

Increased Tau, A β , and Microglial Inactivation in the Middle Temporal Gyrus Is Associated With Lower Cognitive Function in SEA-AD Donors

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Abstract

Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by mechanisms including neuroinflammation, neurofibrillary tangles of phosphorylated tau, accumulation of amyloid plaques, and microglial aggregation. In AD, disease pathology is closely related to cognitive function, measured using the Mini-Mental-State-Examination (MMSE), a clinical screening tool. This study examined relationships among tau burden, amyloid deposition, and microglial activity in the middle temporal gyrus across three MMSE-defined stages of cognitive decline. Data, including quantifications of AT8, 6e10, and IBA1, were obtained from the Seattle Alzheimer's Disease Brain Cell Atlas. Two-way ANOVAs were used to study the change in percent AT8+ area (phosphorylated tau), IBA1+ cells per area and activation (microglia), 6e10+ cells per area (amyloid-beta), and IBA1+/6e10+ colocalized objects per area, alongside MMSE-defined cognitive decline. Our analysis revealed that neuropathological measures of percent AT8+ area, IBA1/6e10 colocalized objects per area, and IBA1 inactive:active ratio reflected a significant overall MMSE effect, while measures of IBA1+/6e10+ colocalized objects per area, IBA1+ cells per area, and 6e10+ cells per area reflected a significant overall layer effect. Although general IBA1+ cell per area counts were comparable across MMSE groups, the inactive:active ratio differed, with the lowest MMSE group having the highest ratios. These findings lead to the hypothesis that lower MMSE scores may be associated with more tau burden, amyloid deposition, and amyloid/microglia colocalization, accompanied by alterations in microglial state. Together, these findings inform a broader understanding of the link between pathological burden and cognitive function in a multidimensional model of AD.

Keywords: Alzheimer's disease (AD), Seattle Alzheimer's Disease Brain Cell Atlas (SEA-AD), Mini-Mental State Examination (MMSE), tau pathology, amyloid beta, microglial activation, middle temporal gyrus, neuroinflammation



1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia and is characterized by progressive cognitive decline (Atri, 2019; US Department of Health and Human Services, 2017). It is accompanied by the accumulation of extracellular amyloid- β (A β) plaques, intracellular neurofibrillary tangles composed of hyperphosphorylated tau, and widespread neuroinflammation (Khachaturian, 1985; Reitz, 2012). Although these pathological hallmarks have been studied extensively, the mechanisms linking such protein aggregation to cognitive impairment are still unclear.

The accumulation of extracellular amyloid plaques, formed from self-aggregating peptides, has long been regarded as the first hallmark of Alzheimer's disease, inhibiting neuronal synaptic function (Reitz, 2012). Amyloid deposition is known to begin years before the onset of clinical symptoms (Wu et al., 2022). On the other hand, tau accumulation, presenting as neurofibrillary tangles toxic to neurons, is implicated to more closely track the timeline of dementia-related neuronal dysfunction, synapse loss, and worsening cognition (Cody et al., 2024). Consequently, increasing tau burden is commonly considered one of the strongest neuropathological indicators of disease severity (Murray et al., 2022). Previous studies have also suggested that tau pathology is more strongly associated with cognitive impairment than amyloid burden alone (Guo et al., 2026). Amyloid pathology nevertheless remains an important marker, as it is commonly believed to initiate a cascade of downstream events that contribute to tau aggregation, neurodegeneration, and ultimately dementia—a process often referred to as the “amyloid cascade hypothesis” (Reitz, 2012).

In addition to protein deposition, neuroinflammation is another major hallmark of dementia, with emerging studies suggesting that inflammation may be both a consequence and an initiator of AD pathology like tau accumulation and amyloid plaques (Zhang et al., 2023). Under healthy conditions, microglia contribute to tissue maintenance through debris clearance and immune surveillance (McKinney et al., 2026). They are the brain's resident immune cells and macrophages, continuously monitoring the brain environment and responding to accumulating amyloid deposits and neuroinflammation (Muzio et al., 2021). In AD, however, microglia aggregate around amyloid plaques and undergo substantial changes in morphology, gene expression, and function (Bolmont et al., 2008).

Experimental evidence suggests that microglia initially exert protective effects by containing plaques and phagocytosis of pathological proteins, but being exposed to prolonged pathological stress may yet lead to impaired clearance mechanisms and ultimately contribute to neurodegeneration by accelerating the spreading of proteins like A β and tau. (Gao et al., 2023). Furthermore, evidence argues that microglia have specific activation states which may mediate synapse loss, worsen tau pathology, and secrete inflammatory factors that directly harm neurons (Hansen et al., 2018). Microglia are implicated to be able to transform from one phenotype to others—many researchers believe that the M1 microglia, which release inflammatory mediators, and the M2 microglia, which release anti-inflammatory mediators, may provide further insight on the effective activation states of microglia (Guo et al., 2022). However, current understanding has not reached a consensus on whether microglial states can be explicitly divided into simply anti-inflammatory and pro-inflammatory subtypes, due to the complex heterogeneity and heavily context-dependent nature of microglial states (Paolicelli et al., 2022). Despite growing recognition of the importance of neuroinflammation, it still remains unclear whether microglia continue to respond effectively as the disease progresses or whether they transition into altered functional states that contribute to further pathological accumulation. This study therefore investigates how the accumulation of microglia around sites of pathology may change across different stages of neurodegeneration.



In AD, neuroinflammation spreads heavily across the brain to areas like the cerebral cortex, the outer layer of the brain responsible for thinking, memory, and language (Ni & Wu, 2021). The cerebral cortex is organized into distinct layers that all differ in cellular composition, connectivity, and functional roles, meaning pathology may behave differently across layers (Chen et al., 2021). Emerging evidence suggests that neurodegenerative pathology may not be distributed uniformly throughout cortical tissue, and analyses restricted to just gray matter measurements may obscure important layer-specific patterns (Willumsen et al., 2022). Across past studies, AD neuropathology has been known to show strong regional specificity when developing across the brain (Yang et al., 2026). Neurofibrillary tangles typically spread from the entorhinal cortex to the hippocampus, limbic system, and neocortex, amyloid plaques show graded accumulation across brain regions, and microglia in the prefrontal cortex, cerebellum, and hippocampus are known to have significant differences in their highly expressed genes (Asher & Priefer, 2022; Jacobsen et al., 2005; Yang et al., 2026).

The middle temporal gyrus (MTG) is particularly relevant in AD because of its involvement in language processing, semantic memory, and other higher order cognitive functions that become impaired during disease progression (Chen et al., 2022). Therapeutic strategies developed over the years rely heavily on gray matter analysis and often encounter obstacles once in clinical trials (Asher & Priefer, 2022). Investigating pathology across individual cortical layers may therefore provide additional insight into selective regional differences and vulnerabilities.

Despite extensive research conducted on AD biomarkers, critical gaps in the scientific understanding of disease mechanisms still exist. It remains unclear whether layer-specific microglial clustering directly damages local circuitry or merely reacts to underlying synaptic failure and plaque growth, and when microglia transition activation states (Escarião et al., 2025). In the MTG itself, the precise functional organization and the exact division of labor for semantic processing remain unknown, meaning a layer-specific approach to examining the MTG may provide more insight (Escarião et al., 2025).

Accordingly, the present study investigated relationships among tau burden, amyloid deposition, and microglial activity within the MTG across MMSE-defined stages of cognitive decline, where MMSE stands for the Mini-Mental State Examination. Particular emphasis was placed on examining the pathology within individual cortical layers 1 to 6, following initial bulk gray matter analyses.

