EEG-Assessed Network and Signal Variability Features in Males and Females During a Visuospatial Task

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Abbreviations

EEG	Electroencephalography
FC	Functional Connectivity
MSE	Multiscale Entropy
BC	Betweenness Centrality
CL	Clustering coefficient
SPL	Shortest Path Length
SWP	Small-World Propensity
DMN	Default Mode Network
SN	Salience Network
fMRI	functional Magnetic Resonance Imaging
CV	Coefficient of Variation
FFT	Fast Fourier Transform
BCI	Brain-Computer Interface
ICA	Independent Component Analyses
SD	Standard Deviation
FDR	False Discovery Rate

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Abstract:

Background: Understanding sex-specific differences in cognitive processes, such as mental rotation, is important for unraveling the complexities of human brain function and behavior. This study investigated putative sex differences in connectivity and signal variability during a 3D object mental rotation task using electroencephalography (EEG); these EEG-based indices have rarely been assessed in this context.

Methods: Participants included 22 males and 20 females (18-41yr). Participants completed a mental rotation task during which they had to identify whether an object was the same but rotated or different. EEG-based connectivity was assessed using lagged coherence and complementary graph theory analytic methods were applied in assessing coherence [graph metrics: betweenness centrality (BC), clustering coefficient (CL), shortest path length (SPL), and small-world propensity (SWP). Signal variability was assessed using multiscale entropy (MSE).

Results: Females exhibited lower accuracy than males on the task. Regarding coherence, in gamma band, males had greater coherence across multiple channels, in frontal and parietal regions; similar results were noted using regional analyses (greater within-region coherence in the gamma band was noted in the left prefrontal and parietal regions in males). Females, on the other hand, displayed greater theta band coherence in right central, prefrontal, and frontocentral regions. Males exhibited greater inter-hemispheric coherence between various regions than females in alpha and gamma bands. Complementary graph theory analyses indicated that, in alpha band, females demonstrated higher BC in left prefrontal region, while males exhibit higher BC in theta band in right parietal region. Notably, theta band CL was significantly greater in males within left prefrontal region whereas females display higher beta band CL in the right prefrontal region. Finally, females vs. males exhibited greater MSE values, particularly in frontal channels and regions, across all most bands (delta, theta, alpha, and gamma; fewest differences were noted in beta).

Conclusion: Males tended to differ from females predominantly in the gamma band on coherence measures. This is notable given the involvement of gamma in cognition. Graph theory assessments yielded lateralization effects that were not evident with conventional methods. Increased MSE suggests greater variability in frontal regions in females, this might reflect greater frontal 'flexibility' that supports behaviour in females. In sum, this work advances our understanding of sex differences in one facet of cognitive processing, namely, mental rotation, and lays the groundwork for future investigations in this field.

1. Introduction

1.1. Overview

Extensive research has been devoted to exploring putative differences in human behaviour between males and females; these are discussed in the sections below. However, it is first essential to define "*sex*" and "*gender*" to minimize confusion in the context of this thesis. These terms, although historically used interchangeably, refer to distinct concepts. "*Sex*" encompasses the biological and physiological characteristics distinguishing males and females, including the reproductive system, chromosomes, and hormonal profiles[1]. In contrast, "*gender*" relates to one's identity, encompassing socially constructed aspects linked to individuals, such as societal norms, roles, and intergroup relationships, as well as internal constructs of the self[2]. In this thesis, the focus is primarily on the biological construct of sex when assessing putative male vs. female differences as this has been the focus of most existing literature.

Sex is among the most significant risk factors for various human diseases, particularly those affecting the brain, such as neurological disorders like Alzheimer's disease and dementia[3,4]. This underscores the potential importance of sex-specific factors in conferring susceptibility or resilience to different disorders. Investigating putative sex differences in human brain structure and function thus presents an avenue for gaining insights into the mechanisms underlying brain-related diseases, including psychiatric disorders characterized by sex disparities, such as depression (more common in females) or substance use disorders (more common in males)[56].

Although the brains of men and women are highly similar, they also show some consistent differences, in both anatomy and function, that might have important implications for sex-based illness, and, by extension, treatments. That is, sex differences in the brain might contribute to the susceptibility of developing specific disorders and underscore certain sex-specific behaviours[7].

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While a comprehensive assessment of the neural differences between males and females is beyond the scope of this thesis, the focus was on two aspects: *connectivity* and *brain signal variability* in the context of visuospatial processing, which tends to be one domain that most consistently differs between the sexes.

1.2. Behavioural differences during visuospatial processing

While many sex-specific behavioural differences have been overstated and some past work in this field has been riddled with flawed and biased research approaches, some sex-specific behavioural differences have emerged. Sex differences in processing and performance within the *visuospatial domain* have been noted in numerous studies. Generally, tasks involving spatial transformations or visuospatial processing (e.g., mental rotation) produce the most robust sex-based differences, with adult males outperforming females[8]; however, differences have also been reported on spatial perception and visualization tasks[9]. Visuospatial processing is crudely defined as the brain's ability to interpret and interact with spatial information, mental rotation tasks are a specific form of visuospatial processing[10]. They involve mentally manipulating objects or spatial information, and mental rotation is considered to be important for understanding spatial relationships and making spatial judgments, it is essential for both academic and daily tasks[11]. One study found that within a 10-year period, sex differences persist, with men having -on average- better visuospatial abilities (e.g., faster reaction times and increased accuracy) than females[12]. Further, the superiority of males vs. females on visuospatial tasks has been demonstrated in animal work[13]. Thus, this suggests a biological (vs. solely a socio-cultural element) to visuospatial differences between the sexes. Nevertheless, the mechanisms underlying putative differences in visuospatial processing are unclear[8].

1.3. Neural profiles and sex differences during visuospatial processing

The nervous system operates as a communication network, involving signaling and information transfer that spans various temporal and spatial scales, from synaptic transmission between neurons, to interactions among neuronal populations and whole-brain patterns of regional co-activation[14].

One of the key regions/central hubs involved in visuospatial processing is the parietal cortex, which plays a role in mental rotation[15–17]. However, recent review findings emphasize the significance of a shared neural network in mental rotation, with involvement of both *frontal* and *parietal* lobes[18–20]. More specifically, neuroimaging studies have revealed that the following regions are involved in mental rotation tasks: supplementary motor area, premotor cortex and superior and inferior parietal lobule, centering on the intraparietal sulcus (**Figure 1**).

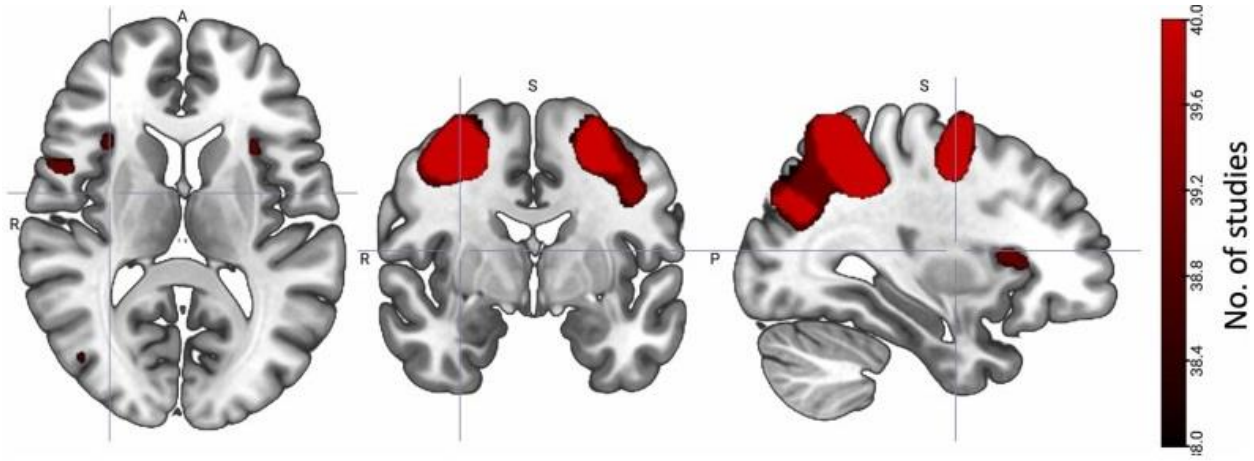

Figure 1. *Regions with strong activation and connectivity in at least 90 % of studies are involved in mental rotation. Figure adapted from Hiew, S., et. al., 2023.*

While there is a general overlap in the key brain regions engaged by males and females during visuospatial processing, including during mental rotation tasks, notable differences exist in their patterns of activation. Specifically, based on a recent review paper by Julieta Ramos-Loyo et

al. (2022), males tend to engage their right hemisphere when engaging in spatial tasks, whereas females tend to activate the left hemisphere or both hemispheres equally[21]. Another study by Haixia Long et. Al. (2021) examined the relationship between sex, mental rotation ability, and resting-state network functional connectivity (FC), as assessed using neuroimaging. They reported that males outperformed females in the mental rotation test, and this difference was mediated by a stronger FC between the default mode network (DMN) and salience network (SN), or large-scale networks involved in introspection and salience/attention focus, respectively[22]. It is worth pointing out that the DMN and SN include some of the areas known to be implicated in mental rotation tasks; these include the precuneus and angular gyrus (part of the parietal lobe) for the DMN[23], and anterior insula which is a key region comprising the SN (the insula is located deep within the lateral sulcus separating the temporal lobe from the parietal and frontal lobes[24]). Thus, assessing connectivity features in the brains of males vs. females during visuospatial processing appears to be an important means of understanding key neuronal features underlying performance differences between the sexes.

1.4. Overview of electroencephalography (EEG)

Progress in the understanding of normal and disturbed brain function, including in understanding putative brain differences underscoring visuospatial processing in males and females, is aided by non-invasive neuroimaging methods such as electroencephalography (EEG). The EEG primarily captures electrical activity generated by groups of pyramidal neurons (primarily post-synaptic neural activity), whose cell bodies are predominantly located in layers III and V of the cerebral cortex[25]. EEG is a measure of voltage fluctuations (i.e., potential difference) in the time domain, picked up by scalp-positioned electrodes based on international 10-20 sytem[26] (**Figure 2**), and provides high temporal resolution[27].

Introduction

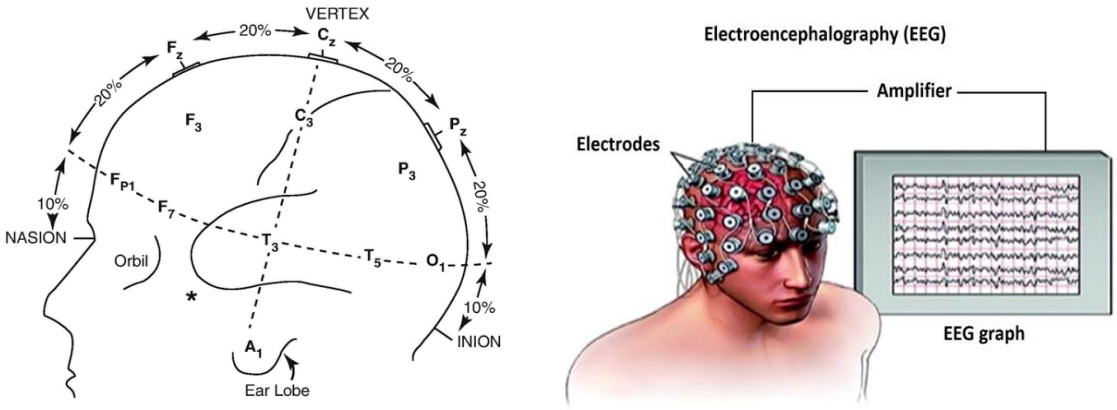

Figure 2. *EEG Recording and electerode placement on the scalp using 10-20 international system. Adapted from Sazgar et. al. 2019* [28].

In time domain, the potential difference (in μV) between two pairs of scalp electrodes or between scalp electrodes and a passive reference electrode can be amplified and recorded for both gaining insight into brain electrical activity for clinical and research purposes[29]. The decomposition of the overall power in the EEG signal into individual bands is commonly achieved through the Fast Fourier Transform (FFT) and related methods for spectral analysis[30]. This focuses on analyzing broad frequency bands (typically power) contained within the EEG[31] (i.e., delta, theta, alpha, beta, and gamma bands). Using this method, the EEG signal is broken down into its component frequency bands, which have been associated with functional characteristics[29–31].

The quantitative analysis of EEG involves the measurement of mean spectral magnitude or power across multiple frequency bands of interest. The resulting numerical parameters are then subject to analysis for various applications, including but not limited to brain disorder detection (e.g. seizures) and insight into cognition[32].

