Neurometabolites in the dlPFC and mPFC during Sleep

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This project represents not just my work, but the work and support of everyone who helped me achieve this goal. Thank you to everyone involved.

Abstract

Introduction: The medial prefrontal cortex (mPFC) and dorsolateral prefrontal cortex (dlPFC) are critical regions involved in cognitive and emotional regulation, yet their exact roles in sleep are not well understood. Given the First Night Effect (FNE), a phenomenon where sleep is disrupted in a novel environment, this study investigates how neurometabolite levels in the dlPFC and mPFC change during sleep and whether these regions differ in their involvement in sleep regulation.

Methods: Two separate groups were analyzed for this study. The dlPFC group contained 14 participants in total, however, the amount of participants included in the data varied based on outliers. Similarly, the mPFC group contained 20 participants, however, outliers were excluded, with the participant being excluded varying. Participants completed two separate afternoon 90-minute nap sessions spaced one week apart. Magnetic resonance spectroscopy (MRS) was used to measure Glx (glutamine/glutamate) and GABA concentrations in the dlPFC and mPFC, while polysomnography (PSG) monitored sleep stages.

Results: mPFC Glx levels significantly decreased from Day 1 to Day 2 while dlPFC Glx and GABA levels did not significantly change from wakefulness or differ between days. Higher mPFC Glx was associated with longer SOL, while greater dlPFC GABA was correlated with shorter SOL. There was a significant difference in Glx levels between the dlPFC and mPFC on Day 2. Last, age was positively correlated with dlPFC Glx levels.

Conclusions: Results indicate that the mPFC and dlPFC play distinct roles in sleep, with their functions diverging overtime. While the mPFC appears to regulate excitatory activity in the transition to sleep and may contribute to wakefulness and delayed sleep onset until reaching NREM sleep, the dlPFC's inhibitory function may facilitate sleep initiation.

Introduction

The medial prefrontal cortex (mPFC) and dorsolateral prefrontal cortex (dlPFC) are critical regions in the prefrontal cortex (PFC), involved in emotional and cognitive regulation, yet little is known about their role in sleep. While there is some evidence that the prefrontal cortex is involved in sleep, there is minimal, if any, evidence for the dlPFC and mPFC being involved in sleep-wake processes (Mashour et al., 2022; Muzur et al., 2002). In sleep disorders, it has been shown that there are disruptions in the balance of inhibitory (GABA) and excitatory (glutamate/glutamine, Glx) neurotransmitters, particularly in insomnia (Plante et al., 2012; Winkelman et al., 2008). Additionally, one study showed that ligand-gated ion channels, which regulate excitatory and inhibitory neurotransmission, play a critical role in the sleep-wake transition (Arnold et al., 2023). By investigating neurometabolic changes in the brain, we may be able to determine the neurobiological basis of sleep disturbances and the role of the prefrontal cortex, if any.

Prefrontal Cortex and Sleep Regulation

The PFC is thought to play a role in modulating arousal, executive functioning, and emotional regulation during sleep, and the mPFC and dlPFC may be involved (Mashour et al., 2022). Functional neuroimaging studies have revealed that in older adults, prefrontal activity declines significantly during non-rapid eye movement (NREM) sleep, which is a stage in sleep thought to facilitate memory consolidation (Mander et al., 2013). Individuals with insomnia exhibit increased overall brain metabolism during sleep, which suggests that normal arousal mechanisms aren’t declining (Nofzinger, 2004). While insomnia is linked to hyperarousal, the prefrontal cortex specifically shows reduced metabolism during wakefulness in insomniacs,

possibly contributing to daytime fatigue and cognitive impairments (Nofzinger, 2004). These findings indicate that the PFC may be important in regulating the transition between wakefulness and sleep. Additionally, individuals with habitual ‘sleep credit’ tend to show greater gray matter volume in the mPFC, which is associated with higher emotional intelligence and better mental health (Weber et al., 2013). Neurometabolic characteristics of this region may contribute to sleep-related cognitive and emotional regulation.

The dlPFC is largely involved in executive control, attention, and working memory, and exhibits enhanced activity during REM sleep relative to the prior stage 2 sleep, according to one study (Kubota et al., 2011). This finding suggests that, rather than being entirely inactive during REM sleep, the dlPFC might continue supporting certain cognitive processes, though its exact role is still unclear. Similarly, in primate models, the mPFC– which integrates emotional and cognitive processing– has been shown to have increased neuronal firing during rest and sleep (Gabbott & Rolls, 2013). These findings indicate that the mPFC may remain active during sleep to support emotional and cognitive processes while the dlPFC might contribute to select cognitive functions during REM sleep rather than completely disengaging. Furthermore, when stimulated, the left dlPFC has been shown to be involved in conflict control during insomnia, meaning that it helps manage competing thoughts and emotions that are inhibiting an easy transition to sleep (Ding et al., 2024). The fact that this region is underactive in those experiencing insomnia, contributing to difficulties in managing cognitive conflicts and maintaining sleep quality, implies that the dlPFC may be involved in aiding the transition sleep.

One study found that unrefreshing sleep is correlated with deficits in the mPFC in patients with chronic fatigue syndrome (Shan et al., 2017). This finding suggests that the mPFC plays a critical role in sleep quality and restoration, and disruptions in this region may contribute

to impaired sleep maintenance and unrefreshing sleep. Furthermore, the dlPFC is thought to be involved in regulating whether an emotion is seen as positive or negative, and as such, may contribute to sleep-related arousal states (Nejati et al., 2021).

Neurometabolite Profiles in the mPFC and dlPFC

Changes in GABAergic and glutamatergic neurotransmission in the PFC have been linked to sleep disturbances, highlighting the importance of studying neurometabolite concentrations in the brain during sleep. The primary inhibitory neurotransmitter in the brain, GABA, is necessary for suppressing wake-promoting activity and facilitating sleep onset (Plante et al., 2012; Zhu et al., 2024). Studies have reported significantly reduced GABA levels in individuals with panic disorder, particularly in the anterior cingulate cortex and basal ganglia (Ham et al., 2007). This finding may also be reflected in insomnia, where patients are often too anxious and aroused to fall asleep. Along these lines, a proton magnetic resonance spectroscopy (MRS) study found that older adults exhibiting sleep-disordered breathing show GABA deficits in the dlPFC (Pereira et al., 2017). This reduction in GABA may suggest that there is a diminished inhibitory tone in the dlPFC, possibly contributing to cognitive impairments associated with sleep-disordered breathing and interfering with sleep-dependent memory processes.

In addition to inhibitory signals from GABA, excitatory neurotransmission also plays a critical role in sleep regulation. One study showed that increased glutamatergic activity in the thalamus is associated with disrupted sleep in individuals with restless legs syndrome (Allen et al. 2013). This finding reinforces the importance of maintaining excitatory-inhibitory balance for proper sleep, and shows that slight changes in excitatory neurotransmission can impact sleep

structure. Glutamine/glutamate (Glx) concentrations, representing excitatory neurotransmission, exhibit diurnal fluctuations, with notable decreases during sleep and increases during wakefulness (Yoon et al., 2024). This pattern suggests that Glx concentrations are regulated in accordance with the sleep-wake cycle, possibly reflecting neural activity associated with different states of consciousness. Additionally, cortical GABA and glutamate concentrations have been associated with self-reported sleep quality, as seen in a study showing that higher scores on the Insomnia Severity Index in patients with PTSD were associated with lower GABA and higher glutamate levels in the parieto-occipital cortex (Meyerhoff et al., 2014).

The First Night Effect as a Model for Insomnia

The First Night Effect (FNE) is a phenomenon in which individuals experience worsened sleep quality on the first night of sleep in a novel environment. It is characterized by increased sleep onset latency (SOL), sleep fragmentation, and asymmetric brain activity (Tamaki et al., 2016; Byun et al., 2019). Neuroimaging studies suggest that during FNE, one hemisphere of the brain, particularly in the default mode network, remains more active (Tamaki & Sasaki, 2019). This may be a protective mechanism against unfamiliar surroundings, as sleeping in a novel environment likely increases arousal to a certain extent. This makes the FNE a particularly useful model for studying the neurochemical basis of sleep disorders given that it mimics notable insomnia features, including difficulty initiating and maintaining sleep and lower sleep efficiency (Ding et al., 2022). Additionally, early research showed that the FNE is especially pronounced during the sleep-onset period, making it even more valuable in investigating sleep initiation mechanisms (Tamaki et al., 2005). Last, studies have explored the role of the GABAergic system

in insomnia, highlighting its importance in modulating arousal and ensuring stable sleep (Varinthra et al., 2024).

