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DESCRIPTION OF DOCTORAL DISSERTATION

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Keywords of doctoral dissertation in Polish: przetwarzanie sygnałów cyfrowych; rozwarcie źrenicy; neuropsychologia; neurofizjologia poznawcza; oscylacje theta; rytmy gamma; inżynieria biomedyczna; uczenie maszynowe

Keywords of doctoral dissertation in English: digital signal processing; pupil dilation; neuropsychology; cognitive neurophysiology; theta oscillations; gamma rhythms; biomedical engineering; machine learning

Summary of doctoral dissertation in Polish: Funkcje poznawcze różnią się między osobami oraz zmieniają w czasie. Obiektywne miary fizjologiczne odzwierciedlające te zmiany pozostają jednak ograniczone. W niniejszej rozprawie zbadano czy aktywność elektrofizjologiczna oraz dynamika rozmiaru źrenicy mogą stanowić skuteczne wskaźniki funkcji poznawczych. Przeprowadzono dwa komplementarne badania z udziałem pacjentów z padaczką. W pierwszym badaniu analizowano zależności pomiędzy aktywnością elektroencefalograficzną (EEG) a funkcjami poznawczymi podczas standaryzowanej oceny neuropsychologicznej z wykorzystaniem Cambridge Neuropsychological Test Automated Battery (CANTAB). Aktywność w paśmie theta w przedniej korze przedczołowej była związana z funkcjami pamięciowymi i wykonawczymi, co sugeruje, że odzwierciedla ona ogólną sprawność poznawczą. Drugie badanie dotyczyło pamięci na poziomie pojedynczych prób z wykorzystaniem jednoczesnych pomiarów śródczaszkowego EEG (iEEG) i pupilometrii podczas zadania pamięci werbalnej. Analizy czasowe zmian rozwartości źrenicy pozwoliły rozróżnić dobrą i słabą skuteczność pamięci podczas kodowania i odtwarzania informacji. Ta cecha pupillometryczna wykazała skuteczność klasyfikacji porównywalną z cechami spektralnymi mocy sygnału iEEG w określonych pasmach częstotliwości, co wspiera jej potencjalne zastosowanie jako nieinwazyjnego biomarkera funkcji pamięciowych. Łącznie uzyskane wyniki dostarczają narzędzi do przetwarzania i analizy sygnałów elektrofizjologicznych oraz pupillometrycznych w celu obiektywnej oceny, monitorowania i modulacji funkcji poznawczych z wykorzystaniem rozwijających się neurotechnologii interfejsów mózg– komputer.

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Summary of doctoral dissertation in English: Cognitive performance varies across individuals and over time. Objective physiological measures reflecting these fluctuations remain limited. This thesis investigated whether electrophysiological activity and pupil dynamics can provide reliable indicators of cognitive performance. Two complementary studies were conducted in epilepsy patients. The first study examined relationships between electroencephalography (EEG) activity and cognitive performance during standardized neuropsychological assessment using the Cambridge Neuropsychological Test Automated Battery (CANTAB). Theta-band activity in the anterior prefrontal cortex was associated with memory and executive functions, suggesting that it reflects general cognitive performance. The second study investigated trial-level memory performance using simultaneous intracranial EEG (iEEG) and pupillometry during a verbal memory task. Time-resolved analyses of pupil size discriminated good and poor memory performance during memory encoding and retrieval. This pupillometric feature showed classification performance comparable to iEEG power-in-band spectral features, supporting its potential use as a non-invasive biomarker of memory performance. Together, these findings provide electrophysiological and pupillometric signal processing and analysis tools for objective assessment, monitoring and modulation of cognitive performance with the emerging brain-computer interface neurotechnologies.

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List of Abbreviations

ANOVA	Analysis of Variance
CANTAB	Cambridge Neuropsychological Test Automated Battery
CT	Computed Tomography
DMS	Delayed Match to Sample
DRE	Drug-Resistant Epilepsy
EEG	Electroencephalography
EMU	Epilepsy Monitoring Unit
FC	Frontal Cortex
FDR	False Discovery Rate
FIR	Finite Impulse Response
GMM	Gaussian Mixture Model
ICA	Independent Component Analysis
iEEG	Intracranial Electroencephalography
IIR	Infinite Impulse Response
IPD	Interpupillary Distance
L	Limbic
LC-NE	Locus Coeruleus–Norepinephrine
LMM	Linear Mixed-Effects Model

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LPO-CV	Leave-Pairs-Out Cross-Validation
MOT	Motor Screening Task
MRI	Magnetic Resonance Imaging
OC	Occipital Cortex
PC	Parietal Cortex
PIB	Power-in-Band
RBF	Radial Basis Function
SSP	Spatial Span
STFT	Short-Time Fourier Transform
SVM	Support Vector Machine
SWM	Spatial Working Memory
TC	Temporal Cortex

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Chapter 1

1. Introduction

Cognitive function, including memory, attention, and executive control, is a major determinant of independence, quality of life, and long-term outcomes across neurological disorders. Impairments in these domains are frequently observed in conditions such as epilepsy, neurodegenerative diseases, and traumatic brain injury, and are strongly associated with reduced functional capacity and decreased quality of life[1–4]. Despite their clinical importance, cognitive functions are still primarily evaluated using behavioral and neuropsychological assessments, which are often time-consuming, context-dependent, and may not adequately capture rapid fluctuations in cognitive state[5]. Moreover, these assessments typically provide overall measures of performance while offering limited insight into the physiological processes underlying cognition.

Recent advances in physiological measurement technologies have created new opportunities for studying cognitive function using objective and measurable signals. Techniques such as electroencephalography (EEG), intracranial EEG (iEEG), and pupillometry make it possible to investigate brain activity and pupil-related physiological changes associated with cognitive processes. EEG and iEEG measure electrical activity generated by neuronal populations8,9, whereas pupillometry measures changes in pupil size associated with cognitive effort, arousal, and attention[6,7]. These techniques provide complementary information about processes related to memory, attention, and executive control and may help identify measurable patterns related to differences in cognitive performance[8–10].

Throughout this thesis, the term physiological signals refers to both brain activity and pupil-related physiological responses. EEG and iEEG are considered measures of brain electrophysiological activity, whereas pupillometry reflects pupil-related physiological changes associated with cognitive processing.

Against this background, this thesis investigates physiological indicators of cognitive performance using EEG, iEEG, and pupillometry. Specifically, it examines electrophysiological activity together with pupil dynamics to identify measurable patterns associated with memory and executive functioning. Through computational and statistical analysis of these signals, this work aims to contribute to the development of objective approaches for assessing cognitive performance and understanding variability across individuals and between trials.

1.1 Background and Motivation

Understanding the physiological mechanisms underlying cognitive performance remains a central challenge in contemporary neuroscience. Cognitive processes such as memory, attention, and executive control depend on coordinated activity across distributed brain networks, making accurate assessment of these functions an important challenge in neuroscience[3,11,12]. Conventional neuropsychological assessments remain important tools for evaluating these functions in both clinical and research settings[13]. However, because they primarily rely on observable performance, they may not fully capture rapid fluctuations in cognitive state or the underlying behavior[14,15].

Neurophysiological recordings provide valuable tools for investigating the biological basis of cognition. EEG provides noninvasive recording of neural activity with high temporal resolution, enabling investigation of oscillatory dynamics relevant to cognitive processing. Neural oscillations across distinct frequency bands have been shown to reflect coordinated communication among distributed brain regions and play a key role in cognitive information processing[16]. Studies of frontal cortical activity have demonstrated that oscillatory signals, especially within the theta frequency band, are closely associated with cognitive control and working memory performance[11,17].

Despite these advances, linking physiological signals to cognitive performance remains challenging because of variability across individuals, experimental conditions, and the complex nature of cognitive processes[18,19]. Physiological recordings also require computational approaches capable of extracting meaningful information from noisy and high-dimensional data. These challenges highlight the importance of structured analytical frameworks for relating physiological measurements to behavioral performance.

1.2 Biomarkers: Definition and Need for Quantitative Cognitive Assessment

Biomarkers have become increasingly important in neuroscience and clinical research, particularly for improving the assessment and monitoring of cognitive dysfunction. In general, a biomarker refers to a measurable indicator of a biological process, physiological state, or pathological condition that can be objectively quantified using standardized methods. The growing use of biomarker-based approaches reflects a broader shift from symptom-based evaluation toward approaches grounded in measurable biological signals.

In the context of cognition, biomarkers are of particular interest because cognitive functions arise from complex physiological processes that cannot be directly assessed through behavioral performance alone. While behavioral assessments provide important indicators of cognitive performance, physiological measurements

may provide complementary insight into mechanisms underlying variability in behavioral outcomes. Consequently, physiological biomarkers may complement behavioral assessments by improving sensitivity to variability in cognitive performance.

For a biomarker to be useful, it should demonstrate reliability, sensitivity to cognitive changes, robustness to noise, and interpretability in relation to established physiological mechanisms. Advances in physiological signal acquisition, statistical modeling, and machine learning have increasingly enabled extraction of reproducible features from complex biological signals, supporting the development of quantitative and computational approaches to cognitive assessment.

Within this framework, physiological signals derived from electrophysiological recordings and pupil dynamics represent promising candidates for investigating variability in memory, attention, and executive functioning. These concepts form the basis for the methodological approaches explored in this thesis.

1.3 Physiological Biomarkers of Cognitive Function

Cognitive functions are supported by dynamic activity within distributed brain networks. These processes unfold over time through coordinated patterns of neuronal activity, which can be measured using electrophysiological recordings. In this context, physiological recordings provide measurable information about neural and behavioral processes underlying cognition[20,21].

Electrophysiological activity is commonly examined through neural oscillations across distinct frequency bands. Oscillatory activity reflects rhythmic changes in neuronal excitability and has been associated with communication among distributed brain regions[22]. Different frequency bands have been linked to different aspects of cognition, including perception, memory formation, attention, and decision-making[23]. Theta-band activity is strongly associated with memory encoding, retrieval, and cognitive control, particularly within frontal and temporal regions[17,24–28]. Alpha-band activity has been related to attentional regulation and sensory selection[29–31], whereas beta- and gamma-band activity have been associated with cognitive control, working memory maintenance, and information integration[30,32].

Beyond individual oscillatory components, cognitive performance depends on interactions across distributed neural networks and localized brain regions. Network-level communication supports the integration of sensory, memory, and executive processes, allowing flexible adaptation to changing task demands[20,21,33]. At the same time, local activity within specialized structures, such as the medial temporal lobe and prefrontal cortex, contributes to memory formation, retrieval, and cognitive control. Direct intracranial recordings have shown that localized high-frequency activity is associated with successful encoding and retrieval, supporting the relevance of iEEG-derived features for studying memory-related processes[34,35.]

Pupil dynamics provide complementary information about cognitive state. Changes in pupil diameter are associated with cognitive effort, attentional allocation, arousal, and decision-making[10,36]. Although pupillometry does not directly measure neural activity, pupil responses provide an indirect physiological indicator of processes that influence cognitive performance. Importantly, pupil responses may occur before observable behavioral outcomes, suggesting that pupil dynamics can provide useful information about trial-by-trial variability in cognitive state and performance[37].

Increasing evidence suggests that cognitive performance cannot be fully understood by examining isolated physiological processes alone. EEG, iEEG, and pupillometry provide complementary perspectives: EEG captures non-invasive electrophysiological activity with high temporal resolution, iEEG provides localized electrophysiological information from cortical and subcortical regions, and pupillometry reflects pupil-related physiological changes associated with cognitive effort and arousal. Combining these signals may therefore provide a more comprehensive representation of cognitive performance than single-modality measurements alone[38–41].

From a computational perspective, the main challenge is to extract meaningful and reproducible features from complex physiological data and relate them to behavioral outcomes. Statistical modeling and machine learning approaches provide tools for identifying physiological patterns associated with cognitive performance and evaluating their predictive value[18]. This forms the theoretical basis for interpreting electrophysiological activity and pupil dynamics as physiological biomarkers of memory, attention, and executive function in this thesis.

This thesis approaches cognitive assessment at two complementary levels. First, it examines whether scalp EEG spectral features can identify inter-individual differences in clinically measured memory and executive performance. Second, it investigates whether pupil dynamics can capture trial-to-trial variability in immediate memory performance and compares these non-invasive measures with iEEG-derived spectral features. Together, these studies examine whether physiological signals can provide objective markers of both between-person differences in cognitive performance and trial-by-trial fluctuations in memory performance.

Despite growing evidence linking electrophysiological activity and pupil dynamics to cognition, important limitations remain. Prior studies have often focused on isolated physiological modalities, group-level behavioral outcomes, or descriptive associations, with limited emphasis on quantitative characterization and classification of cognitive performance and trial-by-trial variability. In particular, direct comparison between non-invasive pupillometry and intracranial electrophysiological features in relation to memory performance remains limited. These gaps motivate the present dissertation, which investigates physiological biomarkers of cognition across both inter-individual and intra-individual levels of variability.

1.4 Research Objectives, Main Thesis, and Research Hypotheses

The main thesis of this dissertation is that physiological signals recorded using EEG, iEEG, and pupillometry can be transformed through digital signal processing, statistical analysis, and machine learning approaches into quantitative biomarkers that enable objective measurement of cognitive performance and memory-related variability across individuals and trials.

This dissertation approaches cognitive performance assessment from both inter-individual and intra-individual perspectives. At the inter-individual level, it investigates whether electrophysiological features derived from scalp EEG differ systematically between individuals with different levels of cognitive performance. At the intra-individual level, it examines whether trial-by-trial physiological variability, reflected in pupil dynamics and iEEG-derived electrophysiological activity, is associated with successful and unsuccessful memory performance during free recall.

To address the main dissertation thesis, several interconnected research objectives are pursued. First, this work aims to identify electrophysiological features discriminating different cognitive performance levels using spectral EEG features across distinct frequency bands and to evaluate their relationship with standardized cognitive outcomes. Second, it investigates pupil dynamics as a non-invasive physiological measure of memory performance, with particular emphasis on whether pupil-derived features contain discriminative information associated with successful and unsuccessful memory retrieval. Third, this dissertation compares pupil dynamics with iEEG-derived electrophysiological features in relation to trial-by-trial variability in memory performance. Finally, it develops and evaluates computational frameworks for physiological signal analysis, including structured preprocessing pipelines, time-resolved statistical analysis, and machine learning approaches for classifying cognitive performance states.

To evaluate the main dissertation thesis, the following research hypotheses are formulated:

H1. Spectral EEG features differ systematically between groups characterized by different levels of cognitive performance.

H2. Pupil dynamics reflect trial-by-trial variability in memory performance.

H3. Physiological features derived from EEG, iEEG, and pupil responses contain information about cognitive and memory performance that can be extracted using statistical and computational approaches.

H4. Pupil-derived physiological features provide information about memory performance with timing and discriminative properties approaching those of simplified univariate iEEG power-in-band features.

These hypotheses collectively support the evaluation of the main dissertation thesis through the empirical studies presented in subsequent chapters.

1.5 Structure of the Thesis

This thesis is organized into six chapters that collectively present the theoretical background, methodological framework, empirical investigations, and interpretative discussion of neurophysiological biomarkers of cognitive performance.

Chapter 1 introduces the conceptual background and motivation for the thesis. It outlines the importance of cognitive function in neurological contexts, discusses the need for objective physiological biomarkers in cognitive assessment, and presents the research objectives and hypotheses that guide the empirical studies.

Chapter 2 provides a comprehensive overview of cognitive functions and memory systems relevant to the present work. This chapter introduces major cognitive domains, including memory, attention, and executive control, and discusses the interactions between these processes that support complex cognitive behavior.

Chapter 3 focuses on neurophysiological measurement techniques used to investigate cognitive processes. The chapter describes electrophysiological recording methods, including EEG and iEEG, as well as pupillometry. It further introduces the transformation of physiological signals into operational biomarkers.

Chapter 4 presents the methodological and analytical framework used in empirical studies. This chapter describes the study of populations, experimental paradigms, and procedures for defining cognitive performance groups in two studies. It provides further details about the recording procedures, signal preprocessing pipelines, feature extraction methods, statistical analysis strategies, and classification approaches used to evaluate physiological indicators of cognitive performance.

Chapter 5 presents the empirical investigations conducted in this thesis. Study I examines spectral EEG features associated with differences in cognitive outcomes across groups classified as good, normal, and poor performance. Study II investigates pupil dynamics and iEEG-derived electrophysiological activity in relation to trial-by-trial variability in memory performance. Author-specific contributions to each empirical study are provided directly after the corresponding publication.

Chapter 6 interprets the findings across both empirical studies, discusses their scientific and practical implications, identifies methodological limitations, verifies the dissertation theses, and outlines directions for future research.

Chapter 2

2. Cognitive Functions and Memory Systems

Understanding cognitive performance requires a conceptual framework for the cognitive functions examined in this thesis. Cognitive functions such as memory, attention, and executive functioning form the foundation of goal-directed behavior, enabling individuals to learn, adapt, and respond effectively to environmental demands. Rather than operating independently, these functions interact through coordinated neural systems, supporting the flexible integration of information across time and context[20,42,43].

The empirical investigations presented in this thesis focus on cognitive domains central to adaptive behavior and memory performance. Study I examines inter-individual differences across visual memory, spatial memory, working memory, and executive functioning, whereas Study II investigates trial-to-trial variability in episodic memory performance within a verbal free recall paradigm. Accordingly, this chapter provides an overview of the cognitive systems most relevant to these investigations, with particular emphasis on memory systems, executive functioning, attentional processes, and retrieval mechanisms.

Establishing this theoretical foundation is essential for interpreting later findings, in which neural and pupil-related signals are examined as measurable indicators of cognitive performance and memory-related variability.

2.1 Cognitive Function

Cognitive functions encompass the mental processes that enable individuals to perceive, interpret, store, and use information to guide behavior. These processes support learning, decision-making, problem solving, and behavioral regulation across changing task demands.

Among the major domains of cognition, memory, attention, and executive functioning are particularly important for cognitive performance. Memory supports the encoding, storage, and retrieval of information across time. Attention determines which information is prioritized for further processing, thereby influencing perception and memory formation. Executive functions coordinate these processes by maintaining task goals, regulating behavior, and supporting flexible adaptation to changing demands[12,44,45].

Cognitive performance is inherently dynamic and can fluctuate across time, even under consistent task conditions. Moment-to-moment changes in attention and task engagement may contribute to differences in performance, both across individuals and across repeated trials within the same individuals[46,47]. Such variability is

particularly relevant to the present thesis, which investigates both inter-individual differences in cognitive performance and trial-to-trial fluctuations in memory performance.

Understanding cognition as a dynamic set of processes provides an important foundation for examining how measurable biological signals relate to cognitive and behavioral outcomes. The following section focuses specifically on human memory systems, which are central to the empirical paradigms examined in this thesis.

2.2 Human Memory System

Human memory enables the acquisition, maintenance, and retrieval of information across time, forming the basis for learning, adaptation, and decision-making. Rather than representing a single unified system, memory consists of multiple interacting subsystems that support different forms of information processing and retention[3,48]. The present thesis focuses particularly on working, visual, spatial, and episodic memory, which are central to the cognitive paradigms examined in subsequent chapters.

Working memory refers to the temporary maintenance and manipulation of information according to task demands. It plays a critical role in reasoning, learning, problem solving, and goal-directed behavior by supporting the maintenance of task-relevant information during ongoing cognitive processing[12].

Neurophysiological studies have demonstrated that working memory depends on coordinated activity across frontal and parietal cortical networks, reflecting interactions between storage and executive control processes[49].

Visual memory supports the retention and recognition of visual information, enabling individuals to identify previously encountered objects, patterns, or scenes. This form of memory contributes to perception and recognition by allowing comparison between incoming sensory input and stored representations. Spatial memory supports the encoding and maintenance of spatial relationships and locations, enabling individuals to temporarily retain and manipulate visuospatial information. These memory systems are essential for tasks involving visuospatial processing and depend on interactions among occipital, parietal, and medial temporal regions[50].

Episodic memory enables recollection of previously experienced events and information. Successful episodic memory depends on the ability to encode, maintain, and later retrieve previously experienced information. This form of memory is particularly relevant to free recall paradigms, in which individuals retrieve previously presented information following a short retention interval and distraction period without external retrieval cues. Variability in episodic memory performance may therefore arise even under consistent task conditions, reflecting differences in encoding and retrieval processes as well as fluctuations in internal cognitive state[51–53].

The medial temporal lobe, particularly the hippocampus and surrounding cortical regions, plays a central role in episodic memory formation and retrieval. Experimental and clinical observations have shown that hippocampal dysfunction impairs the formation of new memories while leaving some previously stored information relatively preserved[54–56]. Interactions between hippocampal and cortical networks are therefore considered fundamental for successful memory performance and retrieval processes[57,58].

Memory processing is commonly described as a sequence of stages that include encoding, consolidation, storage, and retrieval. Encoding involves transforming sensory input into neural representations that can later be accessed. Consolidation refers to processes that stabilize newly formed memory traces and support long-term retention. Retrieval involves accessing stored information and reconstructing previously encoded representations in response to internal or external cues[52,59]. Differences in the efficiency of encoding and retrieval may substantially influence memory outcomes, contributing to variability in cognitive performance across individuals and across trials.

Understanding the organization of memory systems and the processes supporting encoding and retrieval provides an essential foundation for interpreting behavioral and physiological variability in cognitive performance. The following section examines executive functions and cognitive control, which regulate cognitive processes involved in memory and goal-directed behavior.

2.3 Executive Functions

Executive functions refer to higher-order cognitive processes that support the regulation of behavior according to task goals and environmental demands. These processes enable planning, behavioral control, problem solving, and flexible adaptation to changing situations. Executive functions allow individuals to maintain relevant information, ignore distractions, and adjust strategies when needed[44,60,61].

Executive functioning involves several interconnected processes, including working memory, inhibitory control, and cognitive flexibility. Working memory supports the temporary maintenance and manipulation of information required for ongoing tasks. Inhibitory control helps suppress irrelevant information or inappropriate responses, reducing interference during cognitive processing. Cognitive flexibility enables individuals to adjust behavior and switch strategies in response to changing demands[62–64]. Together, these processes support efficient goal-directed behavior and cognitive performance.

Executive functions are strongly associated with activity in frontal brain regions, particularly the prefrontal cortex, which plays a key role in behavioral regulation and decision-making[43,65]. Neurophysiological studies have shown that frontal brain activity increases during tasks involving working memory, executive processing, and cognitive effort. In particular, theta oscillatory activity in frontal regions has been

linked to working memory and executive functioning[66,67]. These findings are relevant to the present thesis, in which frontal electrophysiological activity is examined in relation to cognitive performance.

Executive functions also contribute to memory performance. During encoding, executive processes help organize relevant information and reduce interference from distractions. During retrieval, they support strategic search and maintenance of task goals, contributing to successful recall performance[68,69]. Variability in executive engagement may therefore influence differences in cognitive performance across individuals and across trials.

Understanding executive functions is important for interpreting cognitive performance in tasks involving memory and behavioral regulation. The following section focuses on attentional processes, which further influence information processing and memory performance.

2.4 Attention

Attention refers to the cognitive processes that enable individuals to selectively focus on relevant information while filtering out irrelevant stimuli. By prioritizing certain information for further processing, attention supports efficient perception, learning, and task performance. Rather than functioning as a single mechanism, attention involves processes that regulate the selection and maintenance of information according to current goals and task demands[45,70,71].

Attention plays an important role in memory performance. Information that receives greater attentional resources is more likely to be successfully processed and later remembered, whereas reduced attention may weaken memory formation and decrease recall success[72,73]. Attentional processes therefore contribute substantially to differences in cognitive performance across tasks involving memory and executive functioning.

Importantly, attentional state can fluctuate across time, even under consistent task conditions. Such fluctuations may contribute to variability in behavioral performance across individuals and across trials within the same individual[74,75]. Understanding attentional processes is particularly relevant to the present dissertation because fluctuations in attentional engagement may contribute to variability in both cognitive task performance and trial-by-trial memory outcomes. Such variability provides an important context for interpreting physiological signals examined in subsequent chapters. The following section focuses on retrieval regulation, which supports controlled access to stored information during memory retrieval.

2.5 Retrieval Regulation

Memory retrieval refers to the process of accessing previously stored information and bringing it into conscious awareness. Successful retrieval depends not only on how effectively information was encoded and stored, but also on cognitive processes

that support memory search and recall[68,76]. These processes are particularly important in free recall tasks, in which individuals must retrieve previously learned information without external cues.

During free recall, individuals may successfully retrieve some items while failing to recall others, even when information is presented under similar conditions. Such variability may reflect differences in retrieval efficiency, retrieval strategy, or fluctuations in cognitive state[25,77]. Successful memory performance therefore depends not only on effective encoding but also on efficient retrieval processes.

Retrieval processes are supported by coordinated activity across distributed neural systems, particularly interactions between prefrontal and medial temporal regions involved in memory organization and access. Prefrontal regions contribute to strategic memory search and organization, whereas medial temporal structures, including the hippocampus, support access to stored memory representations. These interactions are especially relevant in tasks requiring sustained recall over time[56,78].

Importantly, retrieval success is not uniform across trials, even under comparable task conditions. Variability in retrieval performance may reflect fluctuations in attentional engagement, retrieval strategy, neural readiness, or physiological state. Understanding these sources of variability is particularly relevant to the present dissertation, in which trial-by-trial physiological dynamics are examined in relation to successful and unsuccessful memory performance during free recall.

Understanding retrieval processes is important for interpreting variability in memory performance, particularly in free recall paradigms where successful performance depends on both encoding and retrieval. The following section focuses on variability in cognitive performance, emphasizing factors that contribute to differences in performance across individuals and across trials.

2.6 Variability in Cognitive Performance

Cognitive performance is not static and may vary both across individuals and within the same individual over time. Even under similar task conditions, performance may fluctuate due to differences in cognitive state, task engagement, memory efficiency, and executive processes. Such variability contributes to differences in behavioral outcomes and may influence performance in tasks involving memory and executive functioning.

Inter-individual variability reflects relatively stable differences in cognitive performance across individuals and may arise from differences in neural functioning or neurological status. In contrast, within-individual variability refers to moment-to-moment fluctuations in performance observed across repeated trials, even when task demands remain constant. Variability across trials may reflect differences in encoding efficiency, retrieval success, or changes in cognitive state during task performance.

Understanding variability in cognitive performance is particularly relevant to the present thesis. Study I examines inter-individual differences in cognitive functioning, whereas Study II investigates trial-to-trial fluctuations in memory performance during free recall. Physiological signals may therefore provide objective indicators of both relatively stable cognitive differences and dynamic changes in cognitive state across time.

Although cognitive processes such as memory, attention, and executive functioning are well characterized behaviorally, their underlying physiological mechanisms remain difficult to assess objectively and dynamically. Variability in cognitive performance across individuals and across trials suggests a need for measurable physiological indicators capable of capturing these processes in real time. The following chapter therefore focuses on neurophysiological measurement techniques and physiological biomarkers relevant to cognitive performance assessment.

Chapter 3

3. Physiological Measurement of Cognitive Processes

Neurophysiological measurement techniques provide access to the biological activity underlying cognitive performance. While behavioral assessments reflect observable outcomes of cognitive processing, physiological recordings enable investigation of the neural and physiological mechanisms associated with cognition at fine temporal scales[79–81]. Such measurements provide insight into processes involved in memory, attention, and executive functioning and support investigation of variability in cognitive performance across individuals and over time.

Among available neurophysiological techniques, electroencephalography (EEG), intracranial electroencephalography (iEEG), and pupillometry have emerged as valuable tools for studying cognitive processes. These methods provide complementary information about neural and physiological activity, ranging from cortical electrophysiological dynamics to pupil-related responses associated with cognitive state. Together, they enable objective investigation of cognitive and memory performance and form the methodological foundation of the empirical studies presented in this thesis.

This chapter introduces the principal neurophysiological measurement techniques relevant to the present thesis. The chapter begins with EEG as a noninvasive method for recording cortical activity, followed by iEEG, which enables localized measurement of neural activity from specific brain regions. Subsequent sections examine pupillometry as a physiological indicator of cognitive state and discuss the transformation of physiological recordings into quantitative biomarkers of cognitive performance.

3.1 Electroencephalography (EEG)

EEG is a noninvasive neurophysiological technique used to record electrical activity generated by neural populations in the cerebral cortex. EEG signals arise primarily from synchronized postsynaptic activity in cortical pyramidal neurons, producing measurable voltage fluctuations detectable at the scalp surface. Because these signals reflect ongoing neural activity, EEG provides valuable information about the temporal dynamics of cognitive processes[82,83].

One of the principal advantages of EEG is its high temporal resolution. Neural activity can be recorded on the order of milliseconds, enabling investigation of rapid cognitive processes involved in perception, attention, memory, and decision-making[79,81,84]. This temporal precision makes EEG particularly suitable for studying

cognitive events that evolve over short time intervals and for identifying neural activity associated with behavioral performance.

EEG recordings are typically obtained using multiple scalp electrodes arranged according to standardized systems, such as the international 10–20 electrode placement system. This arrangement enables measurement of activity across cortical regions and comparison of neural signals across individuals and experimental conditions.

Neural oscillations represent a major feature of EEG activity and are commonly categorized into frequency bands, including delta, theta, alpha, beta, and gamma oscillations. These oscillatory patterns are associated with different aspects of cognitive function. In particular, theta-band activity has been consistently linked to memory encoding, retrieval, and executive functioning, especially within frontal cortical regions[24,27,31].

EEG has been widely used to investigate cognitive performance in domains including attention, working memory, and episodic memory. Changes in oscillatory power across trials may reflect variability in neural activity and cognitive state, providing insight into differences in behavioral performance[85]. This capability is particularly important for studying variability in cognitive performance across individuals and across repeated task conditions.

Despite its strengths, EEG has several methodological limitations. Due to volume conduction effects, scalp-recorded signals provide limited spatial precision compared with invasive recording techniques. EEG recordings are also susceptible to artifacts arising from eye movements, muscle activity, and environmental noise, requiring careful preprocessing procedures to ensure signal quality[86,87].

Nevertheless, EEG remains one of the most widely used approaches for investigating cognitive processes because of its high temporal resolution, noninvasive nature, and suitability for studying dynamic changes in neural activity. These characteristics make EEG particularly valuable for investigating physiological correlates of cognitive performance.

In the present dissertation, EEG is used to examine whether spectral electrophysiological features are associated with inter-individual variability in memory and executive performance.

The following section introduces intracranial electroencephalography (iEEG), an invasive recording technique that provides highly localized measurements of neural activity from specific brain regions.

3.2 Intracranial Electroencephalography (iEEG)

iEEG is an invasive neurophysiological technique that enables direct recording of electrical activity from cortical and subcortical brain regions[8,34,88]. Unlike scalp EEG, which records signals transmitted through multiple tissue layers, iEEG electrodes are placed directly on the cortical surface or inserted into specific brain regions. This

configuration enables highly localized measurement of neural activity with improved spatial precision and signal fidelity.

In clinical practice, iEEG is primarily used to localize epileptogenic regions in patients undergoing presurgical evaluation. Beyond its clinical role, intracranial recording has contributed substantially to understanding the neural mechanisms underlying cognitive processes. Because recordings are obtained from anatomically defined brain regions, iEEG enables detailed investigation of neural activity associated with memory and executive functioning[89,90].

A major advantage of iEEG is its ability to capture localized electrophysiological activity across a broad range of frequency bands, including high-frequency oscillations associated with local neuronal processing. Increases in gamma activity have been linked to neural processes involved in memory encoding and retrieval[91–93]. Intracranial recordings have also demonstrated that electrophysiological activity within frontal and temporal regions differs according to memory performance during encoding and retrieval tasks[94,95].

Despite its strengths, intracranial recording is inherently limited to clinical populations and cannot be applied to healthy individuals without medical indication. Electrode placement is determined by clinical requirements rather than experimental design, resulting in variability in anatomical coverage across participants. Additionally, because iEEG involves surgical implantation, its use is restricted to specialized clinical settings. Nevertheless, the high spatial and temporal precision of intracranial recordings provides insights that cannot be obtained using noninvasive methods alone[8,88].

Overall, intracranial electroencephalography provides a powerful approach for investigating localized neural mechanisms underlying cognitive function. By enabling direct recording of region-specific neural activity, iEEG offers detailed insight into electrophysiological processes associated with memory and executive functioning. In the present thesis, intracranial electrophysiological measures further provide a reference for contextualizing the timing and predictive characteristics of non-invasive pupil-derived physiological signals.

3.3 Pupillometry

Pupillometry is a physiological measurement technique that quantifies changes in pupil diameter over time. It has become an increasingly valuable tool in cognitive neuroscience due to its sensitivity to cognitive processes. Variations in pupil size have been associated with cognitive effort, attentional engagement, and arousal. Because pupil responses can be recorded noninvasively and continuously, pupillometry provides a practical method for monitoring physiological changes associated with cognitive processing during task performance[36,96,97].

Studies have demonstrated that changes in pupil size are associated with differences in attention, task engagement, and cognitive performance[9,10,98]. The

relationship between pupil dynamics and cognition has been examined across a wide range of experimental paradigms, demonstrating that pupil responses vary according to task demands and behavioral performance.

During memory encoding and retrieval tasks, variations in pupil size have been associated with differences in cognitive effort and memory performance[99–102]. Such findings suggest that pupil responses provide indirect information about internal processes related to cognitive performance. In addition, changes in pupil dynamics have been observed prior to behavioral responses and before the presentation of task-relevant stimuli, suggesting that pupil activity may be associated with subsequent task performance[101,103].

Variability in pupil diameter has also been linked to differences in performance across trials, indicating that moment-to-moment physiological changes may contribute to behavioral variability[104,105]. Because pupil signals can be recorded continuously, pupillometry provides a physiological measure that can be examined in relation to fluctuations in cognitive and memory performance over time.