A previous study of these markers in the SEA-AD dataset investigated the APOE4 genotype and an individual's genetic predisposition to dementia (Wang, 2025). However, this present study uses MMSE grouping to better understand the relationships between disease pathology and an individual's ongoing cognitive capacities.

I hypothesized that lower MMSE scores would be associated with higher levels of tau burden and amyloid deposition, as well as accompanied alterations in microglial activation. More specifically, the microglia were expected to increasingly associate with sites of pathology whilst displaying signs of change in activation. Furthermore, layer-specific analyses were expected to reveal patterns that would not be apparent from the gray matter measurements alone.

2. Materials and Methods

Previously collected data were accessed and reanalyzed in the present study. De-identified postmortem human brain data and quantitative neuropathological measurements of the middle temporal gyrus were accessed from the Allen Brain Institute's publicly available SEA-AD dataset (Hawrylycz et al., 2024). All initial experimentation and data collection were

conducted by the Kaiser Permanente Washington Health Research Institute ACT Study researchers and the University of Washington ADRC at the UW BioRepository and Integrated Neuropathology (BRaIN) laboratory. The dataset focused on different patients' histological pathology, obtained via immunolabeling and microscopy across cortical layers and protein markers.

For my study, the data from the Quantitative Neuropathology Summary Data (MTG) and Donor Metadata from the Allen Brain Institute's SEA-AD data set were aligned and compiled in an Excel spreadsheet in order to match neuropathology data to donor MMSE status. Donors were stratified into three groups based on their respective Mini-Mental State Examination (MMSE) scores: 27–30, 23–26, and 0–22.

2.1. MMSE-Based Cognitive Stratification

Cognitive status was classified into donor groups according to Mini-Mental State Examination (MMSE) scores obtained from SEA-AD donor metadata. The MMSE is a widely used clinical screening tool for cognitive impairment, with scores ranging from 0 to 30, where lower scores indicate greater cognitive dysfunction (Creavin et al., 2016; Yang et al., 2024).

Donors were stratified into three groups based on established thresholds used in dementia research: MMSE 27–30 (cognitively normal or minimally impaired), MMSE 23–26 (mild cognitive impairment), and MMSE 0–22 (moderate to severe cognitive impairment consistent with dementia).

Across studies, there exist many different categorizations of MMSE scores, with no consensus yet on what threshold may be truly significant in terms of cognitive status. The Mini-Mental State Examination (MMSE) faces significant diagnostic inconsistency because fixed cutoff scores fail to account for demographic variations—while a score of 24 serves as the traditional clinical threshold, its diagnostic accuracy is heavily confounded by a patient's age and education level, often yielding false negatives in highly educated individuals and false positives in those with limited schooling (Creavin et al., 2016). Consequently, researchers and clinicians increasingly employ stratified thresholds ranging from 21 to 27 to enhance sensitivity and specificity across diverse populations (Salis et al., 2023). Therefore, MMSE grouping is heavily dependent on the dataset being analyzed. Based on this framework, the present study used MMSE stratification to approximate stages of cognitive decline and to enable comparison of neuropathological burden across disease severity.

Due to the nature of dementia disease progression, age at death is an essential consideration when analyzing the severity of pathological burden. Thus, when deciding the MMSE ranges for the three experimental groups, instead of solely relying on numbers used in past studies, I used a one-way ANOVA to run a replicate data column analysis with multiple comparisons of donor ages at death. The group mean of 0–22 was 88.76 with a SD of 7.289, the group mean of 23–26 was 89.29 with a SD of 7.349, and the group mean of 27–30 was 89.87 with a SD of 7.315. Results of pairwise contrast tests ran between groups yielded the following adjusted pairwise p-values: 0.971 for 0–22 vs 23–26, 0.968 for 0–22 vs 27–30, and 0.960 for 23–26 vs 27–30. The analyses yielded no significant difference between groups ($F_{2,76} = 0.1247$, $p = .883$). Thus, these cutoffs provided a more balanced partition of donor data rather than relying on arbitrary and inconsistent thresholds.

In my dataset, the group with MMSE scores 27–30 consisted of 31 donors, the group with MMSE scores 23–26 consisted of 31 donors, and the group with MMSE scores 0–22 consisted of 17 donors.



All downstream analyses were performed using these MMSE-defined groups to evaluate relationships between cognitive status and pathological markers in the middle temporal gyrus.

2.2. Neuropathological Quantification

Detailed tissue processing and staining protocols have been previously described in a published paper as well as an online procedural overview (Gabbitto et al., 2024; Seattle Alzheimer's Disease Data Set, n.d.).

Briefly, postmortem human brain tissue was obtained through the Adult Changes in Thought (ACT) study conducted by the Kaiser Permanente Washington Health Research Institute in collaboration with the University of Washington. Researchers collected middle temporal gyrus (MTG) samples, fixing and embedding each in paraffin prior to sectioning. Sections underwent duplex immunohistochemical staining to detect various hallmark Alzheimer's disease proteins (Hawrylycz et al., 2024). Phosphorylated tau was labeled using the AT8 antibody, while amyloid- β (A β) deposits were identified using the 6E10 monoclonal antibody. Quantification of percent AT8-positive area, 6E10-positive objects per area, and IBA1-associated measures was performed on digitized images of the MTG cortical layers. IBA1-positive cells were classified as active or inactive according to dataset-provided morphological criteria.

Cortical layers are established by the SEA-AD dataset annotations, in which Layer 5 and Layer 6 are combined. Thus, the layers are analyzed accordingly as L1, L2, L3, L4, and L5/6.

The Allen Brain Institute collected and compiled the data for access to the general public. Data were accessed June 6th, 2026, via

<https://portal.brain-map.org/explore/seattle-alzheimers-disease/seattle-alzheimers-disease-brain-cell-atlas-download?edit&language=en>.

2.3. Outlier Removal

To preserve the statistical validity and reproducibility of my pathological assessments, outlier detection was performed for all pathological measurements using the ROUT method (Q = 1%) in GraphPad Prism. No outliers were identified consistently across all measures, therefore no donors were excluded from my analyses.

2.4. Statistical Analysis

Statistical analyses were largely performed using two-way mixed effect model ANOVAs with repeated measures, with MMSE group as the between-subjects factor and cortical layer as the repeated within-donor factor. This design is required because multiple cortical layers are sampled from the same individual, violating the assumption of independent observations necessary for standard one-way ANOVAs. By modeling donor identity as a random effect, this approach accounts for the inherent statistical dependency within each subject, properly partitions the variance, prevents the artificial inflation of the degrees of freedom, and allows for the critical evaluation of the interaction effect between MMSE group and layer (Wilcox & Rousselet, 2023). A one-way ANOVA was performed for the AT8 measure due to the fact that it is a plain gray matter analysis with no cortical layer analyses.

Multiple comparisons were performed for all analyses by comparing cell means with others in its row and column. Šidák's



multiple-comparisons correction was used to control the family-wise Type I error rate arising from multiple pairwise comparisons while providing greater statistical power than the more conservative Bonferroni correction. (Abdi, 2007) Sphericity was tested (Mauchly) when applicable and G-G correction applied when violated.