Overall, the FNE model offers an opportunity to investigate whether neurometabolite imbalances in the mPFC and dlPFC are linked to difficulties in sleep initiation and maintenance.

Aims of the Present Study

This present study aims to explore neurotransmitter levels in the dorsolateral prefrontal cortex (dlPFC) and medial prefrontal cortex (mPFC) in the insomniac and non-insomniac state to understand the roles of these brain regions in sleep. The insomniac state was simulated by using the First Night Effect (FNE), where the first nap session in a novel environment is expected to result in worsened sleep. The primary goal is to determine whether the dlPFC and mPFC are involved in the wake-sleep interaction and non-rapid eye movement (NREM) sleep, and whether the two regions behave similarly or not. This was assessed by examining the percentage of neurometabolites, GABA and Glx, on Day 1 versus Day 2 of sleep and relationships with sleep onset latency (SOL), a common measure of sleep quality. As an exploratory aspect of the study, I analyzed correlations between neurometabolite percentages and age.

This study demonstrates that the mPFC and dlPFC play distinct roles in sleep, with their neurometabolite activity diverging overtime. The mPFC displayed significant fluctuations in Glx across sleep sessions, while the dlPFC's inhibitory neurotransmission, indicated by GABA, was more closely linked to SOL. Higher mPFC Glx levels were associated with prolonged SOL, which suggests that excitatory activity in this region may contribute to difficulty falling asleep. On the other hand, increased dlPFC GABA correlated with shorter SOL, indicating a role of the dlPFC in promoting sleep onset. Interestingly, the differences between the dlPFC and mPFC only

became apparent on the second day, implying that functional differentiation between these regions emerges with sleep adaptation. Additionally, age was positively correlated with dlPFC Glx, suggesting that excitatory balance in this region may change with aging and potentially influence sleep quality. These findings highlight how the prefrontal cortex contributes to sleep regulation, adaptation across nap sessions, and age-related changes in sleep physiology.

Methods

Subjects

Fourteen healthy adults (10 females, 4 males) aged 18-30 participated in the dlPFC study and twenty healthy adults (12 females, 8 males) aged 18-30 participated in the mPFC study. Some data was excluded due to extreme outliers, which is explained in more detail at the end of this section. Subjects were recruited using flyers and Today@Brown and were compensated $25 for each nap session. An experimenter described the purpose and procedures of the experiment to subjects in detail, and they were asked to complete questionnaires about their sleep-wake habits for screening. This included information on their usual sleep and wake times, the regularity of their sleep-wake habits, and information on their physical or psychiatric health, which included any sleep complaints. Participants were excluded if they had a physical or psychiatric impairment, were currently receiving medical treatment, had a suspected sleep disorder, and if they had a habit of consuming alcoholic beverages before sleep. Eligible participants had regular sleep-wake cycles, meaning their sleep varied less than 2 hours day to day, and their average sleep duration ranged from 6 to 9 hours on a regular basis. Participants were asked to not use conditioner in their hair to ensure proper electrode adhesion, take excessive naps, consume alcohol, or engage in any vigorous physical activity the day before the nap sessions. They were also instructed to refrain from caffeine consumption the day of their nap. Data collection was performed at the MRI facility at Brown University.

MRS and Experiment Setup

The experiment involved two afternoon nap sessions (Day 1 and Day 2) separated by approximately one week. All subjects took a nap for the first time in the MRI. The reason for

spacing the sleep sessions one week apart was to avoid the effects of napping during the first sleep session from carrying over into the second sleep session, given that participants are used to a normal sleep schedule without regular naps. It was expected that participants would experience the First Night Effect on Day 1 but not Day 2. During each session, participants slept inside the MRI while a 90-minute polysomnography (PSG) was conducted. This MRS data collection consisted of a simultaneous PSG and MRI setup at Brown's MRI suite using a 3T Siemens Prisma scanner. The PSG was compatible with MRI and had 32 electrodes (23 EEG, 6 EOG, 2 EMG, 1 ECG). A 25x25x25 mm^3 voxel for the MRS was placed in the left dorsolateral prefrontal cortex (dlPFC) or left medial prefrontal cortex (mPFC) depending on the study group. Within the 90-minute scan period, nine 10-minute MRS scans were performed and were used to measure the glutamate (Glx) and GABA concentrations in the dlPFC and mPFC. The first two scans were used for baseline measurements during wakefulness, while the other seven scans were for exploring the percent change in Glx or GABA relative to baseline. PSG data was being recorded at the same time. The changes in Glx and GABA concentrations during NREM sleep were calculated relative to the wakefulness baseline. Sleep onset latency (SOL) was used as an indicator of sleep quality. After the nap session, PSG was sleep staged every 30 seconds according to a standard methodology (Keenan & Hirshkowitz, 2011). The content of these methods are partially adapted from Yamada et al., 2024, as my study examines separate questions within this larger experiment.

To avoid potential artifacts in the data, given how sensitive the MRI scanner is, participants were asked to avoid movement during the scan. They were told to rest in their most comfortable position prior to starting the scan and nap session. To ensure maximal comfort, we

placed participants in a head coil and cushioned it with gauze, gave them blankets, raised their feet with a foam wedge, and gave them earplugs to block out as much sound as possible.

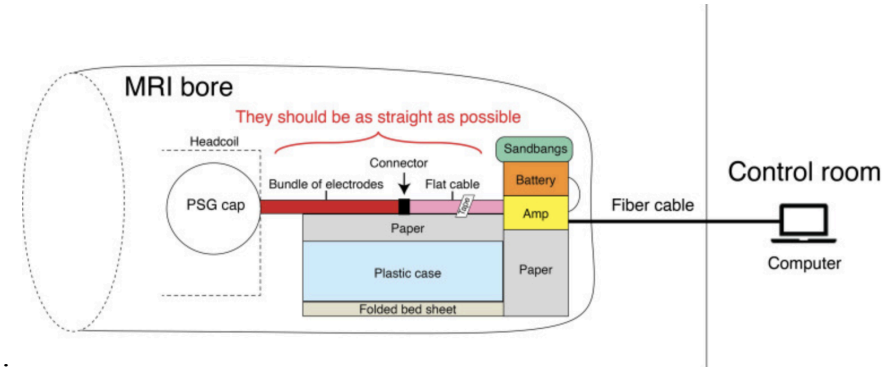

Figure 1 – MRS setup based on Tamaki et al., 2021. The setup is slightly adjusted due to recent changes in MRI procedure and introduction of new equipment.

Data Analysis

I performed all data analysis and constructed all graphical representations using GraphPad Prism. Statistical significance was determined to be $p<0.05$ for this study, and I performed standard paired, two-sample, and one-sample t-tests, and Pearson's correlation tests on the data. Dr. Takashi Yamada performed the appropriate calculations and calibrations for percentages of Glx and GABA and sleep staging, as this required an advanced skill set. The results of this study were taken from a larger study looking at visual perceptual learning in these brain regions, however, this additional data was not relevant to my experiment.

Importantly, certain but not all data for two participants were excluded from the dlPFC study due to extreme outliers. Similarly, in the mPFC study, some data from two participants were excluded due to extreme outliers. This resulted in complete data sets from 12 participants in the dlPFC study and 18 participants in the mPFC study. In both cases, the outliers were determined using the Grubbs test to determine whether the most extreme value in the list of data

points was a significant outlier from the rest. It was not always the case that these participants were outliers, and therefore their data were kept for those data points which did not have significant outliers.

Results

The following figures represent data collected from two separate groups of participants, one with data for the dlPFC and the other with data for the mPFC. Glx and GABA levels during NREM sleep were measured and are illustrated in Figure 2, Figure 6, Figure 9, and Figure 10. Additional analyses were done to determine the relationship between age and neurometabolites in NREM sleep as well as sleep onset latency (SOL) and neurometabolites in the dlPFC and mPFC. The exact results are described following each figure.