Despite its strengths, pupillometry also presents several methodological considerations. Pupil measurements are sensitive to environmental factors such as luminance changes, which can introduce variability unrelated to cognitive processes. Additionally, pupil responses may be influenced by fatigue, emotional state, and pharmacological effects. Careful experimental design, including control of lighting conditions and baseline normalization procedures, is therefore essential to ensure reliable interpretation of pupil data[102,106,107].

Overall, pupillometry provides a sensitive and noninvasive method for investigating physiological processes associated with cognitive function. By capturing continuous changes in pupil diameter, this technique enables investigation of variability in cognitive and memory performance across time. These characteristics make pupillometry particularly valuable for examining relationships between physiological activity and cognitive performance.

In the present dissertation, pupillometry is investigated as a non-invasive physiological measure of trial-by-trial memory variability, with particular emphasis on whether pupil dynamics provide temporally informative signals associated with successful and unsuccessful recall performance.

The following section introduces the concept of neurophysiological biomarkers of cognitive performance, focusing on how physiological signals can be transformed into quantitative indicators of cognitive function.

3.4 From Signals to Features: Biomarkers of Cognitive Performance

While physiological recordings provide continuous measurements of activity associated with cognitive processes, their direct interpretation requires

transformation into structured quantitative measures. This process enables identification of neurophysiological biomarkers, defined as measurable features extracted from physiological recordings that reflect aspects of cognitive functioning and behavioralvariability[41,108].

In electrophysiological recordings such as EEG and iEEG, quantitative features are commonly derived from oscillatory activity within specific frequency bands. Spectral decomposition methods are frequently used to characterize neural activity by estimating signal power across frequency ranges, including delta, theta, alpha, beta, and gamma oscillations[23,80,81]. Because different frequency bands have been associated with distinct cognitive functions, frequency-specific measures provide interpretable indicators of neural activity related to memory, executive functioning, and cognitive performance.

Cognitive processes evolve over time and may involve rapid changes in neural activity. Time-resolved approaches therefore enable examination of how electrophysiological activity changes during different stages of task performance[109,110]. Such approaches support investigation of variability in cognitive processing across repeated task conditions and provide insight into dynamic changes associated with behavioral performance.

In addition to electrophysiological signals, quantitative features can also be extracted from pupil recordings. Measures derived from pupillometry commonly include changes in pupil diameter over time and trial-related fluctuations associated with cognitive performance[10,36,101]. Because pupil responses can be recorded continuously, they provide information about variability in cognitive and memory performance across repeated trials.

Transforming continuous physiological recordings into structured quantitative features provides important advantages for cognitive assessment. While behavioral outcomes reflect observable performance, physiological signals may provide additional information about processes occurring during task performance and variability that is not directly observable through behavior alone. Such measures therefore contribute to objective investigation of cognitive performance across individuals and across time.

In the present thesis, quantitative features derived from EEG, iEEG, and pupillometry are examined in relation to cognitive and memory performance. Together, these approaches provide complementary perspectives on physiological activity associated with cognitive function and variability in performance. The following chapter introduces the computational and statistical approaches used to analyze these physiological signals.

Chapter 4

4. Methodological and Analytical Framework

This thesis employs two complementary methodological approaches to investigate physiological biomarkers of cognitive function. The first approach examines scalp electroencephalography (EEG) during structured computerized neuropsychological assessment to investigate electrophysiological correlates of cognitive performance across multiple domains, including memory and executive functioning. The second approach combines intracranial electroencephalography (iEEG) and pupillometry during a verbal free recall task to investigate trial-to-trial variability in immediate memory performance. Together, these approaches address the central aim of the thesis across complementary levels of analysis, spanning inter-individual differences in cognitive performance and within-individual variability in memory-related processes. This chapter outlines the methodological and analytical framework underlying these investigations, while detailed experimental procedures are presented in the included publications.

4.1 Study Population

This thesis integrates data from two clinical cohorts of patients with epilepsy to investigate physiological biomarkers of cognitive function across complementary levels of analysis. The use of epilepsy populations enabled simultaneous acquisition of physiological recordings during cognitive task performance and provided access to intracranial neural activity that cannot be obtained in healthy individuals for ethical reasons.

Study I included 86 patients with epilepsy recruited from the Epilepsy Monitoring Unit (EMU) at the Mayo Clinic (Rochester, MN, USA). The cohort consisted of 45 males and 41 females, with a mean age of 36.0 ± 12.8 years. The mean age at seizure onset was 21.4 ± 15.6 years, with an average epilepsy duration of 14.0 ± 12.3 years. During routine EEG and video monitoring sessions, participants completed selected tasks from the Cambridge Neuropsychological Test Automated Battery (CANTAB), enabling simultaneous acquisition of behavioral and scalp EEG data. This cohort was used to investigate electrophysiological correlates of inter-individual differences in cognitive performance across visual memory, spatial memory, working memory, and executive functioning.

Study II included seven patients with drug-resistant epilepsy undergoing intracranial monitoring for clinical seizure localization at the Mayo Clinic. Participants had a mean age of 21.4 ± 4.2 years, and four were male. Depth and subdural electrodes had been implanted exclusively for clinical purposes prior to participation in the

experimental protocol. During monitoring, participants completed a verbal free recall task while simultaneous iEEG and pupillometry recordings were obtained. This cohort enabled investigation of trial-to-trial variability in immediate memory performance using localized electrophysiological activity and pupil dynamics.

Participants in both studies met clinical eligibility criteria for epilepsy monitoring and could complete cognitive tasks during recording sessions. In Study I, participants completed structured computerized neuropsychological assessment during scalp EEG monitoring, whereas in Study II, research procedures were conducted only after clinical stabilization to ensure that participation did not interfere with medical care.

The use of epilepsy populations provided important methodological advantages for the present thesis. Clinical monitoring environments enabled physiological recordings during structured cognitive tasks, while intracranial monitoring provided access to localized neural activity with high temporal and spatial precision. At the same time, important limitations should be acknowledged, including clinical heterogeneity, medication effects, variability in electrode placement, and the relatively small sample size of the intracranial cohort. These factors should be considered when interpreting generalizability of the findings.

4.2 Experimental Paradigms

The experimental paradigms used in this thesis were selected to investigate complementary aspects of cognitive function. Study I employed standardized computerized neuropsychological assessment using the Cambridge Neuropsychological Test Automated Battery (CANTAB), enabling investigation of electrophysiological correlates of cognitive performance across multiple domains. Study II used a verbal free recall paradigm designed to investigate trial-to-trial variability in memory performance using simultaneous pupillometry and iEEG recordings. These paradigms were selected because they provide structured behavioral variability that can be examined in relation to physiological signals.

4.2.1 Cambridge Neuropsychological Test Automated Battery (CANTAB)

In Study I, cognitive performance was assessed using selected tasks from the Cambridge Neuropsychological Test Automated Battery (CANTAB), a computerized neuropsychological testing platform widely used in clinical neuroscience research. CANTAB provides standardized administration, automated scoring, and precisely timed stimulus presentation, making it suitable for integration with electrophysiological recordings.

Three tasks were selected to assess cognitive domains relevant to the objectives of the thesis: Delayed Match to Sample (DMS), Spatial Span (SSP), and Spatial Working Memory (SWM).

Delayed Match to Sample (DMS) assessed visual recognition memory. Participants were presented with a complex abstract visual pattern and were subsequently required to identify the matching stimulus from four alternatives following delay intervals. This task evaluated visual memory performance while minimizing verbal encoding strategies.

Spatial Span (SSP) assessed visuospatial working memory capacity. Participants observed a sequence of spatial locations presented in a specific order and were required to reproduce the sequence after presentation. Sequence length progressively increased, allowing estimation of working memory span.

Spatial Working Memory (SWM) assessed working memory and executive functioning. Participants searched for tokens hidden across multiple locations while avoiding previously selected positions. Task performance reflected both memory updating and strategic planning processes.

These tasks were selected because they collectively assessed visual memory, spatial memory, working memory, and executive functioning, which represent cognitive domains central to the aims of the thesis. Their standardized structure provided objective and reproducible measures of cognitive performance that could be examined in relation to scalp EEG spectral features across participants.

4.2.2 Verbal Free Recall Memory Task

In Study II, memory performance was investigated using a verbal free recall paradigm during simultaneous pupillometry and intracranial EEG recording. Unlike standardized neuropsychological testing, the free recall task enabled investigation of trial-to-trial variability in memory performance under controlled experimental conditions.

Each trial consisted of four sequential phases: countdown, encoding, distractor, and recall. During the countdown phase, participants viewed a visual countdown from 10 to 1, which prepared them for the upcoming encoding period and provided a stable pre-task interval before stimulus presentation.

During the encoding phase, participants viewed a list of 12 nouns presented sequentially on a computer screen. Each word was displayed for 1500 ms, followed by a 1000 ms interstimulus interval. This phase enabled investigation of physiological activity associated with memory encoding.

Following encoding, participants completed a brief distractor phase involving arithmetic problems designed to reduce active rehearsal of the word list and separate encoding from retrieval processes.

The final recall phase consisted of a 30-second free recall period during which participants verbally recalled as many previously presented words as possible in any order. Vocal responses were recorded and temporally aligned with physiological recordings to enable analysis of neural and pupil dynamics associated with memory retrieval.

Memory performance was quantified as the number of correctly recalled words per trial. Based on recall performance, trials were categorized as good or poor memory performance, enabling investigation of trial-level variability in physiological activity associated with differences in memory performance.
The free recall paradigm was particularly suitable for the objectives of this thesis because it enabled investigation of both encoding- and retrieval-related physiological activity while generating substantial variability in performance across repeated trials. This structure provided a framework for examining how electrophysiological and pupil-related physiological signals relate to trial-to-trial variability in memory performance.

4.3 Definition of Cognitive Performance Groups

A key methodological step in this thesis involved transforming behavioral outcomes into structured performance groups suitable for quantitative physiological analysis. Because the objective was to investigate relationships between physiological signals and cognitive performance, behavioral measures obtained during task execution were operationalized into performance conditions that enabled systematic comparison of electrophysiological and pupil-related physiological activity.
Performance grouping was implemented differently across the two studies to reflect differences in experimental design and behavioral structure while maintaining conceptual consistency across paradigms. In Study I, behavioral outcomes were grouped at the participant level using standardized neuropsychological measures across cognitive domains. In Study II, behavioral outcomes were grouped at the trial level to capture variability in memory performance across repeated free recall trials.

4.3.1 Performance Grouping in the CANTAB Paradigm (Study I)

In Study I, cognitive performance was evaluated at the participant level across visual memory, spatial memory, working memory, and executive functioning domains using standardized outcomes derived from CANTAB. To enable meaningful comparison across individuals, behavioral outcomes were converted into age- and gender-normalized z-scores according to standardized CANTAB procedures.
Participants were grouped into three performance conditions based on normalized z-score thresholds:

- Good performance: z-score > +1
- Normal performance: −1 ≤ z-score ≤ +1
- Poor performance: z-score < −1

These thresholds enabled categorization of cognitive performance relative to normative distributions while accounting for demographic variability. Because cognitive domains were evaluated independently, participants could belong to different performance groups across tasks. For example, strong performance in

visual memory did not necessarily correspond to strong executive-function performance.

Although three performance groups were defined, analyses primarily emphasized comparisons between good and poor performance because these categories represented the largest behavioral contrast and therefore provided clearer separation for examining physiological variation associated with cognitive performance.

4.3.2 Performance Grouping in the Free Recall Paradigm (Study II)

In Study II, performance grouping was conducted at the trial level to capture variability in memory performance across repeated free recall trials. Unlike the participant-level grouping used in Study I, this approach enabled investigation of within-individual fluctuations in performance during the memory task.

Memory performance was quantified as the number of correctly recalled words following presentation of each 12-word list. Based on recall outcomes, trials were grouped into two performance conditions:

- Good memory performance: ≥5 recalled words
- Poor memory performance: ≤4 recalled words

These thresholds were selected to provide sufficient separation between stronger and weaker recall performance while preserving an adequate number of trials in each condition across participants. Recall performance was z-scored within participants, with values greater than zero representing relatively stronger recall and values less than zero representing relatively weaker recall. The selected cutoff additionally reflected the observed distribution of recall performance and facilitated balanced comparison between relatively successful and less successful memory states.

Trials with zero recall were excluded because they may reflect multiple non-memory-related factors, including reduced task engagement, attentional lapses, or incomplete encoding, making interpretation of physiological differences less specific to memory-related processes.

The use of trial-level grouping enabled physiological signals to be examined in relation to fluctuations in memory performance across repeated task conditions. By comparing electrophysiological and pupil-related physiological activity between good and poor memory performance conditions, it became possible to investigate physiological differences associated with variability in memory performance.

Together, these performance definitions established the behavioral framework for subsequent analyses by enabling physiological recordings to be examined in relation to differences in behavioral performance across cognitive domains and memory tasks.

4.4 Recording Procedures

This thesis employed three physiological recording modalities across the two studies: scalp EEG, iEEG, and pupillometry. These modalities were selected to capture complementary aspects of physiological activity associated with cognitive performance. EEG provided noninvasive measurements of large-scale cortical electrophysiological activity, iEEG enabled localized recording from clinically implanted brain regions, and pupillometry provided continuous measurement of pupil dynamics associated with cognitive processing.

4.4.1 Electroencephalography (EEG)

In Study I, scalp EEG recordings were acquired using the Quantum system (Natus, Middleton, WI, USA). Signals were recorded using a 31-channel electrode configuration arranged according to the international 10–20 system, with Cpz serving as the reference electrode. Data were sampled at 256 Hz, and a hardware high-pass filter with a cutoff frequency of 0.1 Hz was applied during acquisition.

EEG data were recorded continuously during cognitive task performance and subsequently segmented to include active task periods corresponding to the selected CANTAB paradigms. EEG was selected because it enables noninvasive recording of electrophysiological activity with high temporal resolution, making it suitable for investigating neural activity associated with cognitive performance across distributed cortical regions.

4.4.2 Intracranial Electroencephalography (iEEG)

In Study II, intracranial EEG recordings were acquired from patients undergoing clinical monitoring for drug-resistant epilepsy. Signals were recorded from depth and subdural electrodes implanted exclusively for seizure localization purposes. Data were sampled at 32 kHz to enable high-resolution recording of localized electrophysiological activity.

Electrode locations were identified through co-registration of pre-implantation magnetic resonance imaging (MRI) with post-implantation computed tomography (CT) scans using Statistical Parametric Mapping (SPM) software, followed by manual verification procedures.

iEEG was selected because it enables direct recording of electrophysiological activity from anatomically localized brain regions with high spatial and temporal precision. In the present thesis, iEEG recordings were used to investigate physiological activity associated with memory encoding and retrieval during the verbal free recall task.

4.4.3 Pupillometry

In Study II, pupil diameter measurements were acquired using the i4tracking infrared eye-tracking system (Medicton Group Ltd., Prague, Czech Republic). Pupil signals

were recorded at a sampling rate of 150 Hz under controlled lighting conditions to minimize luminance-related variability. Participants were instructed to maintain fixation and minimize head movement during task performance.

Pupillometry was selected because pupil dynamics provide a continuous physiological measure associated with cognitive processing. In the present thesis, pupil recordings were analyzed in relation to trial-to-trial variability in memory performance during the free recall task.

Together, these recording procedures provided complementary physiological measurements that enabled investigation of cognitive performance across participant-level and trial-level analyses. EEG supported assessment of large-scale cortical electrophysiological activity during structured cognitive testing, whereas iEEG and pupillometry enabled examination of localized neural activity and pupil dynamics during memory performance.

4.5 Physiological Signal Processing Pipelines

Raw physiological recordings contain information relevant to cognitive processes but are also affected by noise and artifacts originating from physiological, environmental, and technical sources. Therefore, preprocessing procedures were applied to improve signal quality and ensure that subsequent analyses reflected physiologically meaningful activity. Because EEG, iEEG, and pupillometry differ in their signal properties, modality-specific preprocessing pipelines were implemented in this thesis.

4.5.1 EEG Preprocessing

EEG preprocessing in Study I was performed using MATLAB (R2022b) in combination with the EEGLAB toolbox. The preprocessing pipeline consisted of filtering, artifact removal, and segmentation procedures designed to isolate electrophysiological activity associated with cognitive task performance.

First, EEG signals were filtered using a finite impulse response (FIR) band-pass filter between 1 and 57 Hz. This filtering range reduced low-frequency drift and high-frequency noise while minimizing contamination from power-line interference.

Following filtering, Independent Component Analysis (ICA) was applied to identify and remove physiological artifacts, including ocular activity associated with eye blinks and eye movements. Artifact-related components were identified using automated procedures and verified through visual inspection.

After artifact removal, EEG recordings were segmented to include task-relevant periods corresponding to active cognitive testing intervals. Signals outside task periods were excluded from further analysis to ensure that subsequent feature extraction reflected cognitive task performance.

The resulting cleaned EEG signals were used for spectral analysis to examine electrophysiological activity associated with differences in cognitive performance across CANTAB domains.

4.5.2 Intracranial EEG (iEEG) Preprocessing

Intracranial EEG preprocessing in Study II was performed using Python-based signal analysis tools. Because iEEG provides high-resolution recordings of localized neural activity, preprocessing focused primarily on noise reduction and spectral decomposition, followed by identification of task-responsive electrodes for subsequent analyses.

A notch filter centered at 60 Hz was applied using a second-order infinite impulse response (IIR) filter (Q = 30) to reduce power-line interference while preserving surrounding frequency content.

Following noise removal, electrodes were selected based on task-related spectral responses using a Gaussian Mixture Model (GMM) approach. Spectral activity induced during task performance was quantified for each electrode, and electrodes exhibiting minimal task-related activity were excluded from further analysis. This procedure enabled identification of electrodes exhibiting task-related neural responses while excluding channels with minimal induced activity. Restricting analyses to active electrodes reduced the influence of non-informative signals and improved sensitivity to physiological activity associated with cognitive task performance.

Spectral decomposition was subsequently performed using a Short-Time Fourier Transform (STFT) with a 0.5-second Hanning window and 25% overlap between consecutive windows. Power spectral density estimates were computed across time and transformed using a log10 transformation to reduce skewness and stabilize variance across frequency bands. Spectral power values were then normalized using z-score transformation to facilitate comparison across electrodes, frequency bands, and participants.

Finally, iEEG power time series were interpolated to 150 Hz to match the temporal resolution of pupil recordings, enabling temporal alignment between electrophysiological and pupillometric signals during subsequent analyses.

4.5.3 Pupillometry Preprocessing

Pupil data in Study II were preprocessed in MATLAB R2023b to characterize pupil dynamics while minimizing artifacts associated with blinks, temporary signal loss, and tracking instability.

Raw pupil measurements were extracted using an ellipse-fitting algorithm applied to infrared eye-tracking recordings. Horizontal and vertical pupil diameters were used to estimate pupil area, which served as the primary pupil measure. To improve

comparability across participants, measurements were converted from pixels to millimeters using individual interpupillary distance (IPD) calibration.
Short periods of missing data caused by blinks or temporary signal loss were corrected using linear interpolation. Interpolation was applied only to gaps shorter than 90 ms (approximately 14 samples at 150 Hz), whereas longer gaps were excluded from analysis to avoid distortion of temporal dynamics.
Following interpolation, pupil area values were z-score normalized within participants to reduce inter-individual baseline variability and emphasize task-related changes in pupil dynamics.
Together, these preprocessing procedures ensured that electrophysiological and pupil-related physiological signals were suitable for subsequent statistical analyses examining relationships between physiological activity and cognitive performance.

4.6 Feature Extraction

Feature extraction involved transforming preprocessed physiological recordings into quantitative variables. While preprocessing improved signal quality and reduced noise, feature extraction defined how electrophysiological and pupil-related physiological activity was represented for examining relationships with cognitive performance.
In this thesis, features were extracted from neural recordings (EEG and iEEG) and pupil recordings. These features were selected based on their relevance to cognitive and memory performance.
Two principal feature categories were defined:

- Spectral neural features derived from EEG and intracranial EEG signals
- Time-resolved pupil features derived from pupillometry recordings

4.6.1 Neural Spectral Features (EEG and iEEG)

Neural features were derived from frequency-specific electrophysiological activity recorded using scalp EEG and iEEG. Because oscillatory activity within distinct frequency bands has been associated with cognitive and memory processes, spectral power measures served as the principal neural features examined in this thesis.
In Study I, neural features were represented by spectral power estimates extracted from scalp EEG recordings across seven frequency bands:

- Delta: 1–4 Hz
- Theta: 4–8 Hz
- Alpha: 8–12 Hz
- Low Beta: 12–20 Hz
- High Beta: 20–30 Hz
- Low Gamma: 30–40 Hz

- High Gamma: 40–55 Hz

Spectral power values were examined across scalp electrodes in relation to cognitive performance in visual memory, spatial memory, working memory, and executive-function domains assessed using CANTAB.

In Study II, neural features were represented by intracranial power-in-band (PIB) estimates extracted across six frequency bands:

- Delta: 2–4 Hz
- Theta: 4–8 Hz
- Alpha: 8–12 Hz
- Beta: 12–30 Hz
- Low Gamma: 30–60 Hz
- High Gamma: 60–150 Hz

For intracranial recordings, PIB features were analyzed as time-resolved trajectories aligned to task events during memory encoding and retrieval. During encoding, electrophysiological activity was aligned to word presentation, whereas retrieval-related activity was aligned to vocalization onset during recall. To facilitate regional analyses, electrodes were grouped according to anatomical regions, including frontal, temporal, parietal, occipital, and limbic areas.

Neural spectral features were subsequently examined in relation to differences in cognitive and memory performance across studies.

4.6.2 Pupillometry Features

Pupil-related physiological features were derived from continuous pupil area recordings obtained during the verbal free recall task in Study II. In contrast to electrophysiological features represented by frequency-specific spectral power, pupillometric features were analyzed as time-resolved trajectories associated with memory task events.

For encoding analyses, pupil signals were aligned to word presentation during the encoding phase, enabling examination of physiological changes associated with memory formation. For retrieval analyses, pupil signals were aligned to vocalization onset during the recall phase, allowing investigation of pupil dynamics associated with memory retrieval.

Pupil trajectories were examined in relation to memory performance across trials categorized as good or poor memory performance. This event-based framework enabled assessment of temporal changes in pupil activity occurring before and during memory-related task events, allowing investigation of how pupil dynamics varied across encoding and retrieval periods.

4.7 Statistical Analysis Strategy

Statistical analyses were conducted to evaluate relationships between physiological activity and cognitive performance across studies and to identify temporal intervals during which neural and pupil-related physiological signals differed according to variations in memory performance. Because cognitive processes unfold dynamically over time, statistical methods were designed to preserve temporal resolution and enable characterization of transient physiological effects associated with task performance.

All statistical analyses were implemented using MATLAB (R2022b–R2023b) and Python-based analysis tools, depending on signal modality. Statistical procedures were applied to both neural spectral features and pupillometric measures, ensuring consistent evaluation across physiological modalities. The statistical framework of this thesis addressed three primary objectives:

1. Evaluate physiological differences between performance conditions
2. Identify temporal patterns of physiological activity associated with cognitive and memory performance
3. Assess robustness and statistical reliability of observed effects

4.7.1 Analysis of Group-Level Differences

In Study I, statistical analyses were performed to evaluate relationships between EEG spectral features and cognitive performance across cognitive domains assessed using CANTAB, including visual memory, spatial memory, working memory, and executive function. Comparisons were conducted across participant groups representing good, normal, and poor cognitive performance as defined by standardized behavioral outcomes.

To evaluate electrophysiological differences associated with cognitive performance, two-way analysis of variance (ANOVA) models were applied to EEG spectral power features across electrodes and frequency bands. The analysis incorporated behavioral performance group and electrode location as experimental factors, enabling assessment of performance-related electrophysiological variation across spatial recording sites.

When significant effects were identified, post hoc comparisons were conducted using Tukey–Kramer correction procedures to evaluate differences between specific performance groups while accounting for multiple comparisons. These analyses enabled identification of frequency bands and electrode locations exhibiting significant associations with performance in visual memory, spatial memory, working memory, and executive-function domains.

In addition to group comparisons, correlation analyses were performed to evaluate relationships between EEG spectral features and continuous cognitive performance measures. Correlation-based analyses enabled assessment of how variations in

electrophysiological activity related to behavioral performance across cognitive domains, providing complementary information beyond categorical group comparisons.

Together, these analyses were designed to identify large-scale electrophysiological patterns associated with variation in cognitive performance across individuals, providing an initial characterization of neural features related to cognitive functioning.

4.7.2 Time-Resolved Statistical Analysis

In Study II, time-resolved statistical analyses were performed to examine physiological variability associated with memory performance during encoding and retrieval. Because electrophysiological and pupil signals evolved dynamically over time, analyses were designed to preserve temporal information while accounting for inter-subject variability.

Physiological trajectories were examined at the temporal resolution of signal acquisition using approximately 7 ms time bins corresponding to the 150 Hz sampling rate of pupil recordings and temporally aligned intracranial EEG signals. At each time point, normalized physiological activity was compared between good and poor memory performance conditions to characterize temporal patterns associated with differences in memory performance.

To evaluate temporally sustained physiological effects while controlling for multiple comparisons across time, participant-level cluster-based permutation analysis was performed on subject-wise physiological trajectories. Cluster formation was based on temporally contiguous intervals exceeding the cluster-forming threshold of $p < 0.05$, and statistical significance was evaluated through permutation testing.

Because cluster-based inference was conservative in the context of limited sample size and temporally correlated physiological signals, temporally sustained intervals identified through cluster analysis were subsequently used to define windows of interest for participant-level statistical inference. Within these intervals, subject-wise average good–poor differences were computed and evaluated using one-sample t-tests, Wilcoxon signed-rank tests, and exact sign-flip permutation testing.

This analytical framework enabled identification of temporal intervals during which physiological activity differed between memory performance conditions while preserving temporal resolution and accounting for inter-subject variability.

4.7.3 Robustness Analysis

In Study II, to evaluate the robustness of observed pupil-related physiological effects and determine whether results were disproportionately influenced by individual participants, leave-one-subject-out sensitivity analyses were performed in Study II. This procedure was particularly important given the limited sample size and inter-individual variability characteristic of intracranial monitoring studies.

For each identified pupil-related temporal effect, analyses were repeated iteratively after excluding one participant at a time. Subject-wise physiological differences between good and poor memory performance conditions were recomputed for each iteration, enabling assessment of whether the observed effects remained consistent across participant subsets.

In addition to statistical significance across iterations, the direction of pupil-related effects was examined to determine whether physiological differences between good and poor memory performance remained stable after removal of individual participants. This approach reduced the likelihood that observed pupil effects were driven disproportionately by a single participant or a small subset of participants.

Consistency of pupil-related physiological effects across leave-one-subject-out iterations was interpreted as evidence of increased robustness and reliability of observed memory-related physiological patterns.

4.7.4 Statistical Significance and Interpretation

Statistical significance was evaluated using a threshold of $p < 0.05$. In Study I, significance testing focused on identifying differences in EEG spectral features across cognitive performance groups using analysis of variance, post hoc comparisons, and correlation analyses. In Study II, significance testing emphasized temporally sustained physiological effects associated with differences in memory performance during encoding and retrieval.

Because time-resolved analyses in Study II involved repeated comparisons across time, subject-level cluster-based permutation analysis was used to control for multiple comparisons and identify temporally contiguous physiological effects. To further strengthen inference in the context of the limited sample size, complementary statistical procedures, including one-sample t-tests, Wilcoxon signed-rank tests, and exact sign-flip permutation testing, were applied within temporal windows identified through cluster-based analysis.

In addition to statistical significance, interpretation of physiological effects considered the magnitude and temporal consistency of observed differences. t-statistics were examined to compare the relative strength of physiological effects across neural and pupil-related features, while temporally sustained effects were interpreted more strongly than isolated transient differences.

Together, these statistical procedures provided the analytical framework for examining relationships between physiological activity and cognitive performance across studies. Group-level statistical analyses were applied in Study I to examine associations between EEG features and cognitive performance across CANTAB cognitive domains, whereas Study II incorporated time-resolved statistical inference and robustness analyses to evaluate physiological variability associated with memory performance.

4.8 Classification Strategy

In addition to statistical analyses, classification approaches were implemented in Study II to evaluate whether physiological features contained predictive information associated with memory performance. Whereas statistical analyses examined physiological differences between performance conditions, classification analyses evaluated whether electrophysiological and pupil-related physiological features could distinguish variations in memory performance at the trial level.

4.8.1 Time-Resolved Feature Windows

To preserve temporal information during classification analyses, physiological signals were segmented into overlapping temporal windows. This approach enabled evaluation of how the discriminative information contained within electrophysiological and pupil-related physiological signals evolved across encoding and retrieval periods.

Feature extraction for classification was performed using a sliding-window approach with a 67 ms window size and 33 ms step size, resulting in approximately 50% overlap between consecutive windows. These parameters were selected to balance temporal resolution and signal stability while preserving short-term physiological dynamics. Shorter windows may be overly sensitive to noise, whereas larger windows may obscure transient physiological changes associated with memory processes.

For each temporal window, physiological features were computed independently and evaluated separately for encoding and retrieval phases. During encoding, features were aligned to word presentation, whereas retrieval-related features were aligned to vocalization onset. This event-based structure enabled examination of temporal intervals during which physiological activity exhibited the strongest separability between memory performance conditions.

The use of overlapping temporal windows allowed characterization of how physiological information evolved across time, enabling identification of periods during which neural and pupil-related signals showed the greatest differentiation between good and poor memory performance.

4.8.2 Classification Model

Classification analyses in Study II were performed using a support vector machine (SVM) classifier to evaluate whether physiological features could distinguish differences in memory performance at the trial level. SVMs are widely used in physiological signal analysis because they are effective in high-dimensional settings, robust to noisy data, and capable of identifying discriminative patterns within complex physiological measurements.

A radial basis function (RBF) kernel was selected to allow modeling of nonlinear relationships between physiological features and memory performance conditions. Separate classification analyses were performed independently for each physiological feature, including pupil-related and intracranial electrophysiological features, enabling direct comparison of their relative ability to distinguish good and poor memory performance.

Classification models were applied separately across temporal windows during encoding and retrieval phases. For each time window, feature values were used to classify trials into

good or poor memory performance conditions, allowing evaluation of how physiological separability evolved across time.

Hyperparameter optimization was performed within the training data during cross-validation to minimize overfitting and improve generalizability of classification performance estimates. This approach ensured that model evaluation reflected the discriminative information contained in physiological signals rather than optimization bias.

4.8.3 Cross-Validation Strategy

To evaluate classification performance while reducing overfitting, a leave-pairs-out cross-validation (LPO-CV) strategy was implemented in Study II. Cross-validation is essential in physiological signal classification because it enables assessment of model performance on unseen data, thereby improving the reliability of performance estimates.

In each cross-validation iteration, one pair of trials consisting of one good and one poor memory performance trial was excluded from model training and reserved for testing. The remaining trials were used to train the classification model. This procedure was repeated iteratively across all possible good–poor trial pairs, ensuring that classification performance was evaluated comprehensively across the dataset.

The leave-pairs-out strategy was selected because it preserves class balance during testing while maximizing use of the available data. This consideration was particularly important given the limited sample size and unequal numbers of good and poor memory trials across participants. By repeatedly evaluating performance on unseen trial pairs, the procedure reduced the likelihood that classification results reflected overfitting to specific observations.

To prevent information leakage, hyperparameter optimization was performed exclusively within training data during each cross-validation iteration. Test data remained completely independent until final evaluation, ensuring unbiased estimation of classification performance.

4.8.4 Performance Metrics

Classification performance was evaluated using multiple complementary metrics to assess how effectively physiological features distinguished differences in memory performance. Because the number of good and poor memory trials was not always balanced across participants, performance measures were selected to reduce sensitivity to class imbalance.

The primary evaluation metric was the macro-F1 score, which was selected because it evaluates classification performance equally across classes by averaging F1 scores computed independently for good and poor memory performance conditions. This metric provides a balanced assessment of classification quality and is particularly appropriate when class distributions are unequal.

In addition to macro-F1, weighted-F1 score and balanced accuracy were computed to provide complementary perspectives on classification performance. Weighted-F1 accounts for differences in class frequencies by weighting class-specific F1 scores according to the number of observations in each class. Balanced accuracy reflects the average sensitivity across performance conditions and provides an additional measure robust to class imbalance.

Classification performance was evaluated independently across temporal windows during encoding and retrieval phases. Peak performance values were subsequently identified to determine temporal intervals during which physiological features exhibited the strongest separability between memory performance conditions.
To facilitate interpretation, classification performance was additionally compared with baseline expectations, enabling evaluation of whether physiological signals distinguished memory performance beyond chance levels.

4.8.5 Permutation-Based Validation

To evaluate whether observed classification performance exceeded chance levels while accounting for repeated temporal testing, permutation-based validation procedures were implemented in Study II. This approach was particularly important because classification analyses involved repeated evaluation across overlapping temporal windows, increasing the possibility of inflated performance estimates due to multiple comparisons and peak selection.
Permutation testing was performed by randomly shuffling memory performance labels within participants while preserving the original trial structure. Following label permutation, the complete classification pipeline was repeated, including temporal windowing, model training, hyperparameter optimization, leave-pairs-out cross-validation, and performance evaluation. This procedure was repeated across multiple permutations to generate null distributions of classification performance under the assumption that no systematic relationship existed between physiological activity and memory performance. Peak classification performance observed in the original data was subsequently compared with the permutation-derived null distributions.
To account for repeated testing across temporal windows, statistical inference was based on the distribution of maximum classification performance values obtained across permutations. This max-statistic approach controlled for inflation of performance estimates arising from repeated temporal scanning and enabled more conservative evaluation of classification significance.
Permutation-based validation therefore provided an additional safeguard against overfitting and improved confidence that observed classification performance reflected meaningful physiological differences associated with memory performance rather than random variation.