To ensure valid cross-layer comparisons within the MTG, normalized counts were used for analysis of all measures. This normalization is statistically required to control for the prominent anatomical variations in laminar thickness inherent to the MTG (Zachlod et al., 2022). Failing to account for these structural volume differences would conflate a higher absolute cell count with a true increase in cellular density, thereby introducing systematic bias into our regional comparative analyses. Normalized measures are presented as AT8 percent area (%), IBA1+/6e10+ colocalized objects per area ($\#/\mu\text{m}^2$), IBA1+ objects per area ($\#/\mu\text{m}^2$), 6E10+ objects per area ($\#/\mu\text{m}^2$), and IBA1+ inactive microglial cells per area : IBA1+ active microglial cells per area (#).

Data are presented as bars with mean $\pm$ SEM. Statistical significance thresholds were defined per APA style 7th edition as:

$$*p < .05, **p < .01, ***p < .001.$$

I calculated the overall main effect of MMSE, layers, and the MMSE x layer interaction for all two-way ANOVAs. A significant overall main effect of MMSE group (M) demonstrates that MMSE grouping has a statistically significant effect on the dependent variable. Similarly, a significant overall main effect of cortical layers (L), then, means that the layers of the MTG have a statistically significant effect on the dependent variable. A significant MMSE x cortical layer interaction indicates that there is a statistically significant interaction between both the effects of MMSE grouping and layer. Thus, while the main effects describe overall differences associated with either cognitive status or cortical layers, the interaction significance provides evidence that the two factors jointly influence the distribution of the biomarker across the middle temporal gyrus.

3. Results

3.1. AT8 Pathology in the Middle Temporal Gyrus Higher with Cognitive Decline

To assess the relationship between tau pathology and cognitive decline, percent AT8-positive area was quantified across the gray matter of the middle temporal gyrus (MTG) and stratified by MMSE category (Figure 1). My analyses compared donors with low, medium, and high MMSE scores, where a standard one-way ANOVA revealed a significant difference between groups ($F_{2,76} = 14.31$, $***p < .001$). Šídák-adjusted post-hoc tests showed significance of $***p < .001$ for both MMSE 27-30 vs. MMSE 0-22 and MMSE 23-26 vs. MMSE 0-22.

AT8 burden was higher in donors with decreased MMSE scores, with the lowest MMSE group (0-22) exhibiting a greatly elevated and significant percent AT8-positive area. The intermediate group (23-26) showed values between the high and low MMSE groups, indicative of a graded relationship between tau accumulation and cognitive impairment.

This trend was consistent across donors within each group, with statistical comparisons demonstrating a significant increase in AT8-positive area in the 0-22 group relative to both higher MMSE groups ($***p < .001$).



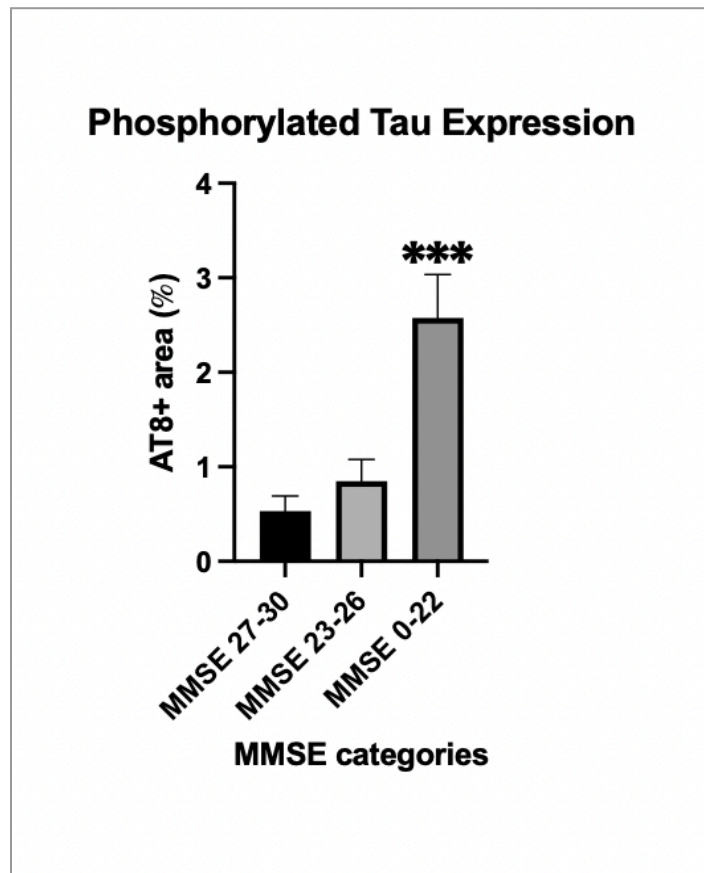


Figure 1: Percent AT8-positive area in MTG gray matter across MMSE categories.

Note. Significance (***) $p < .001$ was found between MMSE group 0-22 and all other MMSE groups..

3.2. Increased Microglial Association with Amyloid Pathology Across Cortical Layers

To evaluate microglial interaction with amyloid pathology, the number of IBA1/6E10 co-localized objects per area was quantified across cortical layers of the MTG (Figure 2). Co-localized object per area counts were higher with decreased MMSE scores, with the greatest values observed in the 0-22 group. The progressive increase in co-localized objects per area across MMSE groups may indicate an enhanced microglial presence in proximity to amyloid deposits as cognitive impairment worsens.

Two-way ANOVA analyses with MMSE group as the between-subjects factor and cortical layer as the repeated within-donor factor revealed a significant overall layer effect ($F_{2,528,192.1} = 14.58$, $***p < .001$), a significant overall MMSE effect ($F_{2,76} = 3.290$, $*p = .043$) and a significant overall interaction effect of layer and MMSE group ($F_{5,056,192.1} = 2.367$, $*p = .041$). However, no statistical significance was found between individual layers.