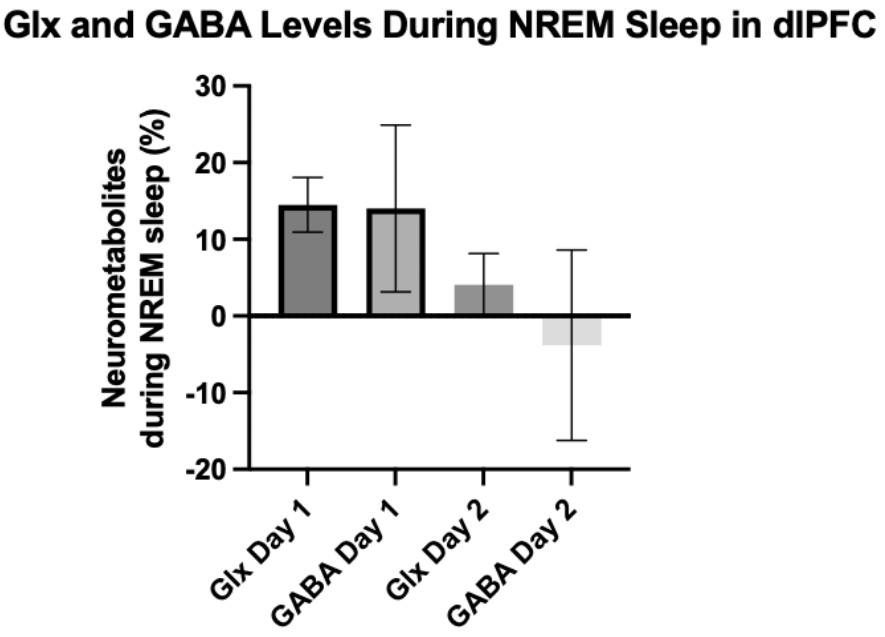

Figure 2: One-sample and paired comparisons of Glx and GABA levels during NREM sleep in the dlPFC. Statistical tests were run to determine whether neurometabolite levels were significantly different from wakefulness baseline and whether there were differences between Day 1 and Day 2. Error bars represent the standard error of the mean (SEM).

To determine whether neurometabolites significantly differed from wakefulness or changed across nap sessions, we first analyzed Glx and GABA concentrations in the dlPFC. In Figure 2, for the subset of participants with complete data for both Day 1 and Day 2 (n=3),

one-sample t-tests were conducted to determine whether Glx and GABA levels deviated from wakefulness on each day. The tests for Glx on Day 1 (p=0.0555, SEM ± 3.53%) and Day 2 (p=0.4279, SEM ± 4.16%), as well as for GABA on Day 1 (p=0.3257, SEM ± 10.87%) and Day 2 (p=0.7873, SEM ± 12.42%), were not statistically significant. Similarly, paired sample t-tests comparing Glx levels between Day 1 and Day 2 (p=0.0685) and GABA levels between the two days (p=0.5190) revealed no significant differences. These results indicate that, in this small sample, neurometabolite concentrations did not significantly differ from baseline wakefulness across days. The large error bars indicate a higher variability between the individuals, meaning that different participants may have responded differently to the sleep conditions. While we can still follow the general trends of the data, as illustrated in the discussion, these interpretations must be done with caution.

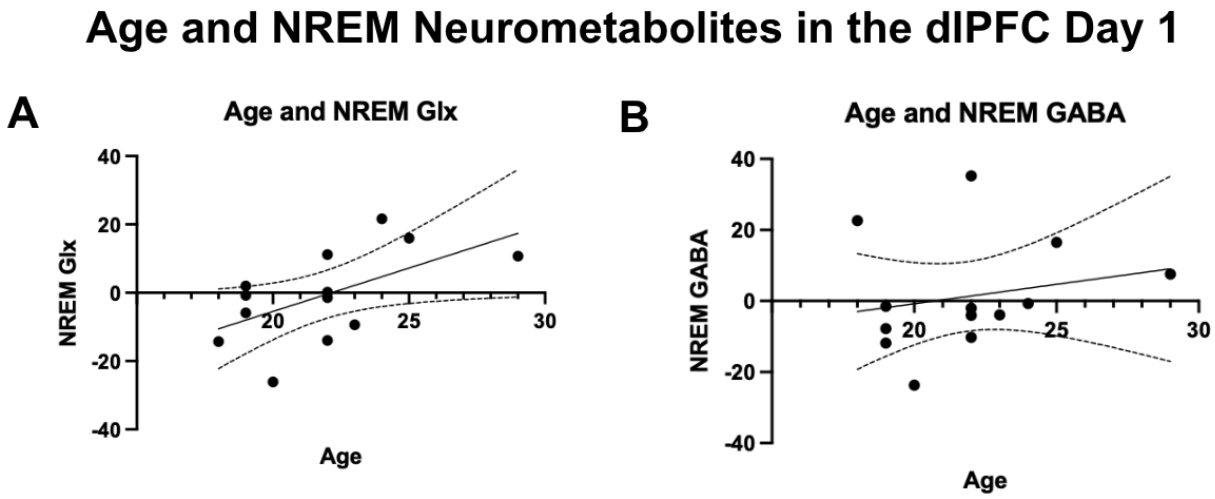

Figure 3: Correlations between age and NREM neurometabolites in the dlPFC on Day 1. Sample size on Day 2 (n=3) was too small for any meaningful results. Dashed lines represent the 95% confidence interval.

Next, we examined the relationship between neurometabolites and participant age. In Figure 3A, after excluding one participant identified as an extreme outlier (resulting in n=13), a

significant positive correlation was observed between NREM Glx and age (r=0.5722, p=0.0410). In contrast, Figure 3B, which excluded the same participant (n=13), did not reveal any significant correlation (r=0.2099, p=0.4912). These findings suggest that, when outliers are removed, there is a significant correlation between NREM Glx levels and age. Therefore, older individuals appear to have higher excitatory activity during NREM sleep. It is important to note that these results do not indicate any causation, as it is unclear whether being older causes more excitatory neurometabolites in the brain during NREM sleep.

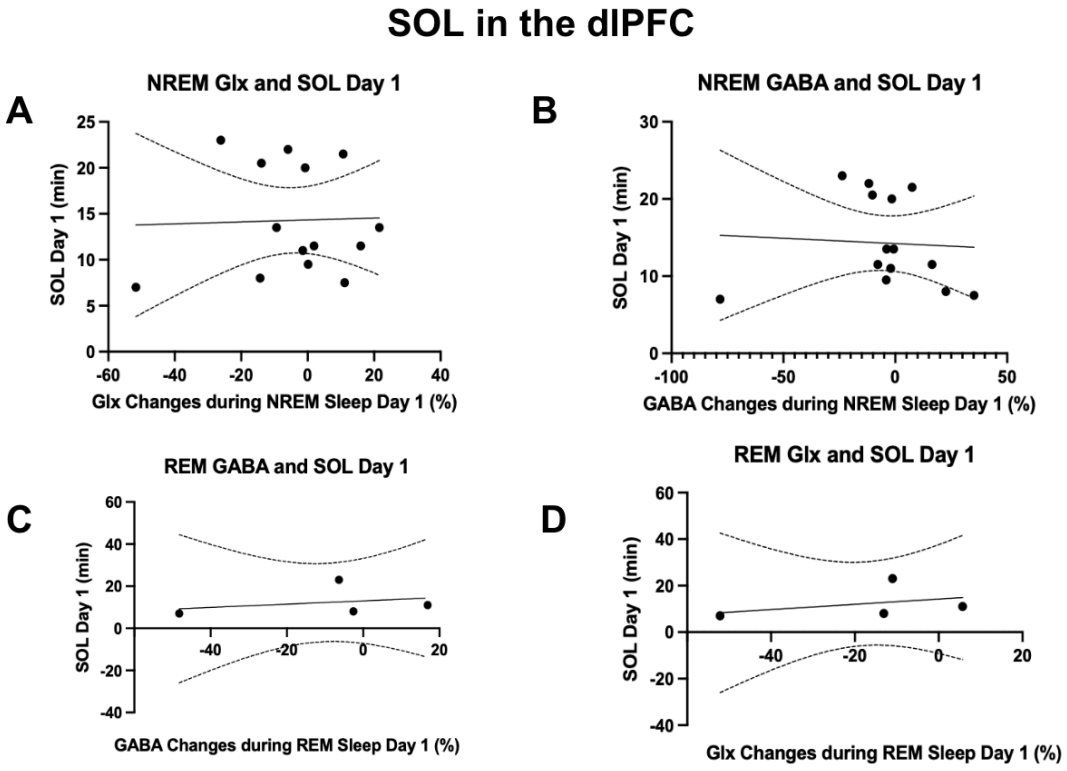

Figure 4: Correlations between sleep onset latency (SOL) and neurometabolites in the dlPFC in NREM and REM sleep on Day 1. No meaningful results could be drawn from Day 2 with the limited sample size (n=3). Dashed lines represent the 95% confidence interval.