1.5. Overview of EEG-based findings in the context of visuospatial processing:

EEG-indexed features have been studied in the context of visuospatial processing and have provided insight into neuronal processes associated with this operation. Previous work in brain-computer interface (BCI) designs has utilized features from EEG power and asymmetry ratios across delta, theta, alpha, and beta bands to classify various mental tasks, including those related to visuospatial processing[33]. The incorporation of high-frequency band features, in particular, was found to significantly enhance the accuracy of mental task classification in BCI systems, underscoring the importance of high-frequency components in scalp-recorded EEG in visuospatial processing[34]. As such, there is evidence for neural features -as assessed by the EEG- that are key during visuospatial processing. For instance, mental rotation tasks have been associated with stronger event-related desynchronization ERD, and a complete blocking of alpha frequency band oscillations during task execution, indicating the involvement of alpha oscillations in parietal lobe, in particular, during mental rotation tasks[35]. Additionally, significant differences in EEG-assessed functional connectivity (further details in **Section 1.5** on EEG-based functional connectivity) and network structure in the alpha and beta frequency bands during visuospatial memory maintenance between individuals with mild cognitive impairment and healthy elderly individuals[36]. As such, these data further implicate the involvement of the alpha and beta bands in the context of visuospatial processing in frontoparietal regions. Furthermore, the mu frequency band (i.e., alpha band associated with sensorimotor source localization) has been linked to motor skill acquisition[37] and voluntary movements[38]. Complex cognitive operations (and fatigue associated with these), tend to implicate alpha and gamma bands, including higher frequency beta bands[39]. As such, existing data implicate alpha with visuospatial and motor processing; however, cognitive operations typically evoke higher-frequency bands such as beta and gamma. Taken

together, these data implicate the alpha and higher-frequency bands, such as beta and potentially gamma in mental imagery tasks.

1.6. Overview of the brain Functional Connectivity (FC):

Brain connectivity, crudely defined as the co-activation/co-occurrence of activity in one brain aspect in relation to another[40], can be assessed while participants are *at rest* (not engaging in any specific task-orientated operations) or during a *task* (task-related connectivity). Broadly, resting-state (i.e., task-free) connectivity reveals intrinsic, spontaneous networks (or regional connections) not tethered to an external stimulus. Task-based connectivity, on the other hand, provides information as to how our brain is modulated by (or engaged in) while doing a task (i.e., certain networks and brain connection structures subserve certain tasks).

In the brain, connectivity can be described as structural, functional, or effective. Structural connectivity denotes a network of anatomical links, typically assessed using diffusion tensor imaging. Effective connectivity reflects directed or causal relationships between elements of the system (i.e., activation in region A is responsible/drives activation in region B)[40]. Functional connectivity (FC) denotes the degree of association between elements of the system (i.e., the co-activation of one region with another).

EEG-indexed connectivity involves identifying statistically significant synchronizations in specific frequencies between scalp electrodes/clusters of electrodes[41]. Brain connectivity can be characterized at different scales[42]: micro-scale (between neurons), meso-scale (between cortical columns), and macro-scale (between brain voxels/regions)[43]. When assessing connectivity profiles in humans via EEG, one is tapping into different macro-scale measures. Several techniques can be used to perform EEG connectivity analyses.

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Coherence is one method for assessing EEG-indexed connectivity. Briefly, coherence is a mathematical method that can be used to determine if two or more sensors (i.e., electrodes) have similar neuronal oscillatory activity with each other[44]; as such, it is a measure of EEG-based FC. Coherence between two signals consists of complex numbers in the form of $a+ib$, where a is a real number (including rational numbers like positive and negative integers, fractions, and irrational numbers) and ib is an imaginary number. Also, a and b belong to real numbers and $i = \sqrt{-1}$. Coherence between two EEG signals at a specific frequency consists of two components: a real part (includes information about the amplitude of the signal) and an imaginary part (includes signal phase information). The coherence coefficient is a normalized quantity wherein a value of 1 represents a perfect linear relationship between signals, and a value of 0 represents no linear relationship (coherence values therefore range from 0-1).

There are several approaches to measuring coherence; one such measure is *lagged coherence*, which evaluates the synchronization patterns between brain regions while considering/accounting for time delays between sensors. There are some advantages to using lagged coherence over classical coherence or phase lag index. Classical coherence measures the degree of the linear correlation between two time series at the same frequency, while the phase lag index assesses the actual differences in phase between signals[45]. Lagged coherence represents the coherence between two signals and their lagged version at a specific frequency (**Figure *3***)[46]. Lagged coherence, in particular, excludes the non-lagged part of coherence, which is comprised of volume conduction effects; as such, it is less affected by instantaneous dependencies, making it more suitable for removing confounding effects related to volume conduction and low spatial resolution compared to the imaginary part of complex-valued coherency[47]. As such, lagged coherence can be a valuable tool for providing insights into the temporal dynamics and coordinated

activity across distinct regions, making it applicable for studying brain network connectivity in various brain-related disorders and or mental operations, such as visuospatial processing[48–51].

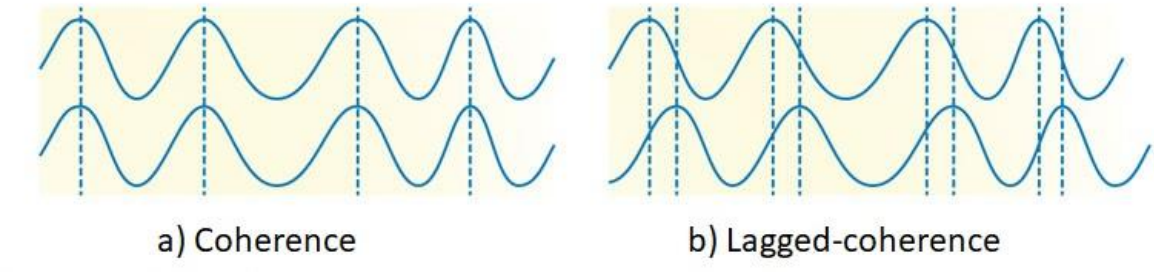

Figure 3. *Example of coherence and lagged coherence between two signals. Adapted from Siegel et al. 2012*[52].

1.7. Overview of EEG-based FC findings in visuospatial processing

A handful of EEG-based coherence (not necessarily utilizing lagged coherence measures) assessments have been applied to study neural activity during visuospatial processing. For example, one study in 2013, investigated electrocortical synchronization patterns during a mental rotation task, and reported increased coherence in the alpha band for letter rotations, and decreased coherence across alpha and gamma bands during higher angel of rotations, suggesting a divergence in the mental rotation process for various types of mental rotations, namely abstract and "verbal"[19]. Another study reported that higher mental rotation speed and accuracy were negatively correlated with FC in pre-frontal, frontal and parietal electrodes[20]. One study by Plaza-Rosales (2023) using a combined behavioural, EEG and eye-tracking data with participants in early-stage Alzheimer's disease and healthy controls engaged in a spatial navigation task, revealed marked occipital beta band activity (15-20 Hz) during early visual processing in the control group[53]. As such, based on existing data, EEG-based connectivity measures tend to implicate connections within/between pre-

frontal, frontal, and parietal regions, and highlighting the potential role of beta band activity and connectivity in early visual processing and perhaps mental rotation.

To our knowledge, there only been a handful of assessments utilizing EEG-based measures of connectivity to assess neural profiles in males vs. females during a visuospatial processing task, such as during a mental rotation task. However, connectivity profiles have been investigated using other brain imaging approaches. For instance, one study[54] focused on interactions between the visuospatial attention allocation network and mental rotation network (central brain region and parietal lobe involvement) using high density EEG by applying a direct transfer function, which describes causal influence of channel *j* on channel *i* at frequency *f*. The findings revealed hemispheric lateralization, indicating that males and females exhibit differences in spatial working memory utilization and attentional processes during varying levels of difficulty in a 3D mental rotation task.

To the best of our knowledge, the current study represents the first exploration of sex differences in EEG-indexed functional connectivity (FC), by way of lagged coherence, during a mental rotation task (i.e., a task that engages cognitive and spatial domains). While previous research has delved into the mechanisms underlying mental rotation and putative differences among various object categories, our investigation uniquely focuses on elucidating sex-related variations in males and females using EEG. This can fill a critical research gap and contribute to a more comprehensive understanding of the electrocortical synchronization patterns involved in mental rotation tasks, particularly in the context of sex-related differences.

1.8. Overview of graph theory and primary network graph features: Applications to EEG analyses

There is a growing demand for appropriate tools and methods for modelling and analyzing brain network data as a complex network[55], especially in high-density EEG arrays; one such approach is *graph theory*[56]. Graph theory is a branch of mathematics that dates back to the 18th century[56]. Today, applications of graph theory pervade all scientific disciplines as well as many modern information and computing technologies. The brain is a natural fit for graph theory approaches as it is readily represented as a network (a graph) of elements (nodes or vertices) and their pairwise interconnections (edges, connections, or links). Comprehensive maps of brain connectivity have given rise to the emerging field of connectomics[42,57], a central focus of which is the systematic and quantitative study of brain networks and graphs.

Brain nodes may be individual neurons or entire brain regions, depending on the measurement technique. Edges can take on binary or weighted values, and they can be directed or undirected, depending on how interactions are estimated from empirical data. Recent advancements in network analysis tools have led to a growing interest in understanding the role of brain network *segregation* and *integration* underscoring cognitive processes, for instance[58] (presented in **Figure 4**). The concept of segregation and integration might also be useful in the examination of potential sex differences during visuospatial processing.

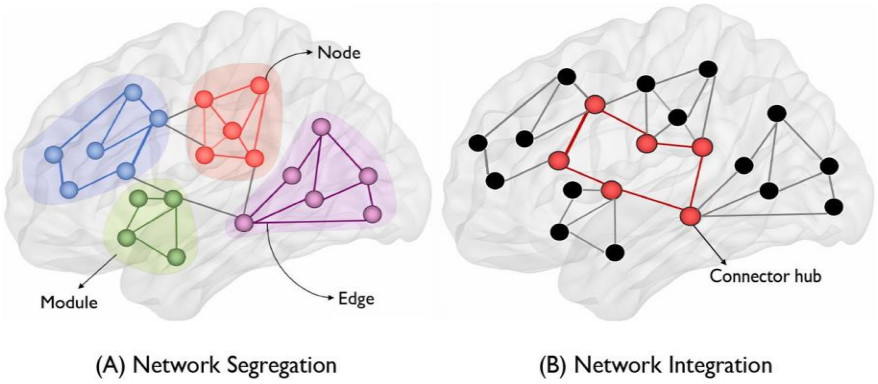

Figure 4. *Nodes, links, (B) integration and (A) segragation of a network: Module or clusters and connector hubs. Adapted from Handiru et. al.,2020* [59].

Integration refers to the efficiency of global information communication or the ability to combine distributed information, while measures of *segregation* quantify the presence of densely interconnected groups of brain regions potentially underscoring an operation. These measures provide insights into how brain regions communicate and process information within distinct clusters or modules[60].

Graph theory methods can offer important new insights into networked brain systems[56]. Emphasizing integration and segregation, graph theory allows us to discern the balance between global connectivity and localized processing. By employing graph theory, we can represent FC as a network, allowing for a comprehensive exploration of brain network organization. The most commonly utilized parameters to describe network/brain feature characteristics in graph theory are the ***clustering coefficient*** (for segregation), ***shortest path length*** (for integration), ***global efficiency*** (for network efficiency), ***betweenness centrality*** (for finding hubs in the network) and ***small-world propensity*** (combination of integration and segregation). These will be the focus within this thesis.

Graph theory-based FC analysis can unveil differences into how brain networks are organized and how information "flows" between regions (i.e., regional communication) in males and females. This information can contribute to understanding sex-related differences in cognitive processing, including visuospatial processing tasks such as mental rotation. For example, in the context of graph theory measures, the shortest path in a graph with matrix-valued edges has been introduced, which could be relevant for analyzing mental rotation tasks within a network framework[61,62]. One recent study employed EEG and graph theory to investigate human brain networks during different sub-stages of a mental rotation task, revealing alterations that provide evidence for the utility of graph theory in elucidating the intricacies of brain connectivity during mental rotation[63]. The study utilized intra-frequency and cross-frequency phase-phase coupling in multiple brain networks (with 11 patients and 11 healthy volunteers) to assess the impact of stroke on temporal and spatial brain information interaction during a mental rotation task. The graph theory analysis of the entire task stage indicates that patients with stroke exhibit a longer feature path length and a smaller clustering coefficient in their brain networks compared to healthy controls; thus, these features might be important considerations when assessing visuospatial processing differences between males and females. To our knowledge, graph-theory analyses have not been applied to EEG-derived FC to explore sex differences during visuospatial processing.