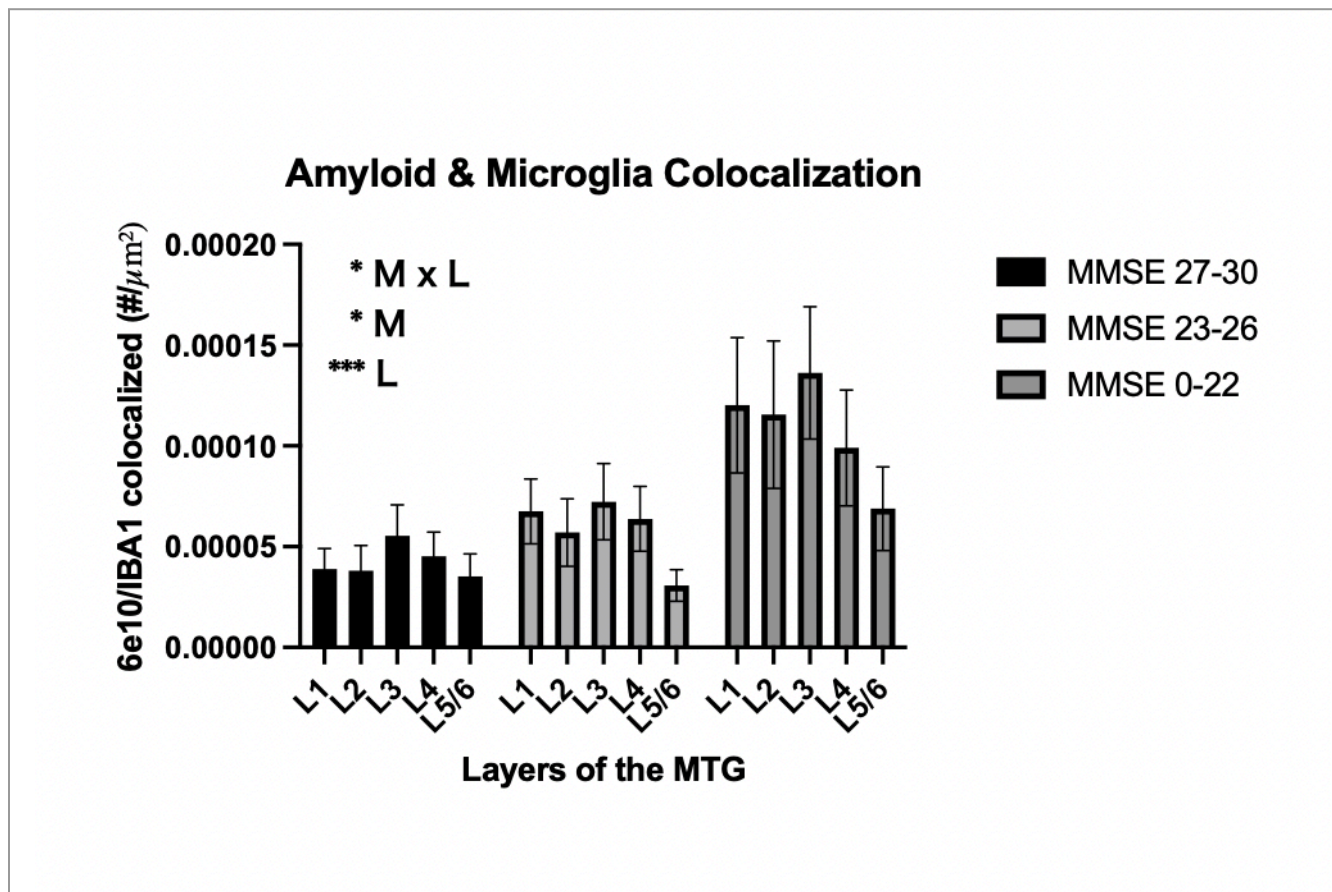


Figure 2: Number of IBA1/6E10 co-localized objects per area.

Note. Significance was observed for the overall interaction effect of layer and MMSE group (* M x L), the overall MMSE group effect (* M), and the overall layer effect (***) L).

3.3. Total Microglial Presence Appears Comparable Across Cognitive Status

To assess whether the survival of microglial cells affected amyloid colocalization, the amount of IBA1-positive cells per area was first compared across cortical layers and MMSE groups (Figure 3). Notably, layer-specific multiple comparison analysis revealed significance between layers within each MMSE group, but not across MMSE groups themselves.

Two-way ANOVA analyses with MMSE group as the between-subjects factor and cortical layer as the repeated within-donor factor revealed a significant overall layer effect ($F_{1,618,122.9} = 14.62$, $***p < .001$), but no significant overall MMSE effect or significant overall interaction effect of layer and MMSE group.



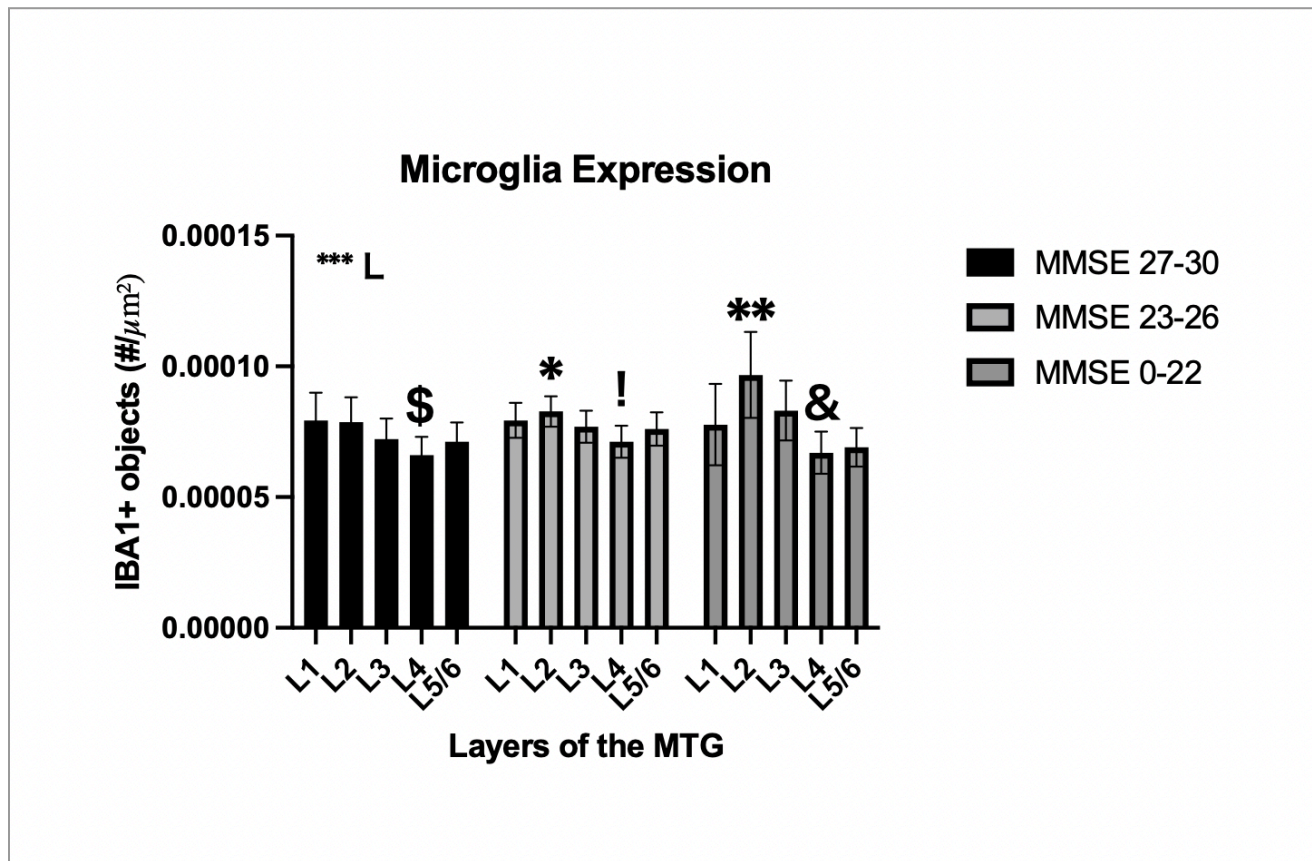


Figure 3: Number of total IBA1 positive cells per area.

Note. Significance was observed for the overall layer effect (***) L. In MMSE 27-30, significance marked by “\$” was found between L4 and L2 (**p < .01), L4 and L5/6 (**p < .01), and L4 and L3 (***p < .001). In MMSE 23-26, significance was found between L3 and L2 (*p < .05). Significance (***p < .001) marked by “!” was found between L2 and L4, and L3 and L4. In MMSE 0-22, significance was found between L1 and L2 (**p < .01). Significance (*p < .05) marked by “&” was found between L3 and L4.

3.4. Layer-Specific Distribution of Amyloid- β Deposition

To determine whether the observed layer-patterns from Figure 2 were driven by amyloid distribution, 6E10-positive objects per area were quantified independently across MTG layers (Figure 4). A consistent significance of 6E10-positive objects per area was observed in layer 3 across all MMSE groups.