4.9 Computational and Methodological Contribution of the Analytical Framework

The methodological contribution of this thesis lies in the integration of complementary computational approaches for transforming physiological signals recorded using EEG, iEEG, and pupillometry into quantitative biomarkers of cognition across multiple levels of variability. Although Study I and Study II employed different experimental paradigms and analytical procedures, both were designed to address the central dissertation thesis: that physiological signals can be processed through digital signal processing, statistical analysis, and machine learning

approaches to support objective characterization of cognitive performance and memory-related variability across individuals and trials.

Study I focused on inter-individual variability by transforming scalp EEG recordings into frequency-specific spectral features and examining whether these features differentiated individuals with varying levels of memory and executive functioning. Through digital signal processing and statistical modeling, EEG-derived biomarkers were evaluated for their ability to characterize cognitive differences between participants. In contrast, Study II focused on intra-individual variability by analyzing trial-by-trial changes in pupil dynamics and intracranial electrophysiological activity during a verbal free recall task to identify physiological signatures associated with memory-related variability across trials.

Because the characteristics of the physiological recordings and behavioral paradigms differed across studies, modality-specific analytical procedures were implemented. Nevertheless, both investigations applied structured computational pipelines involving physiological signal preprocessing, feature extraction, time-resolved statistical inference, robustness evaluation, and machine learning approaches for physiological classification associated with behavioral outcomes. In Study II, additional methodological emphasis was placed on participant-level inference, leave-one-subject-out sensitivity analysis, and permutation-based validation to improve robustness in the context of limited sample size.

The novelty of the analytical framework lies in the integration of complementary physiological signal analysis across both inter-individual and intra-individual levels of cognitive variability. Rather than relying on a single physiological modality or level of analysis, this thesis combines electrophysiological and pupil-related signal processing with digital signal processing, statistical inference, and machine-learning-based validation to investigate cognitive performance and memory-related variability. Across two complementary experimental paradigms, the dissertation establishes a unified and reproducible computational framework for transforming physiological recordings into quantitative indicators of cognitive and memory-related variability, while enabling comparison between non-invasive and invasive physiological measurements.

Chapter 5

5. Empirical Studies: Cognitive Performance Biomarkers

The methodological framework described in the preceding chapter established the analytical procedures used to transform physiological signals into quantitative indicators of cognitive performance. Building upon this foundation, the present chapter presents two empirical studies that investigate relationships between physiological activity and cognitive function using complementary recording modalities and analytical approaches.

The first study examined whether spectral EEG features recorded during standardized neuropsychological assessment were associated with inter-individual variability in memory and executive performance. The second study extended this investigation to trial-level variability in memory performance by combining intracranial electrophysiological recordings and pupillometry during a verbal free recall task. Together, these studies address the central aim of the dissertation by evaluating whether physiological signals contain measurable information associated with variability in cognitive and memory performance across individuals and trials.

The following sections introduce each empirical study and its contribution to the overall dissertation framework before presenting the associated published work.

5.1 Study I — Anterior prefrontal EEG theta activities indicate memory and executive functions in patients with epilepsy

This study investigated whether non-invasive electrophysiological activity recorded using scalp electroencephalography (EEG) was associated with variability in cognitive performance across individuals. As the first empirical investigation of this dissertation, the study addressed whether physiological signals contain measurable information related to differences in memory and executive functioning.

Using simultaneous EEG recordings during standardized neuropsychological assessment, the study examined relationships between neural oscillatory activity and performance across cognitive domains related to visual memory, spatial memory, working memory, and executive functioning. By evaluating electrophysiological variability across participants, the study provided an initial framework for identifying physiological features associated with cognitive performance.

Beyond its empirical findings, Study I established an important conceptual and methodological basis for the second study. While the present investigation focused on inter-individual variability in cognitive performance, it motivated subsequent examination of intra-individual, trial-by-trial physiological variability using multimodal recordings in Study II.

The study additionally attracted scientific commentary emphasizing its potential translational relevance for objective cognitive assessment in epilepsy. In particular, reduced anterior prefrontal theta activity associated with poorer cognitive performance was highlighted as a promising electrophysiological marker of memory and executive dysfunction, with potential implications for diagnosis, monitoring, and future cognitive-targeted interventions[111].

The following publication presents the complete methodology, analyses, and findings of Study I.

5.1.1 Publication I

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Epilepsia®

RESEARCH ARTICLE

Anterior prefrontal EEG theta activities indicate memory and executive functions in patients with epilepsy

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Abstract

Objective: Cognitive deficits are one of the most debilitating comorbidities in epilepsy and other neurodegenerative, neuropsychiatric, and neurodevelopmental brain disorders. Current diagnostic and therapeutic options are limited and lack objective measures of the underlying neural activities. In this study, electrophysiological biomarkers that reflect cognitive functions in clinically validated batteries were determined to aid diagnosis and treatment in specific brain regions.

Methods: We employed the Cambridge Neuropsychological Test Automated Battery (CANTAB) tasks to probe memory and executive functions in 86 patients with epilepsy undergoing clinical electroencephalography (EEG) monitoring. EEG electrode signals during performance of particular battery tasks were decomposed to identify specific frequency bands and cortical areas that differentiated patients with impaired, normal, and good standardized performance according to their age and gender.

Results: The anterior prefrontal cortical EEG power in the theta frequency band was consistently lower in patients with impaired memory and executive function performance (z-score < -1). This effect was evident in all four behavioral measures of executive, visual, spatial, and working memory functions and was confined to the cortical area of all four frontal pole electrodes (Nz, Fpz, Fp1, and Fp2).

Significance: Theta EEG power in the anterior prefrontal cortex provides simple, accessible, and objective electrophysiological measure of memory and executive functions in epilepsy. Our results suggest a feasible clinical biomarker for diagnosis, monitoring, and treatment of cognitive deficits with emerging targeted neuromodulation approaches.

KEYWORDS

cognition, neural oscillations, neurofeedback, neurophysiology, neuropsychology

1 | INTRODUCTION

Cognitive functions encompass a broad range of domains, including memory, perception, learning, attention, language abilities, problem solving, and decision-making, which are impaired in a range of neurological and psychiatric diseases. Such impairments affect general performance and quality of life.[1,2] Hence, individuals with brain disorders often seek treatments to address cognitive impairments as much as other primary disease symptoms, such as seizures, in the case of epilepsy.[3] Limited treatment options for cognitive deficits, such as systemic drugs, have driven the development of more focused and innovative interventions for therapy, targeting specific brain regions and neural activities. Multiple neuroimaging, electrophysiological, and behavioral methods can be used to target new therapy, among which electroencephalography (EEG) offers non-invasive localization of neural activities involved in cognitive functions.

As the demand for safe and effective neuromodulation therapy rises, there is an increasing need for objective functional metrics to assess cognitive and other brain functions. Such physiological and behavioral measures would play a fundamental role in diagnosing and treating cognitive deficits.[4,5] Specific frequency ranges within the EEG spectrum can serve as biomarkers of cognitive abilities. For instance, enhancing resting spectral power in the upper alpha frequency range through neurofeedback training was associated with improved cognitive performance.[6] Other studies have associated specific EEG features, such as spectral power in the delta, theta, and alpha frequency range, with particular cognitive functions.[7–9] Moreover, neural activities in theta and gamma EEG frequencies have been proposed to play critical roles in various aspects of memory and executive functions,[10–16] suggesting their potential use as biomarkers for therapeutic interventions. Despite these advancements, biomarker integration into the clinical practice of diagnosing and treating cognitive impairments remains limited[17] due to inconsistent EEG frequency bands and brain regions engaged in various—often clinically unvalidated—tasks.

Previous studies have shown that different cognitive tasks induced EEG activities in multiple frequency bands and cortical regions,[18–20] indicating the demand for a more comprehensive approach to determine consistent EEG biomarker in the EEG frequency spectrum and anatomic space. An ideal biomarker would target a given neuromodulation therapy or diagnosis in one frequency band and cortical region for a spectrum of cognitive functions.

Here, we conducted EEG recordings during performance of a battery of tasks probing memory and executive functions. Given the results of our previous study with intracranial brain recordings,[21,22] we hypothesized that theta activities in the anterior prefrontal cortical regions will reflect patient performance in the tasks. Theta frequency activities[7,11,23–25] in frontal cortical regions[23–25] have been shown to play essential roles in memory and executive functions, but only during specific tasks. Our goal was to identify a consistent EEG activity across multiple frequency bands and cortical regions that can discriminate between patients with epilepsy with impaired and healthy cognitive performance.

Key points

- Clinically validated battery of touch screen tasks effectively identified epilepsy patients with impaired memory and executive functions.
- Patients with memory and executive function deficits showed significantly decreased electroencephalography (EEG) theta power in the anterior prefrontal cortex.
- Theta power from all four frontal pole EEG electrodes correlated with the behavioral measures of memory and executive functions.
- Anterior prefrontal theta power provides a feasible electrophysiological biomarker for the diagnosis and treatment of cognitive deficits in epilepsy and potentially other brain disorders.

2 | MATERIALS AND METHODS

2.1 | Participants

Eighty-six epilepsy patients with confirmed electrographic seizures in the epilepsy monitoring unit (EMU) at Mayo Clinic were recruited for this study. All participants were recruited and tested under a standardized protocol to ensure consistent experimental conditions throughout their EMU stay. Patients were enrolled in the study within 24 h from their first clinically confirmed electrographic seizure recorded during the monitoring. The cohort consisted of 45 male and 41 female participants with an average age of 36 years (±12.8). These patients experienced their first seizure on average at the age of 21.4 years (±15.6), with a mean epilepsy duration of 14 years (±12.3). Participants reported ~476 mean seizures annually. During an EEG and video examination in the EMU, the patients were asked to complete a series of Cambridge Neuropsychological Test Automated Battery (CANTAB) tasks.[26] The data were collected at the Mayo Clinic (Rochester, MN, USA). The research protocol was approved by the respective

institutional review board (IRB), and written informed consent was obtained from each participant.

2.2 | Experimental paradigm

The data consists of EEG signals and behavioral performance on the CANTAB battery of selected cognitive tasks. We selected CANTAB tasks that assess three primary cognitive domains: attention, memory, and executive function. The attention domain probed by CANTAB involves the capacity to focus selectively on pertinent information while disregarding irrelevant stimuli. Memory tasks probed the patients' capacity to hold information during short and long delays. Executive functions assessed strategic problem-solving and decision-making. The three selected battery tasks that probed these cognitive domains are summarized as follows and visualized in Figure 1A with the average task duration under each schematic (mean ± standard deviation [SD]).

Delayed match to sample (*DMS*): The participants were presented with a complex, abstract, non-verbal visual pattern (sample), followed by a brief delay, and then the presentation of four similar patterns to choose (test). The task required the participants to identify and touch the test pattern that exactly matched the sample. Occasionally the sample and choice patterns were presented simultaneously, whereas in all other trials, a delay of 0, 4, or 12 s was applied before the four choices appeared. The first trial type assessed visual matching ability, whereas the others tested short-term visual recognition memory for patterns that were difficult to describe verbally. Subject performance in this task was quantified as percentage of correct responses, defined as "visual memory" performance.

Spatial span (*SSP*): White squares were displayed on a touchscreen, changing color in a variable order sequence (sample phase). Following the presentation of the entire sequence, the patients were required to touch the boxes in the exact order in which they changed their color in the sample phase (test phase). The sequence begins with two boxes and gradually increases to nine

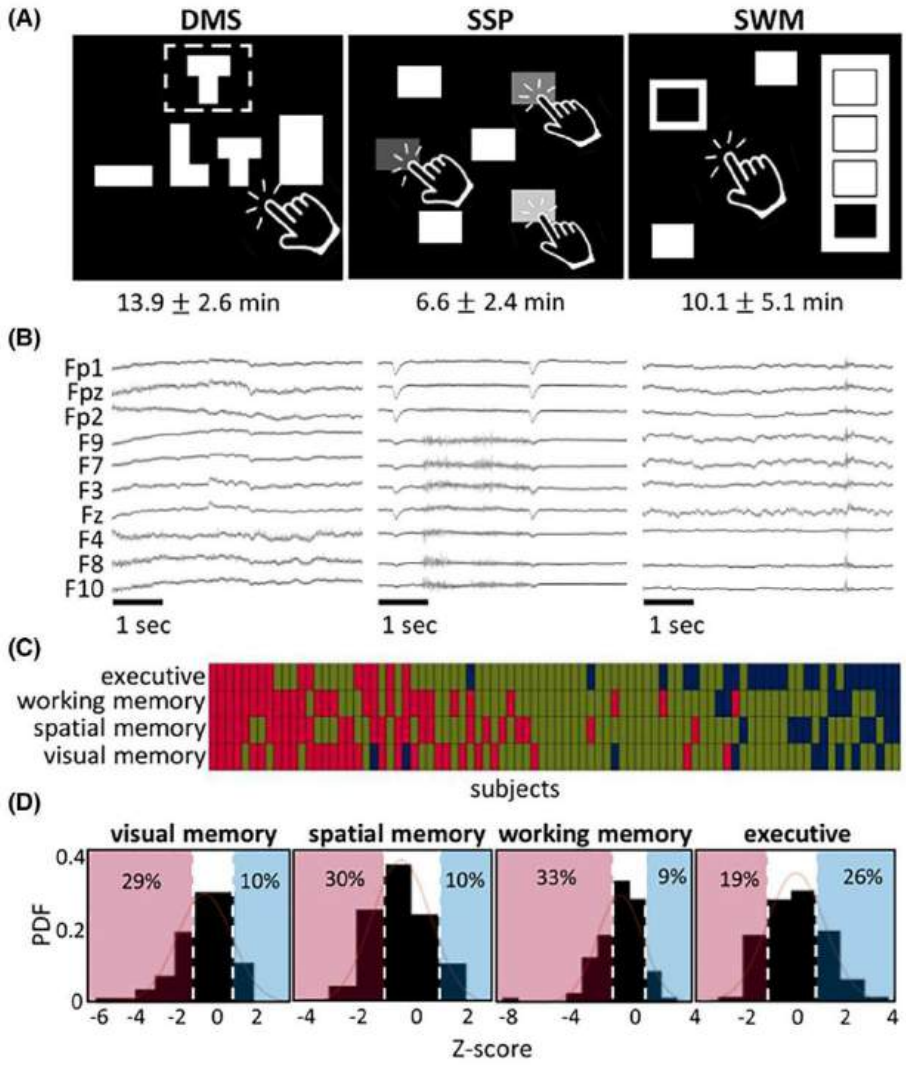

FIGURE 1 EEG recordings during performance of CANTAB tasks were used to identify epilepsy patients with poor, normal, and good memory and executive functions. (A) Selected CANTAB tasks were performed on a touch-screen tablet with a range of time frames during video-EEG monitoring. Under each task's schematic, the average time taken to complete the task among all participants is provided. (B) Example signals recorded from frontal EEG electrodes were taken from entire segments of performing a given task. (C) Participants were arranged in ascending order from the poorest to the best performance across the four measures (poor performers in red, normal performers in green, and good performers in blue) based on their overall scores. (D) Age- and gender-normalized behavioral scores of task performance identified patients with good (blue) and poor (red) memory and executive functions. CANTAB, Cambridge Neuropsychological Test Automated Battery; EEG, electroencephalography.

as the test progresses, with variations in both sequence order and color. Once a sample sequence is successfully replicated in the test phase, the patient advances to the next stage with an additional box in the sequence. In the case of a mistake in the test, the patient has two more attempts at that stage until the task is finished with a score of 1–9 corresponding to the maximum number of boxes in a sequence remembered. This test assesses visuospatial working memory capacity. The final score of the longest sequence recalled was defined as the "spatial memory" performance.

Spatial working memory (*SWM*): The participants were presented with squares randomly located on the screen. The objective was to find which one of them contained a hidden colored "token" by pressing a square and then collecting the found tokens in a column on the right hand side of the screen. The difficulty level of the test gradually increased from three to nine squares. The colors and positions of the boxes on the screen changed in each trial to discourage the use of the same search strategy. However, using a consistent search strategy, for example, from the left to the right side or from top to bottom, to avoid visiting the same box twice, was assessed as a measure of executive planning. This task assesses both the retention and capacity to hold visuospatial information, placing notable demands on executive functions, and providing a metric for both strategy and working memory errors. Two measures were used to evaluate the participants' performance. The former tracked the number of times a participant reopens a box where the token was previously found, to indicate "working memory" performance. The second tracked the search strategy, as explained above, to indicate "executive" task performance.

Moreover, each subject started with completing a control motor screening task (MOT), which tested basic sensorimotor performance by requiring subjects to touch a cross appearing at random locations on the screen. This task, included in the CANTAB and administered at the start of each session, did not engage memory or executive functions, while maintaining a baseline level of attention.

2.3 | EEG data

Data were acquired using the Quantum system (Natus, Middleton WI, USA) at the Mayo Clinic (Rochester, MN, USA). The system utilized a 31-channel setup following the 10-20 electrode placement system. The reference electrode was placed close to the "CPz" lead, and a referential montage was used in all cases. The data were sampled at a rate of 256 Hz, and a hardware-based high-pass filter with a cutoff frequency of 0.1 Hz was applied to the data (see Figure 1B for an illustrative 5-s segment of the signal recorded from frontal EEG electrodes). Data were recorded continuously during the entire experiment but only the EEG epochs related to the tasks were extracted, and periods around the battery performance were removed. Some patients had additional electrode leads recording electrocardiography (ECG) and electrooculography (EOG) signals; these electrodes were not included in the analysis to maintain a standardized process.

2.4 | Behavioral analysis

Each task of the battery was performed once by each subject, and four behavioral measures were computed (visual memory, spatial memory, working memory, and executive function), and expressed as z-scores normalized to the age and gender (see Table S1 for a summary of the patients' scores). This allowed us to group each task score into "good," "normal," or "poor" performance based on the z-score distribution (good: z-score $> +1$; normal: $-1 < z$-score $< +1$; poor: z-score < -1). Since each task was evaluated independently; a subject could be labeled as a good or poor performer in different tasks (Figure 1C). The number of patients ($n = 86$) in the study was sufficient to ensure more than eight patients in any group (poor, normal, and good) to power the statistical comparisons. Figure 1D shows the probability density function (PDF) of the z-scores for all four behavioral measures.

2.5 | EEG analysis

EEG signal processing was conducted using the MATLAB academic version R2022b software package (MathWorks Inc.). The following pre-processing was applied to the entire signal using the EEGLAB toolbox.[27] The first step involved applying a band-pass finite impulse response (FIR) filter within the frequency range of 1–57 Hz to eliminate power-line noise (at 60 Hz) and non-neural signal artifacts. Independent component analysis (ICA) was employed to remove physiological artifacts, such as eye blinks and heartbeats, using both an automated approach[28] and visual examination of the components.

Subsequently, the signals were segmented with the complete duration of each task considered, and the frequency domain features of the signal were extracted by computing the spectral power within seven distinct frequency bands: δ (1–4 Hz), θ (4–8 Hz), α (8–12 Hz), β_1 (12–20 Hz), β_2 (20–30 Hz), γ_1 (30–40 Hz), and γ_2 (40–55 Hz). The signal power acquired from the 31 electrodes was estimated across seven frequency bands and then normalized

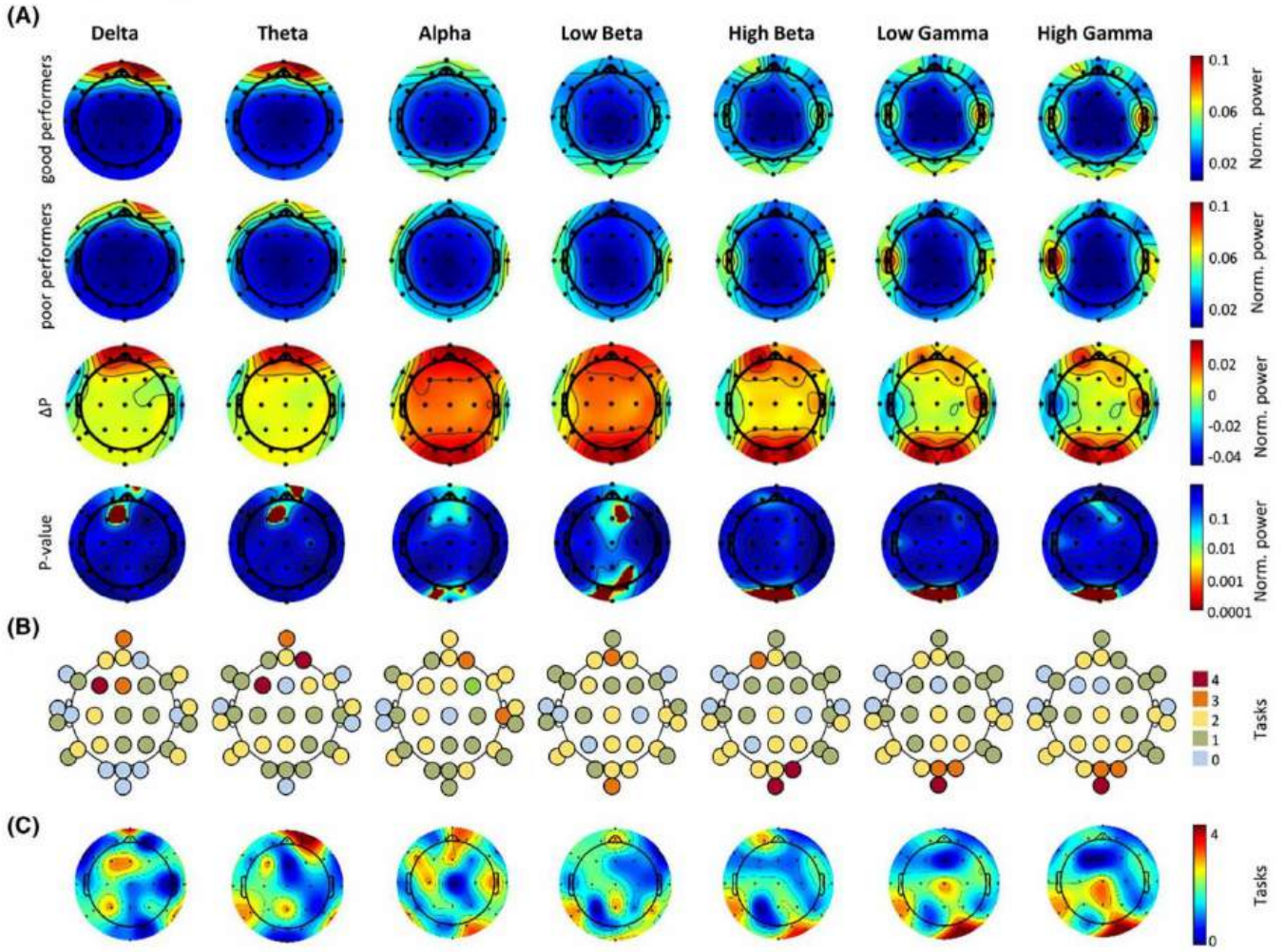

FIGURE 2 The increased spectral power of low-frequency frontal and high-frequency occipital EEG activities differentiates between patients with good and poor task performance. (A) Patients with a good visual memory task performance (see Figure S1 for the other measures) showed significantly greater power in the anterior low-frequency bands and posterior high-frequency bands. (B) EEG electrodes with significantly greater low- and high-frequency powers in up to four memory and executive performance measures were localized in the anterior prefrontal and posterior occipital cortical areas. (C) Electrodes with significant power-in-band differences (ANOVA, $p \leq .05$) in up to four tasks, as displayed in "b", revealed the low- and high-frequency "hotspots" in the corresponding anterior prefrontal and the occipital cortical areas on the interpolated heatmaps. ANOVA, analysis of variance; EEG, electroencephalography.

using an ℓ_1-normalization.[29] Having the normalized power for all patients, the final feature was determined as the average spectral power for a given behavioral measure, which can be seen as a grand average of the spectral power across subjects (Figure 2A, Figure S1).

In addition, we derived the difference in spectral power (ΔP), which serves as a metric of neural processing, defined as the difference in the magnitude of activation in a specific brain region between the good and poor patient performance from the task measure. This was calculated by subtracting the normalized signal power in the poor condition from that in the good condition. ΔP was first computed at the resolution of each electrode within specific frequency bands, as illustrated in Figure 3C, and then averaged among the electrodes within a particular cortical region, as shown in Figure 3D.

2.6 | Statistics

All statistical tests were conducted using the MATLAB academic version R2022b software (MathWorks Inc.). A two-way analysis of variance (ANOVA) was conducted to compare spectral features across all good and poor performers in each task, examining the effects of performance type (1 df) and channel location (30 df) on the normalized signal power. One-way and two-tailed ANOVA with Tukey–Kramer post hoc testing of median and confidence intervals (CIs) was employed to test differences in variance across frequency bands and brain regions and to identify significantly different groups. The spectral features in specific brain regions and frequency bands during the performance of a given cognitive task were compared between groups of patients showing good and poor performance.

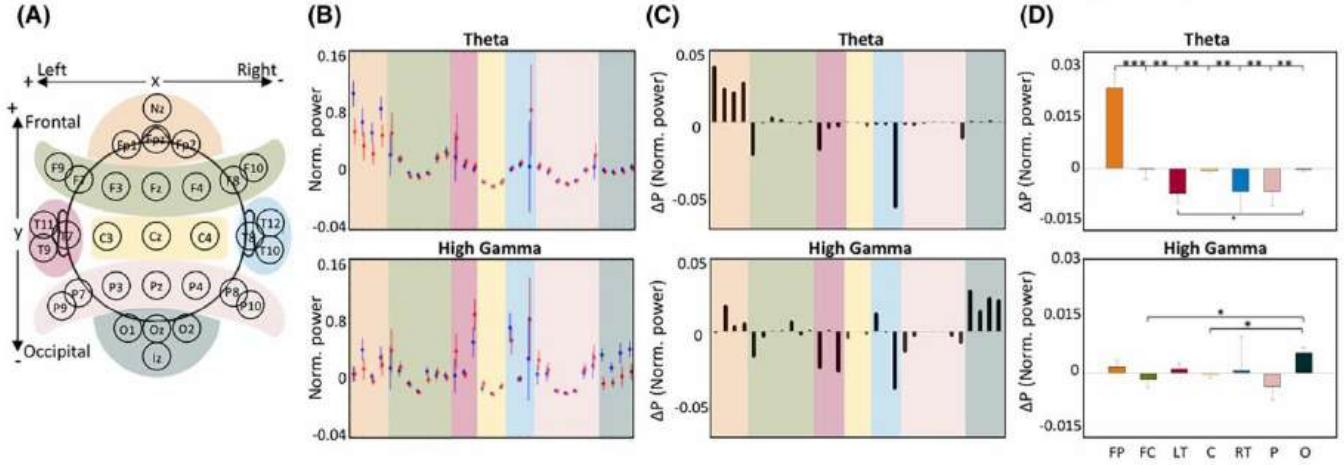

FIGURE 3 Anterior prefrontal EEG theta activities show the greatest spectral power and differences in poor and good memory and executive function performance. (A) EEG electrodes were anatomically grouped into seven color-coded cortical areas of interest in a normalized 2D Cartesian system, derived from spherical coordinates, with "Cz" marking the origin. (B) Average spectral power of the theta band activities in the anterior prefrontal cortex was greater than in any other area, including the gamma band activities in the occipital cortex (bars indicate 95% confidence interval). Notice that the theta power was greater both in case of the good (blue) and the poor (red) patient performance, which was higher than the background range of increasing power away from the "Cz" center of the 2D plane (see Figure S3 for the other three task measures). (C) Spectral power difference (ΔP) between the good and poor patient performance from the task measure plotted in (B) was consistently positive (good > poor) for all four anterior prefrontal electrodes in the theta band (upper panel) and all occipital electrodes in the gamma band (lower panel). Note: the anterior theta was consistent across all four task measures. (D) Mean Δ*p*-values from all the task measures reveal significantly greater anterior prefrontal theta power differences (Tukey–Kramer post hoc, error bars indicate SEM; * $p < .05$, ** $p < .01$, *** $p < .001$) than in any other area (top panel) with fewer significant differences in the gamma band (lower panel). 2D, two-dimensional; EEG, electroencephalography; SEM, standard error of the mean.

ANOVA was used to compare these groups using ΔP magnitude averaged for each cortical region, with a specific focus on the theta and high gamma frequency bands (Figure 3D). Finally, prefrontal theta power was analyzed across the three performance groups (good, normal, and poor) in each task using an analysis of variance (Figure 5C and Figure 5D).

3 | RESULTS

To identify the specific cortical areas and EEG frequency bands that indicate behavioral differences between good and poor performers we used the EEG data of 86 patients with epilepsy performing the CANTAB tasks with standardized behavioral measures of four cognitive domains: visual memory, spatial memory, working memory, and executive functions (Figure 1). Within each domain, patients were stratified into three groups (good, normal, and poor performers) normalized by age and gender according to the CANTAB database. Behavioral results effectively separated the good and poor performance groups (below and above 1 SD from the average) in each of the four domains tested. Most participants belonged to either the good performers or the poor performers group, with only 10% performing both well and poorly across any of the four behavioral measures (Figure 1C). There were consistently more patients with poor (29%–33%) than with good (9%–10%) memory performance (Figure 1D). Executive function performance was more evenly distributed, with 19% poor performers and 26% good performers (Figure 1D). These CANTAB results confirm the profile of cognitive impairments observed in epilepsy, which is focused on memory more than executive functions. A significant correlation was found between all these measures (Spearman, $r > .28$, $N = 86$, $p \leq .01$) and was particularly strong between the executive and working memory scores, as well as between working memory and spatial memory scores (Spearman, $r > .6$). Visual memory scores showed the weakest correlation with the other three measures, which likely include a stronger executive function component in both working and spatial memory functions (Table 1).

We compared spectral EEG power features to examine the effects of the performance group (good and poor; 1 *df*) and the channel location (31 standard scalp EEG leads; 30 *df*). Analysis of variance showed significant effects of the group, channel location, and their interaction across all frequency bands and in each task (ANOVA, *p*-value ≤ .01). The average spectral power in the seven EEG frequency bands was mapped across the neocortex (Figure 2). We found that the most prominent disparities between good and poor cognitive performers were localized in the frontal and occipital regions in the low- and high-frequency

	Visual memory	Spatial memory	Working memory	Executive
Visual memory	1	.3773	.3013	.3269
Spatial memory	.3773	1	**.6093***	.4746
Working memory	.3013	**.6093***	1	**.6678***
Executive	.3269	.4746	**.6678***	1

TABLE 1 Summary of the correlation coefficients across all cognitive measures.

Note: Values marked with asterisks indicate strong correlations ($r > .5$).

bands, respectively (Figure 2A, Figure S1). We identified the specific electrodes that contributed the most to these differences by assigning the number of behavioral task measures (from 0 to 4) for which a given electrode's power-in-band exhibited a significant difference (Figure 2B). Based on our grouping of EEG electrodes into anatomic regions (Figure 3A)—prefrontal [N, Fp], frontal [F], central [C], temporal [T], parietal [P], and occipital [O, I]—we found that prefrontal theta and occipital gamma spectral powers effectively distinguished between good and poor performance across most of the cognitive domains studied. The occipital gamma contribution explains the visual aspect of the CANTAB tasks performed, whereas the prefrontal theta activities point to the memory and executive functions engaged in the tasks. We focused our subsequent analyses on these two spectral activities. We also compared the EEG spectral estimates obtained during the MOT task between good and poor performers in the memory and executive function measures (Figure S2). The results indicate that some statistical differences between patients impaired in memory and executive functions can be detected even using a simple sensorimotor task. However, these differences were less consistent than those observed during the memory and executive function tasks.

The prefrontal theta power values stood out from the other areas, more than the occipital high-gamma activities (Figure 3B, Figure S2). There was a consistent pattern of gradually greater power on the lateral electrodes and lower power on the medial electrodes within each anatomic area (Figure 3B). This pattern shows that the lowest power is observed on the central electrodes and increases away from these reference electrode locations. The prefrontal theta power stood above this background trend across the cortical areas. It is important to note that the elevated theta power showed consistent differences between good and poor performers across nearly all prefrontal electrodes, whereas gamma power differences were observed in the occipital electrodes (Figure 3B, Figure S3). The most reliable trends were seen in the theta and high gamma bands, with consistent effects across subjects and electrode locations, underscoring the robustness of these findings. We compared the magnitude of the theta and gamma spectral power differences between good and poor performers (ΔP) for a given behavioral measure. ΔP across all four measures of memory and executive functions showed high positive ΔP theta values for all prefrontal electrodes (Figure 3C, Figure S4), indicating a greater signal power in the good than in the poor performance condition. The grand average of ΔP from all electrodes in a given area differed significantly across the seven areas (ANOVA, $F = 11.27$, 6 df, $p \leq .001$). The prefrontal theta ΔP was significantly greater than that in any other area (Tukey–Kramer post hoc test, $p \leq .001$) (Figure 3D). These grand average values were not significantly different for the high gamma ΔP.

To visualize the spatial distribution of these spectral patterns across the brain, we generated surface plots of the averaged theta and gamma power for the poor and good performers and their ΔP difference across the four behavioral measures (Figure 4A, Figure S5 for each individual measure). These plots confirm a central low-power valley with peaks rising proportionally to the distance from the "CPz" electrode in both the lateral and anterior-posterior directions. The highest and most consistent peaks are concentrated in the theta activities of the anterior prefrontal cortex. These theta power peaks in the anterior prefrontal cortex, also known as the frontal pole, were greater than would be expected from the background pattern of gradually increasing power away from the central "CPz" electrode. However, we did not see a similar pattern in the high-gamma activity. As shown in Figure 3A, although there is a significant difference in high-gamma power between good and poor performers in the occipital brain region, the power magnitudes are not distinctly higher compared to those in electrodes from the other brain regions.