1.9. Overview of signal variability and sex differences in EEG signal variability

Brain signal variability refers to the spontaneous fluctuations in neural activity that occur over time, reflecting the dynamic nature of the brain's function[64]. These fluctuations can be observed at different spatial and temporal scales, providing insights into the underlying neural processes[65]. Research has shown that brain signal variability is not only a fundamental aspect of brain function but also plays a crucial role in various cognitive processes, such as attention,

memory, and aging[66,67]. Furthermore, brain signal variability has been linked to functional connectivity between brain regions, indicating its potential role in information integration and processing[68]. Greater signal variability, similar to enhanced flexibility within a system, is thought to support adaptive learning processes, contrasting with the potential drawbacks of system rigidity[69]. Indeed, numerous studies consistently reveal a positive correlation between within-person signal variability (as measured with various imaging approaches) and cognitive performance; higher variability tends to be associated with better performance in younger adults and its decrease is predictive of cognitive decline in older individuals[65,67,70]. Moreover, increased brain signal variability, particularly in neocortical regions, is linked to better cognitive performance[67]. The modulation of signal variability in response to task demands may reflect the neural signal's integrity, including flexibility or "nimbleness", which appears to be related to task performance. As such, current studies indicate that the variation observed in brain signals is an important factor that reflects the capacity for information processing and the overall functional integrity of the brain[71–73]. Task-dependent assessments of EEG signal variability (i.e., variability in brain electrical activity) can therefore provide insight into the state of the brain and its ability to perform cognitive tasks, such as mental rotation tasks.

EEG signal variability, in particular, refers to the fluctuations and variations at different scale factors, such as time, in the electrical brain activity recorded by EEG, reflecting the dynamic nature of the brain's electrical activity. One of the simplest means of calculating EEG signal variability is by measuring the standard deviation (SD) of the amplitude envelope of the filtered EEG time series for each of the standard frequency bands. However, more sophisticated signal variability approaches, such as **multiscale entropy (MSE)** has been used as an alternative form of

assessing signal variability in most of the recent EEG-based studies assessing EEG-based variabilie[69,74–76].

In brief, MSE is a quantitative index of the temporal *irregularity* of brain activity temporal patterns, such as in the EEG signal[77]. MSE is an analytical tool that provides insights into the communication patterns between neural generators in a brain over a variety of timescales, allowing researchers to explore the complexity of the spatiotemporal pattern underpinning particular cognitive processes, when assessed during a particular operation[78]. Increased entropy corresponds to greater signal complexity [79]. Pincus[80] introduced and outlined approximate entropy as a method for scrutinizing signal complexity. Approximate entropy offers a unified measure of complexity for the entire EEG channel for each frequency band, encompassing complexity at various time scales, referred to as MSE[81]. MSE is widely adopted due to its demonstrated higher reliability compared to other signal variability analysis approaches (e.g., sample entropy, fuzzy entropy; measures that capture complexity at different time scales), particularly in handling short signals[79]. The primary distinction between MSE and other measures of variability lies in how MSE calculates the average entropy values across multiple coarse-grained signals within each scale factor, setting it apart from other algorithms (coarse-grained signals consist of fewer and larger components than fine-grained)[74,81,82]. Coarse graining, through the process of progressively smoothing the time series, impacts both the entropy of the signal and its overall variability, resulting in a reduction in standard deviation across different time scales[83]. It appears that during tasks, accurate and consistent behaviour is generally supported by an increase in MSE[84].

Only one known EEG study has documented differences in signal variability between males and females, and notably, this observation was made during a resting-state condition. The authors investigated brain signal variability using two neuroimaging methods, EEG and fMRI, in

healthy individuals at rest[69]. The findings revealed significant sex differences in EEG signal variability across different brain areas in the delta, theta, alpha, and beta frequency bands, with females having higher signal variability across all bands, whereas no significant differences were noted in BOLD signal variability. This result suggests potential sex-specific neural processing patterns, while no corresponding differences in BOLD signal variability indicate a unique contribution of EEG variability analysis; further, difference in the origins of EEG and BOLD signal variability might exist. To the best of our knowledge, there have been no studies assessing putative sex differences in EEG-assessed signal variability during visuospatial processing.

1.10. Gaps in the literature

In sum, there is a limited number of EEG-based FC studies investigating potential sex differences during visuospatial tasks, especially by way of lagged coherence. As such, whether EEG-indexed FC features differ between the sexes during visuospatial processing is unclear. In a similar vein, FC analyses have not been explored using graph theory-based approaches which could provide a more fulsome and comprehensive picture of brain features in males and females during visuospatial processing. Second, EEG-based signal variability studies investigating sex differences during visuospatial tasks are also lacking. Variability analyses might provide a more nuanced exploration of dynamic neural activity in both sexes, thereby contributing to a more comprehensive understanding of the complexity of the brain during a visuospatial processing in males and females.

2. Aims & Hypotheses:

2.1. Aims:

The primary aims for this thesis work are as follows:

1. The first aim is centered on examining EEG-indexed FC via *lagged coherence* between males and females during a visuospatial mental rotation task during which participants indicate whether a figure is the "same", "same but rotated" or "different" relative to a reference image. For the purposes of this thesis, all analyses center on the "same but rotated" and "different" conditions (these are combined) between the sexes, as these are the most complex conditions that engage visuospatial and rotational abilities (vs. recognition as in the "same" condition). As a complementary and exploratory aim, graph theory features, specifically clustering coefficient (CL), betweenness centrality (BC), shortest path length (SPL), and small-world-propensity (SWP) were used to further characterize FC features between the sexes in a more nuanced manner.

2. The second aim was focused on assessing EEG signal variability during the visuospatial mental rotation task between males and females using MSE.

2.2. Hypotheses:

1. Though the literature on which to draw our inferences is limited, we expected that males would exhibit higher FC/lagged coherence (typically referred to as coherence for the rest of this thesis) than females, particularly within the parietal lobe, and among parietal, frontal and frontocentral lobes, particularly, in the alpha band for its importance in spatial tasks[85,86] and higher-frequency bands, such as the beta and perhaps the gamma bands, given their importance in cognition, including mental/imagery tasks[87].

With respect to the exploratory aim centered on the analyses of graph theory features, we expect higher network hubs, as operationalized by BC in frontal and parietal regions in males vs. females, particularly, in the beta and gamma band based on previous findings during spatial tasks[88].

Aims & Hypothesis

For the CL, we anticipated higher values in frontal and parietal regions, particularly in the beta and gamma bands, indicating increased local connectivity and specialized processing in males. Conversely, we expect SPL, reflecting more efficient information transfer between brain regions, especially in the alpha and gamma bands in males. Finally, in terms of SWP, we anticipated males to have higher SWP globally, and in higher frequencies bands, such as beta and gamma, as higher SWP is often related to better performance in cognitive tasks[89].

2. We anticipated that males vs. females might exhibit elevated EEG signal variability in their EEG data during the visuospatial task, particularly in the parietal and frontal lobes. It is again feasible that most pronounced changes in EEG-indexed signal variability might emerge in the alpha and beta/gammy bands due to their involvement during spatial tasks and cognitive functioning, however, this is speculative due to the dearth of literature.

3. Materials & Methods

3.1. Overview:

The work presented in this thesis was embedded within a larger study assessing sex differences and their relation with neural features and sex hormones. The study included self-report questionnaires, cognitive assessments, and neuroimaging. Notably, EEG and functional magnetic resonance imaging (fMRI) data were collected simultaneously (EEG+fMRI); however, for the purpose of this thesis, the *focus is exclusively on EEG data*. Nevertheless, certain methodological steps were carried out on the EEG that were required given its acquisition in the scanner.

Briefly, participants came in for a one-time session in the Clinical EEG & Neuroimaging Research Lab (neuroimaging data were collected at the Brain Imaging Centre) at the Royal Ottawa Mental Health Centre. Participants were initially screened over the telephone (details of inclusion/exclusion criteria are outlined in **Section 3.2**). Appropriate participants were invited to participate in the study. Prior to testing, participants were instructed to abstain from psychoactive street drugs, including marijuana, for >3 weeks prior to testing. Current smoking status was ascertained by measuring carbon monoxide (CO) levels (individuals exhibiting values >3ppm were excluded; **Section 3.2**); a urine sample for drug screening was also obtained to ensure a drug-free status. Additionally, participants were requested to avoid caffeine consumption on the testing day, with >6hr abstinence (decaffeinated beverages were allowed). These measures were implemented to minimize potential influences of drug, smoking, or caffeine-related factors on the study outcomes. Following screening, individuals were invited into the laboratory for testing; they were hooked up with electrodes and completed the mental rotation task outlined in **Section 3.1**. Other data were collected as part of the session but will not be further discussed as they are not pertinent to this thesis.

3.2. Participants

Participants were recruited for this study through targeted advertisements posted on the Sona system (i.e., research participant management tool), university announcements (e.g., classroom presentations), and community outreach programs (e.g. Iranian students of uOttawa social media channel). Recruitment started in January 2022, and the first participant underwent testing in February 2022, while the last participant included in this study was recruited and tested in December 2023.

The **inclusion criteria** were as follows: (a) Males and females, aged 18-45 years recruited from the community. b) Predominantly right-handed (this was assessed with the Edinburgh Handedness Questionnaire[90] with a laterality score of >+40 required for inclusion). This criterion allowed us to control for brain activity asymmetry; this is particularly important as there is evidence of r lateralized brain activity/engagement in males and females during the processing of visuospatial tasks[91]. The focus on right-handed individuals aligns with the existing literature, thus facilitating comparisons. (c) Participants were recruited who identified with their anatomical sex. (d) Participants were free of psychoactive medication use without a history of psychiatric or neurological problems (further details in the "exclusion criteria" section). (e) Current non-smokers were included (i.e., daily cigarette or e-cigarette use was exclusionary).

Pertinent **exclusion criteria** were as follows: (a) Not currently identifying with their biological sex (e.g., born female, but now identifies as a male and is taking hormone replacement therapy). (b) Have current psychiatric issues* or substance-use problems within 6 months. (c) Have a history of psychotic problems (e.g., schizophrenia, mania). (d) Have a history of neurological problems (e.g., seizures), including loss of consciousness for >5 min. Psychiatric history was established using questions adapted from the Structured Clinical Interview for DSM-V diagnoses

(SCID)[92] and the Mini-International Neuropsychiatric Interview (MINI)[93]. These were administered by trained personnel. Other questionnaires (and a cognitive battery) were administered, but, are not discussed as they are not pertinent to this thesis, apart from the *Edinburgh Handedness Inventory*.

*NOTE: The presence of one, previous not-treatment-resistant (i.e., responded to intervention) depression episode was not exclusionary (the same is true for a past anxiety disorder) so long as participants are in remission, and not currently on antidepressant medications (stopped within >3months) or undergoing regularly psychological treatment for a psychiatric illness.

3.1. Mental Rotation Task:

During the mental rotation task, participants were asked to judge whether two 3D objects were: a) identical, b) the same but rotated, or c) different **(Figure 5)** (~12 min). This task was comparable to that outlined by Sharma G, et. al., 2018[54]. Participants responded with a three-button MRI-compatible response pad. Each trial began with a 400ms fixation cross, followed by the object pairs (5s), and followed by a blank screen indicating which button corresponds to which answer (average 2.5s). Participants could respond during the stimulus presentation and blank screen. A total of 28 trials per stimulus type were presented (i.e., a total of 84 trials), which were randomly distributed throughout the task.

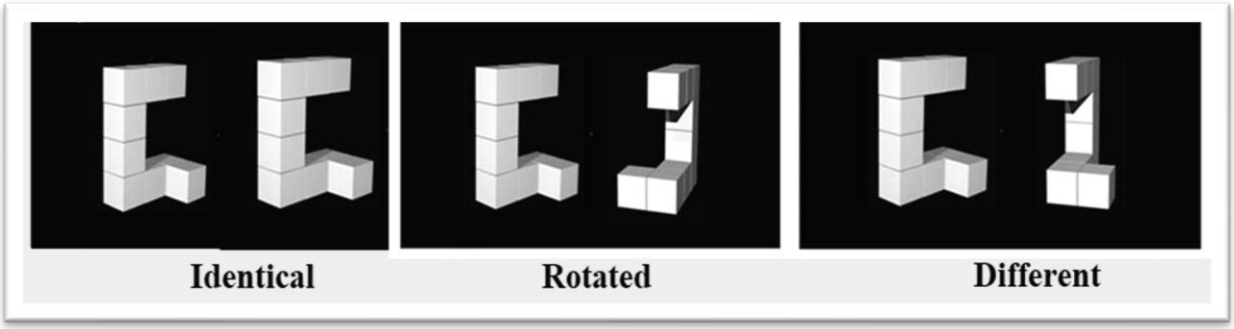

Figure 5. *Mental rotation task images: identical, same but rotated, and different shapes.*

3.2. EEG Recordings

EEG was recorded from a 64-channel MR-compatible EEG-cap (consisted of 64 Ag–AgCl electrodes), along with two MR-compatible amplifiers and a synchronization box (Brain Products/BrainAmp MR plus, Germany) to synchronize the timing of the scanner clock and EEG clock. During the data recordings (sample rate: 5KHz; online filters: 0.016-5000Hz; 63 active channels, AFz as ground [not used in subsequent analyses]; FCz as the reference; and impedances were monitored and kept below 5KΩ before data recording using BrainVision recorder software), one back electrode was used to record electrocardiograph (ECG) activity (for subsequent clean-up of EEG data as described in **Section 3.3**).