Two-way ANOVA analyses with MMSE group as the between-subjects factor and cortical layer as the repeated within-donor factor revealed a significant overall layer effect as well ($F_{2,529,192.2} = 14.45$, ***p < .001), but no significant

overall MMSE effect or significant overall interaction effect of layer and MMSE group.

It is appreciably observed that the low MMSE group exhibits higher densities of 6E10+ objects per area than both other MMSE groups. However, because this is just a descriptive pattern, there is no direct evidence that amyloid density increases with cognitive decline in this analysis.

Notably, the number of 6E10-positive objects per area exceeded the number of IBA1/6E10 co-localized objects per area across all groups and layers, perhaps indicating that only a subset of detected 6E10+ objects per area met the criteria for colocalization.

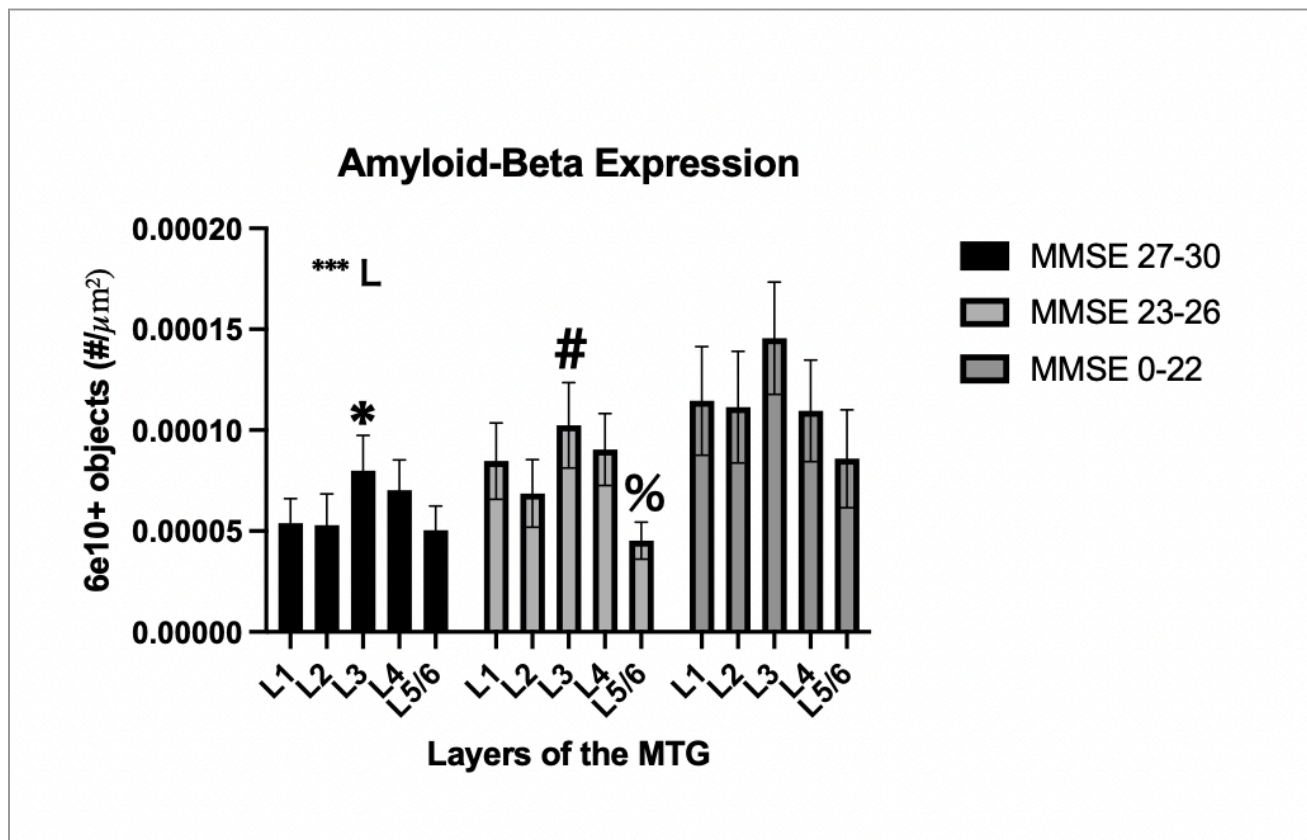


Figure 4: Number of 6E10-positive objects per area.

Note. Significance was observed for the overall layer effect (***) L. In MMSE 27-30, significance was found between L3 and L2 (*p < .05). In MMSE 23-26, significance (***p < .001) marked by “#” was found between L3 and L2 (***p < .001). Significance (*p < .05) marked by “%” was found between L5/6 and L3, and L5/6 and L4. No significance was found between individual layers of MMSE 0-22.

3.5. Microglial Inactivation Is Higher with Worse Cognitive Status

To assess microglial morphology and states, the ratio of morphologically-classified inactive to active IBA1-positive microglial cells per area was calculated (inactive cells per area divided by active cells per area) across cortical layers and MMSE groups (Figure 5). A prominent difference in the inactivation ratio was observed between the 0-22 MMSE group and all other MMSE groups.

Two-way ANOVA analyses with MMSE group as the between-subjects factor and cortical layer as the repeated within-donor factor revealed a significant overall MMSE group effect ($F_{2,76} = 3.959$, $*p = .023$), but no significant overall layer effect or significant overall interaction effect of layer and MMSE group.

