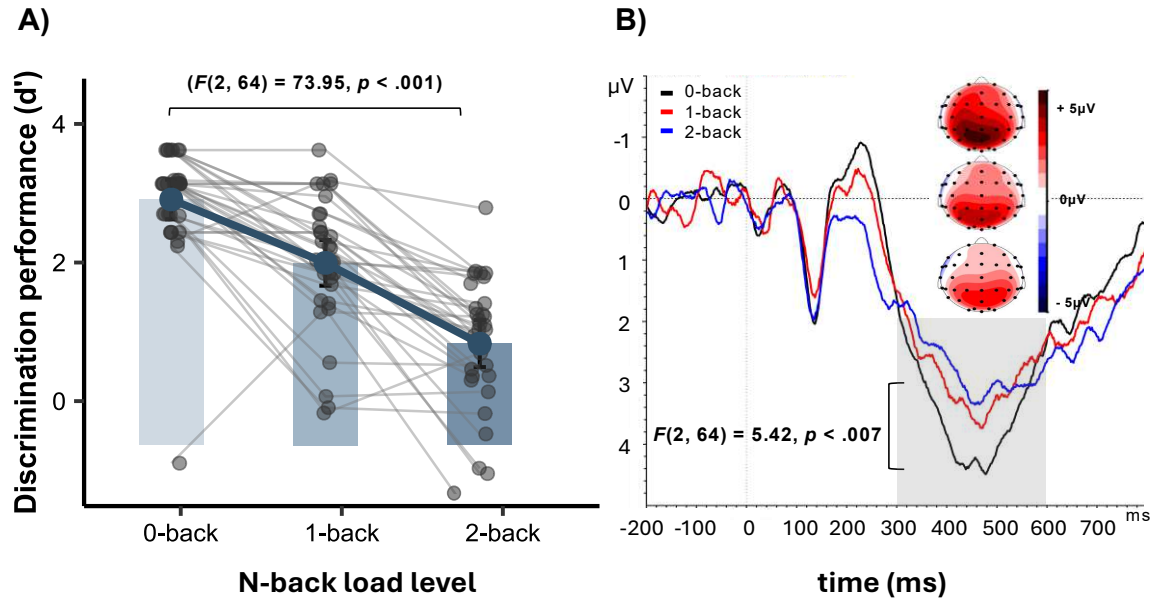


Figure 4. Working memory load modulated behavioral performance and P300b amplitude. (A) Discrimination performance (d') across the 0-back, 1-back, and 2-back working memory load conditions. Individual participants are shown as gray circles connected by gray lines. Blue circles and connecting line represent the group means, and shaded bars indicate corresponding mean values. Increasing working memory load was associated with a progressive reduction in discrimination performance. (B) Grand-average target-locked ERP waveforms recorded at electrode Pz for the 0-back (black), 1-back (red), and 2-back (blue) conditions. The highlighted interval corresponds to the P300b analysis window (300–600 ms). Increasing working memory load was associated with a significant reduction in P300b amplitude.

To next explore inter-individual differences in task-related modulation, exploratory random-slope models were fitted with load coded continuously (0, 1, 2). For both behavioral performance and P300b amplitude, models indicated substantially greater between-person variability in overall levels than in load-related change. Specifically, for d' the estimated random-intercept variance (0.137) exceeded the random-slope variance (0.022); and for P300b

amplitude the random-intercept variance (6.014) greatly exceeded the random-slope variance (0.095). In both cases, model fits were singular, suggesting minimal reliable variability in load-related slopes across participants.

Together, these findings confirm that increasing WM load was associated with both reduced behavioral performance (d') and decreased P300b amplitude. Further, inter-individual differences were expressed primarily in overall levels of behavioral performance and neural responsiveness rather than in the magnitude of WM load-related modulation.