3.3. EEG Data Preprocessing:

As outlined, the EEG data was acquired during simultaneous fMRI data collection, as such, scanner artifacts were removed from the EEG recordings (BrainVision Analyzer 2.1) by an adaptive average template subtraction method and then downsampled (500Hz)[94,95]. Ballistocardiograph artifacts were removed using artifact template subtraction. First, the ECG channel was filtered with a low-pass filter at 15 Hz. Additionally, the heartbeat pulse rate (e.g., ranging from 64 to 94 in the context of this study) was determined by identifying peaks in the signal (followed by visual inspection)[95,96], these were then removed from the data (see Supplementary **Figure 1.** in **Appendix A** for examples of heartbeat pulse detections).

Without any re-referencing (i.e., reference was the same as for the recording session – FCz), EEG was subsequently low pass filtered (70Hz) with a high pass filter of 1Hz and a 60Hz notch filter. Data was segmented using stimuli markers. Out of the three conditions (i.e., “identical”, “same but rotated”, and “different”; with a maximum of 28 segment epochs per stimulus), our analyses focused on epochs derived from the "same but rotated" and "different"

conditions (a combination of both). The stimulus duration was 5 seconds; however, the data was segmented into non-overlapping 2-second epochs (i.e., first 2s after stimulus onset). For most participants, people did not respond to the task within the first 2s (average number of epochs with reaction time >2000ms was 50.65 epoch ranging from 20 to 56 epoch; maximum number of epochs was 56); only epochs without a response were used in the subsequent analyses. Epochs with large voltage fluctuations which could not be cleaned or may have negatively affected the independent component analyses (ICA) were first rejected before applying ICA to increase its performance. ICs related to eye blinks, eye movements, muscular artifacts, and movements were rejected. Channel rejection and interpolation, if necessary, were implemented as part of the preprocessing steps. In the case of having a noisy channel, spherical interpolation with the help of neighbour channels was used (interpolation was used for 3 participants). One female participant did not appear to have understood task instructions (i.e., they did not distinguish between the "same but rotated" and "different" conditions), as such, their data was not included in this study. Subsequently, a final visual inspection, involving the rejection of bad epochs and removal of potential artifacts not identified by ICA, was conducted. The clean and segmented data (see **Figure 2.** in **Appendix A** for an example of cleaned 2-second epoch), with a minimum of 20 and a maximum of 56 epochs, was then exported for subsequent analyses. All clean-up/pre-processing of the EEG data was carried out using BrainVision Analyzer 2.1 on a PC computer (ASUS PC intel core i7, 10700 CPU up to 2.90 GHz, 16 GB RAM, windows 11) located in Clinical EEG & Neuroimaging Research Lab at Royal Ottawa Mental Health Centre.

Data from the two conditions was averaged for all analyses to increase power (i.e., a maximum of 56 epochs was available for all analyses). For inclusion into the study, a minimum of 20 epochs was required (i.e., 40s of data; mean±sd clean data length used for all

participants=87.44±18.31). While the cleaned epoch length employed in the analysis is crucial, it is equally essential to ensure the acquisition of high-quality, artifact-free data[97]. The optimal length of clean data for EEG analysis depends on the specific research question. In our study, we focused on the first 2s of data following the stimulus, concentrating on two out of three conditions to specifically investigate early-stage/response-free mental rotation processing. Clean and segmented EEG data then exported to MATLAB for further analyses in five frequency bands: delta: 1-4Hz, theta: 4-8Hz, alpha: 8-13Hz, beta: 13-30Hz, and gamma: 30-50Hz. All analyses were performed using MATLAB script and functions, and custom scripts; analyses were run on *ASUS* laptop (model: K556, CPU: Intel core i7-7500U, up to 3.5GHz, memory: 12GB).

3.4. Functional connectivity measures and graph theoretical analysis:

In this study, *lagged coherence* was used to investigate FC. The adjacency matrix (i.e., square matrix used to represent a finite graph) consisted of a 63-by-63 matrix in which the rows and columns are electrodes, and the arrays are the mean coherence of all epochs between pairs of electrodes. The classical squared coherence equation for calculating the connectivity matrix is[41,98]:

$$C_{xy}{}^{2}(f) = \frac{|P_{xy}(f)|^{2}}{P_{xx}(f)\ P_{yy}(f)} \quad (1)$$

Where $P_{ij}(f)$ is the cross-spectrum between the two signals *i* and *j* in the time domain.

Commencing with the definition of complex-valued coherence between time series *x* and *y* within the frequency band *w*. This is derived from the cross-spectrum using the covariance and variances of signals. The **lagged linear coherence** in the specified frequency band *w* is expressed by the following equation[47]:

$$LagR^{2}_{xyw} = \frac{[ImCov(x,y)]^{2}}{\mathrm{Var(x)}\times\mathrm{Var(y)}-[ReCov(x,y)]^{2}} \quad (2)$$

where *x* and *y* are the time series of two brain regions or electrodes, *Im* and *Re* are the imaginary and real parts [the FFT results are complex coefficients with real (amplitude information) and imaginary (phase information) parts]; and *Var* and *Cov* are variances and covariance of the signals. This equation and the associated matrices were calculated, and values were extracted between each set of electrodes, and all analyses after preprocessing step was carried out using MATLAB R2021a. Statistical analyses of the coherence is discussed in **Section 3.8**.

After preprocessing, artifact-free and segmented EEG data (63 channels) was exported to MATLAB to calculate connectivity matrix (*lagged coherence*) in each of the frequency bands of interest. For each participant, a 63-by-63 connectivity matrix was calculated per frequency band (five connectivity matrices per participant). Lagged coherence was calculated in three ways: 1. *global connectivity*, represented by the average of the connectivity matrix, was examined across all electrodes for each frequency band. 2. *Coherence per electrode*, i.e., coherence with sample electrode A, was analyzed with all other electrodes (N=62). 3. Lagged coherence *within each of the eight regions* we predefined (details in **Section 3.7**). 4. Finally, the *average connections between the eight regions*, totaling twenty-eight connections, were explored.

Graph theory measures: To supplement the above analyses, four graph theory measures for each participant and each frequency band for the coherence measures using the *BCT* in MATLAB. Specifically, the *shortest path length (SPL)* (i.e., a measure of integration), *clustering coefficient (CL)* (i.e., a measure of segregation), *small-world propensity (SWP)* (i.e., a combination of integration and segregation), and *betweenness centrality (BC)* (i.e., hubs; please refer to **Section 3.4** for further details)

3.5. Quantifying network dynamics: calculating graph features in FC analysis:

A graph for a network that consists of *N* node can be described by representing the *N*-by-N adjacency matrix whose entries are α_{ij} *(i, j= 1, 2, 3, ..., N)* when the link (which can be the coherence between two electrodes) l_{ij} exists (zero otherwise)[55]. The degree k_i of a node *i* is the sum of the number of edges that have a connection with this node and is defined as:

$$k_i = \sum_{j \in N} \alpha_{ij} \quad (3)$$

Shortest Path Length (SPL): It is useful to represent all the shortest path lengths of graph G as a matrix *D* in which the entry d_{ij} is the length of the geodesic from node *i* to node *j*. This geodesic shape is preferred for its compact and systematic encapsulation of pairwise distances, facilitating efficient analysis and computations. The SPL between two nodes is the minimum number of edges that must be traversed to get from one node to another in the optimal path, and the maximum value of d_{ij} is called the diameter of the graph. SPL measures the global connectivity and integration between different nodes of a network. Average shortest path length, also known as characteristic path length, is defined as:

$$SPL_i = \frac{1}{N(N-1)} \sum_{i,J \in N, i \neq j} d_{ij} \quad (4)$$

SPL was calculated per subject, per band for further analysis.

Clustering coefficient (CL): The CL is a parameter that assesses the functional segregation of a network. It measures the degree of local interconnectedness of a node by analyzing the properties of its neighbour nodes. It indicates the possibility that the neighbours of one node become neighbours to each other. The number of triangles around node *i* is denoted by the following:

$$t_i = \frac{1}{2}\sum_{j,h\in N} a_{ij}a_{ih}a_{jh} \quad (5)$$

So, the *CL* of the network can be calculated using this formula:

$$CL_i = \frac{1}{n}\sum_{i\in N} \frac{2t_i}{k_i(k_i-1)} \quad (6)$$

Betweenness Centrality (BC): BC is used to investigate the contribution of each node to all other node pairs on the shortest possible path. Thus, the BC of a node measures not only the importance of the nodes but also the amount of information "flowing" through the node.

$$BC_i = \frac{1}{(n-1)(n-2)} \sum_{\substack{h,j\in N \\ h\neq j,\ h\neq i,\ j\neq i}} \frac{\rho_{hj}(i)}{\rho_{hj}} \quad (7)$$

where ρ_{hj} is the number of shortest paths between *h* and *j*, and $\rho_{hj}(i)$ is the number of shortest paths between h and j that pass through node *i*.

Small-World Propensity (SWP): SWP has been proposed to capture the effect of small-world networks (combination of segregation and integration) into a single statistic[99]. Thus, it conveys integrated information on global and local network characteristics. It involves a double ratio: the numerator is the ratio of the average *CL* to that of a random network, and the denominator is the ratio of the average path length to that of a random network. A small-world network shows more clustering than a random network and remains similar in average path length to that of a random network[100]. The following formula is used to calculate SWP:

$$SWP_i = \frac{CL/CL_{rand}}{SPL/SPL_{rand}} \quad (8)$$

where *CL* and CL_{rand} are the clustering coefficients, and *SPL* and SPL_{rand} are the characteristic path lengths of the respective tested network and a random network.

The above graph features (i.e., *BC*, *CL*, *SPL*, and *SWP*) were obtained using the brain connectivity toolbox (http://www.brain-connectivity-toolbox.net) published by *Mikail Rubinov* and *Olaf Sporns* (2010)[60] in MATLAB R2021a. All graph features were computed for each connectivity/coherence matrix in each frequency band for each subject. We used the average of graph features for each frequency band and each subject to make a comparison in these outcomes between males and females (details in **Section 3.8**).

3.6. Signal variability:

Signal variability was assessed using Multiscale Entropy (MSE). The calculation of MSE was performed utilizing the EEGLAB toolbox using MATLAB script[101]. Clean and segmented data imported from BrainVision Analyzer software was used in MATLAB code to calculate MSE for each electrode and each 2s epoch for each participant. Then, the average MSE for all epochs per channel was calculated for each participant. Coarse-grained signals were used for the analysis of signal variability (for details see **Section 1.9**). According to the literature [102,103] , parameters are defined as follows: model length *m* and a tolerance factor *r*, also called similarity factor, which is used to identify a range of similarities between data points. In this study, *m* and *r* were the default MATLAB values [$m = 2$ and $r = 0.2$ * *standard deviation (x)* were used, wherein *x* corresponds to an epoch of length of 2 second of a specific channel]. Output values of the MSE were obtained per participant, per channel, and for each frequency band and used for statistical analyses in MATLAB (outlined in **Section 3.8**)

3.7. Grouping electrodes/regional clusters:

The following regional clusters were created, in keeping with others' work[19,104] and are visually represented in **Figure 6**. We carried out regional analyses to decrease the dimensional space, and use less conservative statistical approaches (i.e., which did not require stringent multiple

comparison adjustments); regional clustering of electrodes allowed for a more direct comparison with previous literature. These clusters are categorized into four main regions: *prefrontal*, *frontocentral*, *central*, and *parietal* regions in both the left and right hemispheres (i.e., eight regions in total). Within the left prefrontal region, the following electrodes were used: Fp_1, AF_3, AF_7, and F_7, while the right region included Fp_2, AF_4, AF_8, and F_8. The frontocentral region encompassed left electrodes F_1, F_3, F_5, FC_1, FC_3, and FC_5; the right region included F_2, F_4, F_6, FC_2, FC_4, and FC_6. The left central region included electrodes C_1, C_3, C_5, FC_1, FC_3, and FC_5, while the right central region included electrodes C_2, C_4, C_6, FC_2, FC_4, and FC_6. Lastly, the left parietal region consisted of electrodes P_1, P_3, P_5, P_7, PO_3, and PO_5, and the right region of electrodes P_2, P_4, P_6, P_8, PO_4, and PO_6.