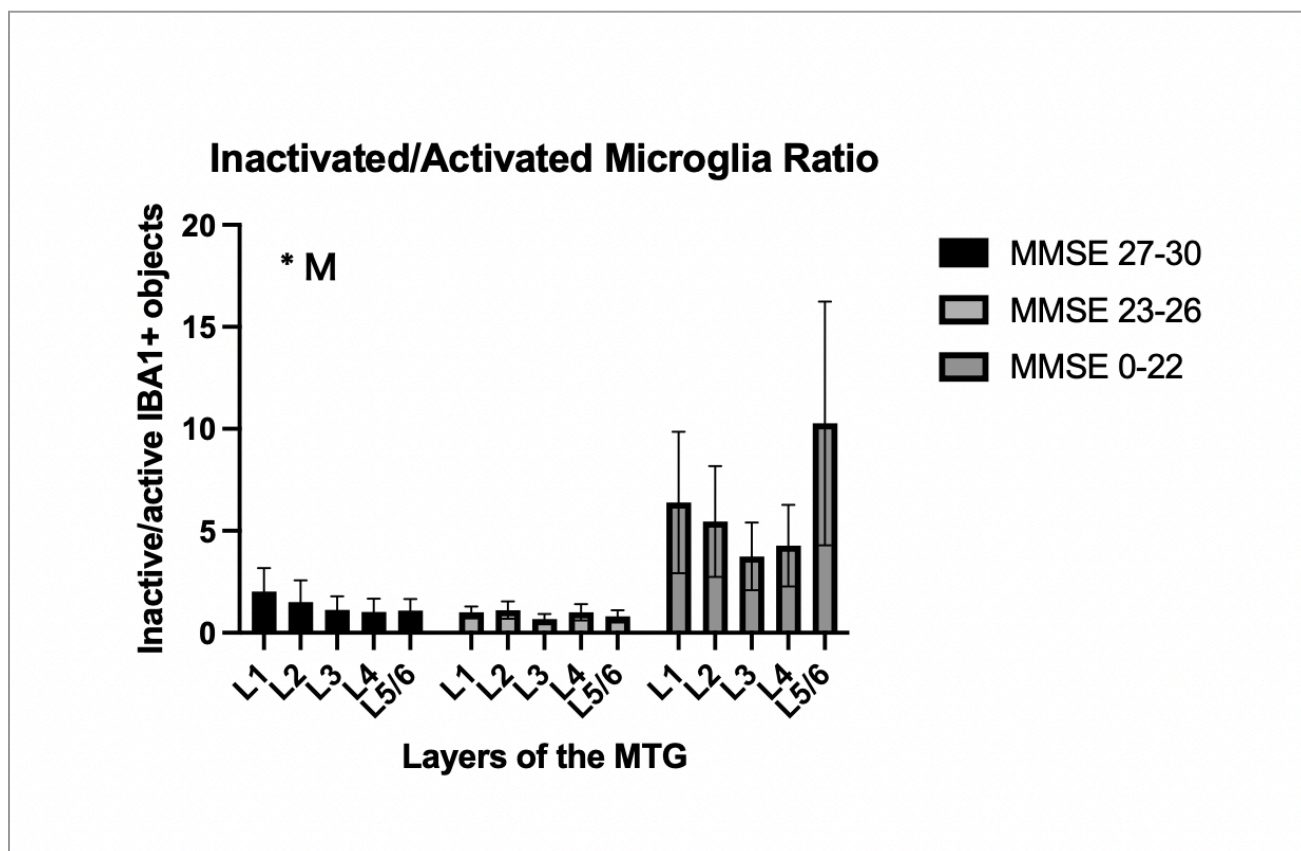


Figure 5: Ratio of inactive to active IBA1-positive cells per area.

Note. Significance was observed for the overall MMSE group effect (*M) only.

Although differences between specific cortical layers and MMSE groups were not statistically significant, an increased inactivation ratio was clear in MMSE group 0-22. In the lowest MMSE group, the ratio indicated a relatively higher amount

of inactivated IBA1 cells, with the highest inactive:active cell per area ratio as compared to both other MMSE groups.

These findings indicate that while microglia remain comparably present in regions of pathology as seen in Figure 3, their activation state may be different for individuals in different stages of disease progression.

3.6. Summary of Pathological Trends Across MMSE Groups

Across measures, lower MMSE scores were associated with higher pathological burden and altered microglial dynamics. AT8-positive percent area, IBA1/6E10 co-localized objects per area, 6E10-positive objects per area, and the IBA1 inactive:active cell per area ratio were highest in donors with an MMSE score between 0-22. IBA1 positive cell per area counts were relatively constant across MMSE scores, indicative of almost no difference related to disease severity. In contrast, the ratio of inactive to active microglia was higher in donors with lower MMSE scores. Overall layer significance was observed in all analyses except for the IBA1 ratio.

4. Discussion

4.1. Progressive Pathological Burden Accompanies Cognitive Decline

The present study investigated relationships between tau pathology, amyloid deposition, and microglial activity within the middle temporal gyrus (MTG) across MMSE-defined stages of cognitive decline. I hypothesized that lower MMSE scores would be associated with higher pathological burden across measures, accompanied by altered microglial activation states. Overall, my findings supported this hypothesis and affirmed expected changes in disease pathology and neuroimmune dynamics in individuals with varying degrees of cognitive deficits.

Tau burden, measured by the percent AT8-positive area in the study, was substantially higher in donors with lower MMSE scores and exhibited a graded pattern across the three cognitive groups. Donors with the lowest MMSE scores (0-22) demonstrated the greatest accumulation of phosphorylated tau within MTG gray matter, supporting the close relationship between tau pathology and cognitive deterioration.

Post-hoc comparisons confirmed significantly greater AT8 levels in the low-MMSE group compared with both the medium- and high-MMSE groups. This relationship between more AT8 pathology and lower MMSE provided an initial indication that cognitive status was associated with meaningful differences in neuropathology within the MTG and motivated further investigation of whether other pathological measures might exhibit similar relationships with MMSE and whether these relationships vary across cortical layers.

Whereas amyloid accumulation is generally believed to precede the onset of clinical symptoms and eventually plateau, tau pathology has been shown to track more closely with disease severity (Mormino & Papp, 2018). The present findings therefore support that tau accumulation is a key pathological feature closely associated with cognitive decline in Alzheimer's disease. Collectively, the statistically significant effect of tau pathology across MMSE groups is consistent with current models of Alzheimer's disease.



4.2. Microglia Associate With Amyloid Plaques

Initially, quantification of IBA1/6E10 co-localized objects per area demonstrated the expected higher microglial association with amyloid deposits in lower MMSE donors. At first glance, this finding suggests a natural heightened immune response accompanying increasing pathological burden.

My analysis of IBA1+/6E10+ colocalized objects per area demonstrated significant main effects of MMSE group and cortical layer, as well as a significant MMSE group x cortical layer interaction. The significant main effect of MMSE group indicates that the density of IBA1+/6E10+ colocalized objects is dependent on the MMSE group. Consistent with this effect, a progressive increase in colocalized objects was observed across the MMSE groups, suggesting greater microglial association with amyloid pathology in the donors with greater cognitive impairment. The significant main effect of cortical layer indicates that the density is also dependent on the layer, which suggests that different regions of the MTG may vary in properties or vulnerability to accumulating amyloid plaques (Leng et al., 2021; Willumsen et al., 2022; Yang et al., 2026).

The significant MMSE x layer interaction indicates that the density of IBA1+/6E10+ colocalized objects is dependent on the interaction between MMSE group and layer. In interpreting this interaction effect, I ran two more secondary multiple comparisons tests to specifically identify where the significant MMSE effect (comparing MMSE means) and the layer effect (comparing layer means) occurred. The secondary MMSE effect analysis highlighted a significance (**p <.001) between the MMSE 0-22 group and both other groups, whereas the MMSE 23-26 group and the MMSE 27-30 group did not significantly differ from each other. The secondary main layer effect analysis then yielded a significance (*p <.05) between L3 and L5/6. Thus, the interaction effect can be interpreted as meaning that the difference between L3 and L5/6 of the MMSE 0-22 group is significantly greater than both the difference between L3 and L5/6 of the MMSE 23-26 group, as well as the difference between L3 and L5/6 of the MMSE 27-30 group. This suggests that the regional variation of amyloid/microglia colocalization may be more pronounced with lower cognition, and therefore differences in regional properties may also be more pronounced in donors with low cognition (Chen et al., 2022; Escarião et al., 2025). These findings therefore prompted further investigation into whether a significant layer effect, MMSE effect, or MMSE x layer interaction effect was also observed for IBA1+ alone or 6E10+ alone.

4.3. Microglia Remain Present but May Transition Towards An Altered State

Despite differences in co-localization with amyloid pathology, IBA1-positive cell per area counts actually remained relatively constant across the three cognitive groups. My analysis of IBA1+ objects per area demonstrated a significant main layer effect, indicating that the density of IBA1+ objects is dependent on the layer. In interpreting this significant layer effect, I ran a secondary multiple comparisons test to specifically identify where the layer effect (comparing layer means) occurred. The analysis highlighted a significance (*p <.05) between L2 and L4, whereas the other layers did not significantly differ from each other. This finding suggests that different regions of the MTG may vary in microglial presence, which could make certain areas more or less vulnerable to injury and accumulation of proteins (Chen et al., 2021). However, no significant main effect of MMSE group or MMSE x layer interaction was observed, indicating that overall IBA1+ object density was not dependent on MMSE or the interaction between MMSE and layer.