Associations between behavioral and neural task response (Hypothesis 2)

As shown in Figure 5A, the association between neural and behavioral modulation was not significant ($\beta = 0.12$, $SE = 0.14$, $t(28) = 0.86$, $p = .396$), indicating that inter-individual differences in load-related changes in P300b amplitude did not predict changes in task performance (d'). Thus, Hypothesis 2a was not supported. Furthermore, as graphically depicted in Figure 5B, absolute d' values and P300b amplitude were not significantly associated ($F(1, 47) = 3.10$, $p = .085$), although the effect was in the expected positive direction. Thus, Hypothesis 2b was not supported, indicating that task-evoked neural responses were not robustly associated with inter-individual differences in task performance.

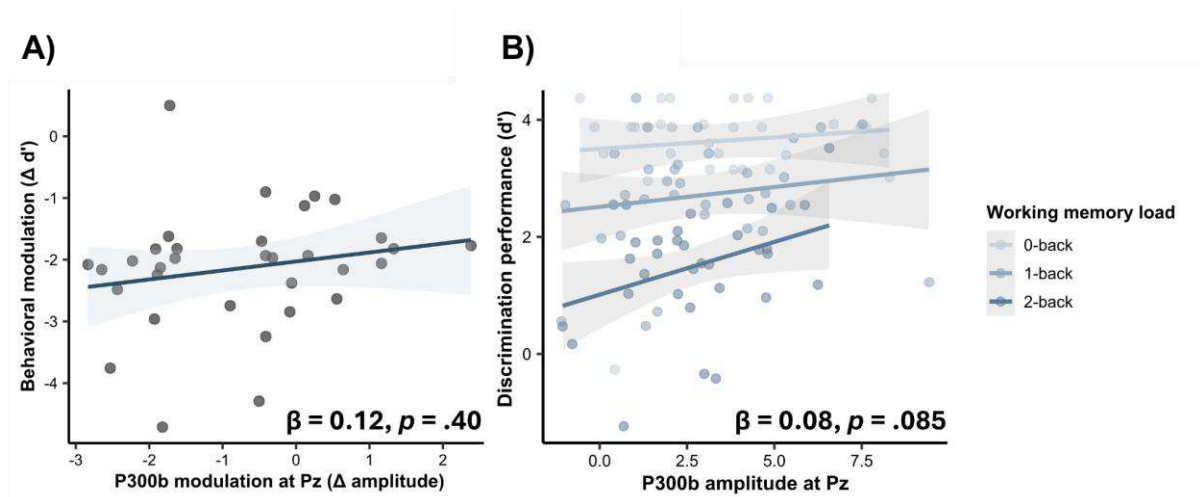


Figure 5. Associations between P300b amplitude and working memory performance. (A) Relationship between load-related P300b modulation (Δ amplitude; 2-back – 0-back) and the corresponding change in discrimination performance ($\Delta d'$). No significant association was observed between neural and behavioral modulation across increasing working memory load. **(B)** Association between absolute P300b amplitude at electrode Pz and discrimination performance (d') across the 0-back, 1-back, and 2-back conditions. Each point represents an

individual participant within a task condition, with colors indicating working memory load. Regression lines and shaded areas represent, respectively, the estimated linear relationship and the 95% confidence intervals for each condition. Although larger P300b amplitude tended to be associated with better behavioral performance, this relationship did not reach statistical significance.

Associations between resting-state microstate duration and task-evoked measures (Hypothesis 3)

As shown in Figure 6A, a significant positive association between microstate C duration and P300b amplitude was observed ($F(1, 33) = 5.62, p = .024$), such that longer mean duration of microstate C was associated with larger P300b amplitude ($\beta = 0.03, SE = 0.01, t(33) = 2.37$). In contrast, as shown in Figure 6B, microstate D duration was not significantly associated with P300b amplitude ($F(1, 33) = 0.61, p = .439$). The main effect of load remained significant ($F(2, 65.15) = 5.59, p = .006$) in this model. As such, Hypothesis 3a was only partially supported by the results.

At the same time, microstate D duration was significantly associated with task performance ($F(1,33) = 7.90, p = .008$), in that longer duration of microstate D was related to lower d' ($\beta = -0.23, SE = 0.008, t(33) = -2.81$; see Figure 6C). In contrast, microstate C duration was not significantly associated with task performance ($F(1, 33) = 0.09, p = .772$; see Figure 6D). Again, the main effect of load remained significant ($F(2, 66) = 76.26, p < .001$) in this model. Therefore, Hypothesis 3b was also only partially supported by the results.