For coherence analysis, as outlined in **Section 3.4**, three approaches were adopted: 1. Lagged coherence per electrode between the sexes per frequency band (i.e., delta, theta, alpha, beta, gamma); 2. Then, using clustering electrodes we assessed connectivity measures for all eight regions of interest per band. 3. Finally, we assessed twenty-eight connectivity between eight regions per band (outlined in **Section 3.8**).

Graph theory measures (BC, CL, SPL, and SWP) were assessed using different approaches: First, average of *BC* and *CL* for eight lobes per frequency band for each participant were assessed. Second, in order to find hubs in the network, BC for each electrode individually was assessed per frequency band and for each participant. Finally, SPL and SWP, which are global connectivity measures and are meaningful when calculated for the whole brain network, were assessed for each participant's brain connectivity matrix per band.

For analysis of MSE, again we used two approaches: a) MSE for each channel and b) for each region of interest, per band for each participant.

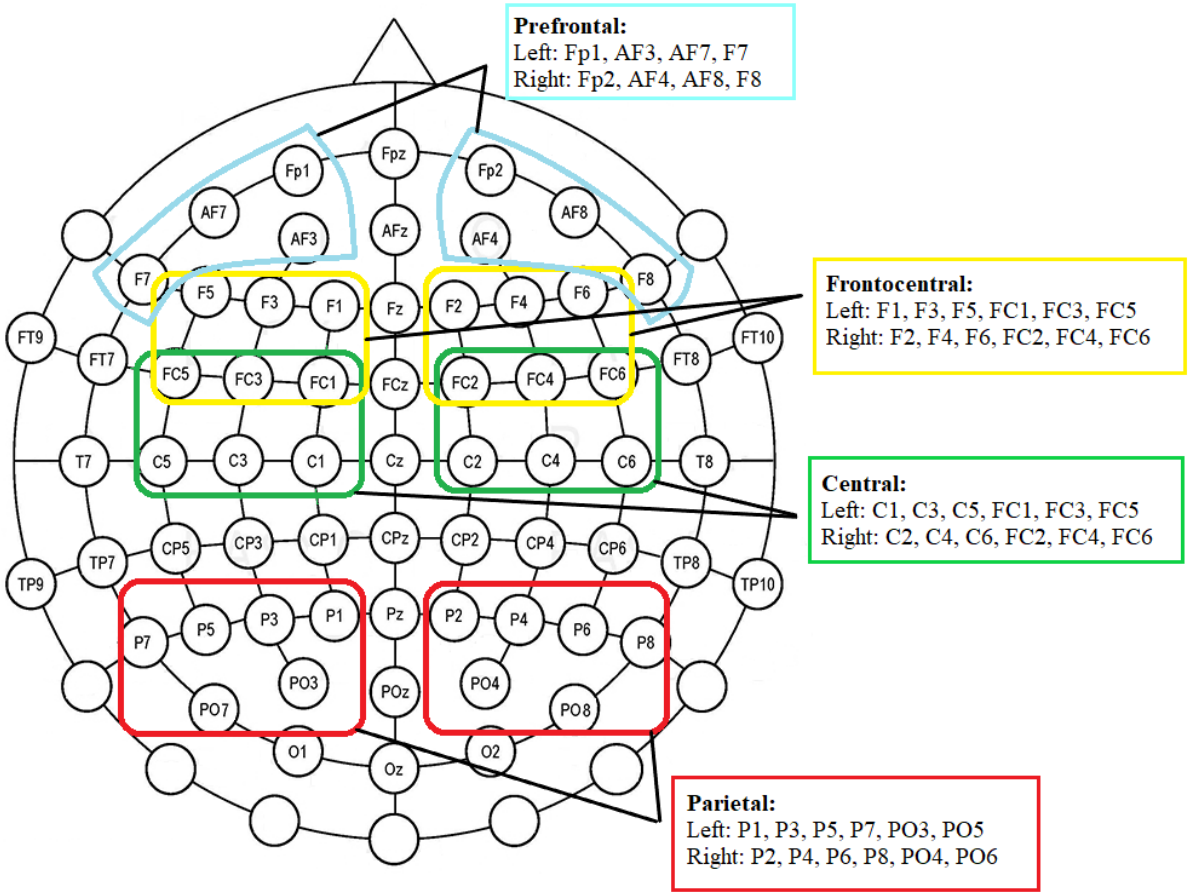

Figure 6. *Electrodes positioning and grouping electrodes for eight regions: Left prefrontal, right prefrontal, left frontal, right frontal, left frontocentral, right frontocentral, left parietal, and right parietal.*

3.8. Statistical analysis:

Participant characteristics & behavioural data:

For the comparison of demographic characteristics (i.e., age and education level) and behavioral data (i.e., age, education, reaction time, and accuracy) between males and females, two-tailed t-tests were applied; significance was $p<.05$. In addition, correlation analyses were carried out by assessing Pearson's correlation (r) between speed and reaction time. Correlation coefficients (r) and p-values are provided in **Section 4.2**. The speed-accuracy trade-off was investigated which refers to the relationship between an individual's willingness to respond slowly and make

comparatively fewer errors compared to their willingness to reply rapidly and make potentially more errors.

Coherence:

Coherence between each channel: First, we assessed between-group differences (i.e., males vs. females) using permutation tests, per each electrode per band (i.e., sixty-three number of tests per band). A non-parametric permutation test was carried out to assess for sex differences in coherence in each EEG channel. It was chosen as it makes no assumptions about data distribution[105]; permutation test was applied using *permtationTest() function* in MATLAB (https://github.com/mickcrosse/PERMUTOOLS) with 10,000 of permutations. Subsequently, a False Discovery Rate (FDR) correction was applied to these analyses, significance level was $p<0.05$.

Coherence within regions of interest: Average coherence for each region per band was assessed between the sexes. For consistency, and due to some normality violations, a Mann-Whitney U test was conducted; significance was $p<0.05$ (with a negative z-value indicating a greater value in females vs. males).

Coherence between regions of interest: Finally, between-sex (i.e., M vs. F) comparisons were carried out in coherence between each pair of the eight regions of interest using a Mann-Whitney U test; significance was again $p<0.05$ (with a negative z-value indicating a greater value in females vs. males). Given the exploratory nature of this analyses, we chose to be liberal with our significance threshold and not apply any corrections for multiple comparisons; granted, this is a limitation (and is outlined as such within the Discussion; **Section 5.3**).

In total, there were 28 possible connections for the region-to-region analyses: left-frontocentral and left-parietal, left-frontocentral and right-parietal, left-prefrontal and left-parietal,

left-prefrontal and right-parietal, right-frontocentral-left-parietal, right-frontocentral and right-parietal, left-central and left-parietal, left-central and right-parietal, right-central and left-parietal, right-central and right-parietal, left-parietal and right-parietal, right-prefrontal and left-parietal, right-prefrontal and right-parietal, left-prefrontal and right-prefrontal, left-prefrontal and left-frontocentral, left-prefrontal and right-frontocentral, left-prefrontal and left-central, left-prefrontal and right-central, left-frontocentral and left-central, left-frontocentral and right-central, right-frontocentral and left-central, right-frontocentral and right-central, right-prefrontal and left-frontocentral, right-prefrontal and right-frontocentral, right-prefrontal and left-central, right-prefrontal and right-central, left-central and right-central).

Graph theory analysis of coherence:

A Mann-Whitney U test was employed to investigate putative sex differences in brain network connectivity graph measures (i.e., *betweenness centrality (BC)*, *clustering coefficient (CL)*, *shortest path length (SPL)*, and *small-world propensity (SWP)*) across the eight predefined regions in each frequency band ($p<0.05$). The *BC* and *CL* for each channel were compared using non-parametric permutation test with FDR correction. Finally Average *SWP* and *SPL* per subject and per band was compared between males and females using Mann-Whitney U test.

All statistical analyses were carried out using MATLAB R2021a, statistical analysis toolbox.

Multiscale Entropy (MSE):

For MSE outcomes, a non-parametric permutation test was used to analyze MSE between males and females for each channel per band ($p<.05_{FDR}$). Finally, a Mann-Whitney U test was used to compare *MSE* per region per band between males and females ($p<.05$).

4. Results

4.1. Participants characteristics:

A total of forty-six participants were recruited and tested (24 males/22 females). However, the data from several participants (N=4) were excluded from the final analyses due to various issues, including participant with very quick responses (<2s), task errors, electrocortical recording issues and the absence of required visuospatial triggers. As a result, data from a total of N=42 volunteers (22 males and 20 females) were used in this study. Participants were all right-handed. The participants age ranged from 18 to 41; details for age and education level (yr) are presented in **Table 1**.

Table 1. *Participants Characteristics*.

	Male (N=22)	**Female (N=20)**	**t-test**
Age (18-41 yr)	25.05 ± 4.41	25.85 ± 6.01	p = 0.68, t = -0.42
Education (12-20 yr)	15.70 ± 1.83	16.35 ± 1.62	p = 0.33, t = -0.98

4.2. Behavioural results:

Behavioural results (i.e., reaction time, accuracy) are presented in **Table 2**. These measures are an average of two non-identical conditions (i.e., “same but rotated” and “different”). There were no sex differences in reaction time (p=0.30) and coefficient of variation (p=0.26). In contrast, the t-test yielded a significant difference (p=0.04, z=2.18) in accuracy rates, with females having a lower accuracy than males. With respect to the speed-accuracy correlation (r), there were no differences between the sexes on the means of the speed-accuracy values. **Figure *7*** illustrates the relationship between speed and accuracy for males and females.

Results

Table 2. *Behavioural data results: Average reaction times (ms) and accuracy (means ± standard deviations [SD]) in males and females. Total number of trials = 56 (28 per condition).*

	Accuracy		Reaction Time (ms)	
	Male	**Female**	**Male**	**Female**
Performance (Mean ± SD)	22.48 ± 3.88 (80.27 %)	19.42 ± 4.97 (69.36 %)	4488.70 ± 1284	4131.11 ± 1385
Coefficient of Variation (%)	17.28 %	25.61 %	18.47 %	22.29 %
Speed-Accuracy Correlation	r = 0.02	r = 0.32		

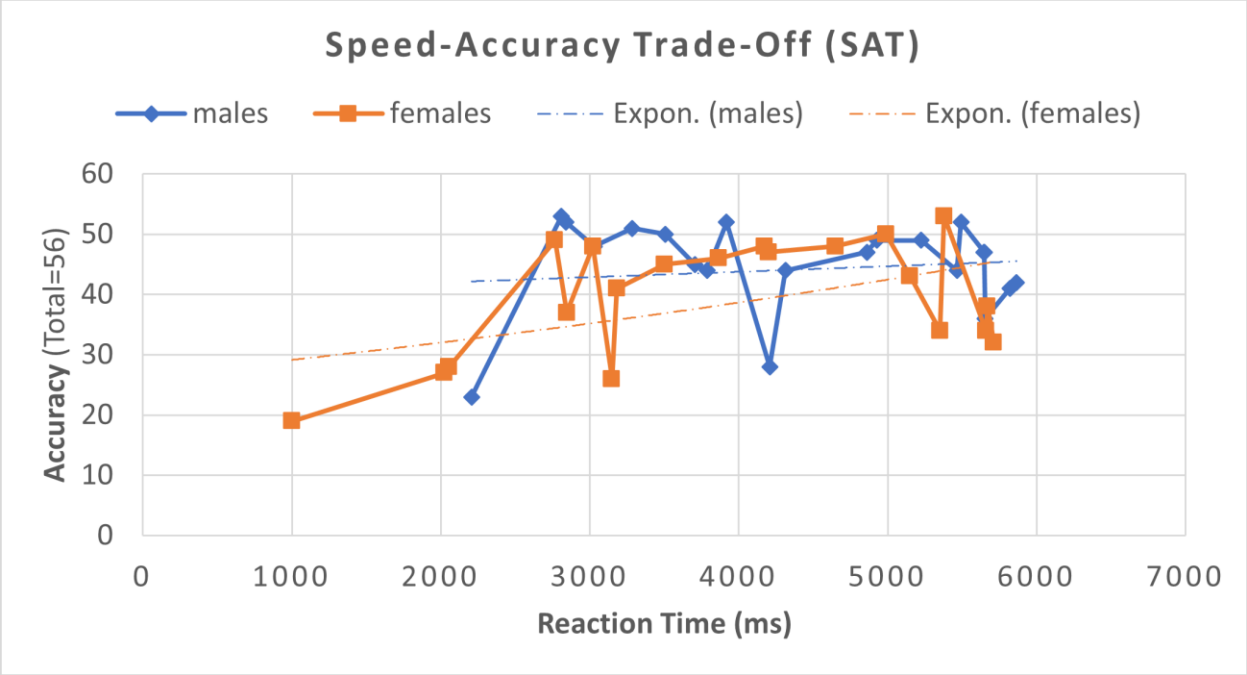

Figure 7. *Speed-accuracy trade-off (SAT) visualization.*

4.3. Lagged-Coherence

Coherence between each channel and all other channels: Sex differences were found in the gamma band in the following channels: Fp1(z=0.81), Fz (z=0.64), P3 (z=0.71), P6 (z=0.71), P8(z=0.70), CP1 (z=0.69), PO4 (z=0.75), PO8 (z=0.87) ; p_{FDR}=0.03 for all electrodes), with males having greater coherence in these channels (i.e., greater coherence between the listed channels and all other channels).