My analysis of 6E10+ colocalized objects per area demonstrated a significant main layer effect, indicating that the amyloid density is dependent on the cortical layer. In interpreting the significant overall layer effect, I ran a secondary multiple



comparisons test to specifically identify where the layer effect (comparing layer means) occurred. The analysis highlighted a significance ($*p < .05$) between L3 and L5/6, whereas the other layers did not significantly differ from each other. This finding further supports that the MTG may exhibit region-specific vulnerability to accumulating amyloid plaques (Leng et al., 2021; Willumsen et al., 2022). No significant main effect of MMSE group or MMSE $\times$ layer interaction was observed, indicating that overall 6e10+ object density did not depend on the donor's MMSE grouping or the interaction between MMSE and layer. Interestingly, 6e10+ object-per-area counts were consistently higher than the corresponding counts of colocalized objects, suggesting that not all amyloid deposits were associated with detectable IBA1-positive microglial colocalization and that microglial association with amyloid deposits may therefore be incomplete.

Nevertheless, while microglia continued to localize around amyloid deposits, the change in inactive divided by active IBA1 cell per area ratios suggested relatively higher microglial inactivation in donors with lower MMSE scores. My analysis of the IBA1+ inactive:active ratio demonstrated a significant main MMSE effect, indicating that the relative proportion of inactive to active microglia differed according to cognitive status. In interpreting the MMSE effect, I ran a secondary multiple comparisons test to specifically identify where the MMSE effect (comparing MMSE means) occurred. The secondary MMSE effect analysis highlighted a significance ($***p < .001$) between the MMSE 0-22 group and both other groups, whereas the MMSE 23-26 group and the MMSE 27-30 group did not significantly differ from each other. Essentially, lower ratios indicate relatively less inactive cells as compared to active, while higher ratios indicate relatively more inactive cells as compared to active. Therefore, the donors exhibiting higher ratios for this measure, namely the low MMSE group, could be experiencing changes in microglial state that cause such a shift in activation (Guo et al., 2022).

However, no significant main effect of cortical layer or MMSE $\times$ layer interaction was observed, indicating that the IBA1+ inactive:active ratio was not dependent on layer or the interaction between MMSE and layer. Thus, unlike the layer-dependent effect observed for IBA1+/6e10+ colocalization, IBA1+ alone, and 6e10+ alone, the pronounced shift in the inactive:active ratio was primarily dependent on MMSE grouping. This suggests an intriguing relationship between lower cognitive status and changes in microglial activation—moreover, this relationship may be observed across regions, independent of layer dynamics.

Rather than representing an entirely absent immune response, however, these results may be indicative of a state of chronic dysfunction or cellular exhaustion. Past research has shown that under conditions of sustained pathological stress, microglia may remain physically associated with amyloid deposits but transition to an altered state (Cheng & Ho, 2025; Gao et al., 2023). This state may include reduced ability to execute protective functions such as plaque clearance, debris removal, or maintenance of tissue homeostasis (Lier et al., 2021). Such a reduced-activation state could be a major contributor to continued pathological accumulation despite persistent microglial presence.

This interpretation is consistent with emerging literature describing differing disease-associated microglial (DAM) states in Alzheimer's disease (Cheng & Ho, 2025; Kuhn & Proctor, 2025). Although the present study does not directly measure DAM signatures, they provide an interesting conceptual connection that may inform future research. Several studies have reported that microglia surrounding plaques exhibit altered transcriptional profiles, including impaired phagocytic activity and reduced homeostatic signaling (Hassan et al., 2025). Experimental models have similarly demonstrated that prolonged exposure to amyloid pathology can shift microglia from protective to maladaptive states (McKinney et al., 2026).



The present findings contribute to this ongoing discussion by suggesting that physical proximity to pathology does not necessarily indicate effective functional engagement. However, although changes in inactive to active ratios based on morphology may provide insight into altered microglial states, the current data cannot conclusively measure or affirm microglial function.

4.4. Selective Layer Enrichment

A notable feature across multiple analyses was the significant overall layer effect. Significance was observed in L3 of the 6e10+ measure and L2 of the IBA1+ measure. Additionally, in the secondary multiple comparisons tests (comparing layer means) for both IBA1+/6e10+ colocalized objects per area and the 6e10+ objects per area, significance (* $p < .05$) was found between L3 and L5/6. Moreover, in the secondary multiple comparisons tests (comparing layer means) for the IBA1+ objects per area, significance (* $p < .05$) was found between L2 and L4.

Cortical layers differ significantly in neuronal composition, connectivity, and functional organization, and these differences may influence selective vulnerability during neurodegeneration (Chen et al., 2021). This is particularly true for the MTG, where there are structural and microarchitectural asymmetries between the left and right hemispheres, regional variations in surface area, and differences in cortical thickness (Zachlod et al., 2022). Analyses limited to bulk gray matter measurements risk masking such spatial patterns. The layer-specific approach used here therefore provides additional resolution and suggests that important disease-related changes may occur within discrete anatomical compartments rather than uniformly throughout cortical tissue.

The enrichment of amyloid pathology within Layer 3 may be particularly significant given the role of this layer in corticocortical communication. Interestingly, a significantly lower 6e10+ object-per-area count was observed in layer 5/6 as well, further suggesting that layer dynamics should be a strong consideration for future studies.

Layer 3 pyramidal neurons form extensive long-range connections with other cortical association areas and are heavily integrated into large-scale neural networks involved in cognition (Planert et al., 2026). Previous studies have suggested that highly connected neuronal populations may be especially vulnerable to pathological protein spread and metabolic stress (Bloomingdale et al., 2022; Brettschneider et al., 2015). Research has also suggested that neurons in layers 2 and 3 of the MTG may be more vulnerable to degenerative mechanisms in early stages of AD (Chen et al., 2021). Consequently, the concentration of amyloid pathology within layer 3 may reflect underlying network properties that increase a region's susceptibility to neurodegeneration (Escarião et al., 2025).

The IBA1+ analyses revealed significant enrichment of microglial presence within Layer 2, and a significantly lower count within Layer 4. The high microglial localization found in Layer 2 and the low microglial localization found in Layer 4 do not necessarily indicate that the microglia themselves exhibit a natural preference towards layers—rather, they may be responding to accumulating pathology in more vulnerable areas of the MTG. This layer-specific angle of researching AD must consider the possibility that certain layers are more susceptible to some biomarkers—and these vulnerable areas may be different for different biomarkers.

Past research has shown that layers 3 and 4 of the neocortex, including the MTG, are the first to accumulate neurofibrillary tangles of tau (Leng et al., 2021). Evidence also suggests that neurons in layers 2, 3, and 5 of the temporal lobe, including the



MTG, may be selectively vulnerable to tau pathology (Chen et al., 2022). Together, these findings raise the possibility that distinct cortical layers may contribute differently to disease progression. Further investigation is needed to confirm whether these layer-specific patterns are unique to the MTG or represent broader cortical inhomogeneity in Alzheimer's disease.

4.5. Findings Extend Existing Models of Disease Progression

The overall findings broadly align with current models of Alzheimer's disease progression while also highlighting potential limitations of simplified frameworks. Descriptive differences in amyloid burden, alongside progressive tau accumulation, is consistent with the amyloid cascade hypothesis, which proposes that amyloid deposition initiates downstream pathological events leading ultimately to neurodegeneration and cognitive decline (Wu et al., 2022).