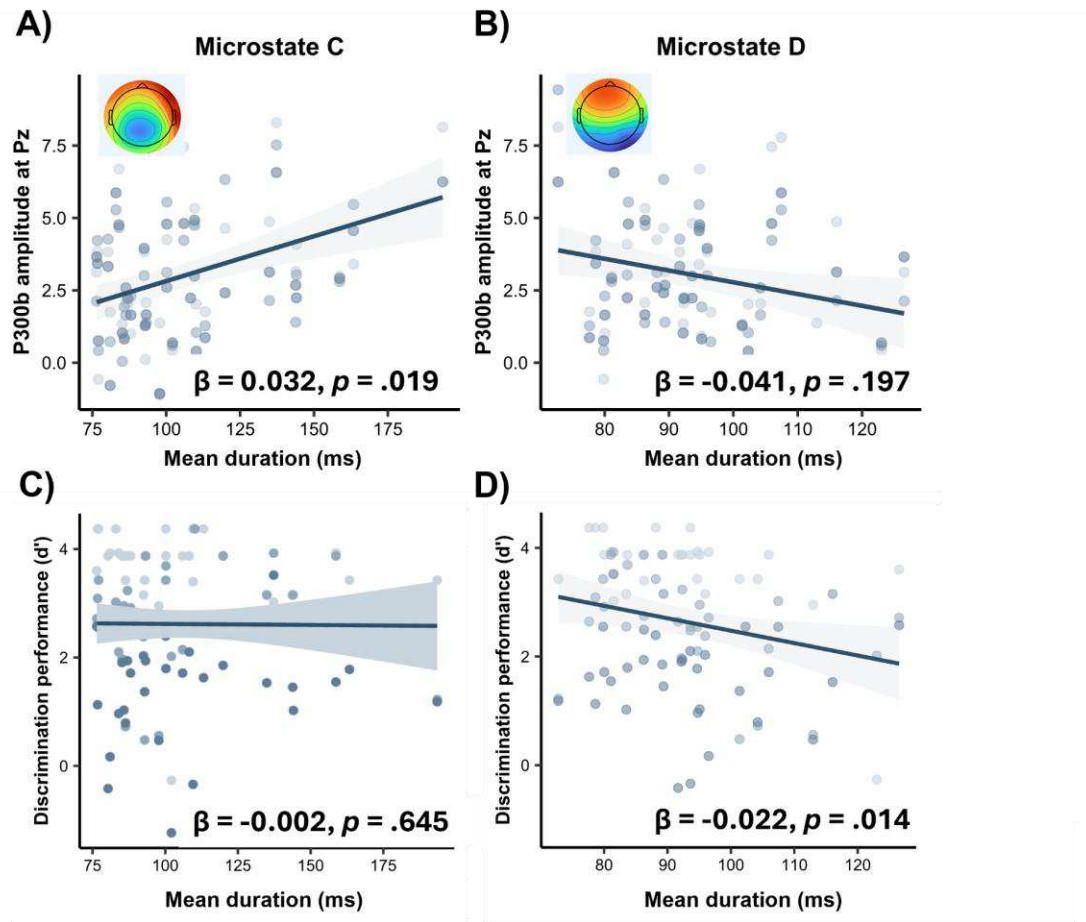


Figure 6. Associations between resting-state microstate duration, task-evoked P300b amplitude, and working memory performance. (A) Longer mean duration of microstate C during eyes-closed resting-state EEG was associated with larger P300b amplitude at electrode Pz during the working memory task. **(B)** Mean duration of microstate D was not significantly associated with P300b amplitude. **(C)** Mean duration of microstate C was not associated with discrimination performance. **(D)** Longer mean duration of microstate D was associated with poorer discrimination performance. Each point represents an individual participant. Regression lines and shaded regions, respectively, represent the estimated linear relationship and 95% confidence intervals obtained from the linear mixed-effects models.