Average regional coherence: Between-sexes (i.e., M vs. F) comparisons were performed in each of the eight regions per band (**Table 3**). Females vs. males exhibited greater coherence in the theta band in the right central region (p=0.01, z=-2.46) as well as in the right prefrontal (p=0.04, z=-2.05) and right frontocentral (p=0.04, z=-2.08) regions. In the gamma band, however, males exhibited higher connectivity than females in the left prefrontal and left parietal regions (p=0.04, z=2.05, and p=0.03, z=2.18, respectively).

Table 3. *Significant lagged coherence differences between males (M) and females (F) within brain regions in specific frequencies.*

Regions	Theta	Beta	Gamma
Left Prefrontal			p = 0.04 z = 2.05, m > f
Left Parietal			p = 0.03 z = 2.18, m > f
Right Prefrontal		p = 0.04 z = -2.05, f > m	
Right Frontocentral		p = 0.04 z = -2.08, f > m	
Right Central	p = 0.01 z = -2.46, f > m		

4.4. Inter- and intra-hemispheric regional coherence:

Inter-hemisphere: Males vs. females exhibited higher coherence between the left prefrontal and right frontocentral region in the alpha band (p=0.03, z=2.13), as well as between the left prefrontal and right central (p=0.02, z=2.33). In the gamma band, males exhibited greater coherence than females between the left prefrontal and right parietal region (p=0.02, z=2.30), as well as between the left and right parietal regions (p=0.03, z=2.13).

Intra-hemisphere: Females vs. males exhibited greater coherence in the beta band between the right prefrontal and right parietal (p=0.03, z=-2.20) regions, and greater beta coherence between right frontocentral and right parietal region (p=0.03, z=-2.15). Furthermore, females vs. males also had higher coherence in the theta band between the right central and right parietal regions (p=0.02, z=-2.35). **Figure *8***. displays connections with significant differences between regions.

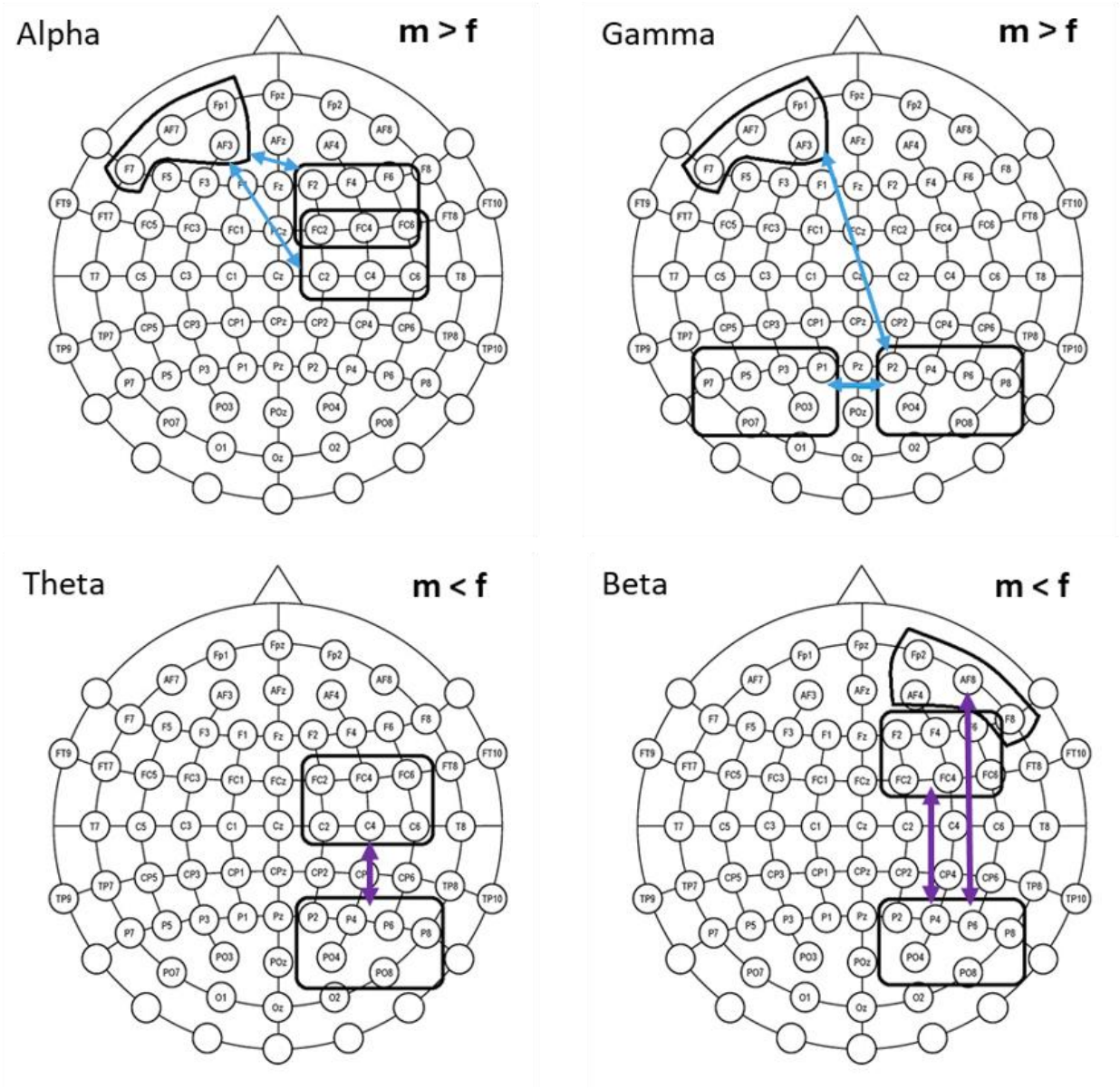

Figure 8. *Significant differences in average lagged coherence between eight brain regions in various frequency bands for males (m) and females (f): Inter- & intra-hemispheric regional connectivity. Males displayed more intra-hemispheric connections in alpha and gamma bands, while females exhibit more inter-hemispheric connections.*

Table 4. *Differences between males (M) and females (F) in average lagged coherence between eight brain regions across frequency bands: Inter- and intra-hemispheric connectivity.*

Connections		**Lagged Coherence differences (across regions)**			
Region 1	**Region 2**	**Theta**	**Alpha**	**Beta**	**Gamma**
L-prefrontal	**R-frontocentral**		p = 0.03 z = 2.13 m > f		
L-prefrontal	**R-central**		p = 0.02 z = 2.33 m > f		
L-prefrontal	**R-parietal**				p = 0.02 z = 2.30 m > f
L-parietal	**R-parietal**				p = 0.03 z = 2.13 m > f
R-prefrontal	**R-parietal**			p = 0.03 z = -2.20 f > m	
R-frontocentral	**R-parietal**			p = 0.03 z = -2.15 f > m	
R-central	**R-parietal**	p = 0.02 z = -2.35 f > m			

4.5. Graph theory measures:

In the left prefrontal region, *BC* analyses revealed that females vs. males exhibited higher centrality (p=0.04, z=-2.02) in the alpha band, whereas in the right parietal region, males vs. females had higher *BC* (p=0.03, z=2.23) in the theta band.

Regarding the *CL*, in the theta band, greater *CL* values in the left prefrontal region were noted in males vs. females (p=0.01, z=2.46). In the beta band, in the right prefrontal region, females vs. males displayed a higher *CL* (p=0.045, z=-2.00).

No significant difference was found for *SWP* and *SPL* between males and females.

Table 5. *Differences between males (M) and females (F) in graph measures: Significant differences in betweenness centrality (BC) and clustering coefficient (CL) across eight brain regions.*

Betweenness Centrality (BC)

Regions	Theta	Alpha
Left prefrontal		p=0.04, z=-2.02, f > m
Right parietal	p=0.03, z=2.23, m > f	

Clustering Coefficient (CL)

Regions	Theta	Beta
Left prefrontal	p = 0.01, z = 2.46, m > f	
Right prefrontal		p = 0.045, z = -2.00, f > m

4.6. Multiscale Entropy (MSE)

MSE for channels per bands: As noted in **Table 6,** females vs. males exhibited greater MSE values across various channels and across various bands ($p_{FDR} < 0.05$), though most results were in frontal.

Table 6. *Significant p-values after FDR correction between males (M) and females (F) for each channel in multiscale entropy (MSE) per frequency band.*

Corrected p-values after FDR (f > m in all channels and bands)					
Channels	**Delta**	**Theta**	**Alpha**	**Beta**	**Gamma**
Fp1	0.02	0.01	0.03	0.04	0.02
Fp2	0.02	0.01	0.03	0.04	0.02
Fpz	0.03	0.01	0.03	0.03	0.02
F1	0.03	0.02	0.04		0.03
F2	0.03	0.02	0.04		0.03
F3	0.03	0.02	0.04		0.03
F4	0.03	0.02	0.03		0.03
F5	0.03	0.02	0.04		0.03
F6	0.03	0.01	0.04		0.03
AF7	0.03	0.02	0.04		0.03
AF8	0.03	0.02	0.04		0.03
FC1	0.03	0.02	0.04		0.03
FC3	0.02	0.01	0.03	0.04	0.02
FC4	0.03	0.006	0.03		0.02
FC6	0.04	0.02			0.04
CP4					0.002
C4	0.03	0.02			0.04
Cz	0.03	0.02	0.04		0.04

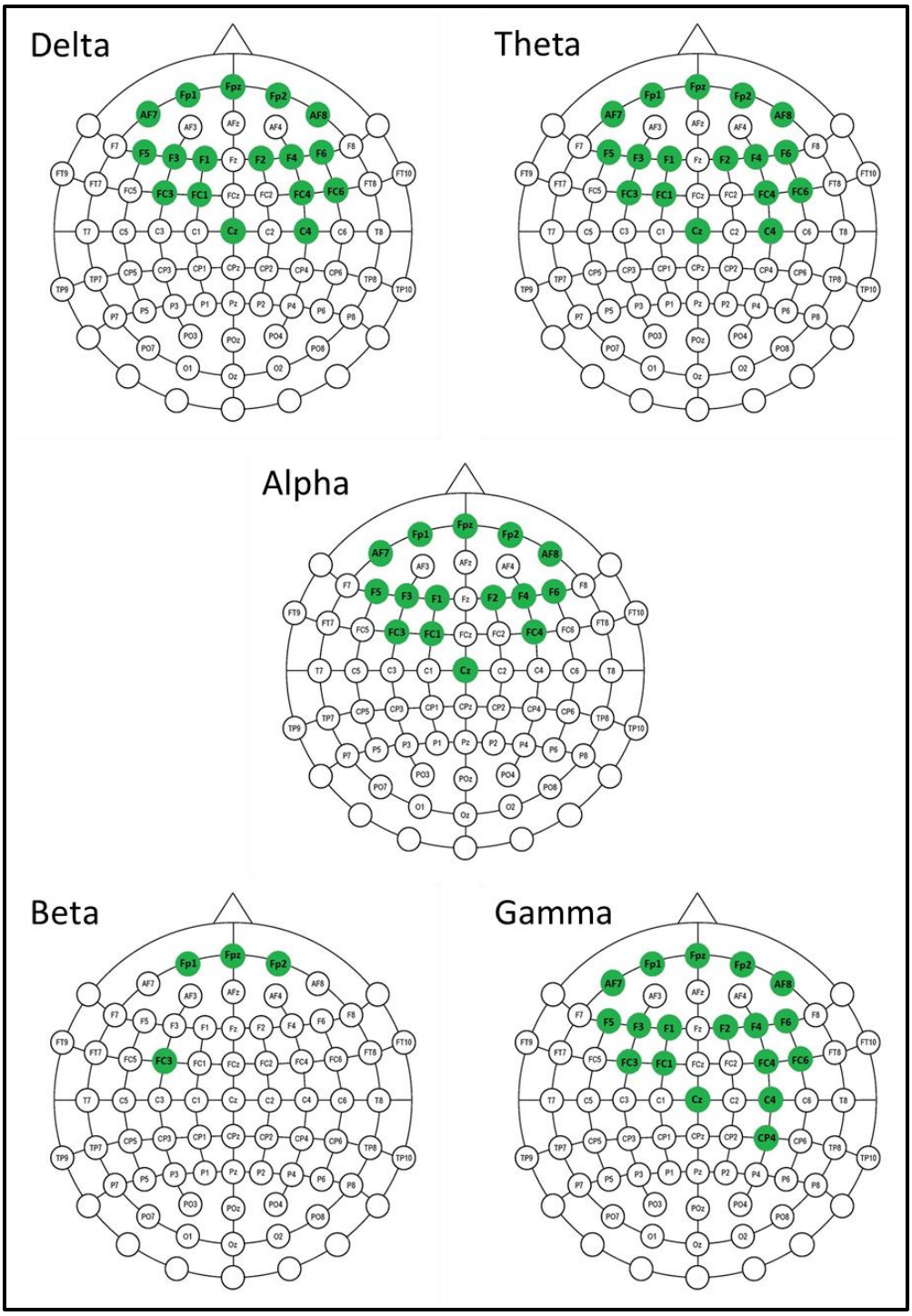

Figure 9. *Electrodes with significant differences highlighted in green between males (M) and females (F) in multiscale entropy (MSE) per frequency band (FDR corrected p-values). Females displayed higher MSE in all highlighted electrodes in all frequency bands.*