The logarithmic distribution of theta and high gamma powers plotted against the Euclidian distance from the "CPz" reference area exhibited an approximately linear correlation (Figure 4B). However, some electrodes showed their distribution away from this background linear power trend, including all four prefrontal ones, with notably greater power values compared even to those positioned farther away from the reference point. It is notable that the four anterior prefrontal electrodes consistently showed a large difference between the good and the poor

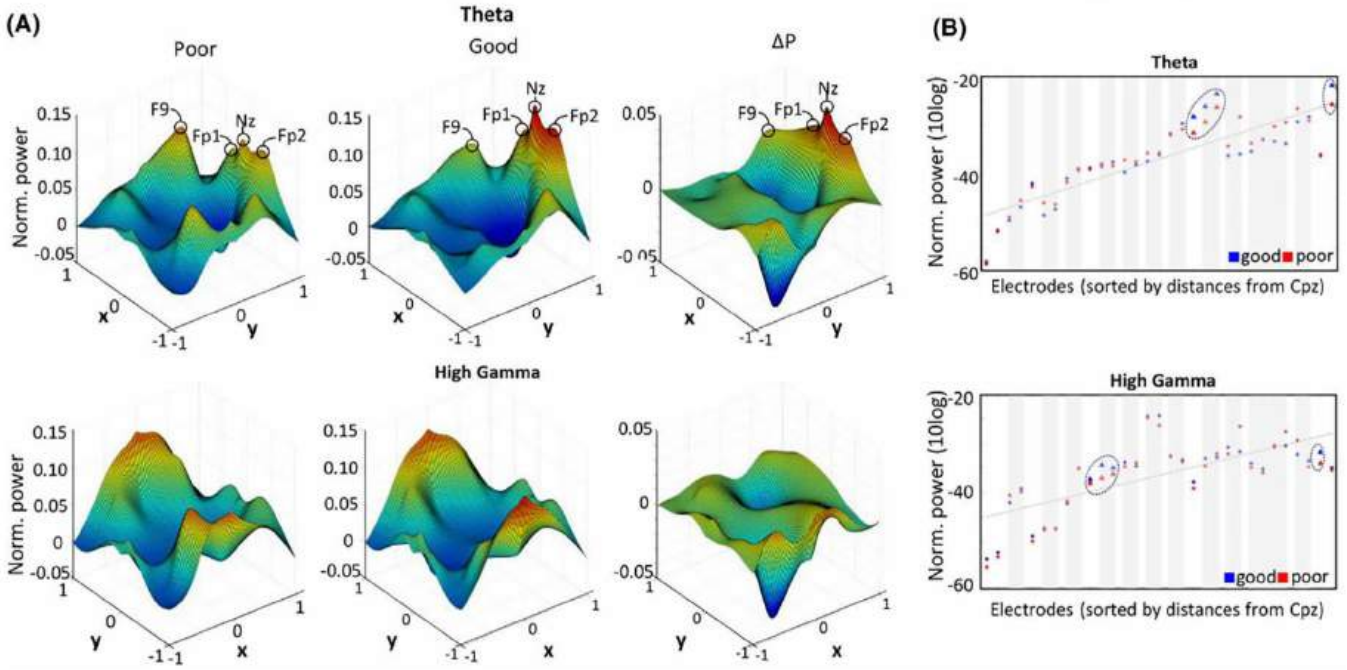

FIGURE 4 Memory and executive function performance are indicated by the anterior prefrontal EEG theta power. (A) 3D surface plots identify global peaks (warmer colors indicate greater magnitudes) of the average spectral power (left and middle column) and the power difference (right column) in the anterior prefrontal theta (upper row) and not in the occipital high-gamma (lower row) band. Notice the characteristic "red hot" peaks at the anterior EEG electrodes rising above the "deep blue" valley around the center of the 3D plane. (B) Logarithmic distribution of the average theta and high gamma power plotted against the Euclidean distance from the reference point "Cpz" confirms the trend of gradually increasing power away from the reference point. Note that the anterior prefrontal and occipital electrodes in the theta and high-gamma bands, respectively, are outlined by a black dotted oval. Only the anterior prefrontal electrodes in the theta band show a rise above this background trend. 3D, three-dimensional; EEG, electroencephalography.

performance conditions (Figure 4B upper panel). This was not the case for the high gamma activities at the occipital electrodes (Figure 4B lower panel).

Finally, focusing on the prefrontal theta band activities, we tested this candidate biomarker activity across the four behavioral measures and among all three performance groups (good, poor, and normal performers) of the 86 participating patients with epilepsy. The results are summarized in Figure 5A, showing that the four prefrontal electrodes (Nz, Fp2, Fp1, and Fpz) have proportionally increased theta power, going from poor to good performance conditions in the four behavioral measures. This pattern is especially evident for the most anterior "Nz" electrode across all four behavioral measures (Figure 5B; see Figure S6A for the other prefrontal electrodes). The theta power recorded from that electrode during individual patient performance in a given task (Figure 5B) revealed a continuous linear ascending trend—the greater the theta power, the better the performance. This analysis was conducted at the level of the entire group of subjects. There was a significant effect of the performance groups on the theta power (ANOVA, $F=48.14$, 2 *df*, $p \leq .001$) and a significant difference between good and poor (Tukey–Kramer post hoc test, $p \leq .001$) as well as between good and normal (Tukey–Kramer post hoc test, $p \leq .001$) performers (Figure 5C; see Figure S6B for the analysis of other prefrontal electrodes).

Breaking down this analysis into individual task measures for the same "Nz" electrode lead confirmed the significant effect of higher theta power with better task performance (Figure 5D; ANOVA, 2 *df*, $p < .05$; refer to Figure S7 for the analysis of other prefrontal leads). A significant difference was observed between good and poor groups across all four measures (Tukey–Kramer post hoc test, $p \leq .05$). Between good and normal groups, a significant difference was found across all measures except visual memory (Tukey–Kramer post hoc test, $p \leq .001$; Table 2 for detailed Tukey–Kramer post hoc test results). Altogether, our results show that increased theta power is associated consistently with improved performance across the individual memory and executive function measures.

Given the consistent correlation between behavioral performance in the task measures and prefrontal theta power on specific electrodes, we predicted that the average theta power across all four electrodes would also correlate with the behavioral measures. Figure 5E shows the

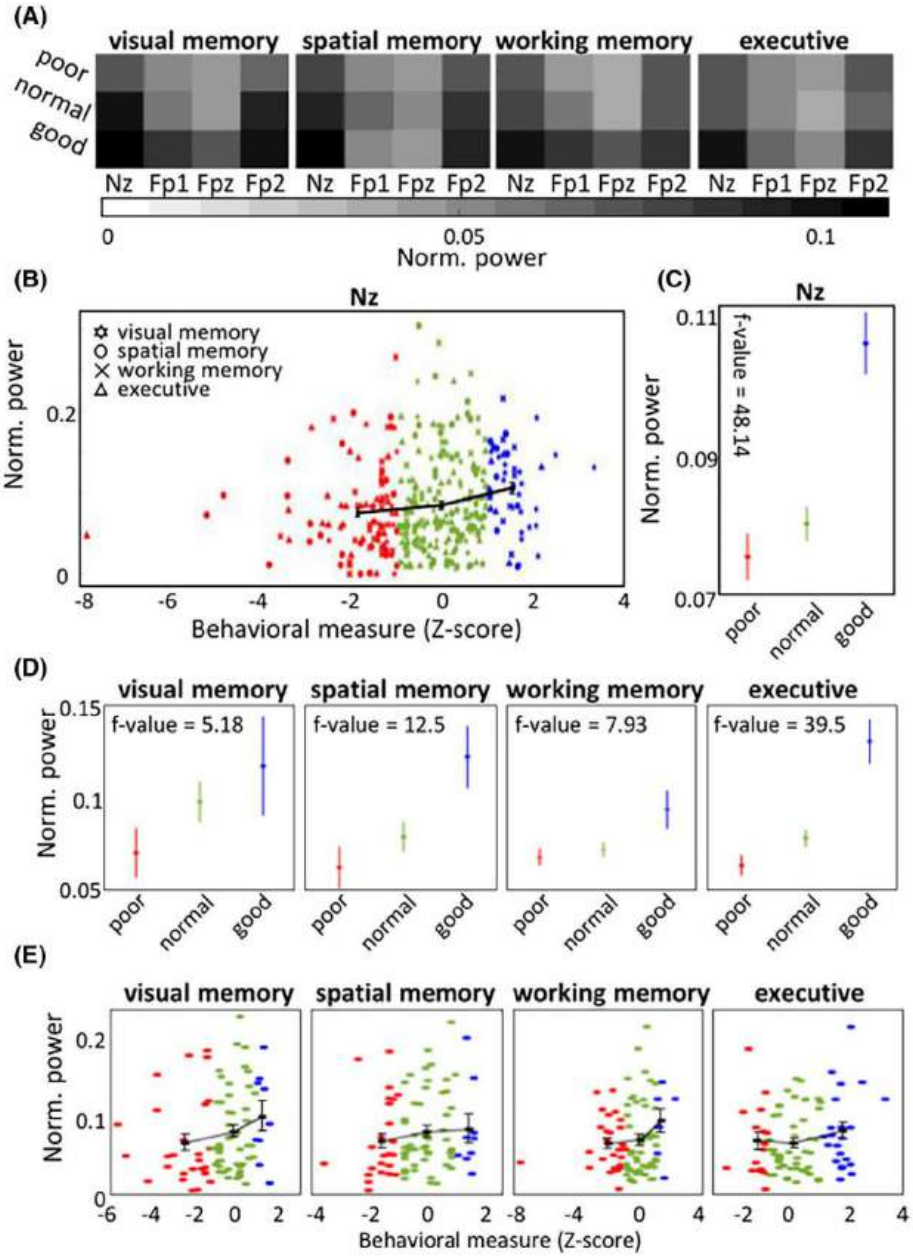

FIGURE 5 Memory and executive function performance are indicated by the anterior prefrontal EEG theta power. (A) Summary of the average theta power across the four anterior prefrontal electrodes and all four task measures shows a consistent pattern of gradually higher power (dark shades) going from poor to good patient performance. (B) Scatterplot of all patient performance scores across the four task measures (shapes) confirms the ascending trend of the grand-average means (black square plot with SEM) with significantly greater theta power on the "Nz" electrode (see Figure S6A for the other frontal pole electrodes) in case of the good (blue) vs normal (green) and poor (red) populations (Tukey–Kramer post hoc *test*, $p < .001$). (C) The average theta power across the four task measures is evaluated as the test statistic (F value) with post hoc group comparisons (95% confidence intervals). (Refer to Figure S6B for the other three electrodes.) (D) The average spectral power of theta band activities on the "Nz" electrode confirms the ascending trend in the grand-average means (bars indicate 95% confidence interval), with effect sizes indicated inside each plot (ANOVA, $df = 2$, $p < .01$); (see Figure S7 for the other three electrodes). (E) Grand-average theta power from all four frontal pole electrodes, plotted as in "B", shows the persistent ascending trend in each task measure of the studied memory and executive functions. ANOVA, analysis of variance; EEG, electroencephalography.

ascending pattern of this averaged prefrontal theta power increasing from poor to normal and good performer conditions. We conclude that theta activity across the entire anterior prefrontal cortical area is indicative of memory and executive functions in patients with epilepsy.

4 | DISCUSSION AND CONCLUSIONS

The goal of this study was to determine the neural activities that would match the behavioral assessment of memory

TABLE 2 Summary of patient group effects on the anterior theta power.

	Nz				Fp1		Fpz		Fp2			
	Visual memory	Spatial memory	Working memory	Executive	Visual memory	Executive	Visual memory	Executive	Visual memory	Spatial memory	Working memory	Executive
Good versus poor	.4520	**.0001**	**.0014**	$<10^{-5}$	$<10^{-5}$	**.0358**	$<10^{-5}$	$<10^{-5}$	.9969	.3752	**.0188**	$<10^{-5}$
Good versus normal	**.0170**	$<10^{-5}$	**.0002**	$<10^{-5}$	$<10^{-5}$	.1765	$<10^{-5}$	.0791	.1680	**.0057**	**.0051**	**.0252**
Normal vs poor	**.0221**	.1225	.5193	.8016	.0715	.9816	.6289	.3304	**.0120**	**.0160**	.6112	.2574

Note: Tukey–Kramer post hoc test results for each electrode and task measure are summarized only for instances of a significant result of the analysis of variance (ANOVA) for any given measure (bold text indicates significant effects).

and executive functions to identify potential electrophysiological biomarkers for cognitive impairments in epilepsy and other brain disorders. Using a clinically-validated CANTAB of cognitive tasks[30–34] we successfully identified patients with impaired, normal, and good performance in visual, spatial, working memory, and executive functions compared to age- and gender-matched healthy populations. These normalized behavioral measures confirmed a predominant impairment of memory functions observed in this patient population.[35] Approximately one-third of the patients tested were impaired across the three memory domains, whereas one fifth of these patients, on average, was also impaired in the executive function measure. Thus, our behavioral results support the use of CANTAB for the assessment and diagnosis of cognitive deficits in epilepsy. However, even this standardized battery is limited to human behavioral performance, which is prone to multiple factors affecting cognition, such as medication, sleep mood, alertness, and other factors related to neurological and psychiatric disorders. Neuropsychological assessment tools are limited not only in terms of the accurate detection of cognitive impairments but also in targeting and monitoring therapeutic interventions. Therefore, there is a need for complementary, more objective, biomarkers of cognitive function that reflect neural activity in specific brain areas.

Our study showed that a relatively simple EEG measure of spectral power in the theta frequency range from four electrodes in the anterior prefrontal cortex correlated with the CANTAB behavioral measures. This is remarkable given that theta power was averaged over the entire period of task performance when patients were likely to be distracted and engaged in other cognitive activities such as mind-wandering. The EEG and behavioral measures were affected by these internal and external factors in the hospital setting; yet they remained closely associated in the three separate tasks, probing four different cognitive measures. The identified EEG biomarker was consistent across all three tasks and cognitive measures and was limited to only one cortical region and frequency band, which stood above the background trend of increasing power away from the reference electrode area. Correlation analyses in individual patients further suggest that, although distinct brain regions support specific memory and executive functions, a shared neural network likely underlies all these processes, with the frontal pole area emerging as a potential region integrating these task-related functions.

This suggests that anterior prefrontal theta activities have the versatility and robustness that would be required for a clinically used biomarker, which suggests that they would not be limited to only a specific task or a cognitive function in the background of other patient-specific "internal" and environment-specific "external" factors.

Although our results show these necessary properties of a potential neural biomarker of memory and executive functions, they are not yet sufficient for clinical use. Likely due to the above-mentioned confounding factors, theta activity was not perfectly correlated with behavioral performance measures. For instance, the poor performance group was differed significantly only from the good performance group, but not from the normal patients in the entire cohort studied (Figure 5). For these reasons, we could not successfully use theta activities to classify patient groups using a machine-learning approach to separate the patient groups (data not shown). A recent study attempted to identify neural biomarkers of chronic pain[36] with similar results in terms of identifying brain areas and a range of intracranial EEG activities but without a clear classification. New studies using intracranial EEG have likewise proposed potential biomarkers of movement impairments[37] or depression.[38] Memory and cognitive functions are arguably more widely spread across networks of brain areas and are thus more challenging to target. Nevertheless, our previous studies with intracranial EEG took advantage of large cohorts of patients[21,22,39] or chronic continuous recordings[40] to identify the theta activities in the anterior cortico-thalamic circuits as a hub for memory functions and a target for therapeutic interventions such as direct electrical brain stimulation.[41] In this study, we demonstrated that the prefrontal theta activities sampled non-invasively with scalp EEG present an accessible, versatile, and robust biomarker for further studies of clinical utility and applications.

Previous studies have confirmed that non-invasive EEG can be used to predict cognitive function performance. Measures as simple as amplitude and latency of particular components of the event-related potential can differentiate good and poor memory performance as subjectively rated by young and old adults.[42] Spectral power in the alpha frequency band during rest is correlated with executive function performance and has been proposed as a biomarker of cognitive training in Parkinson's disease.[43] Theta activity has recently been proposed for diagnosing cognitive impairment in epilepsy.[44] However, most of these EEG studies did not use a clinically validated battery of tasks for a range of memory and cognitive functions, such as CANTAB. The anterior theta activities reported in our study were tested on a range of tasks and behavioral measures that were normalized in a large subject population. Other studies have also shown relevance of the anterior theta activities in particular cognitive functions impaired in various brain disorders.[7,24,44] Hence, they are likely to generalize to other memory and executive functions and extend to other patient populations.

Both theta and gamma frequency bands were associated with memory and executive functions.[2,10,12,13] It has been proposed that these two rhythms play critical roles in coordinating memory representations.[45] In our visual touch-screen cognitive tasks, we consistently showed that better performance is generally related to greater prefrontal theta and occipital gamma activity. Both visual gamma and prefrontal theta are required to coordinate memory representations in all the cognitive tasks that we used. However, in our case, the prefrontal theta signal was even more pronounced than the occipital gamma signal and was solely effective in indicating patient performance (Figures 3 and 4). CANTAB tasks require maintaining increased levels of attention and higher cognitive functions, which may explain greater prefrontal engagement. A greater prefrontal theta signal is promising as a more general biomarker of memory and executive functions that could now be tested in non-visual tasks involving auditory, olfactory, or tactile stimuli.

A study involving adults with self-reported everyday executive function complaints compared two groups: one with attention-deficit/hyperactivity disorder (ADHD) and one without any diagnosis, to a control group using EEG-based cognitive tasks. The analysis focused on the power and functional connectivity in four regions of interest (frontal-midline, fronto-lateral left and right, and parietal regions). Across all executive function domains, dynamic increases in theta power and functional theta connectivity were observed over time in both groups, with notable group differences, especially in conflict monitoring, evident in the frontal-midline and fronto-lateral right regions.[24] Another study was conducted to compare EEG spectral power and theta phase coherence during rest and while performing a working memory task in pediatric individuals with common genetic neurodevelopmental disorders linked to cognitive impairment in a control group of typically developing children matched for age and sex. The findings revealed that the group with cognitive impairment displayed higher resting-state slow-wave power, a lower peak alpha frequency, and elevated theta power, along with increased frontoparietal theta phase coherence during the working memory task. However, these differences disappeared when controlling for the baseline resting-state activity.[25]

Our results revealed a distinct single brain area with significantly greater signal activity than other areas associated with human memory and cognitive function. These functions are widely distributed in the brain between multiple frontal, temporal, and parietal association areas. Single biomarker localization is an attractive target for therapeutic interventions—a hub or a hotspot for memory processing.[21] Other hotspot targets may

exist within the memory and executive function networks, such as the anterior nucleus of the thalamus,[40,41] which could be more suitable in terms of therapeutic efficacy. Nonetheless, anterior prefrontal EEG theta activities provide an accessible biomarker signal in the neocortex, which is more feasible even for non-invasive neuromodulation interventions than the deep thalamic or mesial temporal lobe targets. They also provide an accessible objective biomarker for diagnosing cognitive deficits; suppressed prefrontal theta band power could be a general indicator of memory and executive function impairments.

The accuracy of estimating the reported anterior theta biomarker could be further improved by overcoming several limitations in our study. One is the lack of hemisphere-specific data, which restricted our ability to investigate potential hemispheric lateralization in the studied verbal functions. Due to this constraint, we were limited to identifying a general biomarker for memory and executive functions by averaging measures across all frontal pole electrode leads. Although this approach offers insights into overall frontal activity, it may overlook more precise biomarkers associated with specific functions that could be isolated by focusing on particular leads within the left or right hemisphere. For example, we have found that only the "Fp2" lead in the right hemisphere was showing a significant effect of the patient performance in spatial memory on the anterior theta activities but not the central "Fpz" or "Fp1" leads (see Figure S7). Future studies with hemisphere-specific data may provide more accurate localization of these cognitive biomarkers.

In addition, our analysis was not restricted to specific periods of memory performance, such as encoding or recall, when movement and other artifacts are minimized. Limiting analysis to these artifact-free intervals could improve signal quality and provide more accurate indicators of performance levels (poor, normal, or good), as well as reduce noise contamination, potentially affecting the spectral power estimates. However, implementing this approach in clinical practice poses technical challenges, as it would require precise synchronization between the task tablet and the EEG system. Another possible approach is to segment the task epochs into smaller intervals (e.g., 10 s), estimate power within each segment, and exclude any segments showing abnormally high power levels that could indicate artifacts.

This study was limited to a specific population of patients with epilepsy and may therefore be affected by epileptogenic EEG activities. Although we cannot fully exclude the effects of seizures, epileptiform activities, anti-epileptic medication dosages, or sleep deprivation without complex statistical adjustments—which remain challenging even with this substantial sample size—we at least provided consistent testing conditions for all participants. These potential confounding factors underscore the robustness of the anterior theta biomarker effects that we found. Further research is necessary to assess the generalizability of these results to other brain disorders.

Finally, a comprehensive clinical biomarker of memory and executive functions in brain disorders can be employed to develop new treatments. For example, neurofeedback training to enhance alpha spectral power during rest was shown to improve cognitive performance.[4] Similarly, training to enhance frontal-midline theta activity resulted in improved performance in a memory task testing attention and working memory functions.[46] Therefore, modulating the identified biomarker activities through the neurofeedback of brain stimulation would likely modulate memory and executive functions, leading to new therapy. Future studies will test these predictions for particular clinical applications and patient populations. Building upon recent investigations into memory enhancement through direct brain stimulation,[47–50] we understand that electrical stimulation in specific brain areas can enhance human cognitive performance. Therefore, we anticipate that our findings will represent a promising step toward modulating cognitive functions in the brain.

AUTHOR CONTRIBUTIONS

Nastaran Hamedi drafted the manuscript, directly accessed and verified the underlying data, conducted data analysis, interpreted the results, and revised and approved the manuscript. Jesús S. García-Salinas contributed to data analysis and revised and approved the manuscript. Brent M. Berry contributed to data collection. Gregory A. Worrell designed the study and contributed to data collection. Michal T. Kucewicz designed the study, drafted the manuscript, contributed to data collection, directly accessed and verified the underlying data, contributed to data analysis, interpreted the results, and revised and approved the manuscript. All authors read and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

None of the authors has any conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

All recorded data and metadata are available on DOI: 10.17632/3bt248ppv5.1

ETHICS STATEMENT

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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5.1.2 Supplementary Material for Publication I

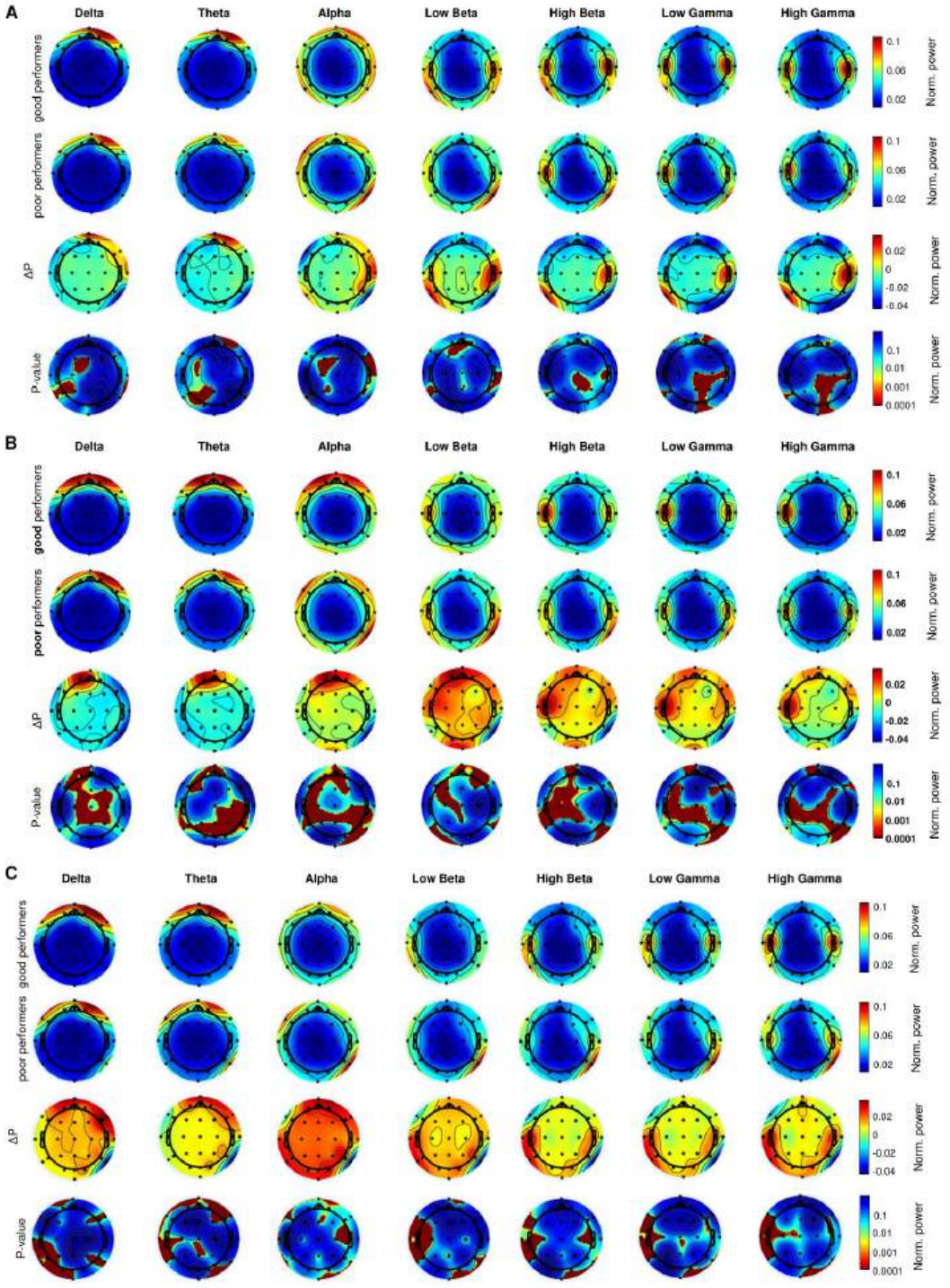

Figure S1. **Patients with good memory and executive performance exhibit significantly higher power in anterior low-frequency bands and posterior high-frequency bands.** The average power across seven frequency bands is displayed for good and poor performers, along with the difference in power and the p-values from the ANOVA test, for (A) spatial memory, (B) working memory, and (C) executive functions.

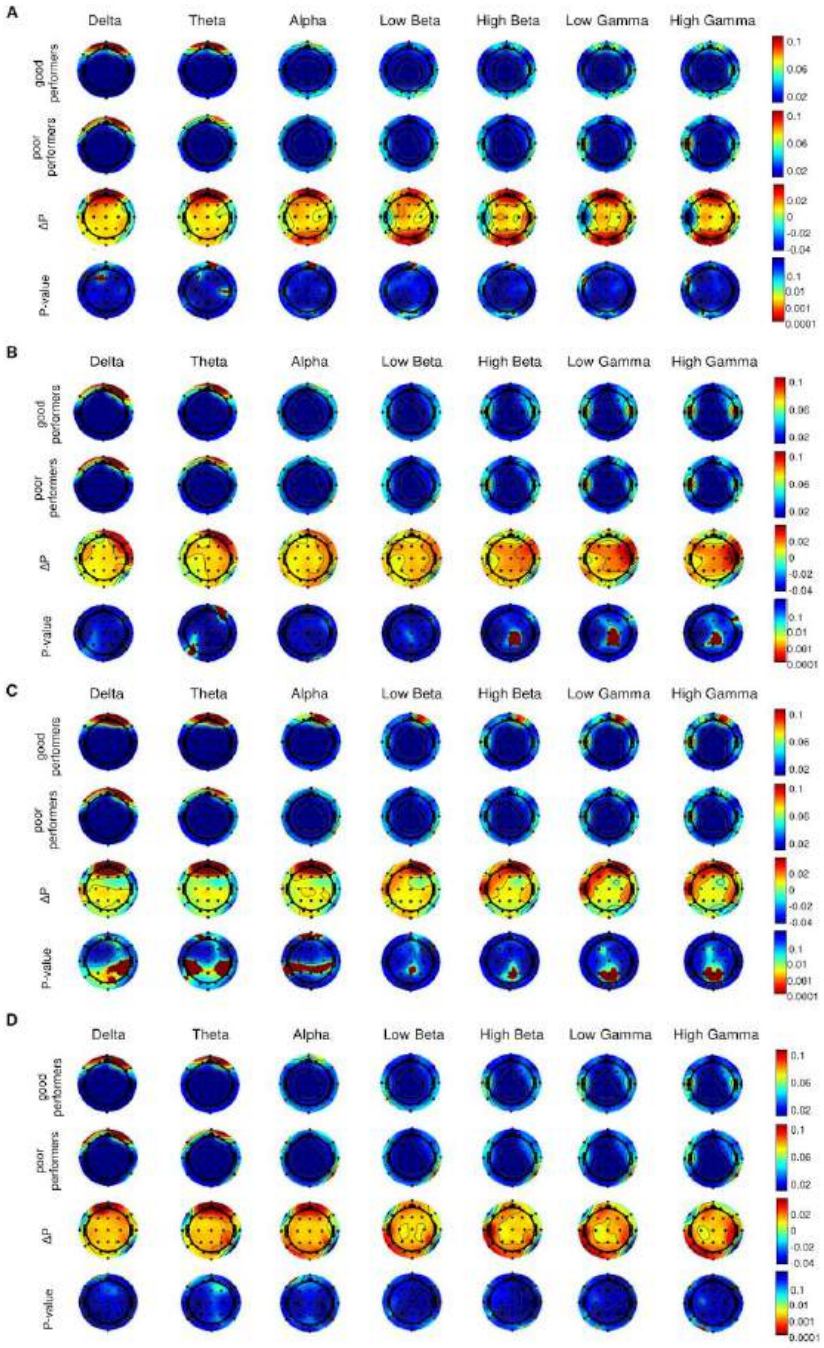

Figure S2. **Patients with good and poor performance in memory and executive function measures exhibit similar trends in the EEG activities during a control MOT task.** The average power across seven frequency bands is shown for good and poor performers, along with the power differences and ANOVA test p-values, for (A) visual memory, (B) spatial memory, (C) working memory, and (D) executive functions but during performance of a control MOT task. Notice similar effects in the anterior theta and posterior gamma activities between the four patient groupings induced even in this different sensorimotor attention task.

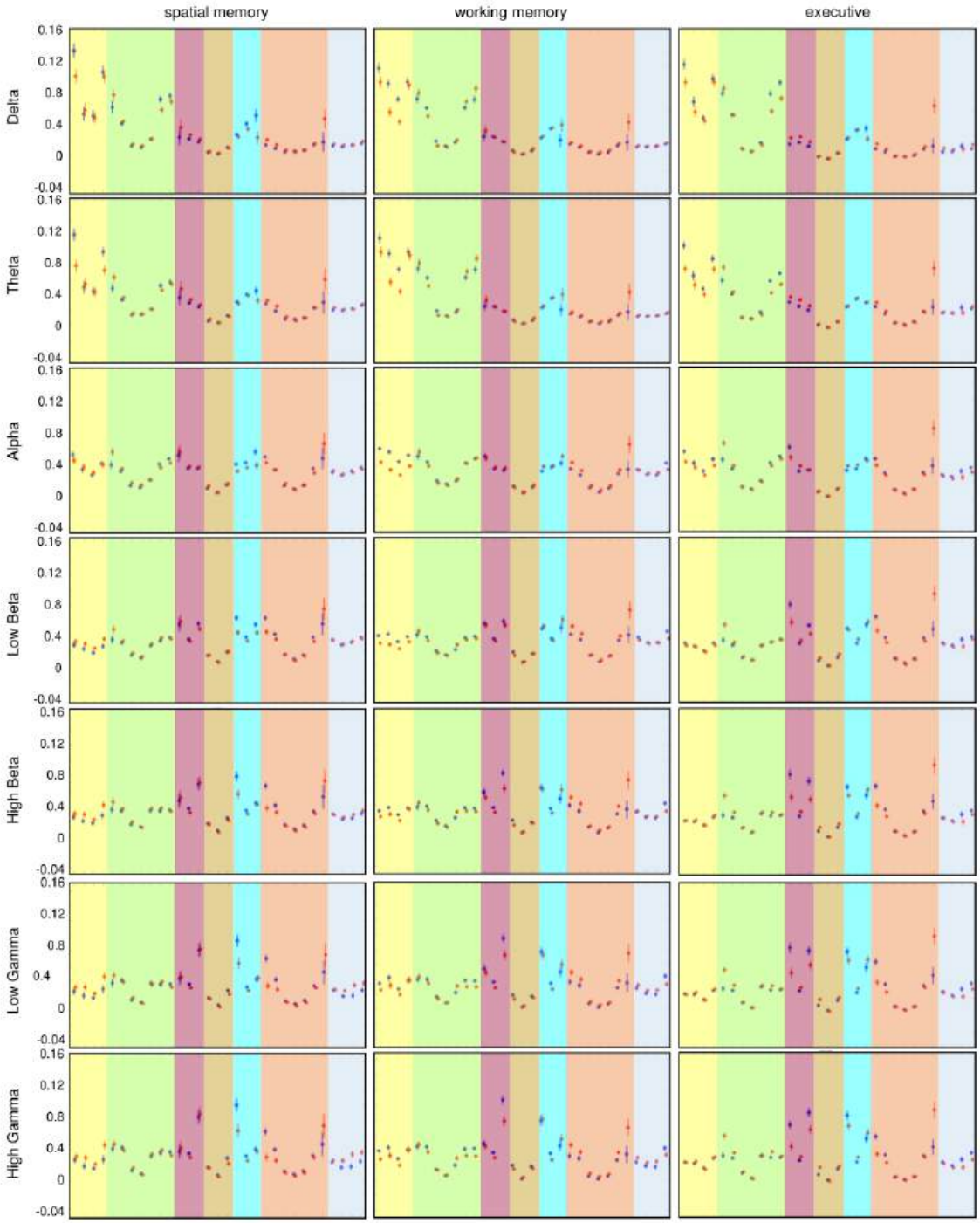

Figure S3. **Anterior prefrontal EEG theta activities show the greatest spectral power and most significant differences between good and poor performers in memory and executive function tasks.** Normalized average power across all frequency bands is compared between good (blue) and poor (red) performers in spatial memory, working memory, and executive tasks, with electrodes grouped from frontal to occipital cortical areas (color-coded as in Fig 3A) and bars indicating 95% confidence intervals. All plots are presented as in Fig 3B.