DISCUSSION

The present study investigated whether inter-individual differences in WM functioning among cognitively healthy older adults are reflected in task-evoked neural and behavioral responses, and in stable characteristics of intrinsic neural organization. Three main findings emerged. First, increasing WM load robustly modulated both behavioral performance and P300b amplitude, confirming that both measures constitute sensitive markers of WM demand among

older adults. Second, despite their common sensitivity to WM load, neural and behavioral modulation were not significantly coupled, suggesting older adults' task-evoked neural responsiveness may not directly translate into inter-individual differences in behavioral performance. Third, resting-state microstate dynamics, as a reflection of intrinsic neural organization, were associated with both neural and behavioral task outcomes, with distinct microstate configurations showing selective relationships with each domain. Together, these findings suggest that while task-evoked measures primarily capture common responses to increasing WM demand, stable characteristics of intrinsic neural organization (as assessed via microstates) offer important insight into inter-individual differences in overall levels of neural responsiveness and cognitive WM performance in cognitively healthy older adults. These novel findings will be discussed in more detail in the following paragraphs.

WM load elicits robust task-related response modulation, with inter-individual differences in overall neural response levels

The present findings replicate a well-established pattern in the WM literature, demonstrating that participants become progressively less accurate in distinguishing targets from non-targets with increasing WM demands (Bopp & Verhaeghen, 2007; Missonnier et al., 2005; Owen et al., 2005). In parallel, we showed that P300b amplitude decreased with increasing WM load, consistent with prior evidence linking the P300b component to the cognitive processes of attentional resource allocation and context updating (Donchin & Coles, 1988; Polich, 2007; Scharinger et al., 2015). These findings confirm that both d' and P300b amplitude provide robust and sensitive markers of WM load processing in older adults.

While the expected WM load effects were clearly detected in our data, a particularly novel finding was the observed pattern of inter-individual variability underlying these effects. That is, participants differed substantially in their *overall* levels of behavioral performance as well as their P300b amplitude, whereas *load-related trajectories* in these measures showed comparatively little between-person variability. This distinction may be particularly informative in the context of the population studied here, namely cognitively healthy older adults. Resource-based accounts of WM in aging propose that increasing task loads place progressively greater demands on a limited pool of cognitive resources, resulting in reduced performance and neural responsiveness under high load, and especially so with greater age (Cabeza et al., 2018; Reuter-Lorenz & Cappell, 2008; Salthouse, 1996). The present findings are consistent with this interpretation, as both behavioral performance and neural

responsiveness in our sample of older adults declined systematically with increasing WM load. However, if inter-individual differences primarily reflected variation in the availability of these resources, one might have expected increased inter-individual variability as a function of greater WM demand. Instead, the relatively homogeneous load-related trajectories observed in this study suggest that increasing WM demand affected participants in largely comparable ways.

Among cognitively healthy older adults, load-related modulation may capture a relatively common response to increasing task demands while providing limited information about why some individuals perform better than others. In contrast, the substantial variability observed in overall level of behavioral performance and P300b amplitude in our data suggests that stable characteristics of the underlying neural system may be informative for understanding inter-individual differences in WM functioning. This interpretation is broadly consistent with theories emphasizing cognitive reserve, neural efficiency, and brain maintenance, which propose that older adults differ in integrity and effectiveness of neural systems supporting cognition, even in the absence of overt cognitive impairment (Cabeza et al., 2018; Nyberg et al., 2012; Stern, 2002). From this perspective, older adults may exhibit similar load-related reductions in performance and neural responsiveness while differing substantially in the overall level at which these systems operate. Consequently, inter-individual differences in WM functioning in older adults may be reflected less in differential responses to increasing cognitive demand but more in stable between-person differences in the organization, efficiency, or integrity of the neural systems supporting cognitive function.