We further explored the link between dlPFC neurometabolites and sleep onset latency (SOL) on Day 1. For Figure 4A, after excluding one extreme outlier (n=13), the correlation was

slightly negative but not statistically significant (r=-0.3507, p=0.2401). In Figure 4B, using the same exclusion criteria (n=13), a significant slightly negative correlation was found (r=-0.6504, p=0.0161). Figures 4C and 4D, derived from a separate analysis with n=4, showed no significant correlations (r=0.2882, p=0.7118 and r=0.3770, p=0.6230, respectively). These results suggest that there is a significant relationship between GABA changes during NREM sleep on Day 1 and sleep onset latency, while no significant relationships exist during REM sleep or for Glx during both REM and NREM sleep. Specifically, increased inhibitory tone in the dlPFC may facilitate quicker sleep initiation.

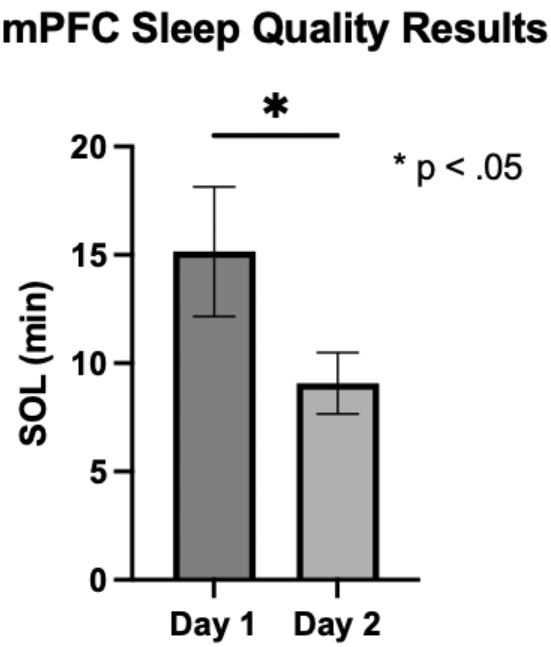

Figure 5: SOL as a measure of sleep quality on Day 1 and Day 2 for all participants (n=20). Error bars represent the SEM.

To confirm the presence of the First Night Effect (FNE), we compared SOL between Day 1 and Day 2 for all participants (n=20). A significant reduction in SOL on Day 2 (p=0.0369) confirmed that sleep improved over time, consistent with expectations under the FNE paradigm.

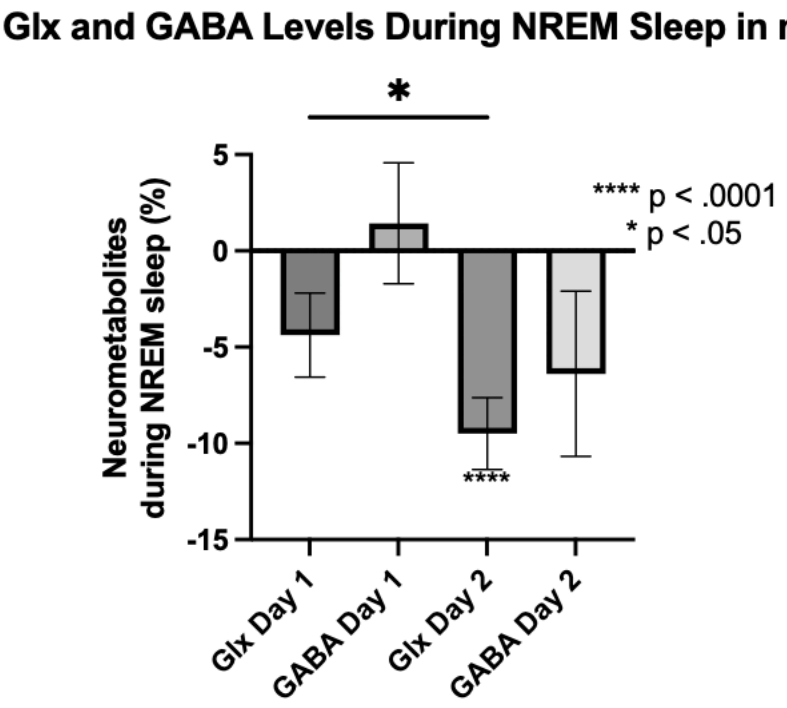

Figure 6: One-sample and paired comparisons of Glx and GABA levels during NREM sleep in the mPFC. Statistical tests were run to determine whether neurometabolite levels were significantly different from wakefulness baseline and whether there were differences between Day 1 and Day 2. Error bars represent the SEM.

We then analyzed mPFC neurometabolites across days. To perform the analysis for Figure 6, one participant was excluded from the Day 1 data for Glx and a different participant for Day 1 GABA, resulting in n=19 for those comparisons. All participants (n=20) were included for Day 2. One-sample t-tests showed that Glx on Day 1 ($p=0.0598$, SEM ± 2.18%), GABA on Day 1 ($p=0.6541$, SEM ± 3.14%), and GABA on Day 2 ($p=0.1530$, SEM ± 4.29%) did not differ significantly from wakefulness. However, Glx on Day 2 was significantly different from wakefulness ($p<0.0001$, SEM ± 1.87%). Furthermore, a paired t-test comparing Glx levels between Day 1 and Day 2 was significant ($p=0.0188$), whereas the paired comparison for GABA levels was not ($p=0.1768$). These findings suggest that, while GABA levels remained relatively

stable, there was a significant change in Glx levels from Day 1 to Day 2 that may indicate a shift in excitatory tone as participants adapted to the sleep environment.

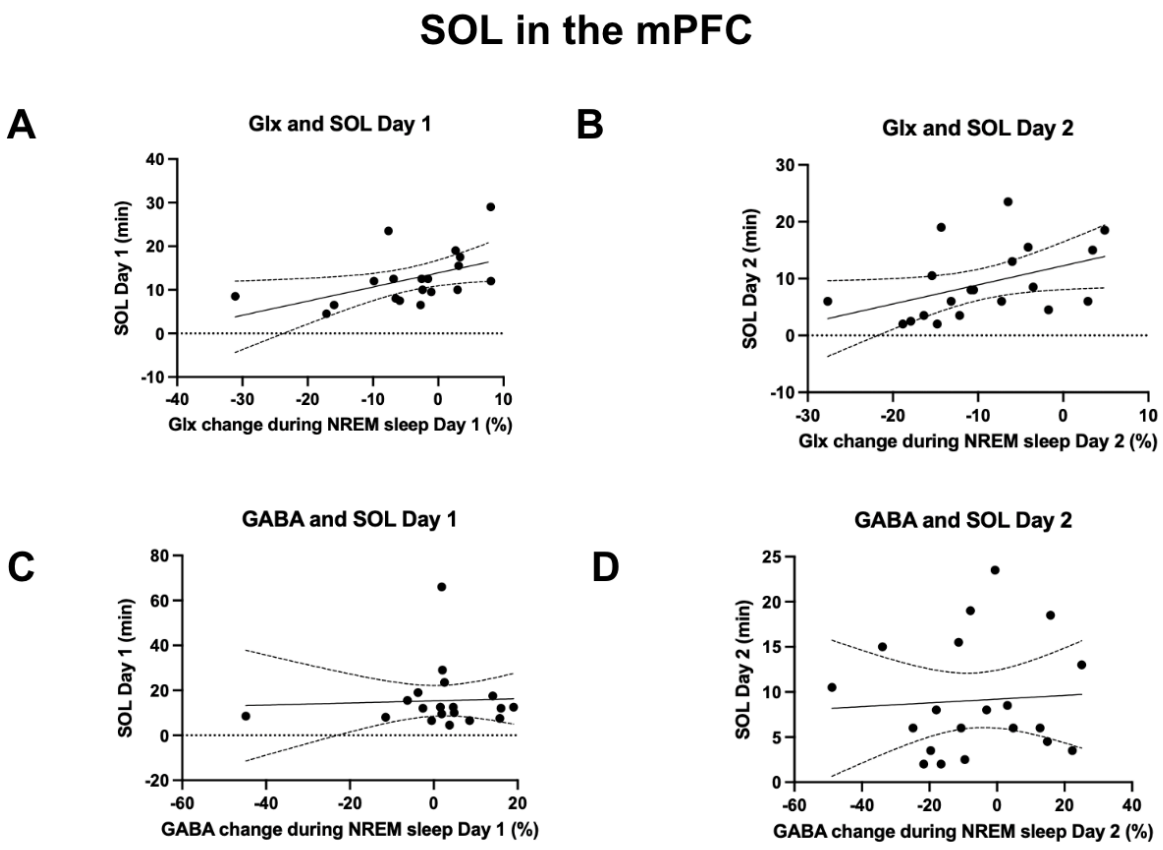

Figure 7: Correlations between neurometabolites, Glx and GABA, and SOL across days. Dashed lines represent the 95% confidence interval.