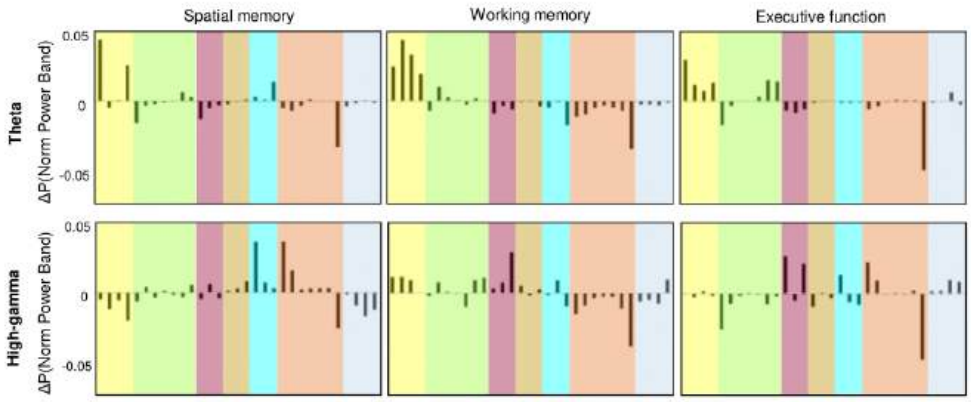

Figure S4. **Anterior prefrontal EEG theta activities show the greatest spectral power and differences in poor and good memory and executive function performance.** Spectral power differences (ΔP) between good and poor patient performers are shown for the three task measures—spatial memory, working memory, and executive functions—along with visual memory (presented in Fig 3C). Electrode groups are arranged from frontal to occipital cortical areas (color-coded as in Fig. 3A), with all plots presented as in Fig 3C.

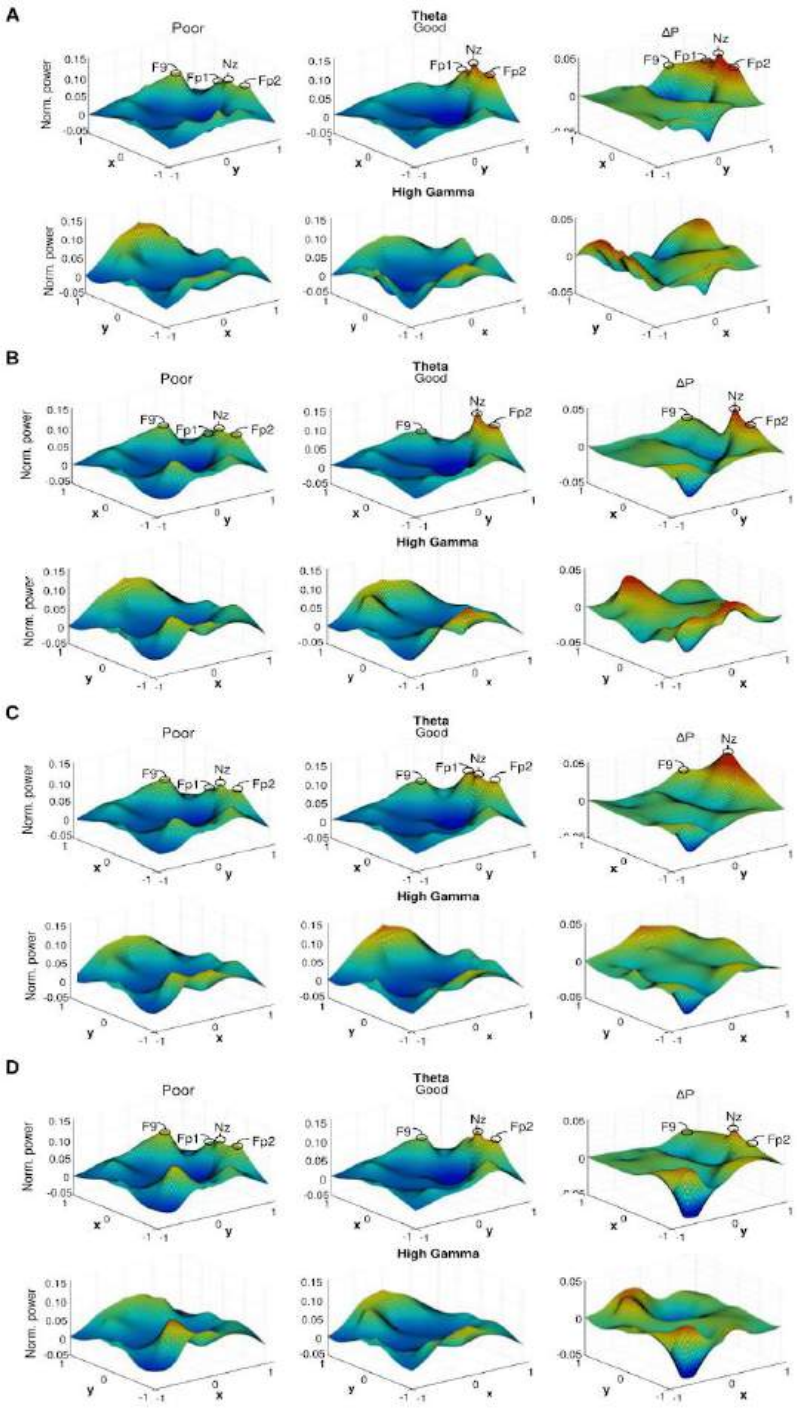

Figure S5. **The anterior prefrontal cortex displays the highest and most consistent theta power peaks, significantly exceeding the gradual power increase observed in the surrounding regions.** 3D surface plots identify global peaks (warmer colors indicate greater magnitudes) of the average spectral power (left and middle column) and the power difference (right column) in the anterior prefrontal theta (upper row) and not in the occipital gamma (lower row) band for the four behavioral measures: (A) visual memory, (B) spatial memory, (C) working memory, and (D) executive functions. Notice the characteristic 'red hot' peaks at the anterior EEG electrodes rising above the 'deep blue' valley around the center of the 3D plane.

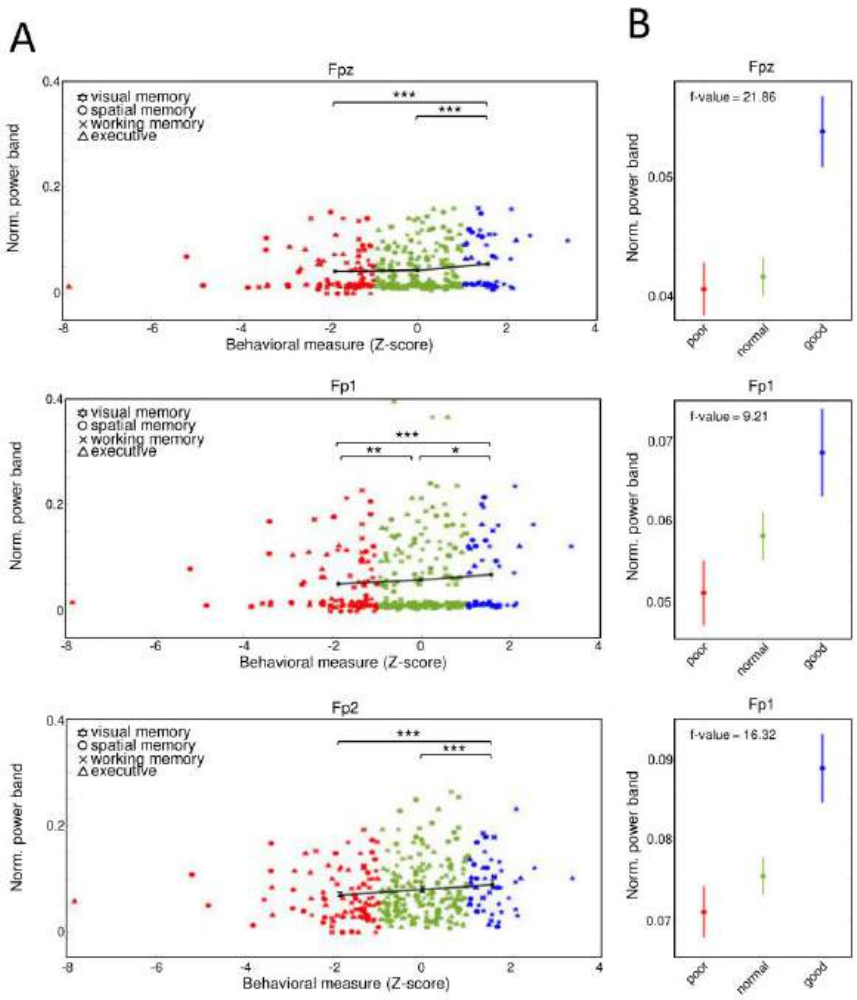

Figure S6. **Increased anterior prefrontal theta power correlates with improved performance in memory and executive functions.** (A) Representation of subjects' theta power from the frontal electrodes ('Fpz', 'Fp1', and 'Fp2'; 'Nz' shown in Fig 5B) is compared with z-scores of performance in: visual memory (hexagons), spatial memory (circles), working memory (crosses), and executive (triangles) functions. Groups marked with a star indicate a significant difference between them (Tukey-Kramer post-hoc test, $p < 0.001$). (B) The average theta power across the four task measures for the frontal electrodes ('Fpz', 'Fp1', and 'Fp2'; 'Nz' in Fig 5C) is evaluated as the test statistic (F value), with post-hoc group comparisons shown as bars indicating 95% confidence intervals.

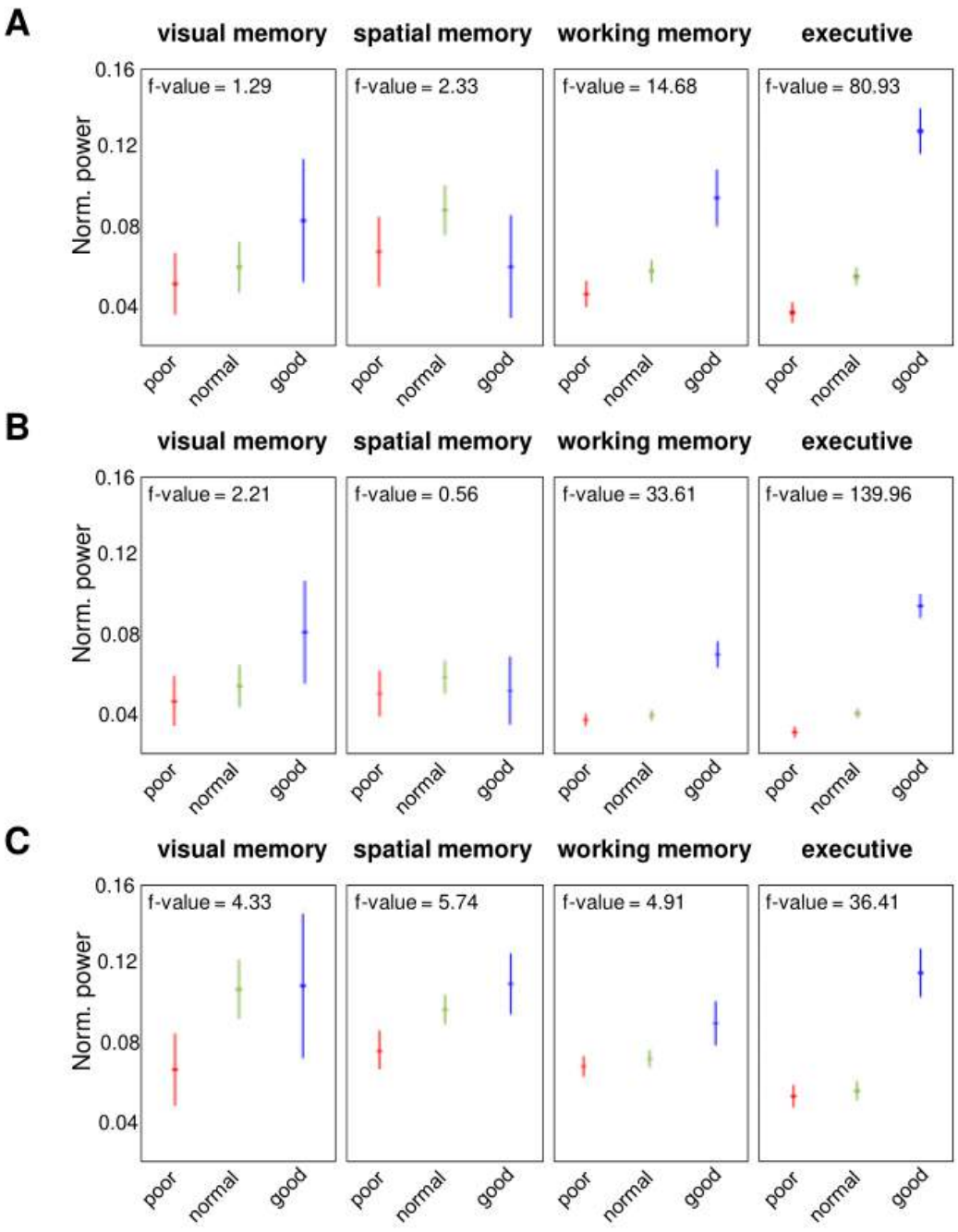

Figure S7. **Increased anterior prefrontal theta power correlates with improved performance in memory and executive functions across the four frontal electrodes.** The average theta power across the four task measures for each prefrontal electrode is evaluated as the test statistic (F value), with post-hoc group comparisons shown as bars indicating 95% confidence intervals for (A) 'Fp1', (B) 'Fpz' and (C) 'Fp2' electrodes. Notice that the ascending trend was not observed in case of the Fp1 and Fpz electrode leads for the spatial memory measure, which may be explained by lateralization of this particular function to the right hemisphere as confirmed in case of the corresponding 'Fp2' electrode lead results.

Table S1. Summary of the patients' task measure scores of their cognitive performance. The scores were normalized by the age and gender according to the CANTAB database of control population.

Patient NO.	Gender (F/M)	Age range	visual memory (z-scores)	spatial memory (z-scores)	working memory (z-scores)	executive (z-scores)
745	M	50-59	-1·81	-0·47	-1·43	-0·14
709	F	40-49	-1·97	-0·09	-0·91	-0·75
701	F	40-49	-3·41	-0·8	0·13	0·4
625	M	70-79	0·19	0·09	1·48	-0·27
657	M	50-59	-0·68	0·51	-0·33	0·96
627	F	24-39	-2·25	-0·81	-2·13	-0·59
727	F	16-23	-0·17	-0·46	-0·8	-0·03
794	F	24-39	-0·78	1·59	0·95	1·58
672	F	16-23	-1·09	-0·46	1·04	1·52
705	M	24-39	-1·01	-0·62	-1·29	-0·91
674	M	60-69	-0·85	-0·14	-0·05	0·59
470	M	24-39	-1·01	-2·05	-0·95	-0·91
659	F	16-23	-0·63	-1·21	-2·64	-1·41
673	M	40-49	-1·25	-0·8	-2·24	-1·13
758	F	40-49	0·20	-0·8	-2·51	-0·56
245	M	24-39	-3·82	-2·05	-0·72	0·89
749	F	40-49	-1·25	-0·8	-2·02	-1·13
622	F	24-39	0·32	-0·81	-0·71	-0·26
636	M	24-39	1·00	-1·34	-1·69	-1·89
744	M	24-39	-1·81	-2·05	-1·46	-1·07
624	M	24-39	0·60	0·09	-0·5	0·08
742	F	24-39	1·06	0·79	-1·2	1·25
610	F	16-23	1·66	0·29	0·63	1·69
780	F	24-39	-4·83	-0·01	-0·59	0·41
665	F	24-39	0·69	1·59	0·89	0·91
621	M	40-49	-1·25	-0·80	-0·14	-1·32
769	F	24-39	0·32	-1·61	-1·76	1·75

644	F	24-39	0·32	0·79	0·34	2·09
739	M	50-59	-2·93	-0·47	-0·83	0·30
740	F	40-49	-0·16	-0·09	-1·02	-1·13
648	F	16-23	1·20	-1·21	-2·89	-1·06
770	F	24-39	-0·41	-1·61	-0·28	-0·59
645	M	40-49	-0·16	0·63	0·52	0·59
774	F	16-23	-1·54	-1·21	-1·8	-0·55
731	M	50-59	1·19	1·49	0·82	0·52
722	M	24-39	0·20	-1·34	0·24	1·38
667	F	24-39	-1·15	-0·01	-0·22	-0·93
737	M	24-39	-0·61	0·81	0·69	1·38
626	F	24-39	-5·19	-1·61	-2·25	-0·93
772	F	40-49	-0·52	0·63	0·36	-0·56
630	M	40-49	0·56	0·63	-0·53	0·02
646	M	24-39	-0·61	-1·34	-0·67	0·89
663	M	40-49	-1·25	-1·51	-1·3	-0·56
655	M	16-23	1·66	0·52	-1·34	0·33
656	M	24-39	-3·42	-1·34	-2·54	-1·23
759	F	60-69	0·44	-0·13	-0·35	0·11
666	F	40-49	-0·16	0·63	0·19	0·02
669	F	60-69	1·13	0·95	2·19	3·35
675	M	16-23	0·75	-0·30	1·07	2·07
664	M	40-49	0·56	1·34	1·35	2·50
704	F	24-39	1·43	-0·01	1·08	0·41
708	F	24-39	-1·15	-2·41	-0·89	0·08
746	F	40-49	-0·52	-0·09	0·25	-0·56
724	M	24-39	0·60	-0·62	0·58	0·24
728	F	24-39	0·32	-0·01	0·89	1·92
700	F	24-39	0·69	-0·81	-2·19	-1·10
720	F	40-49	-0·89	-0·80	-0·14	0·78
717	F	40-49	0·20	-0·09	1·18	1·73

716	M	24-39	1·40	0·09	0·92	1·71
757	F	24-39	-0·04	0·79	-0·03	1·75
715	M	40-49	-0·52	-0·80	-1·36	1·35
748	M	24-39	-2·21	-1·34	0·64	0·08
738	F	16-23	0·75	1·05	1·04	1·69
734	F	16-23	-0·17	-0·46	-3·39	-0·20
741	F	40-49	-0·16	0·63	-0·91	-0·56
736	M	16-23	0·29	-1·11	0·23	-0·76
751	M	24-39	-0·61	-1·34	-0·44	0·40
747	M	24-39	-0·61	0·81	-0·89	0·08
752	M	40-49	-1·61	-0·80	-0·31	2·12
755	M	16-23	1·20	-1·93	-3·38	-2·51
768	M	60-69	0·62	-1·04	-2·04	-1·01
743	M	16-23	-2·92	-3·57	-7·83	-1·42
753	F	24-39	-1·52	-0·01	-1·45	-0·76
803	M	60-69	-2·69	0·77	-0·05	0·01
767	M	24-39	-0·61	-1·34	-1·35	-0·42
761	F	24-39	0·32	0·79	0·34	1·75
773	M	24-39	-1·41	-1·34	-0·78	-1·23
754	M	16-23	0·75	-1·93	-0·97	2·07
771	F	40-49	0·92	1·34	-0·69	-0·75
776	M	24-39	-0·61	-0·62	0·13	-0·42
795	F	40-49	0·20	1·34	0·96	1·54
778	M	24-39	-0·21	1·52	0·07	0·57
798	M	24-39	-2·21	-1·34	-1·57	-1·56
658	M	24-39	-0·61	-1·34	-1·86	-0·25
623	M	24-39	-0·21	0·09	0·75	0·89
729	F	24-39	-0·04	1·59	0·95	1·08

5.1.3 Author's Contribution to Study I

The author was primarily responsible for the computational and analytical aspects of this study and independently conducted the electrophysiological data analyses. This included working directly with the EEG datasets and developing the analytical pipeline used for signal processing, feature extraction, and statistical evaluation of relationships between electrophysiological activity and cognitive performance.

The author designed and performed the statistical analyses used to examine performance-related differences in EEG activity across cognitive domains assessed using CANTAB, including group-level comparisons and correlation-based analyses. The author further contributed to interpretation of the findings within the context of cognitive neuroscience and electrophysiological mechanisms of cognitive function.

The author prepared the manuscript and contributed to revisions in response to reviewer feedback.

5.2 Study II — Pupil size as an early and accessible biomarker of memory performance: A comparative intracranial EEG study

Building Building upon the findings of Study I, the second empirical study investigated whether physiological signals could distinguish variations in memory performance at the level of individual task trials. Whereas the first study focused on electrophysiological correlates of inter-individual differences in cognitive performance, Study II extended the investigation toward dynamic, trial-by-trial physiological variability during memory processing.

To address this objective, simultaneous intracranial electroencephalography (iEEG) and pupillometry recordings were obtained during a verbal free recall task. The combination of these modalities enabled examination of complementary physiological processes associated with memory function. Intracranial EEG provided localized measurements of neural activity from clinically implanted brain regions with high temporal precision, whereas pupillometry provided a non-invasive physiological measure associated with attentional engagement and cognitive state.

A central motivation of this study was to evaluate whether a practical and accessible non-invasive physiological signal, namely pupil dynamics, contains information related to memory performance comparable to that derived from invasive neural recordings. Because intracranial EEG provides highly localized electrophysiological measurements and is regarded as one of the most physiologically precise recording methods available in human neuroscience, it served as an important reference for contextualizing the timing and discriminative characteristics of pupil-related physiological signals.

By incorporating time-resolved statistical analyses and classification approaches, Study II examined whether physiological features derived from neural and pupil-

related signals could distinguish good and poor memory performance during memory encoding and retrieval. This progression from inter-individual characterization to trial-level analysis represents an important methodological extension of the dissertation and advances the broader aim of developing physiologically grounded indicators of cognitive and memory performance.
The following publication presents the complete methodology, analyses, and findings of Study II.

5.2.1 Publication II

Pupil size as an early and accessible biomarker of memory performance: A comparative intracranial EEG study

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ABSTRACT

Noninvasive and real-time methods for tracking the neural dynamics of memory processing remain limited. Here, we tested whether fluctuations in pupil size were associated with trial-to-trial variations in memory performance and how their temporal and discriminative properties compared with intracranial EEG (iEEG) signals. Seven epilepsy patients implanted with electrodes for seizure monitoring performed a free-recall verbal memory task while pupillometry and iEEG signals were recorded simultaneously. Trials were categorized as good or poor based on immediate recall performance, revealing pupil responses that differentiated the trial type 600 ms before word presentation and 200 ms before recall vocalization. These effects showed broadly comparable timing, discriminative strength, and classification performance to simplified iEEG power-in-band features obtained from multiple cortical regions and frequency bands. Our results suggest using pupil dynamics as an accessible biomarker of immediate memory performance in research, clinical and other applications.

Introduction

Memory is the biological process that allows us to retain and reconstruct information acquired throughout life [1]. In humans, this capacity underpins all other cognitive functions like attention, learning and decision-making. Consequently, memory deficits—whether due to aging, neurological disorders such as Alzheimer's disease or epilepsy, or psychiatric conditions such as depression—severely compromise cognition and quality of life [2–4]. Determining physiological biomarkers of memory processing is critical for improved diagnosis, monitoring and development of new therapeutic strategies.

Mechanistic investigations of human memory processing are possible with intracranial EEG (iEEG), which provides superior temporal and spatial resolution but remains confined to clinical populations due to its invasive nature. Decades of iEEG studies identified reliable neural signatures of successful memory encoding and recall, including spectral power in the theta and gamma frequency band [5, 6] or, more recently, high-frequency oscillations in high-gamma and ripple ranges of the iEEG spectrum [7–9].

Noninvasive behavioral metrics offer only indirect insight into memory processing but provide continuous low-cost readouts of these processes. The advantage is in the opportunity to monitor memory performance continuously in everyday settings.This can be achieved using pupillometry, which captures moment-to-moment fluctuations in the pupil size [10]. It reflects the balance of parasympathetic and sympathetic activity and is regulated by the cholinergic and noradrenergic neuromodulatory systems [11–13], indicating the associated cognitive states of attention, arousal, decision-making and memory [14–16]. By providing a noninvasive window into the neural mechanisms of cognition, pupillometry holds promise both for advancing fundamental understanding of these functions and for enabling early detection of their impairments [17, 18].

Thus, pupil dynamics can be leveraged as a screening tool for cognitive dysfunctions, strengthening its role as a potential clinical biomarker for diagnosis and monitoring [17, 19–21]. Previous studies found that pupil responses during memory encoding are predictive of subsequent performance [22–24]. Pupillometry studies, in general, show distinct patterns of constriction and dilation characteristic of memory encoding and recall processes [25, 26]. These characteristic patterns are

reminiscent of iEEG features of successful memory formation and retrieval [5, 27]. Since both the iEEG and pupillometric features predict successful memory processing, it is likely that pupil dynamics not only mirror task engagement but index the neural processes underlying memory encoding and retrieval.

Other noninvasive studies have recently associated pupil dilation with locus coeruleus activity and the reset of hippocampal event boundaries—the moments when the hippocampus resets its internal state to segment a continuous stream of experience into distinct episodic units [28]. Pupil-linked arousal has also been associated with large-scale cortical state changes, reflected in the suppression of EEG alpha and enhancement of gamma activities [29]. Together, these findings underscore the potential of pupillometry as a noninvasive proxy of particular neural activities supporting memory functions.

Building on this growing body of evidence, the present study examines pupillometry as a viable noninvasive alternative to iEEG in tracking trial-by-trial memory performance. Our goal was to compare pupil responses to stimulus encoding and recall with spectral iEEG power across multiple cortical regions and frequency bands. Instead of contrasting specific recalled or forgotten stimuli or general groups of impaired and nonimpaired subjects, this study was focused on trial-to-trial fluctuations of good and poor memory performance in any one subject. The main hypothesis was that noninvasive pupil size fluctuations can predict memory performance with comparable strength and timing of memory-related differences as the invasive iEEG features. If pupil dynamics can predict trial-level memory outcomes with accuracy comparable to iEEG, they may offer a more accessible and noninvasive window into the same neural processes that govern successful remembering—laying the groundwork for real-time cognitive monitoring in both laboratory and everyday life contexts.

Results

To compare the pupillometric and electrophysiological measures of memory processing, we employed a classic verbal free recall paradigm [30] with concurrent high-resolution eye-tracking [31, 32] and intracranial iEEG recordings [33, 34] (Fig. 1a-c). For every subject, trials were labeled as good or poor memory performance based on the number of words recalled (Fig. 1d). Throughout any one trial of encoding and recalling a list of words, pupil size showed stereotypical responses to the task phases, which were highly consistent for the good and poor memory trials across all subjects (Fig. 1e). In general, pupils were relatively more constricted during encoding and gradually more dilated throughout the distractor and the recall phases. This confirms our previously reported effect of gradual pupil dilation with increasing memory load until it is unloaded during the first phase of the recalling period [22] (Fig. 1e). We have found clear differences between the good and poor memory trials in specific task phases (Fig. 1f). Pupil size was the lowest, i.e., most constricted, during the encoding phases, more dilated during the distractor phases when memory was loaded, and most dilated in the first recall phases (Fig. 1f) when most of the words were recalled. This gradual loading trend was not observed for power-in-band (PIB) features (Fig. 1g; see Fig. S1 for all PIB results).

Although the overall temporal profiles were generally similar between trial types, subject-level cluster-based permutation analysis identified candidate temporal windows showing sustained good–poor pupil differences (Fig. 2a). During encoding, the strongest effect occurred 633–407 ms before stimulus presentation, peaking at −547 ms (peak $t = 4.65$; cluster mass = 118.27; cluster-level permutation $p = 0.132$). Subject-level window-average analysis confirmed a significant good–poor difference within this interval (mean good–poor difference = 0.322; $t(6) = 3.55$, $p = 0.012$; exact two-sided permutation $p = 0.039$; Wilcoxon $p = 0.031$; Cohen's $d_z = 1.34$; 95% CI [0.10, 0.54]). During recall, the strongest effect occurred 320–127 ms before vocalization onset, peaking at −240 ms (peak $t = 4.93$; cluster mass = 111.6; cluster-level permutation $p = 0.116$). Because cluster-level correction was conservative in this small sample, these windows were treated as candidate intervals for follow-up participant-level analyses. The corresponding subject-level window-average effect was also significant (mean good–poor difference = 0.194; $t(6) = 4.45$, $p = 0.004$; exact two-sided permutation $p = 0.023$; Wilcoxon $p = 0.016$; Cohen's $d_z = 1.68$; 95% CI [0.087, 0.300]). Together, these findings indicate consistent participant-level differences in pupil dynamics between good and poor memory trials prior to both encoding and recall events.

To assess subject-level robustness, we performed a leave-one-subject-out sensitivity analysis to determine whether the observed pupil effects were disproportionately driven by one or a small subset of participants. During encoding, the pre-stimulus pupil effect remained positive following exclusion of any individual participant, with statistically significant effects preserved across all iterations ($p = 0.0067$–0.034). Similarly, during recall, the pupil effect remained consistently positive after removal of any participant, with statistically significant effects maintained in every iteration ($p = 0.0022$–0.014) (Table. 1). Subject-wise estimates further demonstrated consistency across participants: during encoding, 6 of 7 participants showed positive good–poor pupil differences within the identified pre-stimulus window, whereas during recall all 7 participants showed positive effects within the identified pre-vocalization window (Fig. S2). Together, these findings indicate that the reported pupil effects are not driven by one or a small number of participants, but instead reflect a broadly consistent pattern across subjects.

To test whether pre-event pupil differences could be explained by trial-history or carry-over dynamics, we fitted trial-level linear mixed-effects models separately for encoding and recall (Table. 2). During encoding, current memory performance remained a significant predictor of pre-stimulus pupil size after accounting for previous-trial performance, previous pre-stimulus pupil size, previous response-related pupil activity, baseline pupil size, and trial order ($\beta = -0.304$, $t(453) = -3.03$,

Table 1. Leave-one-subject-out analysis demonstrates consistently positive good–poor pupil differences across all iterations. Leave-one-subject-out analyses were performed separately for encoding and recall. For each iteration, one subject was excluded and the mean good–poor pupil difference was recomputed within the identified temporal window.

ine	Encoding				Recall			
Removed subject	Mean difference	t	p	Direction	Mean difference	t	p	Direction
ine 1	0.3027	2.8873	0.0343	Positive	0.1902	3.7073	0.0139	Positive
2	0.3770	4.4485	0.0067	Positive	0.1714	3.8738	0.0117	Positive
3	0.3312	3.1078	0.0266	Positive	0.2029	4.0343	0.0100	Positive
4	0.3489	3.4157	0.0189	Positive	0.2226	5.7981	0.0022	Positive
5	0.3325	3.1253	0.0261	Positive	0.1883	3.6867	0.0142	Positive
6	0.3074	2.9033	0.0337	Positive	0.2090	4.3403	0.0074	Positive
7	0.2510	3.7335	0.0135	Positive	0.1710	3.8917	0.0115	Positive
ine								

$p = 0.0026$). Previous response-related pupil activity also contributed significantly ($\beta = -0.120$, $p = 0.0219$), indicating the presence of carry-over dynamics. However, previous-trial performance, previous pre-stimulus pupil size, and trial order were not significant predictors. During recall, the current-performance effect was attenuated after accounting for trial-history variables and did not reach conventional significance ($\beta = 0.166$, $t(453) = 1.76$, $p = 0.080$). In contrast, previous-trial performance ($\beta = -0.258$, $p = 0.0070$), previous response-related pupil activity ($\beta = 0.127$, $p = 0.028$), and current baseline pupil size ($p < 0.001$) significantly contributed to the model (Table. 2). These findings suggest that the encoding pre-stimulus effect cannot be fully explained by simple carry-over dynamics, whereas recall-related pre-vocalization effects appear more strongly influenced by trial-history and recovery processes.

Table 2. Memory performance is associated with pre-event pupil size during encoding after controlling trial history and carry-over variables. Linear mixed-effects models were fitted separately for encoding and recall to test whether pre-event pupil size was explained by current memory performance, previous-trial performance, previous pre-event pupil size, previous response-related pupil activity, current baseline pupil size, and trial order. During encoding, current performance remained a significant predictor of pre-stimulus pupil size after accounting for trial-history variables, whereas during recall the current-performance effect was attenuated and previous-trial variables contributed more strongly. β, regression coefficient; SE, standard error.

ine	Encoding				Recall			
Predictor	β	SE	t	p	β	SE	t	p
ine Trial order	0.0007	0.0013	0.55	0.583	0.0013	0.0012	1.07	0.285
Current performance	-0.304	0.100	-3.03	0.0026	0.166	0.095	1.76	0.080
Previous performance	0.146	0.101	1.44	0.151	-0.258	0.095	-2.71	0.0070
Current baseline	0.068	0.028	2.43	0.015	0.090	0.026	3.43	< 0.001
Previous pre-event pupil	-0.060	0.054	-1.10	0.271	-0.041	0.057	-0.73	0.469
Previous response pupil	-0.120	0.052	-2.30	0.0219	0.127	0.058	2.20	0.028
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During the encoding phase, both trial types showed the expected pre-stimulus pupil constriction followed by pupil dilation [22]. This preparatory constriction was prolonged in the good memory trials, extending into the stimulus presentation period. In the poor memory trials, pupils began dilating before the onset of stimulus presentation (Fig. 2a, left). Likewise, during the recall phase, both trial types showed the expected pattern of pupil dilation [22], which was smooth and gradual in the good memory trials but more interrupted in the poor memory trials (Fig. 2a, right).