Results

Multiscale entropy (MSE) across brain regions: In the delta band, females vs. males exhibited higher MSE values in the left prefrontal ($p<0.05$), left frontocentral ($p<0.05$), and right prefrontal regions ($p<0.01$). Similarly, in the theta band, females displayed elevated MSE in the left prefrontal ($p<0.05$), left frontocentral ($p<0.05$), right prefrontal ($p<0.01$), and right frontocentral regions ($p<0.05$). In alpha, females vs. males showed increased MSE in the left prefrontal ($p<0.05$), left frontocentral ($p<0.05$), right prefrontal ($p<0.05$), and right frontocentral regions ($p<0.05$). For beta, females vs. males had higher MSE values in the left prefrontal ($p<0.05$), left frontocentral ($p<0.05$), and right prefrontal regions ($p<0.01$). Last, in the gamma band, females vs. males exhibited greater MSE values in the left prefrontal ($p<0.05$), left frontocentral ($p<0.05$), and right prefrontal regions ($p<0.05$).

Table 7. *Differences in mutiscale entropy (MSE) between males (M) and females (F) in various brain regions and frequency bands*

Multiscale Entropy (MSE) (f > m in all regions and bands)					
	Delta	**Theta**	**Alpha**	**Beta**	**Gamma**
Left Prefrontal	p = 0.004 z = -2.88	p = 0.004 z = -2.91	p = 0.005 z = -2.78	p = 0.005 z = -2.78	p = 0.008 z = -2.66
Left Frontocentral	p = 0.01 z = -2.48	p = 0.02 z = -2.41	p = 0.01 z = -2.48	p = 0.02 z = -2.30	p = 0.02 z = -2.35
Left Central	p = 0.03 z = -2.20	p = 0.04 z = -2.05	p = 0.04 z = -2.08		
Right Prefrontal	p = 0.006 z = -2.76	p = 0.006 z = -2.76	p = 0.007 z = -2.68	p = 0.010 z = -2.58	p = 0.009 z = -2.61
Right Frontocentral	p = 0.01 z = -2.56	p = 0.01 z = -2.53	p = 0.01 z = -2.56	p = 0.02 z = -2.43	p = 0.02 z = -2.41
Right Central	p = 0.01 z = -2.56	p = 0.01 z = -2.53	p = 0.01 z = -2.51	p = 0.02 z = -2.35	p = 0.02 z = -2.25

5. Discussion

5.1. Summary:

The overarching aim of this work was to assess EEG-indexed neural features in males and females during a mental rotation task; focus was on comparing the sexes on coherence (analyzed using conventional methods and complimentary graph theory approaches) as well as signal variability via MSE. These EEG-indexed measures go beyond 'traditional' measures of EEG, such as power, to gain insight into brain processes, including neural network features and neural 'flexibility'.

Briefly, and expectedly, we found that males outperformed females in task accuracy on the mental rotation task. With respect to coherence, males had higher gamma coherence than females in frontal and posterior/parietal channels, (i.e., individual channel's coherence with all other channels/electrodes). Complementing the individual channel results, males also exhibited greater coherence *within* left prefrontal and parietal regions in the gamma band. Furthermore, males vs. females had increased *inter-hemispheric* coherence between various regions in the gamma and alpha bands, contrary to our expectations. Females, on the other hand, exhibited greater coherence than males within the right frontal and central regions in the theta band. Also contrary to our hypotheses, females were found to have higher *intra-hemispheric* coherence between regions in the right hemisphere for both beta and theta bands.

When assessing the exploratory and complementary graph theory measures, higher betweenness centrality (BC) in the alpha band was noted in the left prefrontal region in females and greater BC in the theta band existed in the right parietal region for males. Further, males exhibited increased theta clustering coefficient (CL) in the left prefrontal region, while females showed higher beta CL in the right prefrontal region.

Finally, MSE analysis indicated heightened entropy, particularly in the gamma band, across predominantly frontal and some central regions in females; this was also somewhat inconsistent with our hypotheses.

The above results, their implications and contextualization within the literature are discussed below.

5.2. Sex difference in performance:

The observed performance data aligns with established literature indicating a male advantage in mental rotation tasks, where males typically exhibit faster reaction times and higher accuracy compared to females[11,21,106,107]. This sex difference in visuospatial ability has been extensively documented and attributed to various factors, including differences in neural processing strategies, hormonal influences, and socio-cultural factors[11,107]. While no significant differences in reaction time were observed in the current study, the potential nuances in speed and accuracy warrant consideration. The observation of males being slower, although not statistically significant, raises questions about a potential speed/accuracy trade-off favoring males. This might be associated with the adoption of different strategies by males and females during the mental rotation task, perhaps impacting the observed behavioural outcome patterns. The literature suggests that apparent holistic processing, which refers to the tendency to perceive and process objects as wholes rather than in parts, in mental rotation tasks can be attributed to trade-offs between speed and accuracy, a phenomenon often observed in cognitive processing[108]. Thus, apparent holistic processing might underlie the observed sex differences we observed in the behavioural results and the coherence and MSE data discussed below.

5.3. Sex differences in coherence and coherence lateralization:

The observed greater coherence in frontal and particularly parietal channels/electrodes (Fp1, Fz, P3, P6, P8, CP1, PO4, PO8) within the gamma band for males aligns partially with our initial hypothesis and is in keeping with some existing literature[109,110]. Previous studies have linked higher frequencies, such as gamma band activity, to complex cognitive processes, including mental rotation[33]. This finding suggests that the enhanced coherence in these electrodes (and all other electrodes) in males may be related to their potentially superior performance in mental rotation tasks (in our case, as exemplified by greater accuracy), supporting the notion that gamma band activity plays a role in the neural mechanisms underlying visuospatial processing – especially during the initial stages (i.e., pre-response). The individual channel data is complimented by the regional data wherein males exhibited greater coherence in the gamma band in the left prefrontal and left parietal regions. This is in keeping with some existing literature [37,85,111]. During the mental rotation task, key regions that are engaged (as revealed by neuroimaging) are frontal, frontocentral, and parietal[15,54,110,112,113], thus, while the source-localization of surface/scalp electrodes is suboptimal, it seems that we are observing engagement of comparable regions (at least with respect to gamma oscillations) in males. This might reflect greater regional resource allocation to "solving" the rotation problem and thus maintaining greater accuracy. Surprisingly, the results were noted in the left (versus right) prefrontal and parietal regions; after all, visuospatial processing tends to be right brain lateralized[114,115].

These lateralization findings, i.e., greater right vs. left gamma, in prefrontal and parietal regions in males are surprising considering the often reported right-brain lateralization observed in visuospatial processing, including in mental rotation[114,115]. The results suggest a need for a nuanced understanding of hemispheric involvement in visuospatial processing and may indicate

unique neural strategies employed by individuals in the studied context. After all, studies emphasize that the selection of strategies during a mental rotation task can play a crucial role[11,109]; it is feasible that gamma activity might be involved in this. Finally, most of the lateralization literature during visuospatial processing is centered on activity as measured by neuroimaging or blood flow measures [11,21,109]. EEG results and fMRI BOLD signal findings may not always be directly comparable due to differences in their temporal and spatial resolutions, as well as the distinct neurophysiological processes they capture. Therefore, assessment of coherence in specific frequencies might not be comparable to traditional measures of "activation (i.e., blood oxygen demands, or glucose demands) in a linear fashion.

The observed greater theta coherence *within* the right prefrontal, central, and frontocentral regions in females compared to males is also noteworthy. Theta oscillations (4 - 8 Hz) are known to be associated with stimulus perception in visual tasks and are also known to play a role in memory processes[116,117]. Additionally, theta band activity has been implicated in motor imagery and navigation processing in the brain mainly in central and frontocentral regions[118,119]. In light of the increased gamma activity noted in males (in left prefrontal and parietal regions), this increased theta in females in right regions might reflect different neural processes that are engaged during the initial stages of the processing of the visual stimuli and solving the rotation task. It is feasible that this might underscore different strategies that are used in the mental rotation task, such as wholistic processing. Source-localization of the cortical generators, in future comparable studies, might provide greater clarity. Since -in the current study- the analyses of the sex differences in coherence occurred in the early stages of stimulus processing and cognitive operations (i.e., before a response) the differences in theta and gamma reflect mental operations that are not linked directly with a response/error.

5.4. Intra-hemispheric connectivity

With respect to within hemisphere between-region coherence (*intra-hemispheric between region coherence*), females had greater intra-hemisphere connectivity in the beta band across various regions in the right lobe. Specifically, females exhibited greater beta coherence between the right pre-frontal/frontal and parietal regions. The frontal and parietal regions, as outlined above, are implicated in visuospatial processing; their connections are known to be important in/support visuospatial operations[37,111,120]. As such, increased coherence between pre/frontal and parietal regions in females might reflect more active engagement of the frontoparietal network in conducting the task in the right hemisphere to maintain task performance. Second, increased coherence in the theta band between right central and parietal regions in females vs. males might reflect the involvement of the motor cortex (which has a more central localization); granted source-localization was not carried out, thus, this interpretation is somewhat speculative. Nevertheless, this could suggest an integration of motor-related imagery and spatial perception during the task that is more dominant in females. Males did not have increased coherence within either of the hemisphere in any of the bands compared to females. Previous behavioural work has suggested that the right hemisphere advantage in a mental rotation task shifted to the left hemisphere across blocks of trials[121]. While we did not assess whether there were differences across trials in lateralization, this is certainly a worthy future direction. Further, the lack of lateralization effects that we observed in males could suggest that any lateralization effects might have been "washed out" as a result of averaging (i.e., there might have been more lateralization initially and lateralization might have shifted). Granted, this is speculative and warrants further investigation.

5.5. Inter-hemispheric connectivity

Males vs. females exhibited greater *inter-hemispheric* coherence between various regions in the alpha and gamma bands. Specifically, males exhibited greater coherence between left-prefrontal and right-frontocentral/central regions in the alpha band. Additionally, greater gamma coherence between left-prefrontal and right-parietal regions was noted in males vs. females, as was greater inter-hemispheric gamma coherence in the parietal region in males vs. females. On the other hand, females exhibited no increased inter-hemispheric regional coherence relative to males in any of the bands. In some ways, the fact that females did not exhibit more asymmetry than males is somewhat consistent with literature indicating that females tend to engage both hemispheres in the context of visuospatial processing[21,109].

Increased coherence between the prefrontal regions of the two hemispheres in alpha in males might reflect greater synchronization of frontal regions in males, which are known to be function implicated in executive[122]. The increased alpha coherence between left-prefrontal and right central regions might be involved in motor preparedness/response; however, this is speculative. Since all our participants were right-handed this might reflect coherence that results in "disinhibiting" the left motor region. Alpha tends to be inversely associated with cortical activation [123,124], however, other data shows its importance in visuospatial attention[125]. Greater gamma coherence between the left prefrontal and right parietal regions in males might again reflect a more pronounced asymmetry in males in the visuospatial processing network across the brain. Finally, greater inter-hemispheric coherence in males between the two parietal regions might be an important facet in supporting more optimal performance (at least as measured by eventual accuracy) during the task in males vs. females. In general, the above coherence data -analyzed with relatively conventional approaches- suggest that males predominantly differed from females in the

gamma band, which might play an important role in underscoring sex-specific differences in mental rotation tasks.