To build on our understanding of the role of the mPFC in sleep initiation, we looked at the relationship between SOL and neurometabolite levels in this region. In Figure 7A, after excluding one extreme outlier (n=19), there was a significant positive correlation between Glx and SOL on Day 1 ($r=0.4967$, $p=0.0305$). Figure 7B, which included all participants (n=20) also showed a significant positive correlation between Glx and SOL on Day 2 ($r=0.4449$, $p=0.0494$). In contrast, Figures 7C and 7D examined correlations between GABA and SOL. In Figure 7C, a different participant from 7A was excluded due to an extreme outlier (n=19) and there was no

significant correlation between GABA and SOL on Day 1 (r=0.04746, p=0.8470). In 7D, all participants were included (n=20), and similar to 7C, there was no significant correlation between GABA and SOL on Day 2 (r=0.06297, p=0.7920). These results indicate that changes in Glx may be associated with variations in sleep onset latency, whereas GABA does not show this type of relationship in the mPFC. Specifically, persistent excitatory activity in the mPFC may contribute to sleep onset difficulties.

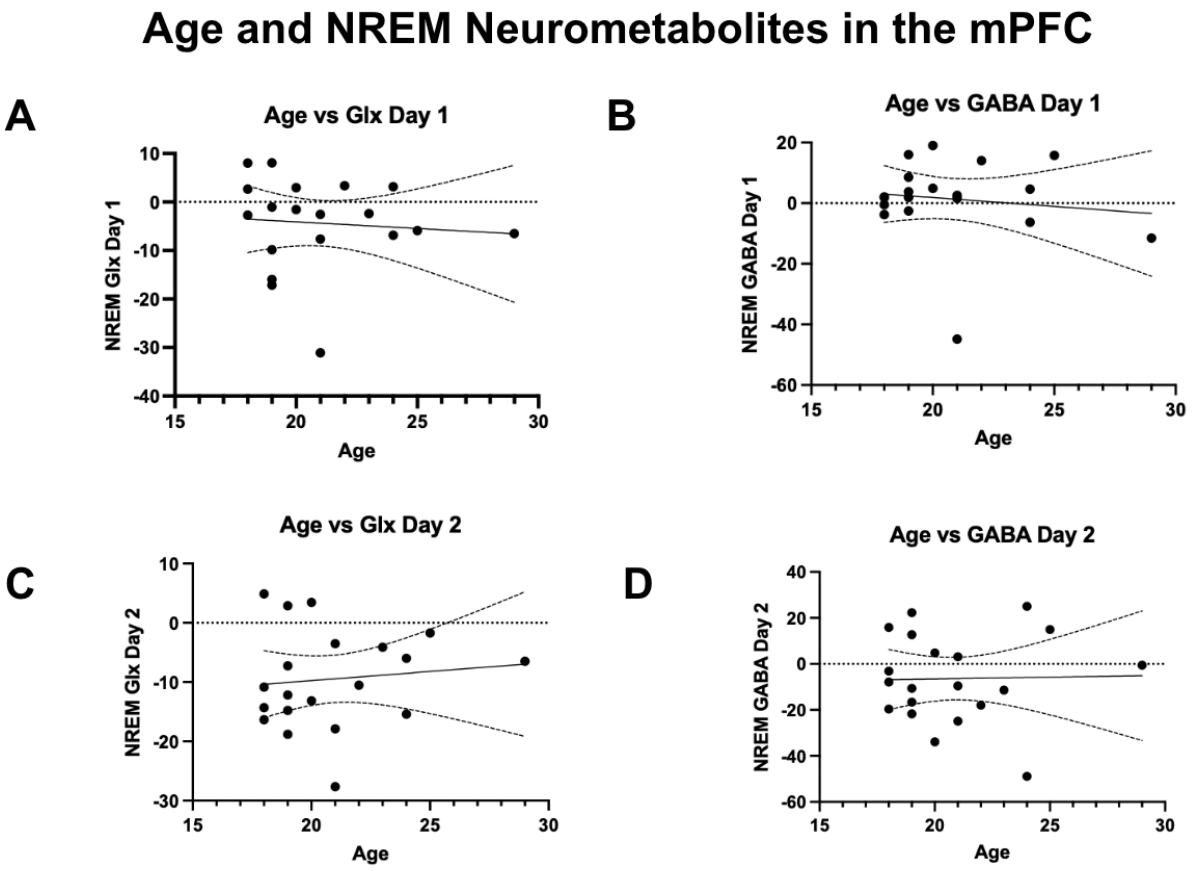

Figure 8: Correlations between age and neurometabolites, Glx and GABA, in the mPFC during NREM sleep. Results include data from Day 1 and Day 2. Dashed lines represent the 95% confidence interval.

For Figure 8, analyses were performed to assess the relationship between age and neurometabolite levels in the mPFC. In Figure 8A, after excluding one extreme outlier (n=19), there was no significant correlation between age and Glx on Day 1 (r=-0.08390, p=0.7327).

Figure 8B, after excluding a different participant from 8A, also showed no significant correlation between age and GABA on Day 1 (r=-0.1253, p=0.6093). All participants were included for Day 2 (n=20). Figure 8C showed no significant correlation between age and Glx on Day 2 (r=0.1076, p=0.6515). Similarly, in 8D there was no significant correlation between age and GABA on Day 2 (r=0.02347, p=0.9218). Taking these results into account, age does not have any correlation with Glx or GABA changes on either day in the mPFC with this sample of participants. This is in contrast to the results for age and neurometabolites in the dlPFC, where age and NREM Glx showed a significant relationship, suggesting a region-specific aging effect.

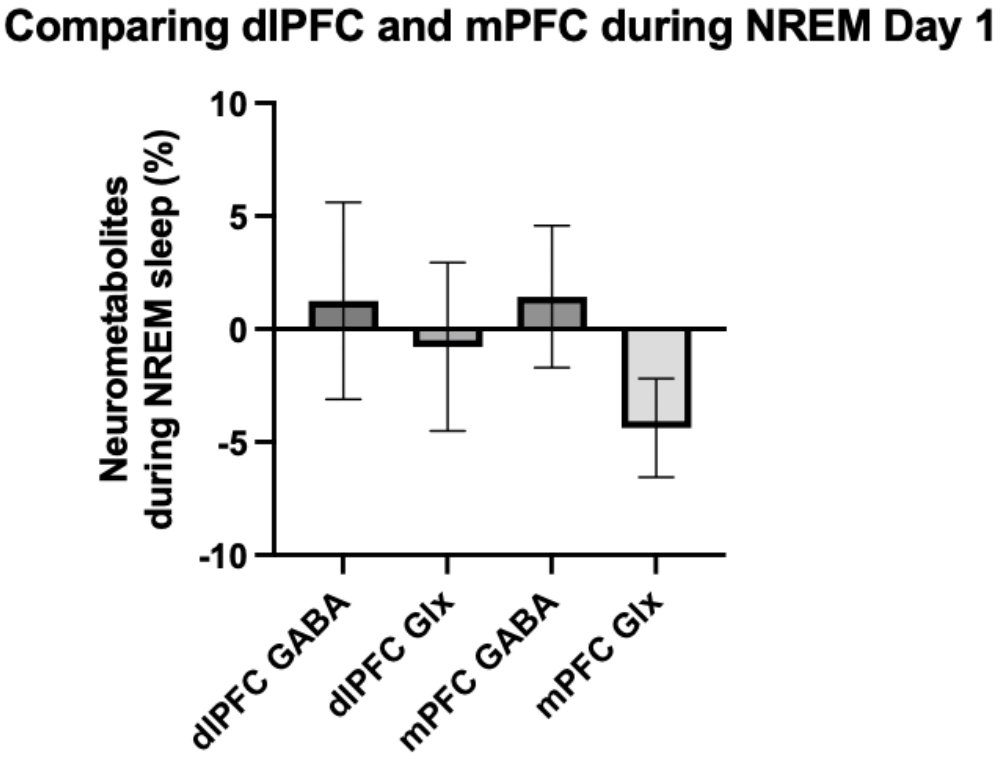

Figure 9: One-sample and unpaired two-sample comparisons of Glx and GABA levels during NREM sleep in the dlPFC and mPFC on Day 1. Statistical tests were run to determine whether neurometabolite levels were significantly different from wakefulness baseline and whether there were differences between the dlPFC and mPFC GABA and Glx levels. Error bars represent the standard error of the mean (SEM).