To quantify these effects, we determined the latency and magnitude of the maximum memory-related separation using subject-level time-resolved t-statistics (Fig. 2a). Both pupil and PIB measures showed peak discriminative strength prior to the onset of encoding and recall. The spectral PIB features revealed heterogeneous temporal profiles of the memory-related separation across six frequency bands and five brain regions (Fig. 2b; Fig. S3).

Head-to-head comparison showed that pupil dynamics was one of the strongest and earliest indicators of immediate memory performance. The magnitude of the pupil effect, quantified as the maximum absolute subject-level t-value, exceeded 92% of PIB features during encoding and 96% during recall (Fig. 3a). The latency of the peak pupil effect occurred earlier than 73% of PIB features during encoding and 54% during recall (Fig. 3b). PIB features with insufficient subject coverage ($N < 4$) were

excluded from these comparisons (Table. S3). Altogether, the pupillometric memory effect was one of the earliest and strongest indicators of memory performance surpassing many of the invasive spectral PIB features (Fig. 3c).

Finally, we assessed how well the pupil size and PIB features could predict the immediate memory performance using univariate support vector machine (SVM) classifiers with leave-pairs-out cross-validation (LPO-CV). Using the maximum values of F1-scores, which quantify the classification performance, we compared the latency and strength of each feature's performance. The pupillometric feature reached its maximum value (F1 = 0.61) 200 ms before the stimulus onset for encoding and 640 ms (F1 = 0.60) before the onset of recall vocalization (Fig. 4a). This classification performance was better than 64% of all PIB features during encoding and 71% during recall (Fig. 4b; see Fig. S4 for all PIB features), and occurred earlier than 46% and 57% of the PIB features, respectively. Percentages were calculated across 28 PIB features for which valid classification metrics were available.

To account for potential optimistic estimates arising from selecting the peak classification performance across overlapping time windows, we additionally performed a max-statistic label-permutation validation in which the complete classification pipeline, including time-window scanning, leave-pairs-out cross-validation, model fitting, and peak macro-F1 selection, was repeated after randomly permuting trial labels within participants. For encoding, the observed peak macro-F1 exceeded the permutation-derived null distribution of maximum macro-F1 values (500 permutations; null 95th percentile = 0.545, permutation $p = 0.002$). Similarly, during recall, the observed peak macro-F1 exceeded the permutation-derived null distribution (500 permutations; null 95th percentile = 0.546, permutation $p = 0.004$), indicating modest but statistically significant above-chance classification performance after accounting for the time-window selection procedure.

These analyses were based on univariate classifiers evaluating each PIB feature independently. Although multivariate approaches that integrate information across frequencies or regions could in principle enhance PIB performance by capturing distributed neural interactions, we adopted a univariate framework to ensure interpretability and to mitigate overfitting given the limited number of trials per subject. Together, these classification analyses provide complementary evidence that pupil dynamics contain predictive information about memory performance and showed above-chance performance when evaluated on previously unseen participants. Importantly, classifier performance was interpreted as a supplementary predictive measure and not as the inferential basis for claims regarding effect timing or statistical significance.

Discussion and Conclusion

This study suggests that pupil dynamics provide a temporally resolved marker of trial-to-trial memory performance, with timing and discriminability comparable to simple univariate spectral power features derived from intracranial EEG recordings. Using subject-level statistical inference, pupil responses differentiated good from poor memory trials hundreds of milliseconds before word presentation during encoding and before vocalization during recall.

These pupillometric effects showed temporal and discriminative correspondence with spectral PIB across canonical EEG frequency bands and cortical lobes, positioning pupillometry as a noninvasive measure associated with memory-related processes that are reflected in the direct electrophysiological recordings of neural activity. It should be emphasized, however, that more complex and multivariate iEEG features—such as measures of functional connectivity, signal complexity, or distributed decoding approaches capable of tracking encoding states in real time [35]—could in principle yield stronger predictive performance than the simple PIB features examined here. The aim of this work was therefore not to exhaustively evaluate all possible iEEG biomarkers, but to benchmark a well-characterized and interpretable spectral measure against a noninvasive pupillometric signal.

Importantly, our analysis focuses on trial-level variability in immediate memory performance across subjects, rather than differences between recalled and forgotten stimuli or impaired and nonimpaired individuals. This framework emphasizes variability at the level of individual trials while characterizing aggregate patterns across the study population, thereby providing a framework that can be tested in broader populations in future studies and aligns with prior evidence that eye-based measures can reveal hippocampal-dependent memory states even in the absence of explicit report [36].

Together, our findings suggest that pupillometric measures contain modest but statistically reliable predictive information, with latencies and discriminative strength comparable to those of the simplified electrophysiological measures analyzed here, underscoring their potential use for noninvasive and real-time cognitive assessment. Previous studies reported that both pre- and post-stimulus pupil size predicted subsequent memory recall performance [22, 37]. Our study was focused on distinguishing good and poor memory states instead of recalling particular stimuli, and identified pupil dynamics as a prospective marker of memory state emerging even before the onset of encoding.

Previous pupillometry studies have consistently linked pupil dynamics to memory processes across task phases. In epilepsy patients performing a free-recall task, structured pupil responses were observed during countdown, encoding, distractor, and recall periods, with successful retrieval accompanied by robust post-vocalization dilations even in the absence of visual input; words that were later remembered were associated with smaller pre-stimulus and larger post-stimulus pupils compared to subsequently forgotten words [22]. Other studies have shown that larger baseline and stimulus-evoked pupil responses predict

successful memory performance [37, 38], proposing that pre-stimulus pupil size reflects a preparatory "readiness to remember" state. Together, these findings support the view that pupil size provides a continuous and sensitive physiological index of memory-related encoding processes that may contribute to subsequent retrieval success.

The early emergence of pupil-based differences before stimulus presentation suggests that preparatory states may contribute to variability in memory outcomes, consistent with prior work demonstrating that eye-movement metrics can track associative memory processes on a trial-by-trial basis [39]. However, the trial-history analyses indicate that pre-event pupil dynamics should not be interpreted as purely preparatory. During encoding, current memory performance remained a significant predictor of pre-stimulus pupil size after controlling for previous-trial performance, previous pre-stimulus pupil size, previous response-related pupil activity, baseline pupil size, and trial order. This suggests that the encoding-related pre-stimulus effect cannot be fully explained by simple carry-over or slow recovery dynamics. At the same time, previous response-related pupil activity and current baseline pupil size also contributed to the model, indicating that inter-trial recovery processes still explain part of the variance. During recall, the current-performance effect was attenuated after accounting for trial-history variables, whereas previous-trial performance, previous response-related pupil activity, and baseline pupil size significantly predicted pre-vocalization pupil size. These findings support a cautious interpretation: pre-stimulus pupil differences during encoding are consistent with preparatory cognitive state, whereas pre-vocalization pupil differences during recall appear more strongly influenced by trial-history and recovery dynamics. Such pre-encoding effects are consistent with the possibility that successful memory performance is influenced not only by stimulus-driven processing but also by endogenous moment-to-moment fluctuations in attention and arousal that may place the brain in a state favorable for memory encoding [37, 38]. Temporal coincidence of these early pupil modulations with intracranial spectral activities suggests that these preparatory states are expressed in both the peripheral and central nervous systems, linking internal fluctuations in attention and arousal to the timing of neural processes that support memory formation [29, 35, 40]. This pattern is consistent with models proposing that transient shifts in brain state influence memory success by modulating cortical excitability before learning begins [41].

The temporal correspondence between the pupil and iEEG features suggests that they may reflect shared neuromodulatory influences associated with memory performance. Specifically, early pre-stimulus pupil constriction and concurrent changes in cortical spectral power during successful trials may reflect preparatory fluctuations in attention and arousal that facilitate effective memory encoding. Such coordinated dynamics are consistent with frameworks involving the locus coeruleus–norepinephrine (LC–NE) system, which has been widely implicated in regulating cortical excitability and hippocampal plasticity [40, 42]. Previous studies have shown that pupil diameter covaries with LC activity and large-scale fluctuations in arousal measured using fMRI and EEG [16, 42–44]. However, the present study did not directly measure LC activity or norepinephrine signaling. Therefore, the LC–NE interpretation should be considered a biologically plausible hypothesis rather than a demonstrated mechanism. Future studies combining pupillometry with pharmacological manipulation or direct neuroimaging of the locus coeruleus will be necessary to test this proposed link explicitly.

By directly comparing pupillometric measures with invasive electrophysiological benchmarks, our study suggests that pupil dynamics carry predictive information about memory state comparable to that obtained from the simplified univariate PIB features analyzed here. Importantly, this comparison does not imply equivalence to optimized intracranial EEG decoding approaches or establish a causal relationship between pupil dynamics and neural activity. Rather, the findings indicate that a simple, noninvasive physiological measure can capture memory-related state information that overlaps in timing and discriminative value with selected invasive electrophysiological features. Free-recall paradigms similar to ours are already used clinically to detect early cognitive decline [45, 46]. Pupil-based measures could extend these assessments by enabling fast, real-time evaluation of ongoing memory states. Abnormal pupil-linked arousal has been reported in Alzheimer's disease and correlates with locus coeruleus integrity [47].

Several limitations should be considered when interpreting these findings. First, the electrophysiological comparison was based on simple univariate PIB measures to allow a direct and interpretable comparison with pupil data, but the present results should be understood relative to simplified univariate electrophysiological features rather than optimized multivariate neural decoding approaches. More advanced EEG measures, such as cross-frequency coupling, phase synchrony, connectivity analyses, signal complexity, or multivariate decoding approaches [48, 49], may provide additional predictive information. Second, spectral decomposition was performed using a 0.5-s window, which represents a trade-off between temporal and frequency resolution. While this choice enabled time-resolved comparisons aligned with pupil dynamics, frequency resolution near the lower boundary of the delta band (2–4 Hz) is inherently limited with this window length. As a result, slow neural dynamics within this frequency range may not have been fully characterized, potentially reducing sensitivity to low-frequency oscillatory processes.

Third, although the statistical framework accounts for participant-level variability and temporal autocorrelation through subject-level cluster-based permutation testing, the small sample size ($N = 7$) limits statistical power. Cluster-level correction was therefore conservative, and the identified temporal windows should be interpreted in conjunction with the participant-level window-average tests and exact sign-flip permutation analyses. While the consistency of the effects across subjects and the

leave-pairs-subject-out analyses support the robustness of the main pupil findings, replication in larger cohorts will be necessary to establish their reliability.

Fourth, the study group was relatively small and consisted of young patients with drug-resistant epilepsy undergoing invasive monitoring. This clinical context limits the generalizability of the findings to broader populations. Each participant included in the present analysis contributed both good and poor memory trials, allowing within-subject comparison of memory-performance states. However, the cohort was not designed to systematically assess interindividual differences in baseline cognitive ability, and such variability may influence physiological markers of memory performance. This issue is particularly relevant because memory impairments have been reported in approximately one-third of patients with drug-resistant epilepsy in larger cohorts [50]. Interictal epileptiform activity is an inherent feature of intracranial recordings in epilepsy patients and cannot be fully excluded. However, the present analyses focused on task-locked temporal patterns aligned to cognitive events, which are unlikely to be systematically produced by sporadic pathological discharges. Averaging trials within each participant before group-level inference further reduces the influence of such non-task-related activity. Nevertheless, epilepsy-related dynamics remain a limitation of studies conducted in clinical populations. The inclusion of pupillometry provides a complementary advantage because pupil responses are not directly contaminated by interictal epileptiform discharges and therefore offer an independent noninvasive measure of cognitive state. At the same time, disease-related effects on arousal, medication status, clinical setting, or baseline cognitive variability cannot be fully excluded and should be addressed in larger and more systematically characterized cohorts. Previous work using the same verbal free recall paradigm in healthy volunteers demonstrated consistent pupil modulation across task phases [22], but larger studies in healthy and clinical populations are required to confirm generalizability beyond epilepsy cohorts.

Another limitation concerns variability in electrode implantation sites across patients. Because electrode placement was determined by clinical needs, cortical and limbic regions were sampled unevenly across subjects. Although each cortical lobe included at least four electrodes across subjects, unequal electrode sampling may still influence regional estimates and limit interpretation of spatial specificity. Some areas relevant to memory and autonomic regulation were relatively undersampled, and electrophysiological features from these regions could potentially provide stronger predictive value than the PIB measures examined here. Future studies with larger cohorts, more systematic electrode coverage, and statistical models accounting for electrode distribution will be required to evaluate regional contributions more fully.

Finally, our analyses identified relationships between pupil and EEG measures associated with memory performance but did not establish causal interactions. While previous studies using pupillometry, fMRI, and EEG point to the involvement of the locus coeruleus–norepinephrine (LC–NE) system in memory-related pupil dynamics [29, 51], future work employing pharmacological or stimulation interventions will be needed to directly test the underlying neurobiological mechanisms. These findings suggest broader neuroscience relevance for pupillary dynamics as potential indicators of dynamic cognitive states and candidate biomarkers of memory-related processes. Because pupil responses reflect both cognitive and affective influences, future studies should investigate how cognitive load, emotional state, and autonomic regulation jointly contribute to pupil-linked neural dynamics across behavioral and clinical contexts. In the present study, emotional influences and luminance changes were minimized by using neutral word stimuli and constant visual display conditions, suggesting that cognitive processing can generate measurable pupil-linked autonomic signatures even within an integrated cognitive–emotional–autonomic framework. Intracranial electrophysiological features were used primarily as benchmark references to evaluate the temporal and discriminative properties of pupil dynamics, rather than to infer direct mechanistic coupling. Future studies combining pupillometry, gaze-tracking, autonomic measures, and electrophysiology will be essential for establishing the mechanistic basis, reliability, and potential clinical applicability of pupil-based biomarkers across neurological populations.

Because pupillometry is relatively inexpensive, portable, and noninvasive, it may also have future potential for integration into wearable or mobile technologies. However, reliable deployment outside controlled experimental settings will require further methodological development to address challenges such as lighting variability, motion, calibration stability, and device resolution.

It remains to be addressed whether the reported pupil measures generalize beyond the verbal memory recall task employed in our study. Similar pupil dynamics have been observed in recognition, associative learning, and working-memory paradigms [28, 52–54], indicating that pupil measures capture more general fluctuations in arousal and vigilance that support diverse forms of memory and attention. This can be directly tested by combining pupillometry with neuroimaging or pharmacological approaches [17, 55]. Longitudinal work in aging cohorts will determine whether long-term changes in these pupil-based measures predict chronic progressive cognitive decline. Pupillometric measures such as those reported here may eventually support continuous, noninvasive monitoring of cognitive state in laboratory, clinical, educational, and training environments. However, this potential will require validation across larger cohorts, broader cognitive tasks, and real-world recording conditions.

Materials and Methods

Data Collection

Seven patients with drug-resistant epilepsy (mean age 21.4 ± 4.2 years; 4 males) participated in the study at the Mayo Clinic, Rochester, MN, USA (see Table S1 for demographic and clinical characteristics of the study participants). All participants provided written informed consent prior to participation. The study protocol was approved by the Institutional Review Board (IRB) of Mayo Clinic, and all procedures were conducted in accordance with the principles of the Declaration of Helsinki.

iEEG data were recorded from depth and subdural electrodes implanted for clinical purposes, sampled at 32 kHz. Electrode placement was determined by the clinical team for seizure localization. Post-implantation electrode localization was performed by co-registering postoperative CT images with pre-implantation MRI using a rigid-body linear transformation with a mutual information–based multimodal registration algorithm implemented in Statistical Parametric Mapping (SPM). The automated alignment was visually inspected and manually adjusted when necessary to ensure accurate anatomical correspondence. Electrode contacts were then manually identified on the co-registered CT images and localized in each subject's native anatomical space without transformation to a standard template [56, 57]. All patients in this study underwent intracranial monitoring prior to any surgical resection; notably, none of the participants had a history of previous mesial temporal surgery or resection. Magnetic resonance imaging (MRI) confirmed preserved amygdala and mesial temporal lobe structures in all patients at the time of recording.

Patients performed a verbal free recall memory task [30] while undergoing simultaneous iEEG and pupillometry. Task events were synchronized with iEEG recordings using TTL pulses generated by the task computer. Testing sessions were conducted during clinically stable periods, and no seizures occurred during the analyzed trials. Interictal epileptiform activity was not explicitly removed, as such discharges occur sporadically and are unlikely to align systematically with task events. Averaging across multiple trials was used to reduce the influence of non-task-related pathological activity. Spoken recall responses were captured via microphone recordings and subsequently extracted for analysis [58]. Pupil size was recorded with the i4tracking system (Medicton Group Ltd., Prague, Czech Republic), an infrared-based eye-tracking device providing ~ 0.1 mm/pixel resolution at 150 Hz sampling rate. The system employs dual infrared light sources to optimize pupil detection while minimizing interference from ambient IR sources [59, 60].

Participants were positioned at a standardized viewing distance from the task monitor placed on an adjustable bedside table. The monitor position remained fixed throughout each session, and patients were instructed to remain still, maintain fixation on the task monitor, and avoid unnecessary movement. These instructions helped maintain consistent task engagement and minimized opportunities for self-monitoring or performance-related rumination during trials Testing was conducted in a dimly lit room with stable illumination that remained constant throughout all sessions. Window blinds were kept closed throughout testing to prevent external daylight exposure. Overhead ceiling lights were maintained at a constant low intensity, and no additional point light sources were present in the room. Ambient luminance and testing times were identical for every participant, who were not facing any windows or direct light sources to ensure that pupil fluctuations reflected internal cognitive rather than external environmental factors. Prior to each session, pupil detection quality was verified and automatic calibration was performed between tasks to ensure accurate tracking [31]. The experimental setup is illustrated in Fig. 1a.

Medication status and potential autonomic confounds were controlled within the clinical constraints of epilepsy monitoring. As part of standard pre-surgical evaluation, patients were tapered off antiepileptic and selected centrally acting medications under medical supervision to facilitate spontaneous seizure occurrence. In addition, intake of common autonomic stimulants such as caffeine and nicotine was restricted, and patients remained on a standardized hospital diet throughout hospitalization. These precautions reduced variability in sympathetic and parasympathetic activity unrelated to the experimental task.

Behavioral Task Paradigm

A well-established verbal free recall paradigm was used to probe memory performance [30]. This paradigm primarily assesses verbal memory recall while engaging cognitive processes commonly associated with episodic memory, including encoding, maintenance across a delay, and retrieval of temporally structured information [61]. Participants studied lists of 12 nouns, each displayed on a laptop screen for 1500 ms, separated by a 1000 ms interstimulus interval. Words were randomly selected from a standardized pool of 300 high-frequency nouns commonly used in human free-recall studies (University of Pennsylvania WordPools; http://memory.psych.upenn.edu/WordPools), consisting primarily of emotionally neutral items to minimize potential affective confounds. The complete set of word lists presented to each subject across all trials is provided in Table S2.

All visual stimuli were presented in dark gray on a light gray background to minimize pupil responses to changes in luminance and contrast. The same light gray background was maintained across countdown, encoding, distractor, and free recall phases. During the free recall phase, a fixation cross positioned at the same vertical location as the presented words remained visible to ensure constant visual input and luminance across all task periods. After each list, they completed a brief arithmetic distractor task to minimize recency effects, followed by a 30-second free recall period during which they verbally recalled as many words as possible. Each session comprised 15 trials, with one word list presented per trial (Fig. 1b). iEEG and

pupillometry were recorded simultaneously throughout each session (see Fig. 1c for an illustration of a single trial showing two occipital channels and corresponding pupil dynamics). Trials were classified according to recall performance. The number of recalled words per trial was z-scored across all trials from all participants, and trials with z-scores above zero were labeled as good, while those below zero were labeled as poor. Because a z-score of zero corresponds to the mean recall level, this threshold separated trials based on the natural distribution of recall performance rather than an arbitrarily chosen number of recalled words. In the present dataset, this resulted in trials with five or more recalled words being classified as good and four or fewer recalled words as poor (Fig. 1d). Trials with no recalls were excluded, as these likely reflected lapses of attention rather than poor memory performance. Subjects included in the present analyses demonstrated variability in recall performance, producing both good and poor trials. Subjects who produced uniformly poor recall across all trials were not included, as representation of both trial types was required to enable meaningful within-subject comparisons and classification analyses. Accordingly, pupil and iEEG data from the good and poor trials were analyzed as good and poor memory performance, respectively.

Analysis of Pupil Signals

Eye-tracking data were analyzed in MATLAB R2023b (MathWorks Inc.). Pupil size was extracted using an ellipse-fitting algorithm applied to the pupil image. Raw pupil diameter values (in pixels) were converted to millimeters by normalizing each subject's interpupillary distance (IPD) to the IPD measured in the camera images. Pupil area was then computed from the fitted vertical and horizontal diameters using the standard ellipse area formula [22, 62]. To address brief signal losses from blinks or artifacts, linear interpolation was applied to preserve temporal continuity. Segments with data loss exceeding 90 ms (14 consecutive samples at 150 Hz) were excluded, as interpolation was deemed unreliable, consistent with recommended preprocessing practices [62]. For between-group comparisons, pupil area was normalized using z-score transformation. Pupil area and PIB values were normalized independently within each subject using z-score transformation prior to pooling trials across participants, thereby reducing the influence of inter-individual baseline differences.

Pupil signals were grouped into good and poor memory performance according to trial labels defined by the recall outcomes (Fig. 1e). For group-level comparisons, pupil area was averaged across task phases: two 15-s intervals for encoding (Encoding 1, Encoding 2), two 10-s intervals for the distractor phase (Distractor 1, Distractor 2), and three 10-s intervals for recall (Recall 1–3), as summarized in Fig. 1f. For event-related analyses, pupil area was extracted from trials time-locked to behavioral events. Specifically, ±1-s segments were analyzed around word presentation during encoding and around word vocalization during recall, restricted to successfully recalled words. These event-related responses were then compared between good and poor memory performance, following prior work demonstrating memory-related pupil modulation in the same free recall task [22].

Analysis of iEEG Signals

The iEEG recordings were analyzed in Python to examine frequency-specific spectral power dynamics. Raw signals were filtered with a 60 Hz notch filter (second-order IIR, Q = 30) to remove line noise. Active electrodes were identified using an automated Gaussian Mixture Model (GMM) classification based on induced spectral power during encoding relative to baseline [63], applied independently for each subject. Importantly, this procedure was performed independently of memory performance labels (good vs. poor trials), thereby reducing the risk of circularity in subsequent performance-based comparisons. The proportion of active electrodes per subject is reported in Table S3. Spectral decomposition was obtained with a Short-Time Fourier Transform (STFT) using a 0.5-s Hanning window and 25% overlap, and power spectral density (PSD) values were log10-transformed [64]. While this window length provides adequate temporal resolution for event-related analyses and reliable estimation of theta and higher frequency bands, frequency resolution at the lower edge of the delta band (2–4 Hz) is inherently limited due to the finite window duration. Power estimates were averaged within six canonical frequency bands: delta (2–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta (12–30 Hz), low gamma (30–60 Hz), and high gamma (60–150 Hz). Power-in-band (PIB) values were normalized with a z-score transformation and averaged across electrodes within anatomically defined brain regions: limbic (L), frontal cortex (FC), parietal cortex (PC), occipital cortex (OC), and temporal cortex (TC). To ensure sufficient regional representation, analyses were performed only for cortical lobes that included at least four active electrodes across two subjects. Because electrode placement was determined by clinical requirements, not all regions were sampled in every subject. Therefore, regional PIB values were computed by pooling electrodes across subjects within each cortical lobe, a commonly adopted approach in intracranial EEG studies with clinically determined implantation targets.

Although raw iEEG data were acquired at 32 kHz, spectral power computation reduced the effective temporal resolution of the PIB time series owing to windowed averaging. The resulting PIB signals capture slow fluctuations in band-limited power rather than high-frequency neural activity. To align with the pupillometric data, PIB time series were interpolated to a 150 Hz sampling frequency—well above the spectral bandwidth of task-related power changes—providing a common temporal framework for multimodal analyses.

PIB values were averaged across the same encoding, distractor, and recall intervals used in the pupil analysis (see Fig. 1g). They were also classified into good and poor memory conditions as described above. For event-related analyses, ±1-s segments were extracted around word presentation (encoding) and word vocalization (recall), restricted to successfully recalled words, to

capture transient task-related changes in spectral activities. The resulting time-resolved PIB features were used for statistical comparisons and classification analyses.

Statistical Analysis

All statistical analyses of the pupillometric features were performed in MATLAB R2023b (MathWorks Inc.) and in Python for the iEEG features. Pupil area and PIBs were analyzed time-locked to the word presentation during encoding, and to the word vocalization onset during recall. Group-level differences between good and poor memory performance trials were visualized as 95% confidence intervals computed across all trials from all subjects (Fig. 1 f–g).

To assess differences between memory conditions, pupil area and PIB signals were first averaged separately for good and poor memory trials within each participant. Statistical inference was performed across participants rather than pooled trials, thereby treating the participant as the unit of inference and accounting for between-subject variability. Time-resolved statistical analysis was performed using a subject-level cluster-based permutation framework. Participant-level t-statistics comparing good and poor memory conditions were computed at each time point, and temporally adjacent time points exceeding the cluster-forming threshold were grouped into contiguous clusters. Cluster mass was evaluated against an exact sign-flip permutation null distribution across participants. This approach accounts for both temporal dependence among neighboring time points and participant-level variability, thereby avoiding the assumption of independent observations (Fig. 2).

Because cluster-level inference was conservative, temporally sustained windows identified by the cluster-based analysis were used to define windows of interest for participant-level inference. Subject-wise average good–poor differences were computed within each window and assessed using one-sample t-tests, Wilcoxon signed-rank tests, and exact subject-level sign-flip permutation testing.

To evaluate the robustness of the observed effects and assess whether they were disproportionately driven by one or a small number of participants, a leave-one-subject-out sensitivity analysis was performed. The primary temporal effect identified in the cluster-based analysis was recomputed iteratively after excluding each participant in turn. In addition, subject-wise estimates of the good–poor difference were extracted within the identified temporal windows to assess consistency across participants (Table. 1).

To determine whether pre-event pupil differences could be explained by trial-history or carry-over dynamics, additional trial-level linear mixed-effects analyses were performed separately for encoding and recall. Pre-event pupil size within the identified temporal windows was modeled as a function of current memory performance, previous-trial performance, previous pre-event pupil size, previous response-related pupil activity, baseline pupil size, and trial order, with subject included as a random intercept to account for repeated measurements within participants (Table. 2).

For visualization and feature comparison, the temporal evolution of subject-level t-statistics was retained to characterize the timing and relative strength of separation between good and poor memory conditions. Comparisons between pupil and PIB features were based on the maximum absolute subject-level t-value and the latency of this peak, enabling direct comparison of temporal discriminability across physiological features (Fig. 3). These time-resolved t-statistic profiles were used as descriptive visualizations rather than independent point-wise inferential tests.

Time-resolved analyses were performed at the native temporal resolution of the signals (150 Hz; approximately 7-ms bins), enabling comparisons between pupil and PIB modalities while preserving temporal precision.

Classification Analysis

We evaluated and compared the predictive power of the pupil-based and iEEG-based features using a time-resolved single-feature classification framework. Continuous pupillometric and the iEEG PIB signals were first converted into temporally localized features using a sliding-window procedure. Mean values were computed within 67-ms windows advanced in 33-ms steps. These parameters were selected to balance temporal resolution with signal stability: a 67-ms window provides ~ 10 samples at 150 Hz, yielding reliable estimates without excessive smoothing, while the 33-ms step ensures dense temporal sampling with $\sim 50\%$ overlap, providing a unified temporal structure across modalities.

Each model was trained on a single feature, e.g., pupil area or a PIB from a specific frequency band and cortical region, to isolate the independent predictive contribution of each signal component. Classification was performed using a Support Vector Machine (SVM) with a radial basis function (RBF) kernel [65], which provides flexibility in modeling potentially nonlinear boundaries even for single-feature data. Hyperparameters were optimized via grid search within the training folds.

Model performance was quantified using the macro-averaged F1-score (F1-score), which balances precision and recall while assigning equal weight to each class, making it suitable for datasets with class imbalance. In our dataset, the ratio of good to poor trials was approximately 2:1; therefore, the macro-averaged F1-score was used to avoid inflated performance estimates driven by the majority class. Robustness and generalizability were assessed with leave-pair-out cross-validation (LPO-CV). In each fold, data from two patients were excluded from training and used exclusively for testing, while the classifier was trained on data from the remaining patients. This procedure was repeated across all possible combinations of patient pairs, ensuring that testing was always performed on data from previously unseen patients.

Classification performance was evaluated independently at each time window across the temporal profile. To summarize predictive performance, we reported the maximum macro-F1 score and its latency for each feature. To account for potential optimistic estimates arising from selecting the peak classification performance across multiple overlapping windows, we performed a max-statistic label-permutation validation. Specifically, the complete classification pipeline—including time-window scanning, leave-pairs-out cross-validation, model fitting, and peak macro-F1 selection—was repeated across 500 label permutations after randomly permuting trial labels within participants. For each permutation, the maximum macro-F1 across all time windows was retained to generate a null distribution of peak classification performance against which the observed peak macro-F1 was compared.

Average F1-scores for PIB-based and pupil-based models were reported separately to enable a direct comparison of predictive capabilities, as summarized in Fig. 4.

Note that low-gamma PIB features in the OC and PC regions were excluded from the LPO-CV analysis because only two subjects had active electrodes in these regions (Table. S3); these features therefore appear blank in Fig. S3.

Data Availability

The data analysed in this study have been published and are freely available on the EBRAINS data repository [60].

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Author contributions statement

N.H. drafted the manuscript, directly accessed and verified the underlying data, conducted data analysis, interpreted the results, revised, and approved the manuscript. L.F.S. revised, and approved the manuscript. M.L., C.T., V.S.M., and B.J. contributed to data collection, directly accessed and verified the underlying data. A.C. and G.A.W. designed the study, contributed to data collection, revised the manuscript. M.T.K. designed the study, drafted the manuscript, contributed to data collection, directly accessed and verified the underlying data, contributed to data analysis, interpreted the results, revised, and approved the manuscript. All authors read and approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

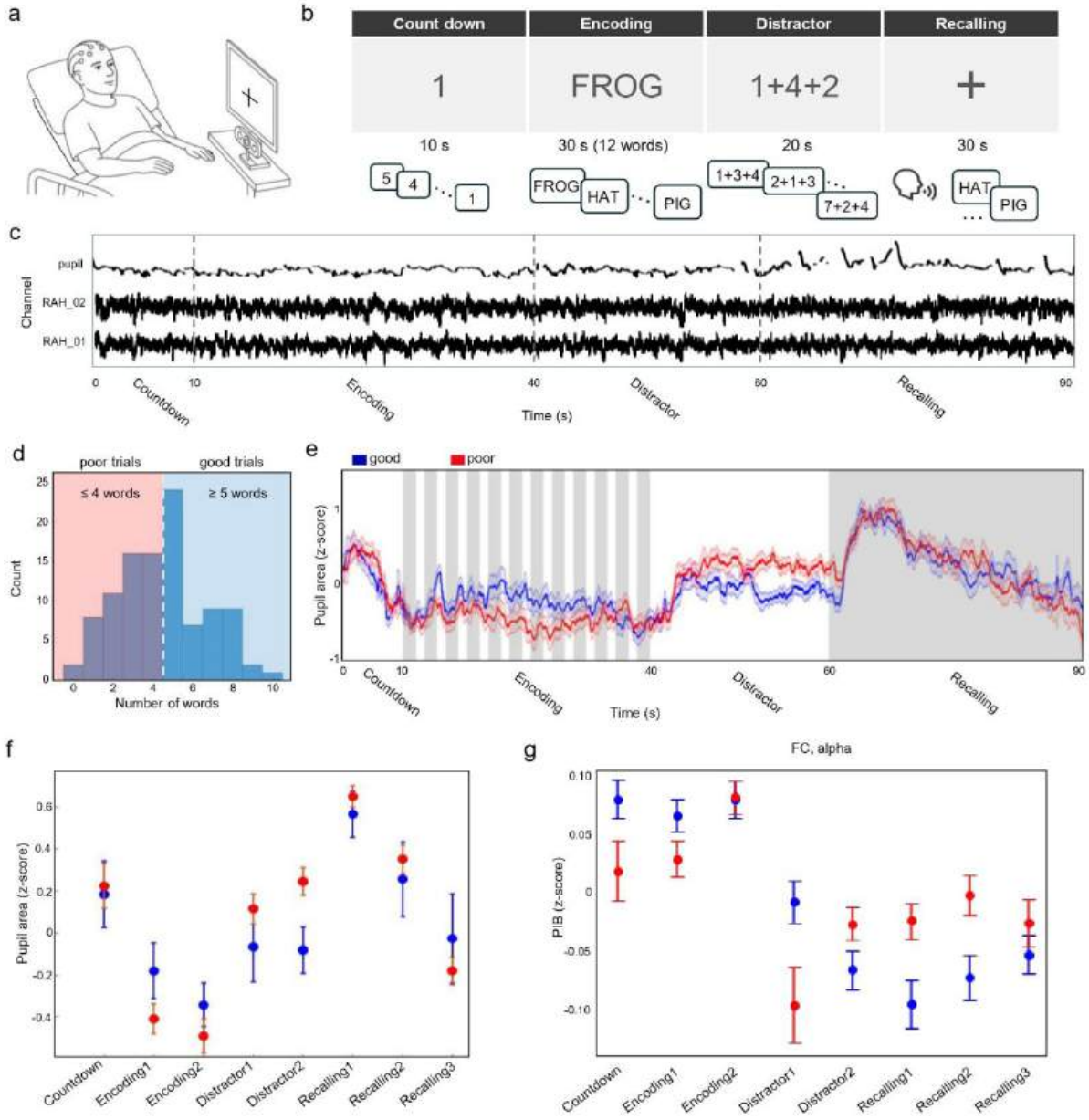

Figure 1. Simultaneous iEEG–pupillometry recordings reveal distinct differences between good and poor performance in a free recall verbal memory task. (a) Patients performed the task while lying in bed with depth and subdural electrodes implanted for clinical monitoring, while pupil size was simultaneously recorded with an infrared-based eye-tracking system. (b)Each session consisted of 15 trials, with one word list presented per trial. The schematic also illustrates the visual displays shown on the monitor during the countdown, encoding, distractor, and recall phases. (c) Example signals recorded from occipital iEEG channels during a single trial, shown alongside the pupil area recorded from the same trial. (d) Trials were labeled according to recall performance: ≥ 5 recalled words defined good memory trials, whereas ≤ 4 defined poor memory trials; trials with no recalls were excluded. (e) Grand-average pupil signals (means computed per subject and then averaged across participants), grouped into the good (blue) and poor (red) memory trials (Shaded areas indicate the standard error of the mean (SEM) computed across subjects (N = 7)). Grey shaded regions mark epochs of word presentation on the screen and the recall period, during which a fixation cross remained visible on the same light gray background. Notice the consistent stereotypical responses of pupil constriction and dilation across all subjects, especially in the encoding and recall phases of the task (gray background marks epochs of word presentations and the recall period). (f) Group-level pupil area averaged across task phases from all subjects: two 15-s intervals for encoding, two 10-s intervals for the distractor, and three 10-s intervals for recall (bars indicate 95% confidence intervals). (g) Group-level PIB (power-in-band) values averaged across the same task phases as in 'e'. This is an example of alpha frequency from all frontal cortex (FC) electrodes.