Task-related neural responsiveness does not directly translate into behavioral performance

Contrary to our hypotheses, neither load-related modulation nor absolute P300b amplitude was significantly associated with behavioral performance in our older adults. While these findings may appear surprising given the robust sensitivity of both measures to increasing WM load, load-related change represented only a relatively small source of inter-individual variability in the present sample. As discussed above, the task manipulation affected both behavior and neural activity at the group level, but the magnitude of these load-related changes was relatively homogeneous across individuals. Therefore, the absence of a behavioral-P300b modulation coupling in our sample may partially reflect limited between-person variability in modulation of the slopes (i.e., the extent of the range in modulation between load levels). However, it may

also indicate that load-related changes in neural responsiveness and behavioral performance capture related, but not identical, aspects of WM functioning.

P300 amplitude is commonly interpreted as an index of attentional resource allocation, stimulus evaluation, and context updating, with larger amplitude reflecting greater neural responsiveness to task-relevant information (Kok, 2001). Consistent with previous research (McEvoy et al., 2001; Scharinger et al., 2015), we found that increasing WM demand was associated with systematic reductions in P300 amplitude. However, the absence of a significant association between neural and behavioral modulation suggests that this neural responsiveness may not translate directly into inter-individual differences in behavioral performance among cognitively healthy older adults.

This interpretation is in line with evidence that the relationship between P300 amplitude and WM performance differs across the adult lifespan. Saliasi and colleagues (2013) reported substantial differences in P300-behavioral coupling in younger and older adults, suggesting that larger amplitude do not necessarily reflect better behavioral outcomes in aging. Similarly, Kok (2001) argued that P300 amplitude primarily reflects the allocation of processing resources rather than behavioral performance itself. More recent work, however, has emphasized that P300 amplitude reflects specific processing operations that do not translate directly into task success (Verleger, 2020). One explanation is that P300 amplitude reflects task engagement, whereas behavioral performance reflects the cumulative outcome of multiple interacting processes. Consequently, larger P300 amplitude may indicate greater responsiveness to task-relevant information without necessarily implying more accurate performance. Likewise, reductions in P300 amplitude under increasing WM load may primarily reflect a common neural response to greater processing demands rather than a proportional decline in behavioral performance.

Furthermore, theories of cognitive reserve, brain maintenance, and compensatory neural recruitment suggest that cognitively healthy older adults may maintain performance through multiple interacting neural mechanisms rather than relying on a single processing component (Cabeza, 2002; Cabeza et al., 2018; Nyberg et al., 2012; Park & Reuter-Lorenz, 2009). Consequently, variation within a single task-evoked neural response may not fully capture the broader neural mechanisms supporting task performance. Load-related P300b modulation may therefore primarily reflect neural processes that are common across participants (and tasks) in response to increasing WM demands, whereas inter-individual differences in task-(-specific)

performance may depend more strongly on stable features of intrinsic brain organization. This study's microstate findings support this interpretation and provide further insight into the neural mechanisms underlying inter-individual differences in WM performance and P300b response.

Intrinsic neural organization contributes to inter-individual differences in neural and behavioral task responses

Consistent with our hypotheses, resting-state microstate dynamics were associated with both neural and behavioral task-evoked responses. Importantly, however, these associations were highly selective across outcome domains. Whereas longer microstate C duration was associated with larger P300b amplitude, longer microstate D duration was associated with reduced behavioral discrimination accuracy. Equally informative was the absence of associations: microstate C duration did not predict behavioral performance, and microstate D duration did not predict P300b amplitude. If a common underlying mechanism drove both neural and behavioral task-evoked responses, one might expect the same microstate characteristics to predict both outcome domains. Instead, the observed pattern in our data suggests that different aspects of intrinsic neural organization contribute to different components of WM task processing in cognitive healthy older adults. Consequently, if neural responsiveness and behavioral performance are influenced by partially distinct intrinsic neural characteristics, the absence of strong neural-behavioral modulation coupling observed in the present study becomes less surprising.

Microstate C has previously been linked to internally directed cognition (Jabès et al., 2021; Pan et al., 2020), salience processing (Britz et al., 2010), and coordination of information across distributed brain networks (Bréchet et al., 2019; Custo et al., 2017), although its precise functional role remains debated (Michel & Koenig, 2018b; Tarailis et al., 2024). In the present study, longer microstate C duration was associated with larger P300b amplitude but not with behavioral performance. This pattern suggests that intrinsic neural organization may influence the responsiveness of neural systems to task-relevant information without necessarily determining behavioral success.