Comparisons between brain regions on Day 1 (Figure 9) revealed no significant differences in Glx or GABA levels between the dlPFC and mPFC, and neither region differed significantly from wakefulness. To perform the analysis for Figure 9, one participant was excluded from the dlPFC GABA and Glx data resulting in n=13 for those comparisons. For the mPFC, one participant was excluded from the GABA data and a different participant from mPFC Glx, resulting in n=19 for those comparisons. One-sample t-tests showed that dlPFC GABA (p=0.7798, SEM ± 4.362%), dlPFC Glx (p=0.8356, SEM ± 3.724%), mPFC GABA (p=0.6541, SEM ± 3.143%), and mPFC Glx (p=0.0598, SEM ± 2.177%) did not differ significantly from wakefulness. Furthermore, an unpaired two-sample t-test comparing GABA levels between dlPFC and mPFC (p=0.9721) and Glx levels between dlPFC and mPFC (p=0.3825) was not significant. These findings suggest that, on Day 1, characterized by worse sleep quality, the dlPFC and mPFC appear to behave similarly to each other in terms of changes in glutamate and GABA levels.

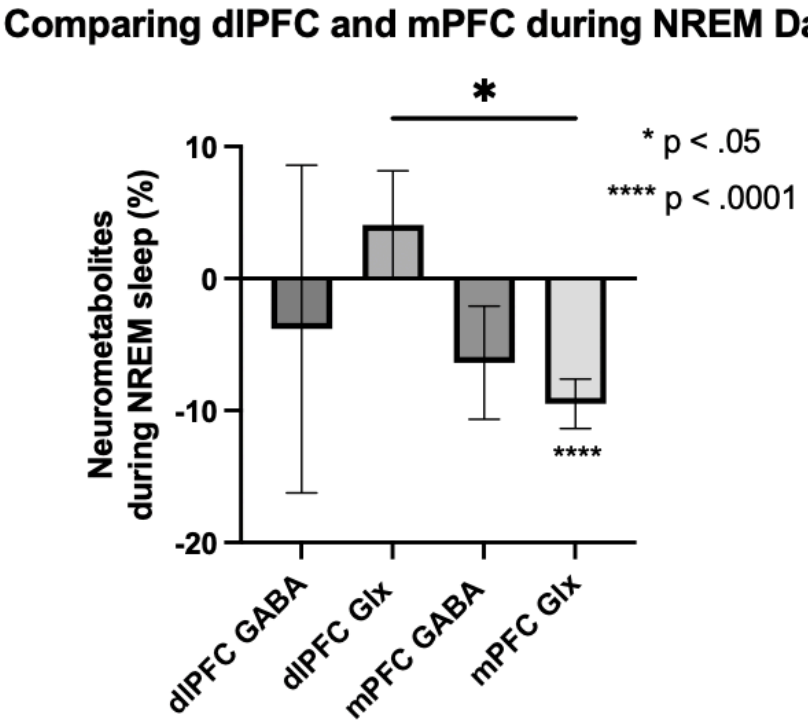

Figure 10: One-sample and unpaired two-sample comparisons of Glx and GABA levels during NREM sleep in the dlPFC and mPFC on Day 2. Statistical tests were run to determine whether neurometabolite levels were significantly different from wakefulness baseline (also shown in Figures 2 and 6) and whether there were differences between the dlPFC and mPFC GABA and Glx levels. Error bars represent the standard error of the mean (SEM).

However, by Day 2, this pattern changed (Figure 10). To avoid redundancy in explaining the results, only the results of the comparisons between dlPFC and mPFC using an unpaired two-sample t-test will be described here. No participants were excluded for either brain region, resulting in n=3 for the dlPFC and n=20 for the mPFC. Comparing dlPFC GABA and mPFC GABA ($p=0.8334$) showed no significant results while Glx levels differed significantly ($p=0.0148$), with lower Glx in the mPFC compared to the dlPFC. This indicates that inhibitory activity remained relatively stable across both regions while excitatory activity differs between the dlPFC and mPFC on Day 2. Additionally, the results reveal a functional divergence, where excitatory activity in the mPFC downregulates more rapidly than in the dlPFC, possibly supporting improved sleep initiation and adaptation.

Discussion

Key Findings and their Implications

This study aimed to investigate whether the dlPFC and mPFC are involved in sleep by analyzing neurometabolite changes (GABA and Glx) compared to wakefulness and whether these regions behave similarly or differently across nap sessions. I also explored the relationship between neurometabolites and sleep onset latency (SOL), and how age may influence prefrontal neurometabolite concentrations.

A primary question of this study was whether GABA and Glx levels in the dlPFC and mPFC significantly differed from wakefulness during NREM sleep. The results indicate that, in the dlPFC, neither GABA nor Glx showed significant differences from wakefulness during either nap session. However, in the mPFC, Glx levels on Day 2 were significantly decreased from wakefulness, and there was a significant decrease in Glx levels from Day 1 to Day 2. These findings align with previous research suggesting that the mPFC may play a key role in sleep regulation, especially in emotional and cognitive integration during sleep (Gabbott & Rolls, 2013). While the dlPFC did not show significant differences across days, this may reflect sample size limitations rather than an absence of functional change.

It still may be useful to notice the general trend that Glx and GABA were both higher on Day 1 than Day 2 in the dlPFC. This finding suggests that the first night of sleep in a new environment (Day 1) may be associated with greater excitatory (Glx) and inhibitory (GABA) activity in the dlPFC. The decline in both Glx and GABA on Day 2 may indicate an adaptation where the dlPFC reduces excitatory-inhibitory activity as the brain becomes more accustomed to the environment. However, given that these trends were not statistically significant, it is difficult to draw concrete conclusions from the data. Additionally, since Glx and GABA levels were not

significantly different from wakefulness, sleep might not induce major changes in these neurotransmitters in the dlPFC, at least in the context of this small sample size. The lack of significant changes in dlPFC neurometabolite levels could indicate that the dlPFC is less actively involved in sleep regulation.

One important factor to note is that research has shown involvement of the dlPFC in REM sleep, but not necessarily in NREM sleep (Kubota et al., 2011). Therefore, the lack of significant results for the dlPFC may be due to the lack of data for REM sleep. Additionally, the small sample size (n=3) for the dlPFC group compared to the mPFC group may be a factor to consider in the insignificance of the results. Still, these preliminary results indicate that the dlPFC might not play a significant role in NREM sleep while the mPFC appears to have some role based on the significant decrease in Glx levels between Day 1 and Day 2.

With regards to the First Night Effect (FNE), Figure 5 offers concrete evidence that the FNE had occurred in the mPFC given a significant decrease in SOL between Day 1 and Day 2. Given that there was a significant decrease in the excitatory neurotransmitter, Glx, between Day 1 and Day 2 in the mPFC during NREM sleep, it is likely that this brain region is involved in maintaining sleep. Previous evidence has indicated that the mPFC may play a role in sleep quality, with deficits in the mPFC being correlated with unrefreshing sleep in chronic fatigue syndrome (Shan et al., 2017). The same analysis couldn't be performed for the dlPFC group as we only had SOL data for Day 1. Given that no significant differences were found between dlPFC Day 1 and Day 2, it is possible that the FNE, if it occurred, did not affect the dlPFC. If the FNE was at play, the insomniac brain on Day 1 still did not show any significant differences from wakefulness in neurometabolite levels nor did the non-insomniac brain on Day 2. We should have at least seen a significant difference between days if the FNE had an impact on the

dlPFC, as this would indicate that the neurometabolites were sensitive to insomniac versus non-insomniac states. This provides further evidence that the dlPFC might not be involved in sleep, at least NREM sleep. Additionally, in contrast to the mPFC, the dlPFC is primarily associated with executive function and attention, and therefore may not be as sensitive to environmental changes during sleep (Kubota et al., 2011; Ding et al., 2024).