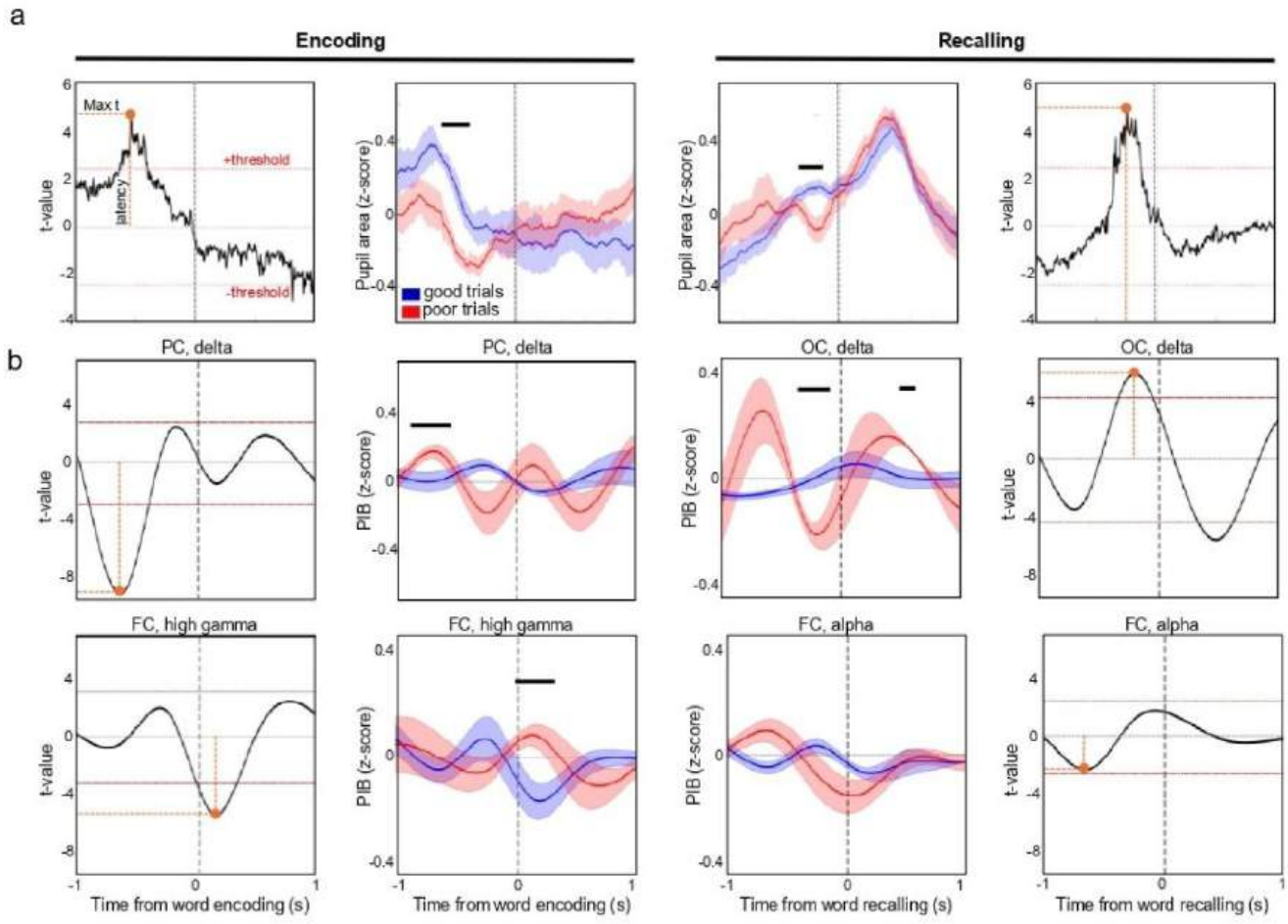

Figure 2. Pupil dynamics and spectral power features show distinct temporal profiles of good and poor memory encoding and recall. (a) Time course of normalized pupil area, time-locked to word presentation and vocalization onset, shown together with subject-level *t*-statistics of the good-versus-poor memory contrast and the latency of the maximum absolute *t*-value. Dashed lines in the statistical plots denote the cluster-forming threshold used in the subject-level cluster-based permutation analysis. (b) Normalized power-in-band (PIB) features across example frequency bands and brain regions are plotted in the same way as in (a). Bars above the plots indicate temporally contiguous time periods in which the subject-level statistics exceeded the cluster-forming threshold used in the cluster-based permutation analysis. Both pupil area and PIB features show temporal differences between good and poor memory trials. Abbreviations: PIB, power-in-band; FC, frontal cortex; PC, parietal cortex; OC, occipital cortex; TC, temporal cortex.

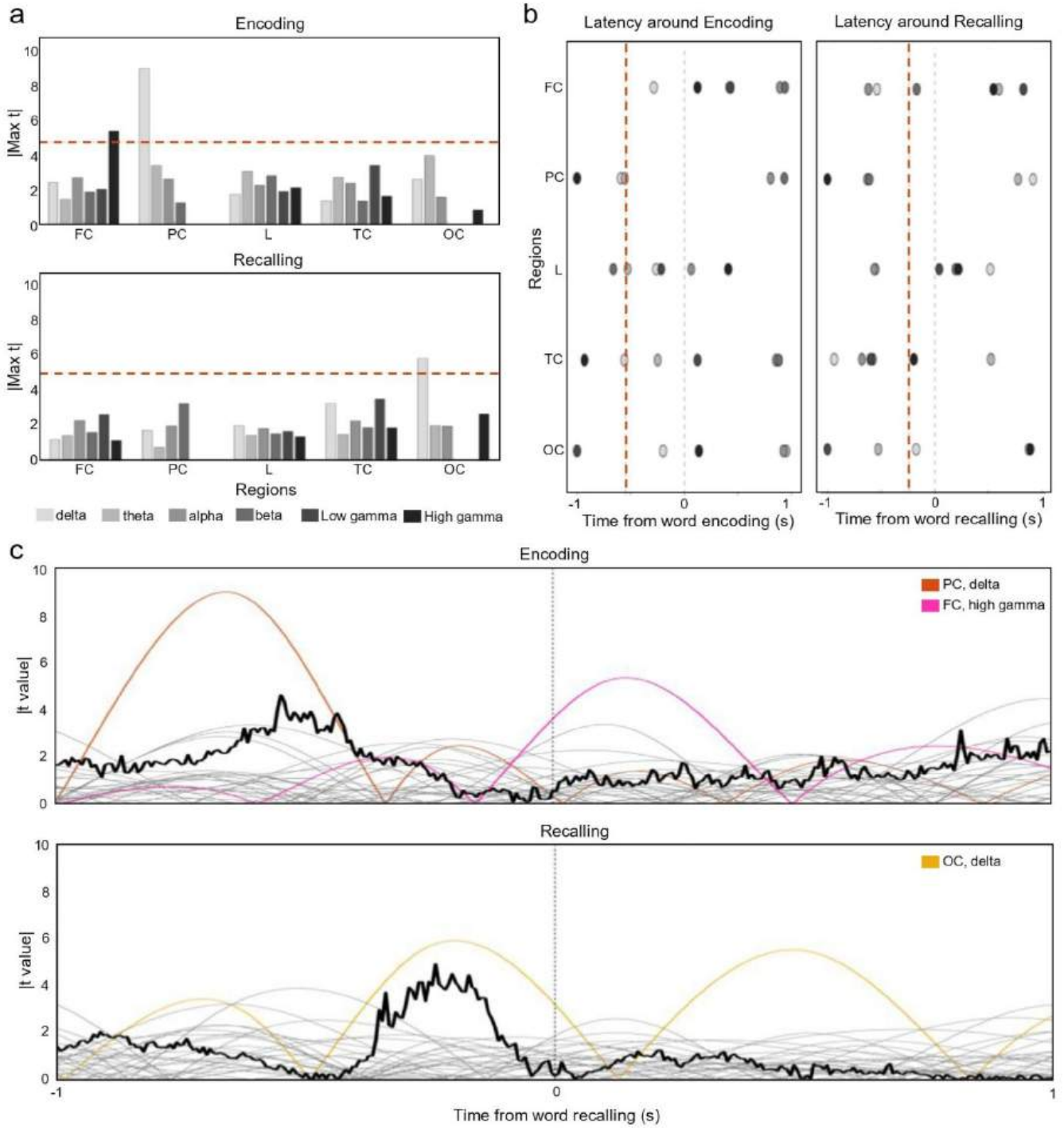

Figure 3. Pupil responses emerge earlier and with greater discriminability than most spectral power features. (a) Pupil discriminative strength (Max *t*; brown dashed line) exceeds the majority of power-in-band (PIB) values (92% during encoding; 96% during recall). (b) Latency of the pupil effect (brown dashed line) occurs earlier than most PIB features before the onset of stimulus encoding (73%) and recall vocalization (54%) marked by grey dashed line. (c) Joint summary of all absolute subject-level *t*-value demonstrates that pupil discriminability is comparable to—and better than—the spectral power features across time and magnitude space. Colored PIB traces indicate features whose Max t exceeds the pupil Max t. Abbreviations: PIB, power-in-band; FC, frontal cortex; PC, parietal cortex; L, limbic; TC, temporal cortex; OC, occipital cortex.

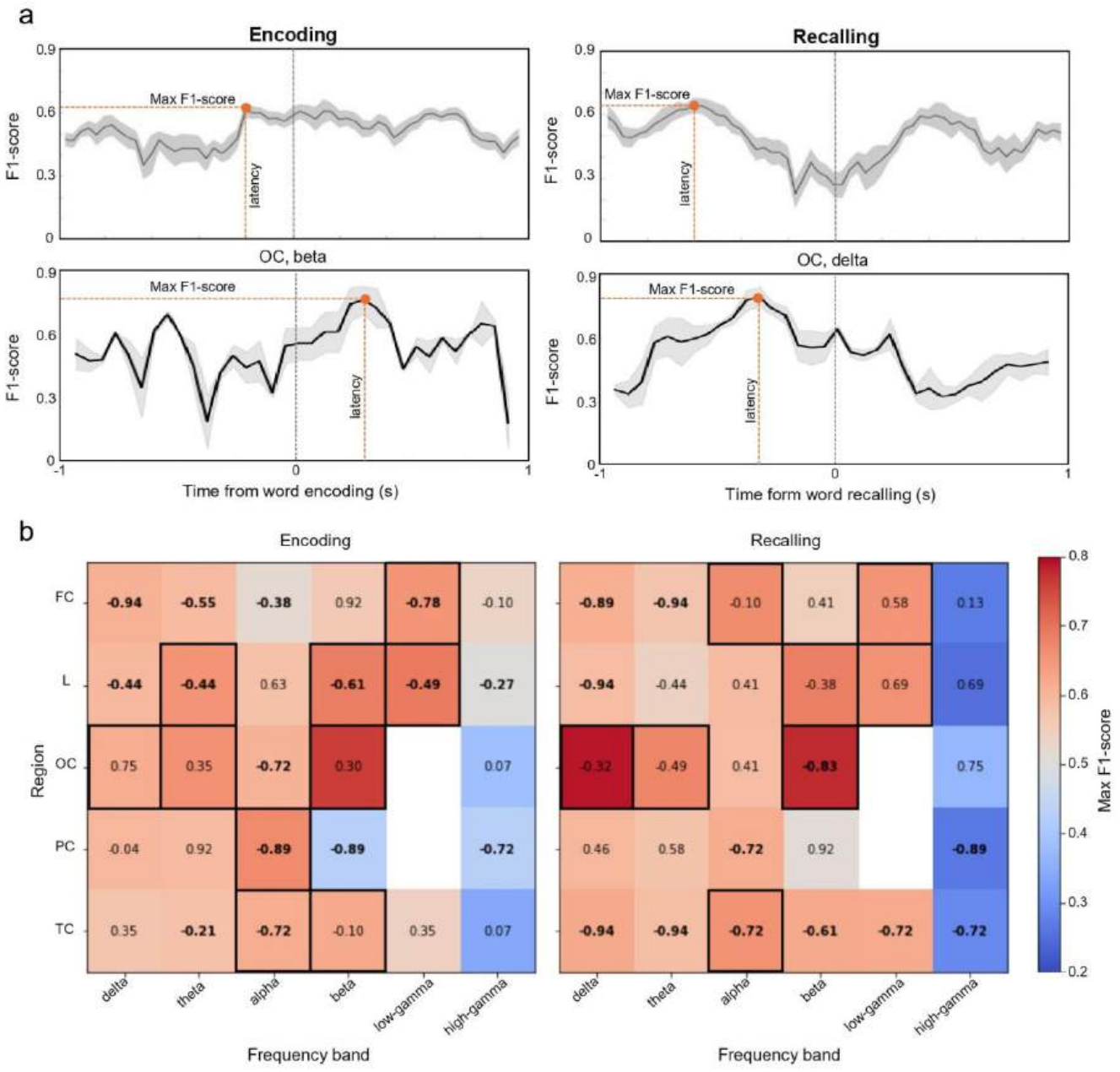

Figure 4. Pupil area predicts memory performance comparably to the spectral PIB features. (a) Time-resolved classification performance for pupil area and example PIB features is identified at the maximum classification performance relative to the word presentation and the vocalization onset (shaded areas mark the standard deviation across folds of the LPO-CV). (b) Heatmaps summarize classification results for all PIB features across six frequency bands and five cortical regions during encoding and recall with each block corresponding to a single PIB feature (color indicates the max F1-score and numbers denote latencies relative to word presentation or recall vocalization). Note: blocks outlined in black mark PIB features with higher Max F1 than the pupil area; latencies shown in bold indicate PIB features peaking earlier than the pupil response. Notice that the pupil area outperformed 64% of the PIBs features during encoding and 71% during recall, while detecting memory performance earlier than 46% and 57% of the PIB features, respectively.

5.2.2 Supplementary Material for Publication II

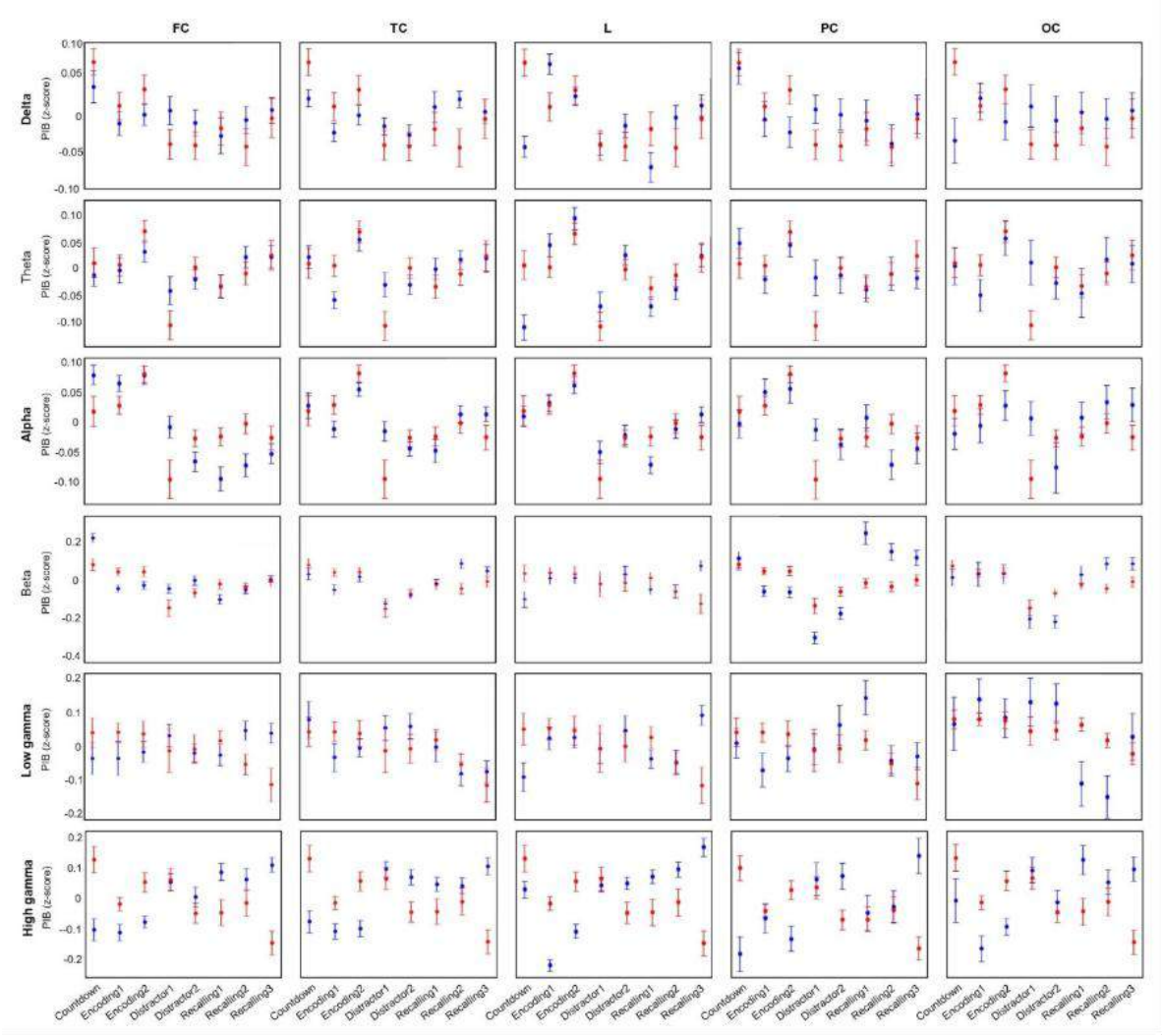

Fig. S1. Group-level PIB dynamics across frequency bands and cortical regions during the free-recall task. Group-level pupil area averaged across task phases from all subjects is plotted in the same way as in Fig. 1e. Abbreviations: PIB, power-in-band; FC, frontal cortex; PC, parietal cortex; OC, occipital cortex; TC, temporal.

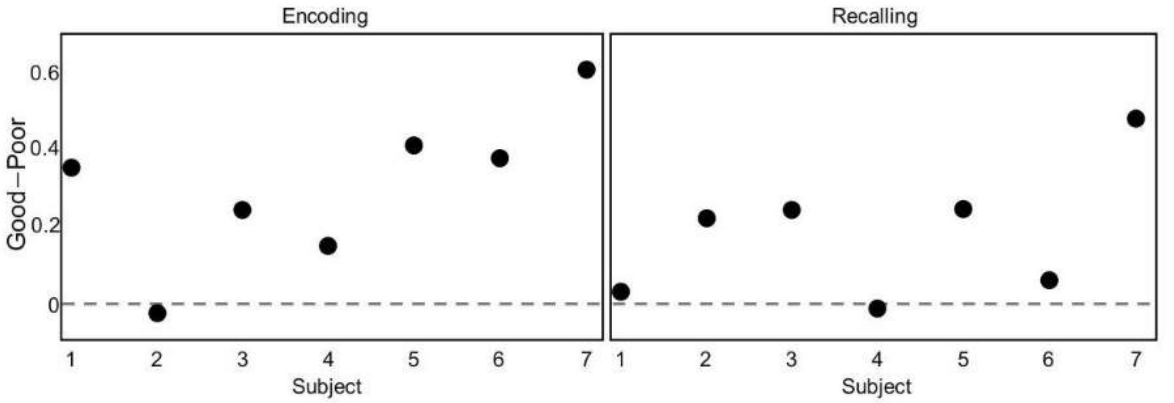

Fig. S2. Subject-wise pupil effects are broadly consistent across participants. Each point shows the mean good−poor pupil difference for one participant within the identified temporal window. Effects were positive in 6 of 7 subjects during encoding and in 6 of 7 subjects during recall. The dashed horizontal line indicates zero difference.

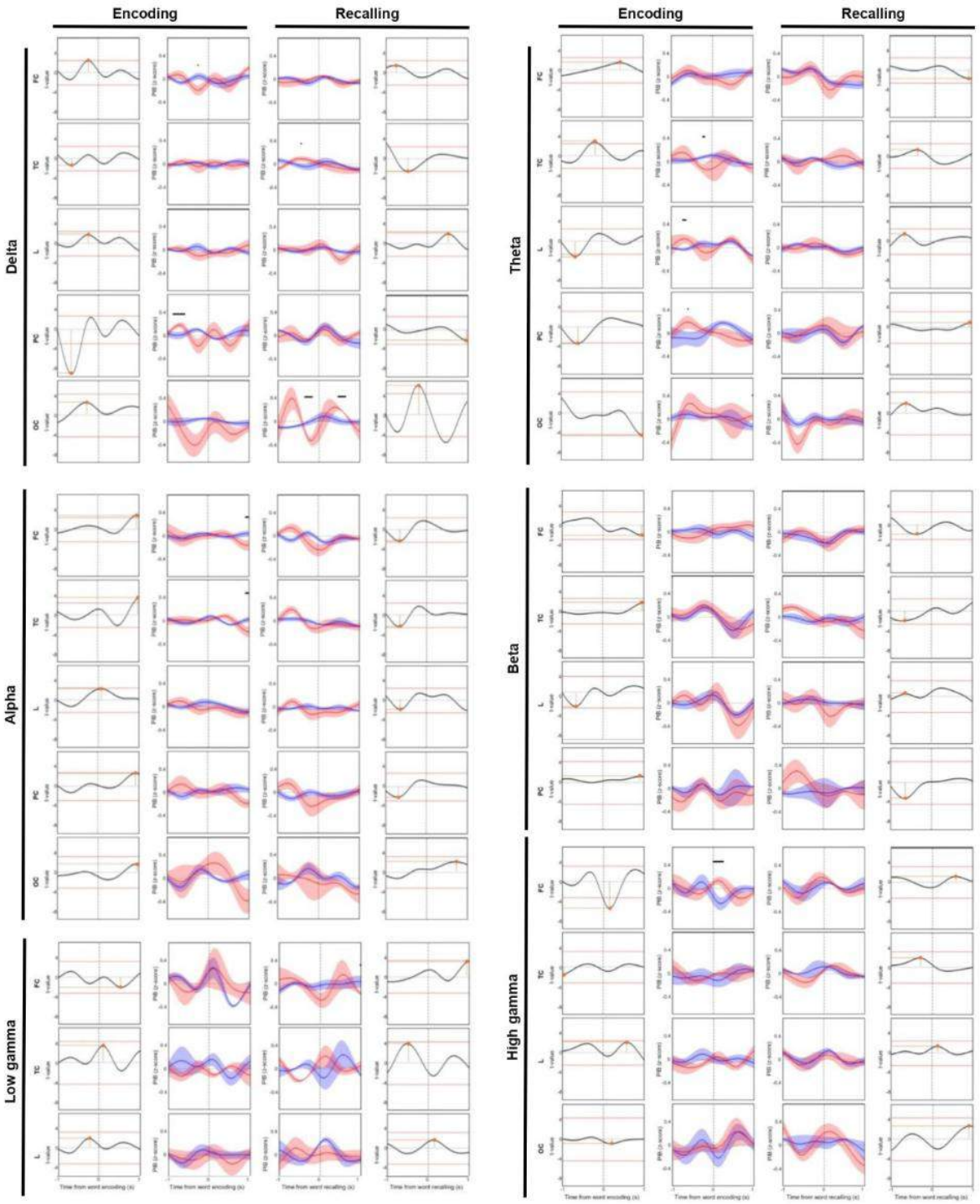

Fig. S3. Time-resolved PIB dynamics across frequency bands and cortical regions during encoding and recalling. Normalized PIB features across example frequency bands and brain regions are plotted in the same way as in Fig. 2b. Abbreviations: PIB, power-in-band; FC, frontal cortex; PC, parietal cortex; OC, occipital cortex; TC, temporal.

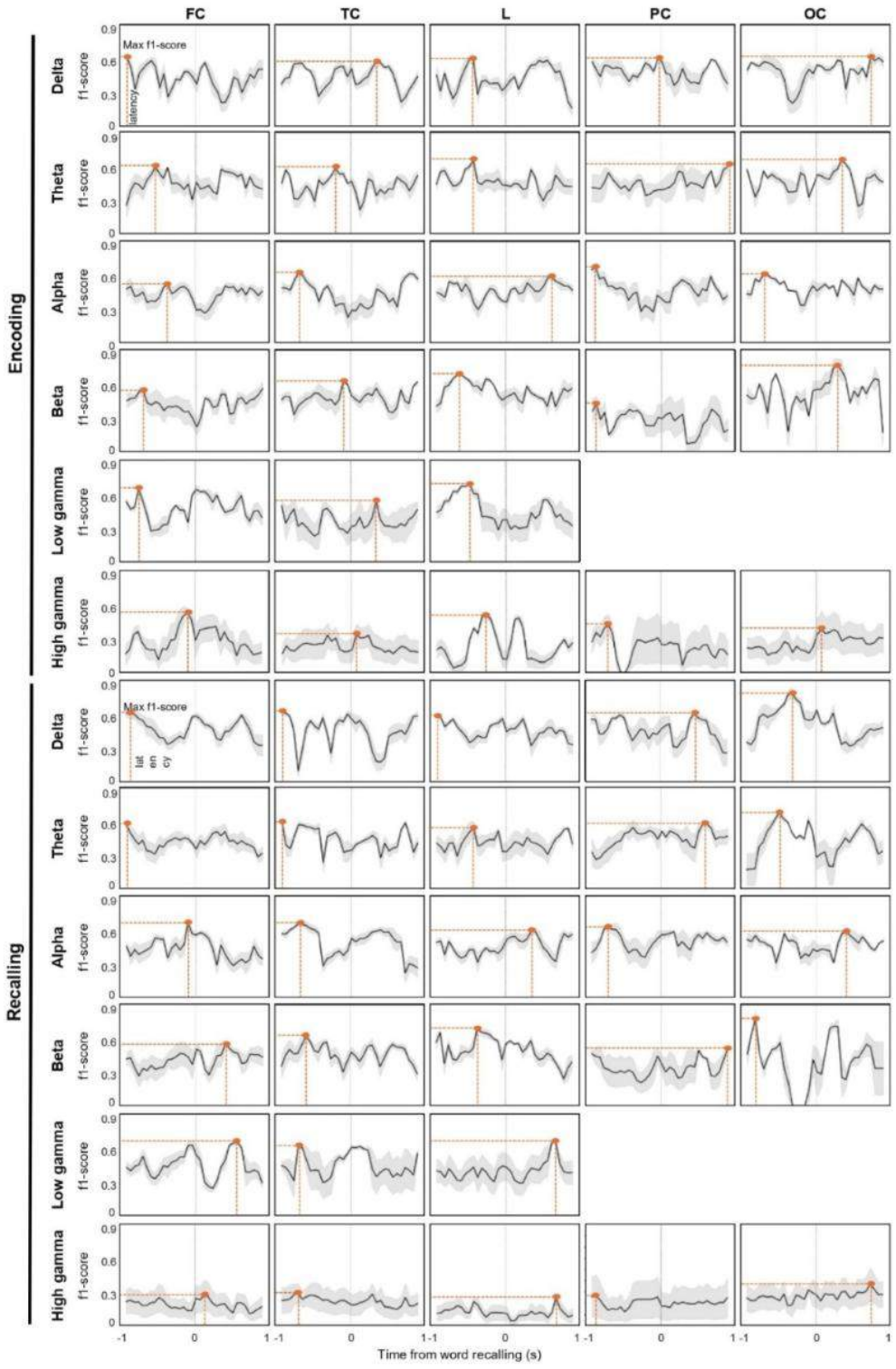

Fig. S4. Extended classification analyses reveal diverse and temporally distinct predictive profiles of PIB features. Across frequency bands and cortical regions, time-resolved single-feature classification showed that PIB features captured memory-related differences with heterogeneous but often comparable predictive strength to the pupil signal; these results are plotted in the same manner as in Fig. 4a. Abbreviations: PIB, power-in-band; FC, frontal cortex; PC, parietal cortex; OC, occipital cortex; TC, temporal.

Table S1. Demographic and clinical characteristics of the study participants. DRE stands for drug-resistant epilepsy.

Subject	Gender (F/M)	Age rang	Ophthalmology	Psychiatry	Medication	Epilepsy	Stimulant	Seizures last 24 hours
Sub1	M	17-25	No	Depression	No	DRE	No	No
Sub2	F	17-25	No	No	No	DRE	No	No
Sub3	F	17-25	No	No	No	DRE	No	No
Sub4	M	26-35	No	No	No	DRE	No	No
Sub5	M	26-35	No	No	No	DRE	No	No
Sub6	M	17-25	No	No	oxycodone 1h before testing	DRE	No	No
Sub7	F	17-25	Contact lenses	No	No	DRE	No	No

Table S2. Word lists presented during the verbal memory recall task. Each subject completed 15 trials, with each trial consisting of 12 words randomly selected from a standardized pool of 300 high-frequency nouns. Words are shown in the order presented during encoding. Words were not repeated within subjects but could overlap across subjects due to sampling from a shared standardized pool.