In contrast, longer microstate D duration was associated with worse behavioral performance. Microstate D has previously been linked to frontoparietal systems involved in attentional control and executive functioning (Tarailis et al., 2024). Although this finding initially appears counterintuitive given the proposed role of microstate D, longer persistence of a neural

configuration does not necessarily imply more efficient processing. One possibility is that prolonged microstate D duration reflects greater stability of attentional control networks. While such stability may support sustained attentional engagement, successful performance in a continuous-updating task such as the *N*-back also requires flexible transition between cognitive states. As such, prolonged persistence of microstate D may interfere with behavioral performance under these task requirements due to limiting cognitive flexibility.

Notably, both findings involved the same temporal microstate parameter, duration, yet were associated with different outcome domains and opposite functional implications. This pattern suggests that longer microstate duration is not inherently beneficial or detrimental. Rather, duration may reflect the persistence of a specific neural configuration, and its functional significance depends on the neural state being maintained, as well as in relation to task-specific demands.

Taken together, our microstate findings suggest that different aspects of intrinsic neural organization contribute to different components of WM processing in cognitively healthy older adults. Importantly, these associations were observed despite relatively homogeneous load-related trajectories across participants. The examination of stable characteristics of intrinsic neural organization is rather novel and may help explain why individuals differ in overall neural responsiveness and behavioral performance even when they respond similarly to increasing task demands. In fact, in healthy cognitive aging, such intrinsic neural characteristics may therefore represent an important source of individual variability that is not fully captured by task-evoked modulation alone.

Limitations

First, although the study was adequately powered to detect medium-to-large effects, sensitivity analyses indicated limited power for detecting small associations. The absence of significant associations, particularly with respect to neural-behavioral modulation coupling and selective microstate effects, should therefore be interpreted with appropriate caution until replicated in a larger sample. Second, our sample consisted exclusively of cognitively healthy older adults. While this was a deliberate strength of the study, as it enabled the investigation of neural and cognitive variability in the absence of overt cognitive impairment, it also restricted the range of cognitive functioning represented in the data. The relatively homogeneous load-related trajectories observed across participants may therefore partly reflect overall preserved cognitive functioning in this (positively selected) population. Future studies including

individuals with mild cognitive impairment or other forms of cognitive decline as well as from more representative and diverse backgrounds may help determine whether neural-behavioral modulation coupling becomes more pronounced when neural resources are increasingly constrained and individual differences in vulnerability become more apparent. Finally, the cross-sectional design precludes conclusions regarding directionality or causal nature of the observed associations between intrinsic neural organization and task-related functioning. Although resting-state microstate dynamics were associated with both neural and behavioral outcomes, it remains unclear whether these characteristics represent stable traits of cognitive functioning, markers of broader neural system integrity, or consequences of other age-related processes. Longitudinal investigations and intervention-based approaches, as well as the determination of intra-individual stability of microstate-based neural markers, will be important for determining whether changes in microstate dynamics precede or accompany changes in cognitive performance and task-evoked neural responses over time.

Conclusion

The present findings demonstrate that WM load robustly modulated both behavioral performance and P300b amplitude in cognitively healthy older adults. However, individual differences were expressed primarily in overall levels of neural and behavioral functioning rather than in load-related modulation, and neural-behavioral modulation coupling was not observed. Resting-state microstate dynamics were associated with both neural and behavioral task outcomes, suggesting that stable characteristics of intrinsic neural organization represent sensitive measures of inter-individual differences in neural and cognitive functioning and contribute to individual variability in WM functioning in cognitively healthy older adults. These findings highlight the importance of considering intrinsic neural organization, as perhaps particularly sensitive measures of individual differences in cognitive functioning, alongside task-evoked measures when seeking to understand variability in healthy aging.

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conceptualization, methodology, validation, project administration, funding acquisition, visualization, writing – original draft, review and editing, data curation, investigation.

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Conflict of Interest: The authors declare no conflict of interest.

Data Availability Statement: All analysis scripts are available from xxx.osf.io.

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