To investigate the potential role of the prefrontal cortex in sleep initiation, I examined correlations between neurometabolites and sleep onset latency (SOL). A key difference in the figures for the dlPFC compared to the mPFC is that the dlPFC has data for only Day 1 and includes both NREM and REM sleep whereas the mPFC has data for both Day 1 and Day 2 for NREM sleep only. These differences are a result of limitations in the available data. In the dlPFC, we found a significant slightly negative correlation between GABA during NREM sleep and SOL on Day 1. This suggests that higher inhibitory activity in the dlPFC may facilitate sleep onset, reducing SOL. Glx in the dlPFC did not significantly correlate with SOL, indicating that excitatory neurotransmission in this region might not strongly influence sleep initiation. In the mPFC, Glx showed a significant positive correlation with SOL on both days, while GABA did not. These results imply that increased excitatory activity in the mPFC is associated with a longer SOL, possibly reflecting arousal-related processes that delay sleep onset. GABA levels in the dlPFC correlated with SOL, while Glx levels in the mPFC correlated with SOL, further supporting the idea that these regions have distinct roles in the wake-sleep transition and sleep regulation. These findings indicate that the dlPFC may have an inhibitory function that is important for sleep initiation, while increased excitatory activity in the mPFC may contribute to prolonged wakefulness and arousal, aligning with its role in cognitive and emotional processing.

A final analysis on this topic was done to directly compare the mPFC and dlPFC on Day 1 and Day 2, looking at how Glx and GABA levels compare between the two brain regions. The results indicate that GABA and Glx levels in the dlPFC and mPFC during NREM sleep on Day 1 are not significantly different from each other, nor are they significantly different from wakefulness. This suggests that, at least in the early stages of sleep adaptation, such as the first night, these two prefrontal regions may not exhibit distinct neurometabolic profiles during NREM sleep. Even though the absolute levels of GABA and Glx are similar between these two regions, they may still serve different functions in sleep regulation, as suggested by their differing relationships with SOL. The negative correlation between dlPFC GABA and SOL suggests that inhibitory tone in the dlPFC may facilitate sleep initiation, whereas the positive correlation between mPFC Glx and SOL may indicate that excitatory activity in the mPFC prolongs wakefulness or cognitive processing. Additionally, the FNE may contribute to the lack of distinct regional differences. Day 1 sleep represents an adaptation period to a novel environment, so it is possible that neither the dlPFC nor mPFC has adjusted to perform a specialized role in sleep regulation. This conjecture is further supported by the fact that Glx levels in the mPFC were significantly lower than in the dlPFC on Day 2, suggesting that overtime, the dlPFC and mPFC may become more functionally distinct in their neurometabolite activity during NREM sleep. The lower Glx in the mPFC might reflect a decrease in arousal-related activity compared to the dlPFC, which may be maintaining a higher Glx for attention and executive function. Although this appears to contradict the SOL-related findings, it is possible that mPFC Glx initially prolongs wakefulness for environmental monitoring, followed by a reduction in activity once NREM sleep is reached to promote restful sleep. If additional significant differences in neurometabolite levels emerge in subsequent nap sessions, this would

suggest that these regions become more functionally distinct with continued sleep adaptation. Thus, a future direction for this study is to examine changes across multiple nap sessions, as the lack of differences on Day 1 could mean that prefrontal involvement in sleep is not yet fully engaged during this adaptation period. Another factor to consider is that prior research suggests the dlPFC becomes more active during REM sleep (Kubota et al., 2011), and if there were sufficient data, this would have been an area of exploration to compare mPFC and dlPFC during REM sleep.

An additional, exploratory aspect of this study looks at whether age influences prefrontal neurometabolite levels. The results showed that in the dlPFC, age is significantly correlated with NREM Glx levels, suggesting that older individuals tend to have higher excitatory activity during NREM sleep. In contrast, age did not significantly correlate with Glx or GABA levels in the mPFC on either day, indicating that, in this sample, there is not a notable relationship between age and neurometabolite levels. These findings are consistent with prior research indicating that glutamate levels change with aging, however they differ in that literature has shown a decrease in glutamate with aging rather than an increase (Chang et al., 2009; Hädel et al., 2013). This inconsistency is likely a result of different brain regions being analyzed and a less robust age difference in the present study, possibly contributing to the varied findings. The lack of significant correlations in the mPFC could suggest that age-related changes in neurometabolite levels occur more prominently in the dlPFC than in the mPFC, highlighting another difference between these two regions.

Limitations and Future Directions

While this study produced some promising findings, there are a couple important limitations to consider that may have impacted the results. First, participants were asked to sleep in an MRI with an EEG cap on. While PSG recordings showed that participants fell asleep, the noises from the scanner and the possible discomfort with the EEG cap or while lying down in the scanner may have made it more difficult for participants to fall asleep or to sleep deeply throughout the entire nap session. Additionally, the sample size was particularly limited for certain comparisons, especially in Figure 2, which may have contributed to the statistically insignificant results. While this study primarily focused on NREM sleep, with no significant relationships found between REM sleep neurometabolites and SOL, it would have been valuable to analyze REM sleep neurometabolites across days if the data were present. In particular, the dlPFC exhibits increased activity during REM sleep (Kubota et al., 2011), and therefore, more comprehensive analyses of REM sleep dynamics are needed to determine the dlPFC's role in this sleep stage. Last, this study establishes correlations between neurometabolites and sleep measures, however, we cannot determine causality from this. For instance, higher mPFC Glx was associated with longer SOL, but it is unclear whether excess excitatory activity prevents sleep onset or if it is simply cognitive/emotional processes interfering with sleep.

There are many future directions for this study, with the first being increasing the sample size to get more significant results. Second, future studies should include direct comparisons of neurometabolites across both NREM and REM sleep to determine if the dlPFC plays a greater role in REM sleep dynamics. Additionally, it may be interesting to look at individuals with clinical sleep disorders such as insomnia and sleep apnea to provide insight into how prefrontal neurometabolite imbalances contribute to disordered sleep. As mentioned previously, extending

the days that this study is conducted may reveal additional differences in neurometabolite profiles of the mPFC and dlPFC. With regards to the analyses on aging, the findings could be enhanced by widening the age range to determine whether age related alterations in Glx and GABA contribute to sleep disturbances in older adults. Last, future studies could look at neurometabolite changes across sleep stages to determine whether prefrontal neurometabolite shifts correspond to specific sleep stage transitions.

Conclusions

This study offers preliminary evidence that the dlPFC and mPFC exhibit different neurometabolic patterns during sleep, supporting the hypothesis that these regions play distinct roles in sleep regulation. The mPFC demonstrated significant decreases in Glx percentages across sleep sessions, while the dlPFC showed a stronger relationship between GABA levels and sleep onset latency. The lack of significant differences between neurometabolites in the mPFC and dlPFC on Day 1 could be a reflection of adaptations due to the First Night Effect rather than a permanent lack of differentiation. This seems to be the case, given that the dlPFC and mPFC showed significant differences in Glx on Day 2, however it is important to note the small sample size for the dlPFC in this analysis. Higher mPFC Glx levels were associated with a longer SOL, suggesting that excitatory activity in this region may contribute to wakefulness and delayed sleep onset. Higher dlPFC GABA levels correlated with a shorter SOL on Day 1, indicating that inhibitory activity in this region may facilitate sleep initiation. The present results indicate that the mPFC may be involved in maintaining sleep once the individual reaches NREM sleep based on the decreased Glx levels on Day 2, even though it seems to delay sleep onset at first. On the other hand, the dlPFC may be involved in sleep initiation but not necessarily sleep maintenance given its lack of significant differences from wakefulness and between days. Additionally, age-related increases in Glx were observed in the dlPFC but not in the mPFC, suggesting that aging may differentially impact excitatory-inhibitory balance in these regions. Overall, these findings contribute to the growing understanding of prefrontal involvement in sleep and aging, and highlight the need for larger sample sizes, causal analyses, and more days in the study, among other limitations, to explore these relationships.