However, the present findings suggest that immune responses may play a more active and complex role than originally proposed. While classical models often frame neuroinflammation as a secondary consequence of pathology, growing evidence suggests that microglial dysfunction itself may contribute meaningfully to disease progression (Cheng & Ho, 2025; McKinney et al., 2026). The present data support this possibility—microglial cells appeared increasingly associated with pathology but simultaneously exhibited changes suggestive of diminished activity.

In their 2025 manuscript analyzing the SEA-AD dataset, Escarião et al. noted that higher PAM (plaque-associated microglia) expression correlated with lower MMSE scores—my finding of greater IBA1+ /6e10 colocalized density in the low MMSE group complements this, while further demonstrating that this may occur alongside an increased inactive:active microglial ratio (Escarião et al., 2025). My study integrates multiple pathological dimensions and specifically examines the relationship between microglial-amyloid colocalization, total IBA1+ density, amyloid density, and morphology-based inactive:active microglial state across MMSE groups.

Presence alone does not necessarily imply effective response, and thus cells may occupy regions of pathology while simultaneously undergoing shifts towards a reduced activation state (McKinney et al., 2026). The observed shift in morphology raises the possibility that altered microglial states may be a critical feature of disease progression and could help inform future explanations of why pathological burden continues to increase despite persistent immune response, although functional and causal relationships are yet to be confirmed (Guo et al., 2022; Lier et al., 2021).

These findings are consistent in conceptualizing Alzheimer's disease as a multifactorial disease involving more complex interactions among protein aggregation, neuroinflammation, synaptic dysfunction, and neuronal loss. Rather than acting solely downstream of amyloid deposition, microglia may well influence other stages of disease progression by affecting neuroinflammation and becoming unable to clear pathological proteins. Consequently, therapeutic strategies targeting microglia may need to focus not only on enhancing microglial activity but also on preserving beneficial, active states and preventing transitions toward chronic inactivation.

4.6. Limitations and Future Directions

Several limitations should be considered when interpreting these findings. Firstly, because the present study relied on observational postmortem data, clear causal relationships cannot be established. Although significant associations were identified between MMSE status and neuropathological measures, it remains unclear whether altered microglial states drive



disease progression or emerge as a consequence of accumulating pathology. These findings should be used to help inform and generate hypotheses for future therapeutic studies, rather than to directly inform treatment.

Additionally, MMSE-based grouping provides only an approximate measure of disease severity. Cognitive decline is a multidimensional process influenced by numerous biological and clinical factors, and thus individuals with similar MMSE scores may actually differ substantially in their true pathological profiles. Future studies incorporating both additional cognitive assessments and clinical data may provide a more comprehensive understanding of the relationship between cognition and neuropathology.

The two microglial activation (active and inactive) states were determined according to morphology-based classifications provided by the dataset, but morphology alone may be insufficient in showing the functional diversity of microglial states. Future studies incorporating transcriptomic, proteomic, and molecular approaches may better help determine whether the observed changes represent dysfunction, adaptive responses, or distinct disease-associated phenotypes.

The layer-specific findings observed in this study also need further investigation. The observed enrichment of amyloid pathology within Layer 3 and microglia within Layer 2, along with the consistent significant overall layer effect in several of the two-way ANOVAs, raises important concerns regarding what truly controls selective cortical vulnerability. Future studies must examine whether differences in neuronal connectivity, synaptic density, or structural communication patterns contribute to the observed distribution of pathology. Further research on layer-specific attributes may provide insight into how pathological processes spread through large-scale brain networks.

Finally, the present analysis focused only on the middle temporal gyrus. Expanding similar layer-resolved analyses to other brain regions commonly affected in Alzheimer's disease, like the hippocampus, entorhinal cortex, frontal cortex, etc., could determine whether the observed relationships between pathology and microglial dynamics represent a generalized feature of disease progression or are a region-specific phenomenon (Leng et al., 2021). Future large-scale studies utilizing automated spatial transcriptomics or volumetric modeling would benefit from nested mixed-effects linear models to further isolate intra-donor laminar variance.

4.7. Conclusions

In summary, lower cognitive function was associated with higher tau and descriptive amyloid-density patterns within the MTG, accompanied by changes in microglial dynamics. Tau burden exhibited a particularly strong graded relationship with MMSE groups, supporting previous evidence that tau pathology closely tracks clinical impairment. On the other hand, greater microglial localization was observed near amyloid deposits in low MMSE scores despite evidence suggesting altered activation states in individuals with varying degrees of cognitive ability.

Together, these findings support a model where pathological burden and neuroimmune changes evolve in parallel with Alzheimer's disease progression. The results further suggest that microglial presence alone may be insufficient to assess neuroimmune function, as cells can remain associated with pathology while potentially undergoing shifts toward less effective or maladaptive states. Additionally, the layer-specific patterns identified within the MTG highlight the importance of considering spatial organization when investigating such neurodegenerative mechanisms. Understanding why certain



cortical layers and regions exhibit heightened vulnerability and the changes in pathology associated with these regions may ultimately lead to the development of more targeted therapeutic strategies.

5. References

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Data Availability Statement

All datasets analyzed and methodology are available through the Allen Institute's BrainMap in the Seattle Alzheimer's Disease Brain Cell Atlas: <https://portal.brain-map.org/explore/seattle-alzheimers-disease/seattle-alzheimers-disease-brain-cell-atlas-download?edit&language=en>.

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Author Biography

Amber Zhou is a high school junior at the Brearley School in New York City with a strong interest in various fields of biomedicine, specifically multiple myeloma genetics and clinical care, because conducting wet-lab research and seeing its real patient impact genuinely intrigues her.

Beyond the classroom, Amber participates in a wide variety of extracurricular activities such as volunteering at a pediatric hospital, working at a podiatric surgery clinic, and teaching taekwondo to younger students. To accompany this, she has a strong commitment to school leadership, seeking roles as co-head of Science Club and The Catalyst, the school's STEM magazine, as she believes that fostering scientific curiosity is integral to a balanced community. Moreover, she regularly engages in service and mentorship to support both younger peers and patients in need, culminating in presenting her research on blood cancer at the prestigious ASH Annual Conference this December.

Amber sees herself as forward-thinking, ambitious, and grounded, helped by her dedication to both advanced scientific inquiry and community impact. Attending a top-tier university and pursuing a career in biomedical research and medicine is one of Amber's long-term goals because, after gaining deep exposure to both clinical environments and advanced laboratory settings, she is ready to harness the resources these institutions have to offer to grow as a scientist.

Mentor Contribution Statement

Dr. Jorge A. Avila provided comprehensive mentorship and scientific guidance throughout the development of this research project.

Dr. Avila introduced the author to Alzheimer's disease research and provided access to the publicly available datasets, analytical framework, computational workflows, and software protocols used throughout the study. Through his instruction, the author learned to organize, analyze, visualize, and interpret high-dimensional transcriptomic and genomic data independently.

Through the research process, Dr. Avila offered continuous feedback on the study design, statistical analyses, interpretation of the results, and scientific writing. His thoughtful critiques strengthened the rigor, clarity, and overall quality of the manuscript while encouraging the author's independent scientific reasoning and problem-solving skills.

Finally, Dr. Avila carefully reviewed multiple manuscript revisions prior to submission, providing constructive and extensive suggestions to improve the presentation and communication of the findings. His mentorship was essential in the successful completion of this project and in the author's personal growth as an emerging researcher.