5.6. Sex differences and graph theory measures:

The graph theory measures were conducted in order complement the above coherence data. The observed increase in betweenness centrality (BC) in the left prefrontal region in the alpha band in females suggests enhanced influence of this region in the broader network, at least within the alpha band, during the mental rotation. Given evidence that alpha tends to be inversely related to cortical activity[124], this might be reflective of -perhaps- an "inefficiency" of this regional hub. On the other hand, alpha has also been related to attention regulation and modulation of task-irrelevant information[123], thus, alternate explanations are possible. For instance, the left prefrontal region in females might reflect that it has a more central role in coordinating information related to visuospatial processing. We also noted heightened theta-band BC in the right parietal region in males vs. females, which again might suggest increased influence of this region in facilitating communication within the network during mental rotation in males. This might also signify a specialization of the right parietal region in spatial processing tasks for males; this information did not emerge assessing more conventional analyses. Theta band activity has been associated with allocation of attentional resources in visuospatial attention tasks[126].

Finally, the sexes exhibited some contrasting clustering coefficient (CL) patterns, with males exhibiting heightened theta-band CL in the left prefrontal region while females showed elevated beta-band CL in the right prefrontal region, suggesting distinct network integration strategies subserved by different oscillatory features between the sexes. While the interpretation of these results is somewhat nebulous, they underscore the potential utility in assessing coherence

using graph theory measures. Specifically, it appears that the prefrontal cortex plays a key role in the integration of activity, but asymmetry and different band contributions might be important differences in males vs. females underscoring early mental rotation processes. Granted, these graph theory analyses were exploratory and complementary, as such, they certainly warrant replication in an independent and larger samples size. Nevertheless, the measures of BC and the CL might be especially useful for assessing specific regional contributions (in terms of network integration) within the brain network during the mental rotation task. Conversely, small-world propensity (SWP) and shortest path length (SPL) provide a broader perspective, offering insights into the brain network's global organization and functioning across the entire brain. Thus, such measures should be considered in comparable future work.

5.7. Sex differences and signal variability:

Similar to the existing literature, which indicates that females generally exhibit higher multiscale entropy (MSE) than males across all frequency bands during rest[69], our study revealed a similar pattern during the early stages of the mental rotation task. However, we anticipated observing higher MSE in males during the task. After all, greater EEG multiscale entropy or signal variability has been related to better performance[71,127]; given that males tend to exhibit superior performance on mental rotation tasks, on average, greater signal variability in males was expected. In short, increased variability is generally thought to be reflective of more adaptability within brain functioning during cognitive tasks.

When examining the increased MSE in females for the per electrode data, predominantly frontal regions were involved; the increase was evident across most of the bands, with the exception of beta. The results were similar when assessing the regional data (predominantly frontal

regions). The increased MSE observed predominantly in frontal regions, known for their role in cognition, suggests a potential link between neuronal flexibility and cognitive processing, which is a key component in mental rotation. This pattern might reflect greater variability supporting maximal flexibility in neural processes during the early stages of the mental rotation task in females. It is also certainly feasible that variability may have decreased during the later stages of the task (i.e., immediately prior to response selection), and perhaps males would have shown some increases in variability relative to females at later time points during the mental rotation task. Granted this is speculative and should be tested in future comparable work. Notably, the relative consistency in beta band MSE between sexes (i.e., it was one of the few bands that did not exhibit increases in females vs. males) suggests that beta band variability might be a common feature in supporting visuospatial processing, regardless of sex. However, it appears that females show increase variability rather consistently in frontal regions regardless of the oscillatory frequency in the early stages of mental rotation processing.

6. Implications and Future Directions:

Understanding sex-related differences in mental rotation tasks has implications for various fields, including education, cognitive psychology, and clinical neuroscience. After all, these findings may guide the development of personalized interventions and educational strategies that account for individual cognitive profiles, including differences that might exist between the sexes. For instance, if our finding of greater MSE in females holds true with replication work, thus reflecting greater neuronal variability/brain 'flexibility' in certain brain regions during mental rotation tasks compared to males, educational strategies -particularly those involving visuospatial processing- could be tailored to accommodate further repetition. It is feasible that more repetition/practice might be necessary to stabilize neuronal features/entrain the pattern and decrease variability. In other words, while variability is generally viewed as adaptive in learning, it might be beneficial to minimize neuronal variability though additional practice/repetition for greater 'automatization' of certain behaviour. Conversely, perhaps a more "variable" brain is more adaptive and the incorporation of different or more diverse learning strategies for females might be advantageous. While this is all speculative, ultimately, understanding sex-related differences in mental rotation, and cognitive processing, more generally could inform the development of targeted interventions for conditions that exhibit sex-specific patterns, ultimately leading to more personalized and efficient therapeutic approaches in healthcare.

While this study is among the first (to our knowledge) to assess EEG-indexed functional connectivity (FC) and signal variability during a 3D object mental rotation task using EEG, and in incorporating graph theoretical approaches in the coherence analyses, certain limitations exist and should be considered in future comparable work. First, we did not consider *what* (if any) specific strategies were employed by participants in solving the mental rotation task. It is plausible that the

above-mentioned variations in coherence and MSE between the sexes might be underscored by differences in strategy utilization, highlighting the need for a more comprehensive exploration of cognitive approaches in future work. Second, the study would benefit from a more nuanced examination of gender identity, extending beyond a binary framework. Previous work has suggested that gender identity plays a role in cognitive processes, including in mental rotation[11,128,129]. Thus, it would be intriguing to assess whether gender versus sex yields comparable results in the context of mental rotation tasks. Third, combining EEG with fMRI in future research holds promise. This was indeed the goal of the broader study in which this work was embedded in. This approach would, and hopefully will, offer enhanced spatial and temporal insights into the neural underpinnings of mental rotation tasks. In other words, combined EEG+fMRI might provide source-localization information to elucidate some of the findings, although it poses challenges related to coherence and MSE analysis. Fourth, a critical aspect to investigate in future studies is the role of sex hormones in relation to the observed neural profiles. Indeed, understanding how different hormone levels, irrespective of sex, are associated with specific neural features aligns with the broader context of the study in which this thesis work was embedded. Fifth, future studies might explore the relation between individual performance variations and corresponding brain features during mental rotation tasks. Analyzing differential responders categorized as "fast" and "slow" could reveal connections between response speed, entropy levels, and distinct connectivity profiles. In a similar vein -and more pertinent to the current work- it would be worthwhile assessing whether neuronal differences might differ throughout the mental rotation processing window. Our analyses centered on the pre-response window; however, it is feasible that there might have been shifts that occurred immediately before or after a response. In other words, assessing temporal dynamics at the beginning and end of the

task could uncover differential lateralization switching between males and females, providing a more comprehensive understanding of sex-specific neural processes during mental rotation. Finally, there are some methodological limitations to the presented work. Most notably, the sample size in the current study was relatively modest when considering the rather extensive statistical assessments. Relatedly, our decision to adopt a lenient significance threshold without correction for multiple comparisons, while reflective of the exploratory nature of the analyses, introduces a potential source of bias and might limit power. In the future, employing more stringent statistical approaches in a larger sample size would enhance the robustness and generalizability of the findings. Nevertheless, the outlined work lays a strong foundation for future EEG-based assessments of coherence and variability in the exploration of sex differences.

7. Conclusion:

In conclusion, this study represents a thorough exploration into sex differences in functional connectivity, as assessed using coherence, and signal variability during a 3D object mental rotation task, incorporating relatively novel methodologies such as multiscale entropy (MSE) and coherence, alongside graph theory analysis. Our data suggest that the contribution of gamma coherence is more pronounced in males in the early stages of visuospatial processing. Inter-hemispheric differences in coherence were dominant in males, speaking to lateralization effects/different involvement of the hemispheres (vs. whole brain) between the sexes. Graph theory measures uncovered aspects that were not readily evident using conventional analytic techniques of coherence speaking to the utility of graph theory measures in uncovering certain nuances in network features. Finally, MSE analyses demonstrated that females generally had higher MSE values across most of the bands (except for only minor differences in beta), particularly in frontal regions. As such, this suggests that females have greater frontal neuronal variability supporting the early stages of a mental rotation task. Many of these results and putative interpretations are novel. Thus, in conclusion, the combination of diverse analyses as conducted herein provides a nuanced understanding of the neural processes involved in mental rotation in males vs. females. This work lays the foundation for further research and potential applications in education and clinical settings when considering different learning and problem-solving strategies between the sexes.

Appendices

Appendix A

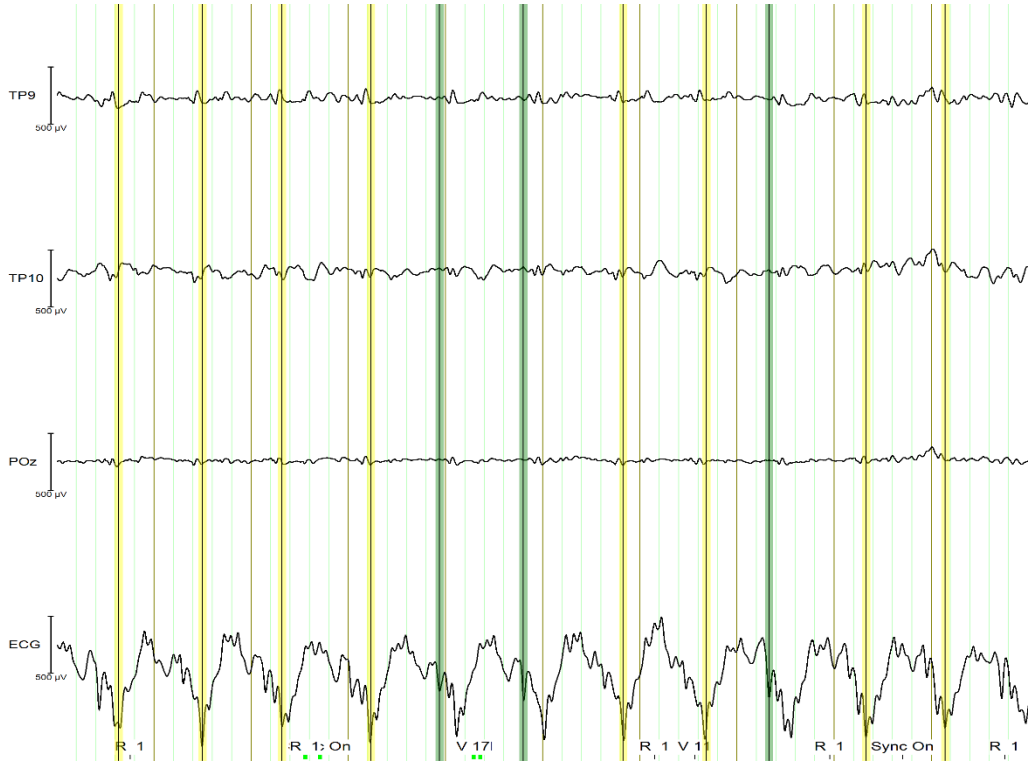

Figure 10. *Pulse artifact detection. Green lines are the automatic peak detection inherent to the BrainVision software, and yellow lines are manual adjustments. After peak detection, cardiobalistic correction based on the peak markers will be applied.*

Appendices

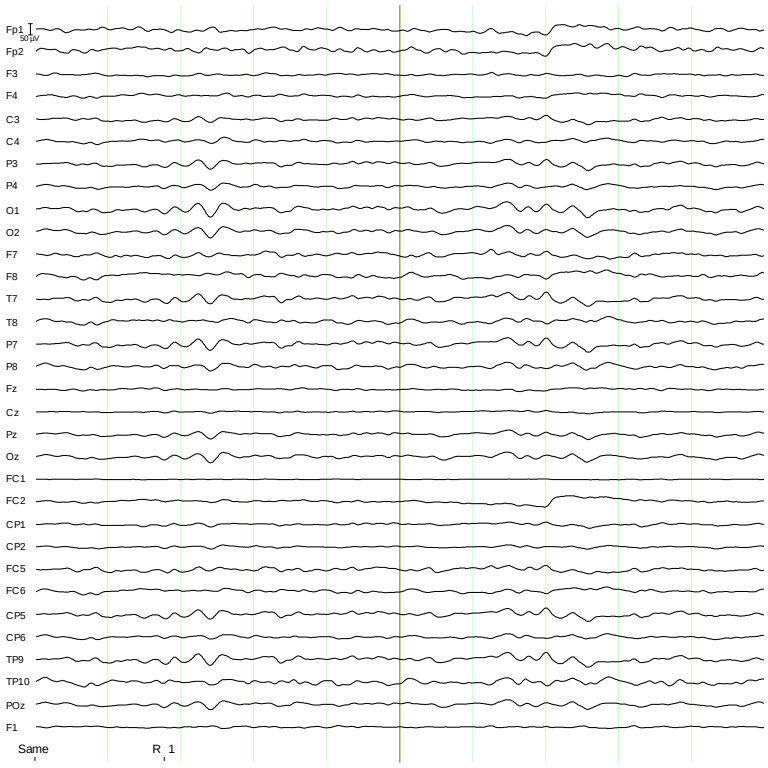

Figure 11. *Exemplary cleaned up EEG traces of 32 channels (as an example) for a 2-second epoch for one participant.*

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