Subject	Trial	Word1	Word2	Word3	Word4	Word5	Word6	Word7	Word8	Word9	Word10	Word11	Word12
Sub1	1	POND	THUMB	FENCE	FUR	TRASH	CONE	FUDGE	PIE	TOOL	STORE	SHIRT	SINK
Sub1	2	SEA	BADGE	DOG	PURSE	TOOTH	TAIL	BOOT	SPONGE	FLAG	CLOTH	WALL	SHARK
Sub1	3	BENCH	MOUSE	CLOCK	BANK	TENT	GOLD	CROW	THREAD	SUN	CAVE	FISH	SAIL
Sub1	4	CAT	GLASS	PEAR	BEACH	HORN	JUDGE	POLE	BOX	HORSE	FROG	FOOD	HAND
Sub1	5	CORN	CANE	SMILE	HEN	PIT	ANT	LAWN	KEY	WAND	PARK	FACE	HEART
Sub1	6	AXE	FOG	SHEEP	NET	PRINCE	STICK	SWAMP	NEST	SHELL	NOSE	LOCK	OAR
Sub1	7	DUCK	BEAR	HOLE	STRING	OAK	STOVE	FILM	SHIP	CORD	SQUARE	STEAK	SLEEVE
Sub1	8	PHONE	STEM	DRESS	TEETH	RUG	STREET	SKUNK	PASTE	BLOOM	COIN	BARN	BOWL
Sub1	9	SHRIMP	BREAD	LAKE	PANTS	LAMP	SOAP	RICE	RAKE	RAIN	CLOWN	BRICK	MULE
Sub1	10	PEN	PEARL	TOY	BOOK	DITCH	MUG	CHIEF	COUCH	LEAF	WEED	VEST	SOUP
Sub1	11	MOOSE	YARD	STOOL	STRAW	SNAKE	TOE	JET	BIRD	BAG	FAN	BUSH	ICE
Sub1	12	GLOVE	SHEET	DEER	HOOF	CAR	CHEEK	BRIDGE	GUARD	SHIELD	SEAL	EEL	BOY
Sub1	13	WOLF	ROCK	FOAM	LIP	BAT	STEAM	PET	SOCK	CHALK	ROOM	BALL	LAND
Sub1	14	WAVE	SPOON	SWORD	SIGN	SALT	BATH	MOLE	ELF	JUICE	HOSE	ROSE	MAT
Sub1	15	FLOOR	SLIME	BED	FLAME	SUIT	MOON	BLUSH	TANK	SCHOOL	SKI	TRAY	CALF
Sub2	1	FROG	GATE	FLOOR	SHIRT	PIN	HAT	SPRING	BLOOM	DEER	ROPE	SUN	WEED
Sub2	2	CAT	EEL	ARM	DOLL	NEST	MAP	BOOK	SPARK	WEB	SWAMP	MOUSE	ANT
Sub2	3	SHELL	TOE	PIG	LAMP	DOOR	FISH	SCHOOL	JAR	CAGE	WOOD	TREE	MAIL
Sub2	4	HOSE	CROW	VEST	BARN	OAK	MOUTH	STEM	GRAPE	BRANCH	CHAIR	STORE	HOOF
Sub2	5	HOLE	FAN	TANK	PEA	LAND	FRUIT	SHEET	LEAF	STEAK	FLAME	ARK	SEA
Sub2	6	SNAIL	FOAM	SPONGE	TRAY	WING	HOOK	STEAM	CHEEK	FOOT	MOON	SQUARE	FARM
Sub2	7	NOSE	BEAR	CROWN	JAIL	BAT	CAPE	THUMB	PEACH	BENCH	CLOWN	BLUSH	WHALE
Sub2	8	ICE	CAKE	WHEEL	SLIME	DOG	LAMB	ROCK	BRIDGE	CRANE	DOCK	DITCH	COW
Sub2	9	SHEEP	THREAD	HORN	ROSE	FORK	ROOM	BIRD	TIE	LIP	PEARL	BAND	INK
Sub2	10	BROOM	FACE	LAKE	SALT	JEEP	KEY	RAKE	PIPE	DRUM	STORM	NAIL	STRAW
Sub2	11	KITE	BEE	TOOTH	TAIL	STONE	SHARK	BOWL	FENCE	SOCK	BAG	SHIP	CART
Sub2	12	SINK	CORN	DESK	HAND	CANE	CLAY	MULE	STRING	BREAD	PEN	ROAD	WORM
Sub2	13	BADGE	WORLD	SAIL	SNAKE	PLATE	RIB	WALL	SWORD	GRASS	PALM	CUP	DUCK

Sub2	14	SKY	CLOTH	BEAN	SKUNK	MAZE	SHRIMP	PLANE	BOX	PANTS	FOG	VAN	COIN
Sub2	15	CLOCK	EAR	LAWN	BOOT	GEESE	RICE	SLUSH	TOAD	HEN	TRASH	STREET	STOVE
Sub3	1	PIE	OWL	BRUSH	STAIR	MULE	OAK	NET	ROOM	SOAP	CAR	ARK	CLIFF
Sub3	2	FOAM	SHRIMP	SAIL	ROCK	LEG	TOAST	STRAW	LAND	SHOE	TANK	CLOTH	DOLL
Sub3	3	FOOD	FARM	VEST	GUARD	CLOWN	GOLD	SNAKE	CLOCK	TRASH	PLANE	PLANT	DOOR
Sub3	4	TEETH	MOUSE	DOCK	JEEP	PRINCE	SHARK	MAIL	POND	HOLE	WAND	VAN	RICE
Sub3	5	FACE	TREE	BOOT	SEAL	BAND	CUP	JAR	SHIP	GATE	STRING	KITE	CUBE
Sub3	6	PHONE	CAPE	JUICE	HAT	FRUIT	STEAK	DEER	SEA	BOWL	HOOF	SOCK	WEED
Sub3	7	POLE	GOAT	STOVE	BAG	FROG	NAIL	STONE	GEESE	FORK	DOG	BOARD	DIME
Sub3	8	DRUM	BENCH	CART	RUG	CASH	TOAD	THREAD	BALL	BEAR	AXE	STORE	LEAF
Sub3	9	DART	BOY	EGG	PIN	SHEEP	CROWN	PANTS	TENT	CAT	CANE	BEE	RAT
Sub3	10	HOOK	JUDGE	GRASS	STEM	BANK	BRANCH	TIE	FUDGE	BLOOM	COW	CALF	FLEA
Sub3	11	ROSE	PEN	OAR	CRANE	NOSE	SNAIL	TOY	RAIN	PARK	HEN	SPONGE	ELF
Sub3	12	FISH	DESK	SHELL	BRIDGE	SKUNK	CORN	BREAD	BUSH	APE	MUG	FOG	SLUSH
Sub3	13	PASTE	BIRD	ANT	WING	CHALK	MOTH	KEY	BATH	WOOD	WORLD	WAVE	CAVE
Sub3	14	SINK	HEART	HILL	JET	FENCE	MAP	BOX	BARN	SEAT	CAGE	PEARL	STEAM
Sub3	15	LAWN	SPEAR	ROOF	TAIL	BROOM	BADGE	FORT	CLAY	FUR	STORM	SHIRT	MOON
Sub4	1	PIE	OWL	BRUSH	STAIR	MULE	OAK	NET	ROOM	SOAP	CAR	ARK	CLIFF
Sub4	2	FOAM	SHRIMP	SAIL	ROCK	LEG	TOAST	STRAW	LAND	SHOE	TANK	CLOTH	DOLL
Sub4	3	FOOD	FARM	VEST	GUARD	CLOWN	GOLD	SNAKE	CLOCK	TRASH	PLANE	PLANT	DOOR
Sub4	4	TEETH	MOUSE	DOCK	JEEP	PRINCE	SHARK	MAIL	POND	HOLE	WAND	VAN	RICE
Sub4	5	FACE	TREE	BOOT	SEAL	BAND	CUP	JAR	SHIP	GATE	STRING	KITE	CUBE
Sub4	6	PHONE	CAPE	JUICE	HAT	FRUIT	STEAK	DEER	SEA	BOWL	HOOF	SOCK	WEED
Sub4	7	POLE	GOAT	STOVE	BAG	FROG	NAIL	STONE	GEESE	FORK	DOG	BOARD	DIME
Sub4	8	DRUM	BENCH	CART	RUG	CASH	TOAD	THREAD	BALL	BEAR	AXE	STORE	LEAF
Sub4	9	DART	BOY	EGG	PIN	SHEEP	CROWN	PANTS	TENT	CAT	CANE	BEE	RAT
Sub4	10	HOOK	JUDGE	GRASS	STEM	BANK	BRANCH	TIE	FUDGE	BLOOM	COW	CALF	FLEA
Sub4	11	ROSE	PEN	OAR	CRANE	NOSE	SNAIL	TOY	RAIN	PARK	HEN	SPONGE	ELF
Sub4	12	FISH	DESK	SHELL	BRIDGE	SKUNK	CORN	BREAD	BUSH	APE	MUG	FOG	SLUSH

Sub4	13	PASTE	BIRD	ANT	WING	CHALK	MOTH	KEY	BATH	WOOD	WORLD	WAVE	CAVE
Sub4	14	SINK	HEART	HILL	JET	FENCE	MAP	BOX	BARN	SEAT	CAGE	PEARL	STEAM
Sub4	15	LAWN	SPEAR	ROOF	TAIL	BROOM	BADGE	FORT	CLAY	FUR	STORM	SHIRT	MOON
Sub5	1	SHEET	ROAD	JAR	PASTE	LOCK	HEART	PLANE	STRING	CHEEK	GLOVE	BOY	SHIELD
Sub5	2	EAR	BLOOM	CAPE	PEAR	STOOL	SHARK	DOCK	SMOKE	ROCK	ROOT	PHONE	CHIN
Sub5	3	HOOF	FORK	POND	SLUSH	PEA	CUBE	SUN	DOOR	WEB	CLOUD	AXE	WOOD
Sub5	4	SPRING	LAWN	VEST	MILK	FLEA	BRANCH	DOG	FENCE	KEY	CAVE	FLAME	FUDGE
Sub5	5	MOTH	JET	GUARD	WHEEL	FOOD	PLATE	JAIL	BATH	BED	DUCK	CAT	CAR
Sub5	6	SOCK	TAIL	EEL	TRUCK	MUG	STORE	SLIME	BOARD	BEAN	CHAIR	SOUP	GATE
Sub5	7	SMILE	SHRIMP	KITE	OAR	HORSE	FLAG	SHELL	DESK	BAND	FAN	DART	CAKE
Sub5	8	SUIT	THREAD	TREE	GEESE	SLEEVE	WHALE	TOY	NAIL	FOOT	SPONGE	SIGN	LAMB
Sub5	9	STORM	FLUTE	SNAKE	PLANT	HOLE	CORN	ELF	GOAT	ROOF	BANK	CONE	PIG
Sub5	10	CASH	THUMB	PRINCE	SKY	OWL	TOOTH	BREAD	MAP	POLE	BRIDGE	BOAT	RAT
Sub5	11	TOE	HOSE	FORT	BLUSH	CUP	DIME	ARM	WEED	WORM	YARD	SEAT	BOOT
Sub5	12	MULE	BOMB	STOVE	STAIR	ZOO	TANK	LEAF	SPEAR	RIB	FROG	CLAY	SWAMP
Sub5	13	FRUIT	CHALK	WAND	HORN	ROPE	DITCH	STEAM	SCHOOL	COW	BROOM	SEAL	WOLF
Sub5	14	TENT	CLOWN	BOX	INK	ARK	DRESS	PET	CLOCK	CLIFF	MUD	DEER	MAIL
Sub5	15	BEAR	FUR	MOOSE	SHOE	RAKE	FLOOR	WAVE	PARK	DOLL	LEG	SKI	BEE
Sub6	1	SHEET	ROAD	JAR	PASTE	LOCK	HEART	PLANE	STRING	CHEEK	GLOVE	BOY	SHIELD
Sub6	2	EAR	BLOOM	CAPE	PEAR	STOOL	SHARK	DOCK	SMOKE	ROCK	ROOT	PHONE	CHIN
Sub6	3	HOOF	FORK	POND	SLUSH	PEA	CUBE	SUN	DOOR	WEB	CLOUD	AXE	WOOD
Sub6	4	SPRING	LAWN	VEST	MILK	FLEA	BRANCH	DOG	FENCE	KEY	CAVE	FLAME	FUDGE
Sub6	5	MOTH	JET	GUARD	WHEEL	FOOD	PLATE	JAIL	BATH	BED	DUCK	CAT	CAR
Sub6	6	SOCK	TAIL	EEL	TRUCK	MUG	STORE	SLIME	BOARD	BEAN	CHAIR	SOUP	GATE
Sub6	7	SMILE	SHRIMP	KITE	OAR	HORSE	FLAG	SHELL	DESK	BAND	FAN	DART	CAKE
Sub6	8	SUIT	THREAD	TREE	GEESE	SLEEVE	WHALE	TOY	NAIL	FOOT	SPONGE	SIGN	LAMB
Sub6	9	STORM	FLUTE	SNAKE	PLANT	HOLE	CORN	ELF	GOAT	ROOF	BANK	CONE	PIG
Sub6	10	CASH	THUMB	PRINCE	SKY	OWL	TOOTH	BREAD	MAP	POLE	BRIDGE	BOAT	RAT
Sub6	11	TOE	HOSE	FORT	BLUSH	CUP	DIME	ARM	WEED	WORM	YARD	SEAT	BOOT

Sub6	12	MULE	BOMB	STOVE	STAIR	ZOO	TANK	LEAF	SPEAR	RIB	FROG	CLAY	SWAMP
Sub6	13	FRUIT	CHALK	WAND	HORN	ROPE	DITCH	STEAM	SCHOOL	COW	BROOM	SEAL	WOLF
Sub6	14	TENT	CLOWN	BOX	INK	ARK	DRESS	PET	CLOCK	CLIFF	MUD	DEER	MAIL
Sub6	15	BEAR	FUR	MOOSE	SHOE	RAKE	FLOOR	WAVE	PARK	DOLL	LEG	SKI	BEE
Sub7	1	TEA	WORLD	BAT	APE	HORN	PIN	CLOCK	STREET	SPARK	BALL	SPONGE	PET
Sub7	2	GATE	FROG	PANTS	WOOD	TRAIN	DRESS	ZOO	WOLF	PURSE	LAND	CAT	PEA
Sub7	3	GLOVE	NOSE	TOAST	WING	SHIELD	JEEP	BARN	GLASS	SLEEVE	BLOOM	ICE	FLAME
Sub7	4	MUD	CROWN	STICK	CALF	TEETH	SQUARE	RIB	DOLL	CLOTH	HEART	MUG	GEESE
Sub7	5	PLANT	DOOR	DOCK	CUBE	SPRING	FORK	HILL	HEN	STEAK	JUDGE	POND	STORM
Sub7	6	SAIL	CHIN	BRICK	FOOD	WHEEL	INK	STAR	FACE	TOAD	DEER	JUICE	SCHOOL
Sub7	7	HORSE	STRAW	BOOK	SPEAR	SOCK	TANK	HAND	WAND	BADGE	MULE	FRUIT	LAWN
Sub7	8	CLAY	SMILE	OAK	HOOK	RICE	BEACH	MAIL	BOWL	PHONE	WHALE	JAIL	SEA
Sub7	9	SEAL	FENCE	DITCH	SIGN	PARK	COW	LAKE	FOX	STAIR	OAR	SOAP	VASE
Sub7	10	CRANE	PASTE	PEACH	TREE	BEAK	RAT	STEM	CAGE	SOUP	POOL	BUSH	CHEEK
Sub7	11	SWAMP	GRAPE	PLATE	SUN	SHIRT	FLEA	BOMB	HOOF	HAT	FUDGE	BOY	MAP
Sub7	12	PIPE	RAIN	FUR	SKY	BIRD	YARD	RAKE	PLANE	WEB	PALM	CAR	BENCH
Sub7	13	JET	MOTH	BATH	TRASH	CAPE	DRUM	TAIL	BED	CHALK	BOAT	SWORD	CHAIR
Sub7	14	PIE	PEAR	PIT	LAMB	KEY	FOG	MOON	SUIT	KITE	BLUSH	FARM	GOLD
Sub7	15	DESK	BEAN	BEE	STEAM	WORM	MILK	CONE	DUCK	COUCH	OWL	HOLE	COIN

Table S3. Number of electrophysiologically active electrodes across subjects, brain regions, and frequency bands. Number of electrodes classified as active by Gaussian Mixture Model (GMM) analysis [61] of induced spectral power during encoding, reported for each participant, brain region (L, FC, PC, TC, OC), and frequency bands (Delta-High gamma). Abbreviations: PIB, power-in-band; FC, frontal cortex; PC, parietal cortex; OC, occipital cortex; TC, temporal.

Frequency band	Region	Sub1	Sub2	Sub3	Sub4	Sub5	Sub6	Sub7	Total subject
Delta	FC	18	15	19	13	15	2	24	7
	TC	37	39	33	15	18	23	7	7
	L	13	16	6	10	16	8	33	7
	PC	7	0	0	9	10	3	6	5
	OC	3	2	2	0	4	0	0	4
Theta	FC	0	4	1	13	7	2	16	6
	TC	2	17	14	1	4	10	10	7
	L	0	1	6	11	8	6	27	6
	PC	0	0	0	5	3	1	3	4
	OC	0	1	3	0	0	1	1	4
Alpha	FC	5	20	20	24	24	5	25	7
	TC	30	31	32	21	22	29	10	7
	L	13	18	14	19	20	8	25	7
	PC	7	0	0	9	11	6	6	5
	OC	3	2	1	0	0	0	2	4
Beta	FC	0	8	0	5	8	2	11	5
	TC	0	22	18	2	2	7	10	6
	L	0	8	3	3	0	0	11	4
	PC	0	1	0	5	1	3	0	4
	OC	0	2	0	0	4	0	5	3
Low gamma	FC	0	4	3	2	0	0	4	4
	TC	0	2	0	1	0	1	6	4
	L	0	1	0	2	0	2	14	4
	PC	0	2	0	2	0	0	0	2
	OC	0	0	0	0	3	0	2	2
High gamma	FC	0	0	1	2	16	0	4	4
	TC	0	11	11	0	21	0	8	4
	L	2	2	2	1	13	2	16	7
	PC	0	3	0	0	1	0	4	3
	OC	0	2	1	0	1	0	1	4

5.2.3 Author's Contribution to Study II

The author played a leading role in the methodological, computational, and analytical components of this study and was primarily responsible for the physiological data analyses. This included working directly with iEEG and pupillometry datasets and developing the analytical procedures used to examine relationships between physiological activity and memory performance.

The author designed and implemented preprocessing and analytical procedures for both pupillometric and electrophysiological data, including signal processing, feature extraction, time-resolved statistical analyses, robustness analyses, and classification procedures for distinguishing good and poor memory performance. The author further developed the computational framework for temporal feature evaluation, cross-validation procedures, and permutation-based validation of classification performance.

The author contributed substantially to interpretation of the findings, particularly regarding the comparison between pupil-related physiological features and intracranial electrophysiological measures as candidate physiological biomarkers associated with memory performance.

The author prepared the manuscript and played a central role in the revision process in response to reviewer feedback.

Chapter 6

6. Discussion, Limitations, and Future Directions

The empirical studies presented in the preceding chapter investigated physiological signals as candidate biomarkers of cognitive performance using EEG, iEEG, and pupillometry together with signal processing, statistical analysis, and machine learning approaches. Across both inter-individual and trial-level contexts, this thesis examined how electrophysiological activity and pupil dynamics relate to variability in cognitive performance.

The purpose of the present chapter is to interpret these findings within a broader scientific and methodological framework. Specifically, this chapter discusses the implications of the identified biomarkers, evaluates methodological strengths and limitations, and considers potential clinical, educational, and technological applications, as well as directions for future research. Through this discussion, the findings of this thesis are positioned within the broader effort to develop objective, accessible, and physiology-informed approaches for cognitive assessment and monitoring.

6.1 Interpretation of Main Findings

The findings obtained across the empirical studies presented in this dissertation provide important insight into how physiological activity reflects variability in cognitive performance and support the interpretation of physiological signals as biomarkers of cognitive function. By examining electrophysiological activity derived from EEG and iEEG alongside pupil dynamics across structured cognitive paradigms, this dissertation demonstrated that cognitive performance is accompanied by measurable physiological patterns associated with both relatively stable differences in cognitive performance across individuals and transient trial-by-trial fluctuations in memory performance. These observations support the interpretation that cognitive performance emerges through interactions between neural mechanisms and broader physiological regulatory processes rather than being reflected solely through behavioral outcomes.

Study I demonstrated that neural oscillatory activity recorded using non-invasive scalp electroencephalography contains measurable information associated with differences in cognitive performance across individuals. Spectral EEG features varied systematically according to performance in memory- and executive-related cognitive domains, suggesting that electrophysiological activity reflects meaningful variation in cognitive functioning. Lower theta activity within anterior prefrontal regions was consistently associated with poorer performance across executive,

visual, spatial, and working memory domains, suggesting that frontal theta oscillations may reflect a shared physiological process supporting multiple aspects of cognitive performance. These findings are consistent with previous evidence linking frontal theta activity to executive control, working memory, and memory-related processes[17,24,26,27].

The observation that anterior prefrontal electrophysiological activity differed according to cognitive performance suggests that variability in memory and executive functioning may be associated with differences in frontal neural activity involved in cognitive and memory-related processes. Specifically, lower anterior prefrontal theta activity was consistently observed in participants with poorer cognitive performance, whereas higher theta activity was associated with better performance. Given the established role of frontal theta oscillations in working memory, attention, and executive control, these findings suggest that frontal electrophysiological activity may reflect differences in the neural processes supporting cognitive performance[19,21,22,42].

Study II extended these findings by demonstrating that physiological signals also capture moment-to-moment variability associated with memory performance at the level of individual task trials. Whereas Study I focused primarily on relatively stable inter-individual differences, Study II showed that physiological activity evolves dynamically during memory encoding and retrieval and differs systematically according to subsequent behavioral outcomes. The ability of physiological features to distinguish good and poor memory performance supports the interpretation that transient physiological states contribute meaningfully to memory-related cognitive processes. These findings are consistent with previous work showing that episodic memory depends on temporally organized neural processes during encoding and retrieval[8,35,48,51,53,56,68,77].

A particularly important finding of this thesis was that pupil-derived physiological features demonstrated classification performance approaching that observed for simplified intracranial EEG power-in-band features. Although intracranial EEG provides highly localized and physiologically precise measurements of neural activity, its invasive nature restricts its use to specialized clinical settings[8,34,88,89]. In contrast, pupillometry provides a practical and non-invasive physiological measurement approach[10,36,37]. The observation that pupil-related physiological features exhibited meaningful discriminative capability supports the interpretation of pupillometry as a candidate non-invasive biomarker associated with memory performance.

Another important interpretation emerging from Study II concerns the temporal organization of physiological activity during memory processing. Time-resolved analyses identified specific temporal intervals during encoding and retrieval in which physiological signals differentiated memory performance conditions. Importantly, temporally informative pupil-related effects emerged before recall responses,

suggesting that physiological indicators of memory performance may become observable before overt behavioral outcomes. These findings support the interpretation that memory-related cognitive processes unfold through temporally structured physiological stages that may be measurable prior to behavioral expression [6,9,10,74,75,101].

An additional important observation concerns the robustness of pupil-related physiological effects across participants. Despite the limited sample size characteristic of intracranial studies, leave-one-subject-out analyses demonstrated consistent directionality of pupil-related effects and preserved statistical significance across participant subsets. These findings strengthen the interpretation that observed pupil dynamics reflect reproducible memory-related physiological processes rather than effects driven disproportionately by individual participants.

Taken together, the findings of this thesis demonstrate that physiological signals capture complementary aspects of cognitive variability operating across different temporal scales. Study I identified electrophysiological patterns associated with relatively stable differences in cognitive performance across individuals, whereas Study II demonstrated that neural and pupil-related physiological activity also reflects dynamic trial-by-trial variability in memory performance. Together, these findings support the interpretation of electrophysiological and pupil-derived features as candidate biomarkers of cognitive function and memory-related variability.

Beyond the empirical findings, an important contribution of this dissertation lies in the complementary investigation of physiological variability across both inter-individual and intra-individual levels of cognition. While Study I examined relatively stable cognitive differences across individuals using scalp electrophysiological recordings during standardized neuropsychological assessment, Study II investigated trial-by-trial physiological dynamics during memory performance using intracranial electrophysiology and pupillometry in a distinct experimental paradigm. Although these studies employed different datasets, modalities, and behavioral tasks, together they provide complementary perspectives on how physiological activity relates to variability in cognitive and memory performance. Furthermore, by contextualizing non-invasive pupil-related physiological features against intracranial electrophysiological activity during memory processing, this work contributes additional evidence regarding the potential of accessible physiological measurements for objective cognitive assessment.

6.2 Scientific and Practical Implications

The findings of this thesis carry important implications for both physiological biomarker research and practical approaches to cognitive assessment. By demonstrating that physiological signals contain measurable information associated with variability in cognitive performance, this work contributes to the development of objective, physiology-based approaches for characterizing cognitive function. More

broadly, the findings support the interpretation that cognitive performance may be assessed not only through behavioral outcomes but also through measurable physiological processes associated with cognition.

From a scientific perspective, this thesis contributes to the growing effort to identify physiologically grounded biomarkers of cognition. Traditional cognitive assessment methods primarily rely on behavioral measures such as accuracy and reaction time, which provide indirect information about underlying cognitive mechanisms. In contrast, electrophysiological and pupillometric signals provide continuous measurements capable of capturing dynamic physiological changes during task performance [36,37,79,81,112]. The identification of physiological patterns associated with cognitive and memory performance therefore supports the development of more comprehensive models of cognition that integrate behavioral and physiological dimensions.

An important scientific implication of this work concerns the complementary role of neural and broader physiological systems in shaping cognitive performance. While electrophysiological recordings provided information about neural processes associated with memory and executive functioning, pupil dynamics reflected broader physiological states associated with attentional engagement and cognitive readiness. The observation that both modalities contained information associated with memory performance supports the interpretation that cognition emerges through interactions between localized neural mechanisms and broader physiological regulatory systems[10,17,43,44].

The findings also have important implications for the development of candidate physiological biomarkers of cognitive performance. In particular, the observation that pupil-derived physiological features demonstrated classification performance approaching that observed for simplified intracranial EEG power-in-band features suggests that meaningful information related to memory performance may be extracted using practical and non-invasive physiological measurements. This finding is particularly relevant because invasive neural recordings remain restricted to specialized clinical settings, whereas pupillometry can be implemented using accessible and scalable technologies.

From a clinical perspective, physiological biomarkers associated with cognitive performance may contribute to improved monitoring of cognitive variability in neurological conditions[111–114]. Memory-related impairment is a common feature across several neurological disorders, and objective physiological measurements may provide complementary information beyond conventional behavioral assessment. Because pupillometry can be acquired non-invasively and continuously, it represents a particularly promising modality for scalable cognitive monitoring in clinical environments.

Beyond clinical applications, the methodological framework developed in this thesis may also have relevance for adaptive technologies, educational systems, and

human–computer interaction. Physiological indicators associated with attentional engagement and cognitive readiness may support the development of systems capable of adapting dynamically to changes in cognitive state[40,74,75,104]. For example, educational technologies may adjust learning demands according to physiological indicators of cognitive load, whereas adaptive interfaces may modify information presentation according to changes in attentional engagement.

This thesis further demonstrates how continuous physiological recordings can be transformed into structured quantitative features suitable for computational analysis. The integration of signal processing, statistical analysis, and machine learning methods illustrates how physiological measurements may be incorporated into quantitative frameworks for cognitive assessment. More broadly, these findings support continued development of computational systems capable of integrating physiological information into adaptive and intelligent technologies.

An additional practical contribution of this thesis concerns the methodological evaluation of physiological biomarkers under conditions of limited sample size and temporally evolving signals. By combining subject-level statistical inference, robustness analyses, and permutation-validated classification procedures, the proposed framework provides an approach for evaluating physiological information while reducing risks associated with overfitting, repeated temporal testing, and participant-specific effects. Such methodological considerations may be particularly valuable for future neurophysiological studies conducted in clinical populations.

Overall, the findings presented in this thesis demonstrate the potential value of physiological biomarkers for advancing objective approaches to cognitive assessment. By combining neurophysiological measurement with computational analysis, this work provides a foundation for future systems capable of monitoring cognitive performance using practical and physiologically meaningful signals.

6.3 Limitations

Although the findings presented in this thesis provide evidence supporting the value of physiological signals as candidate biomarkers associated with cognitive performance, several methodological limitations should be considered when interpreting the results.

First, both empirical studies were conducted in clinical populations of patients with epilepsy. Although epilepsy monitoring provides a unique opportunity to obtain simultaneous physiological recordings during cognitive task performance, neurological pathology, seizure-related factors, and medication effects may influence both physiological activity and behavioral performance[89,111,113,114]. Consequently, the generalizability of the findings to healthy populations and other clinical groups should be interpreted with caution.

Second, Study II involved a relatively small sample size, reflecting the limited availability of participants undergoing intracranial monitoring. Although subject-level

statistical procedures, permutation testing, and robustness analyses were implemented to strengthen reliability, the limited sample size restricts statistical generalization and may reduce sensitivity to smaller physiological effects. Replication in larger cohorts will therefore be important for evaluating the reproducibility and robustness of the identified physiological biomarkers. Therefore, the Study II findings should be interpreted as evidence of feasibility and candidate physiological relevance rather than as definitive proof of generalizable biomarker performance.

An additional limitation concerns intracranial electrode placement in Study II. Electrode implantation was determined exclusively by clinical requirements rather than experimental design, resulting in heterogeneous anatomical coverage across participants. Consequently, some brain regions relevant to memory processing may have been sampled inconsistently or not recorded in all participants. This constraint is inherent to human intracranial research and should be considered when interpreting region-specific electrophysiological findings[8,34,88,89].

The experimental paradigms used in this thesis also impose certain constraints on interpretation. Study I focused on structured neuropsychological testing using selected CANTAB tasks, whereas Study II employed a verbal free recall paradigm designed to investigate episodic memory. Although these paradigms enabled controlled investigation of cognitive processes, the identified physiological biomarkers may be task-dependent and may not necessarily generalize to broader or more naturalistic cognitive contexts.

Another consideration concerns interpretation of classification performance in Study II. Although physiological features successfully distinguished differences in memory performance at the trial level, classification outcomes should not be interpreted as deterministic prediction of cognitive states or behavioral outcomes. Rather, the findings indicate that physiological signals contain meaningful information associated with variability in memory performance under controlled experimental conditions.

Finally, while this thesis demonstrated the complementary value of electrophysiological and pupillometric measurements, the multimodal framework remained limited to a relatively small number of physiological modalities. Future studies incorporating additional physiological measures, such as autonomic or behavioral indicators, may enable more comprehensive characterization of cognitive state and improve the robustness of physiology-based biomarker models.

Despite these limitations, the methodological framework developed in this thesis provides an important foundation for continued investigation of physiology-based cognitive biomarkers. By integrating neurophysiological recording, signal processing, statistical inference, and computational analysis, the present work demonstrates the feasibility of transforming physiological measurements into quantitative indicators associated with variability in cognitive performance.

6.4 Future Directions

The findings presented in this thesis establish a foundation for continued development of physiology-based frameworks for characterizing cognitive and memory performance using measurable biological signals. Although the present work demonstrated that electrophysiological and pupillometric activity contains meaningful information associated with cognitive processes, several opportunities remain for extending both the methodological and translational contributions of this research.

One important direction for future work involves expanding the range of cognitive functions investigated using physiological biomarkers. While the present thesis focused primarily on memory-related performance, future investigations may extend the proposed analytical framework to additional domains including attention, executive control, learning, and decision-making[44,45,49,60,63,64]. Such extensions may contribute to the identification of domain-specific biomarkers and improve understanding of how physiological dynamics vary across cognitive processes and behavioral demands.

Future studies may also investigate larger and more diverse participant populations. Because the present work was conducted in clinical epilepsy cohorts, extending analyses to healthy individuals and broader neurological populations would improve generalizability and enable evaluation of how physiological biomarkers vary across demographic, clinical, and cognitive conditions. Longitudinal investigations may further characterize how physiological activity evolves across learning, fatigue, aging, or disease progression, thereby supporting development of individualized cognitive monitoring frameworks.

Another important direction concerns continued development of noninvasive physiological biomarkers of cognition. A key finding of this thesis was that pupil-related physiological features demonstrated classification performance approaching that observed for simplified intracranial electrophysiological measurements. Future work may therefore focus on optimizing pupillometry-based approaches for scalable cognitive assessment in naturalistic environments using portable and wearable eye-tracking technologies[36,37,95,101,115]. Such developments may facilitate broader implementation of physiology-based monitoring outside specialized research and clinical settings.

Methodologically, future research may further develop the analytical approaches used for physiological signal analysis. Although the present thesis employed preprocessing, time-resolved analysis, statistical evaluation, and classification methods to examine relationships between physiological activity and cognitive performance, future studies may improve these approaches by incorporating more advanced strategies for combining information across physiological measurements and improving characterization of physiological changes over time. Such

developments may support more robust identification of physiological patterns associated with cognitive performance and improve reliability of physiology-based cognitive assessment.

The integration of additional physiological modalities represents another promising direction. While the present work demonstrated the complementary value of electrophysiological and pupillometric measurements, future multimodal frameworks may incorporate additional physiological signals such as heart rate variability, respiration, electrodermal activity, or eye-movement behavior[38–40]. Combining complementary measurements may improve characterization of cognitive state and support more comprehensive biomarker models.

Future investigations may also explore integrated multimodal biomarker frameworks combining electrophysiological and pupil-related features rather than evaluating these modalities independently. Because neural and pupil-related physiological activity likely reflects complementary aspects of cognition, integrated approaches may improve sensitivity to changes in cognitive state and strengthen characterization of memory-related variability.

Another important future direction involves real-time implementation of physiology-based cognitive monitoring systems. The analytical framework developed in this thesis demonstrates that physiologically meaningful features can be extracted from continuously evolving signals during cognitive task performance. Extending these approaches toward real-time processing may support intelligent systems capable of adapting dynamically to changes in cognitive state. Such systems may have applications in adaptive learning environments, cognitive workload monitoring, personalized clinical assessment, and human–computer interaction.

Finally, improving interpretability and transparency of computational biomarker models will remain essential as analytical approaches become increasingly sophisticated. Ensuring that identified biomarkers remain physiologically meaningful and scientifically interpretable will be important for supporting both clinical translation and practical deployment. Future research may therefore focus on explainable computational frameworks capable of identifying the physiological features contributing most strongly to cognitive-state classification.

Overall, the future directions outlined in this section highlight the potential for continued advancement of physiology-based approaches to cognitive assessment. By integrating physiological measurement, computational modeling, and scalable sensing technologies, future work may support development of robust systems capable of monitoring cognitive performance across scientific, clinical, educational, and technological environments.

6.5 Final Conclusion

This thesis investigated whether physiological signals can serve as objective biomarkers associated with cognitive and memory performance. By integrating

electrophysiological recordings, pupillometric measurements, signal processing techniques, statistical analysis, and computational approaches, the work developed a structured framework for examining how physiological activity reflects variability in cognitive function across both inter-individual and trial-level contexts.

The findings demonstrated that neural oscillatory activity recorded using noninvasive scalp electroencephalography contains measurable information associated with differences in memory- and executive-related cognitive performance across individuals. Furthermore, time-resolved analyses of intracranial electrophysiology and pupil dynamics showed that physiological activity differs systematically according to memory performance during encoding and retrieval. Together, these findings indicate that cognitive performance is accompanied by identifiable physiological patterns measurable across complementary physiological systems.

A particularly important contribution of this thesis was the demonstration that pupil-related physiological features exhibited classification performance approaching that observed for simplified intracranial electrophysiological features during memory-related task performance. Given the accessibility and noninvasive nature of pupillometry, this finding highlights its potential as a practical physiological indicator of memory-related cognitive variability and supports further development of scalable approaches to cognitive assessment.

Beyond the empirical findings, this thesis contributes a methodological framework for transforming continuous physiological recordings into quantitative representations suitable for statistical and computational analysis. By combining neurophysiological measurement with signal processing, time-resolved analysis, statistical inference, and classification approaches, the proposed framework demonstrates how physiological data may be transformed into measurable indicators associated with cognitive performance.

Collectively, the findings support the central dissertation thesis that physiological signals derived from electrophysiological activity and pupil dynamics can be transformed into quantitative indicators associated with cognitive and memory performance across both inter-individual and intra-individual levels of variability.

Overall, this thesis supports the broader view that cognition can be examined through measurable physiological processes in addition to behavioral outcomes alone. By integrating electrophysiological measurement, pupillometry, signal processing, statistical inference, and computational analysis, this work demonstrates how physiological recordings can be transformed into quantitative indicators associated with cognitive and memory-related variability. Taken together, the findings provide evidence that physiological signal analysis may support objective and computationally tractable approaches to cognitive assessment across complementary temporal scales.

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