References

Allen, R. P., Barker, P. B., Horska, A., & Earley, C. J. (2013). Thalamic glutamate/glutamine in restless legs syndrome: Increased and related to disturbed sleep. *Neurology*, *80*(22), 2028–2034. https://doi.org/10.1212/wnl.0b013e318294b3f6

Arnold, E. C., Soler-Llavina, G., Kambara, K., & Bertrand, D. (2023). The importance of ligand gated ion channels in sleep and sleep disorders. *Biochemical Pharmacology*, *212*, 115532. https://doi.org/10.1016/j.bcp.2023.115532

Byun, J.-H., Kim, K. T., Moon, H., Motamedi, G. K., & Cho, Y. W. (2019). The first night effect during polysomnography, and patients' estimates of sleep quality. *Psychiatry Research*, *274*, 27–29. https://doi.org/10.1016/j.psychres.2019.02.011

Chang, L., Jiang, C. S., & Ernst, T. (2009). Effects of age and sex on brain glutamate and other metabolites. *Magnetic Resonance Imaging*, *27*(1), 142–145. https://doi.org/10.1016/j.mri.2008.06.002

Ding, L., Chen, B., Dai, Y., & Li, Y. (2022). A meta-analysis of the first-night effect in healthy individuals for the full age spectrum. *Sleep Medicine*, *89*, 159–165. https://doi.org/10.1016/j.sleep.2021.12.007

Ding, X., He, L., Kang, T., Yang, Y., Ji, H., Zhao, H., Lang, X., Sun, C., & Zhang, X. (2024). The role of the left dorsolateral prefrontal cortex in conflict control during insomnia disorder. *Journal of Psychiatric Research*, *171*, 271–276. https://doi.org/10.1016/j.jpsychires.2024.02.010

Gabbott, P. L., & Rolls, E. T. (2013). Increased neuronal firing in resting and sleep in areas of the macaque medial prefrontal cortex. *European Journal of Neuroscience*, *37*(11), 1737–1746. https://doi.org/10.1111/ejn.12171

Hädel, S., Wirth, C., Rapp, M., Gallinat, J., & Schubert, F. (2013). Effects of age and sex on the concentrations of glutamate and glutamine in the human brain. *Journal of Magnetic Resonance Imaging*, *38*(6), 1480–1487. https://doi.org/10.1002/jmri.24123

Ham, B.-J., Sung, Y., Kim, N., Kim, S. J., Kim, J. E., Kim, D. J., Lee, J. Y., Kim, J. H., Yoon, S. J., & Lyoo, I. K. (2007). Decreased GABA levels in anterior cingulate and basal ganglia in medicated subjects with panic disorder: A proton magnetic resonance spectroscopy (1H-MRS) study. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *31*(2), 403–411. https://doi.org/10.1016/j.pnpbp.2006.10.011

Keenan, S., & Hirshkowitz, M. (2011). *Chapter 141 -Monitoring and Staging Human Sleep*. http://apsychoserver.psych.arizona.edu/jjbareprints/psyc501a/readings/Keenan%20Hirshkowitz%202011.pdf

Kubota, Y., Takasu, N. N., Horita, S., Kondo, M., Shimizu, M., Okada, T., Wakamura, T., & Toichi, M. (2011). Dorsolateral prefrontal cortical oxygenation during REM sleep in humans. *Brain Research*, *1389*, 83–92. https://doi.org/10.1016/j.brainres.2011.02.061

Mander, B. A., Rao, V., Lu, B., Saletin, J. M., Lindquist, J. R., Ancoli-Israel, S., Jagust, W., & Walker, M. P. (2013). Prefrontal atrophy, disrupted NREM slow waves and impaired hippocampal-dependent memory in aging. *Nature Neuroscience*, *16*(3), 357–364. https://doi.org/10.1038/nn.3324

Mashour, G. A., Pal, D., & Brown, E. N. (2022). Prefrontal cortex as a key node in arousal circuitry. *Trends in Neurosciences*, *45*(10), 722–732. https://doi.org/10.1016/j.tins.2022.07.002

Meyerhoff, D. J., Mon, A., Metzler, T., & Neylan, T. C. (2014). Cortical Gamma-Aminobutyric Acid and Glutamate in Posttraumatic Stress Disorder and Their Relationships to Self-Reported Sleep Quality. *Sleep*, *37*(5), 893–900. https://doi.org/10.5665/sleep.3654

Muzur, A., Pace-Schott, E. F., & Hobson, J. Allan. (2002). The prefrontal cortex in sleep. *Trends in Cognitive Sciences*, *6*(11), 475–481. https://doi.org/10.1016/s1364-6613(02)01992-7

Nejati, V., Majdi, R., Salehinejad, M. A., & Nitsche, M. A. (2021). The role of dorsolateral and ventromedial prefrontal cortex in the processing of emotional dimensions. *Scientific Reports*, *11*(1), 1971. https://doi.org/10.1038/s41598-021-81454-7

Nofzinger, E. A. (2004). Functional Neuroimaging Evidence for Hyperarousal in Insomnia. *American Journal of Psychiatry*, *161*(11), 2126–2128. https://doi.org/10.1176/appi.ajp.161.11.2126

Pereira, A. C., Mao, X., Jiang, C. S., Kang, G., Milrad, S., McEwen, B. S., Krieger, A. C., & Shungu, D. C. (2017). Dorsolateral prefrontal cortex GABA deficit in older adults with sleep-disordered breathing. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(38), 10250–10255. https://doi.org/10.1073/pnas.1700177114

Plante, D. T., Jensen, J. E., & Winkelman, J. W. (2012). The Role of GABA in Primary Insomnia. *Sleep*, *35*(6), 741–742. https://doi.org/10.5665/sleep.1854

Shan, Z. Y., Kwiatek, R., Burnet, R., Peter Del Fante, Staines, D. R., Marshall‐Gradisnik, S. M., & Barnden, L. R. (2017). Medial prefrontal cortex deficits correlate with unrefreshing sleep in patients with chronic fatigue syndrome. *NMR in Biomedicine*, *30*(10). https://doi.org/10.1002/nbm.3757

Tamaki, M., Bang, J., Watanabe, T., & Sasaki, Y. (2016). Night Watch in One Brain Hemisphere during Sleep Associated with the First-Night Effect in Humans. *Current Biology*, *26*(9), 1190–1194. https://doi.org/10.1016/j.cub.2016.02.063

Tamaki, M., Nittono, H., Hayashi, M., & Hori, T. (2005). Examination of the First-Night Effect during the Sleep-Onset Period. *Sleep*, *28*(2), 195–202. https://doi.org/10.1093/sleep/28.2.195

Tamaki, M., & Sasaki, Y. (2019). Surveillance During REM Sleep for the First-Night Effect. *Frontiers in Neuroscience*, *13*. https://doi.org/10.3389/fnins.2019.01161

Tamaki, M., Watanabe, T., & Sasaki, Y. (2021). Coregistration of magnetic resonance spectroscopy and polysomnography for sleep analysis in human subjects. *STAR Protocols*, *2*(4), 100974. https://doi.org/10.1016/j.xpro.2021.100974

Varinthra, P., Anwar, S. N. M. N., Shih, S.-C., & Liu, I. Y. (2024). The role of the GABAergic system on insomnia. *Tzu Chi Medical Journal*, *36*(2), 103. https://doi.org/10.4103/tcmj.tcmj_243_23

Weber, M., Webb, C. A., Deldonno, S. R., Kipman, M., Schwab, Z. J., Weiner, M. R., & Killgore, W. D. S. (2013). Habitual “sleep credit” is associated with greater grey matter volume of the medial prefrontal cortex, higher emotional intelligence and better mental health. *Journal of Sleep Research*, *22*(5), 527–534. https://doi.org/10.1111/jsr.12056

Winkelman, J. W., Buxton, O. M., Jensen, J. E., Benson, K. L., O’Connor, S. P., Wang, W., & Renshaw, P. F. (2008). Reduced Brain GABA in Primary Insomnia: Preliminary Data from 4T Proton Magnetic Resonance Spectroscopy (1H-MRS). *Sleep*, *31*(11), 1499–1506. https://doi.org/10.1093/sleep/31.11.1499

Yamada, T., LaBonte-Clark, T., Khan, S., Sage, P., Kalyan, P., Berhe, H., Hopkins, S., Cheng, Y.-A., Nakashima, Y., Watanabe, T., & Sasaki, Y. (2024). 0365 Failure to Reduce Glutamate Levels in Medial Prefrontal Cortex During NREM Sleep Could Cause Transient Insomnia. *SLEEP*, *47*(Supplement_1), A157–A158. https://doi.org/10.1093/sleep/zsae067.0365

Yoon, S., Lee, S., Joo, Y., Ha, E., Hong, H., Song, Y., Lee, H., Kim, S., Suh, C., Lee, C. J., & Lyoo, I. K. (2024). Variations in brain glutamate and glutamine levels throughout the sleep-wake cycle. *Biological Psychiatry*. https://doi.org/10.1016/j.biopsych.2024.11.016

Zhu, W., Huang, L., Cheng, H., Li, N., Zhang, B., Dai, W., Wu, X., Zhang, D., Feng, W., Li, S., & Xu, H. (2024). GABA and its receptors' mechanisms in the treatment of insomnia. *Heliyon*, *10*(23), e40665. https://doi.org/10.1016/j.heliyon.2024.e40665