Group 10: Spatial Minds

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I have read and attest to the statement in the BST 210 HW assignment

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Question 2

a. Literature Review

Our group conducted a literature review to inform our project, focusing on research in neuroscience and spatial transcriptomics, particularly studies using the MERFISH technique. Similar questions have been explored, such as the relationship between biological sex, gene expression, and spatial cell distribution. In these studies, researchers frequently apply spatial regression models, generalized linear models, and various spatial statistics to investigate these associations.

We plan to integrate these approaches into our analysis, emphasizing spatial regression techniques and modeling complex interactions (e.g., sex and disease state) to refine our models. One commonly used method identifies the proportion of a specific cell type (cell B) within a defined radius around another cell type (cell A) and compares these proportions under control and diseased states to assess if disease alters cell proximity. However, this technique doesn't differentiate the precise spatial locations of cell B within that radius. It only measures the presence of cell B within the region, rather than how close cell B actually is to cell A.

To establish a background distribution for comparison, the authors generate a null distribution by randomizing the locations of cell type B cells within the spatial area. The proximity enrichment is then expressed as the log2 ratio of true to background. They calculate a Z-score for statistical significance as follows:

$$z = \frac{\text{true} - \text{background}}{\text{background sd}} \tag{1}$$

where `background_sd` is the standard deviation of the background distribution. This Z-score is used to adjust for multiple comparisons across all cell type pairs using the False Discovery Rate (FDR). Inspired by this approach, our method will adapt similar spatial quantification but focus on distance-based metrics to assess the spatial distribution changes under different experimental conditions.

Our approach builds on these insights by measuring the actual distance between cell A and its closest cell B, providing a more nuanced view of cell proximity in relation to disease. This method allows us to better capture changes in cellular spatial relationships in disease contexts, which might be overlooked in simpler proportion-based analyses.

References:

- Karlstad, J., Blasi, T., Busskamp, V., & Zador, A. M. (2022). Spatially resolved single-cell transcriptomics reveals cell type–specific patterns of organization in the mouse brain. *Cell, 185*(1), 92-106.e18.

- Xiaowei Zhuang et al. (2023), MERFISH Spatial Transcriptomics Dataset of a Single Adult Mouse Brain
- Kalish et al. (2021), Maternal Immune Activation in Mice Disrupts Proteostasis in the Fetal Brain.
- Chennuru Vankadara, S., & von Luxburg, U. (2018), Distance-Preserving Generative Models for Spatial Transcriptomics.

b. Peer review

We have received several constructive insights from our peers and the teaching team, which have been instrumental in refining the direction of our project. The feedback can be summarized as follows:

1. **Data Complexity:** The high-dimensional nature of the data poses challenges, especially regarding potential overfitting and computational inefficiencies due to numerous covariates (e.g., gene expression, cell locations). Reviewers recommended incorporating regularization techniques such as LASSO to reduce the number of variables and improve model performance.
2. **Model Simplification:** Reliance on linear models may be a limitation as they might not fully capture the complexity of gene expression and spatial relationships, potentially leading to an oversimplified analysis. Reviewers advised exploring more flexible models such as Generalized Additive Models (GAMs) or splines, especially for continuous covariates like gene expression and spatial distances, to better capture complex relationships.
3. **Biological Interpretation of Spatial Data:** Translating results into meaningful biological insights can be challenging. Advanced spatial statistics or domain-specific interpretations are needed to better understand the biological significance of cell distances and gene expression patterns.

i.

- **Yes**, the feedback provided by both our peers and the teaching team included insights that were directly viable for shaping the direction of our project.

ii.

- We have responded to the feedback and made the following modifications:

1. **Limitations of the Linear Model:** Recognizing that a linear model might not be suitable for our research question, we shifted our analysis towards a Negative Binomial model, which is more appropriate for our count-based outcomes and allows for overdispersion. This change ensures our model aligns better with the data characteristics.
2. **Model Simplification:** By switching to a Negative Binomial model, we streamlined our modeling approach, making it more compatible with our data and addressing computational limitations.
3. **Refined Research Questions:** We redefined our primary and secondary research questions to enhance clarity and relevance:
    - **Primary Question:** Does the colocalization of two cell types of interest change under control versus diseased conditions? We will analyze 552 possible cell pairs (24 cell types).
    - **Secondary Question:** Does cell type enrichment vary across nine brain regions and between control and diseased states? This analysis involves assessing 24 cell types within each of the nine brain regions of interest.

c. Domain expertise

Yes, we have contacted the domain expert, Dr. Brian Kalish (Boston Children's Hospital), to seek guidance on interpreting the spatial distribution and colocalization patterns of the cell types under different conditions. Dr. Brian Kalish's insights have been valuable in refining our approach to analyzing cell-type enrichment across brain regions and ensuring our methodological choices align with current best practices in spatial transcriptomics.

Question 3 Analysis Plan

1. **Calculate the Density Factor d_i**
   For each tissue sample i, compute the density factor by taking the mean of all nearest-neighbor distances d_{ij} between each cell pair j:

$$d_i = \frac{1}{N_i} \sum_{j=1}^{N_i} d_{ij} \tag{2}$$

   where N_i is the number of cells in sample i.

2. **Determine the Hyperparameter n**
   Select the hyperparameter n by performing a sensitivity analysis to scale the density factor, defining the radius r_i as:

$$r_i = n \times d_i \tag{3}$$

3. **Generate Cell Type Pairs**
   Create all possible pairs of distinct cell types to analyze their spatial interactions within each tissue sample.

4. **Count Nearby Cells Y_{ij}**
   For each cell of type B in sample i, count the number of type A cells within the radius r_i, resulting in the count variable:

$$Y_{ij} = \text{Number of type A cells within } r_i \text{ of cell } j \text{ of type B} \tag{4}$$

5. **Fit the Full Negative Binomial Model**
   Model the counts Y_{ij} using Negative Binomial regression, including the experimental condition and, if applicable, random effects:

$$Y_{ij} \sim \text{Negative Binomial}(\mu_{ij}, \theta) \tag{5}$$

$$\log(\mu_{ij}) = \beta_0 + \beta_1 \cdot \text{Condition}_i + u_i \quad \text{(if using random effects)} \tag{6}$$

6. **Extract Rate Ratios and Confidence Intervals**
   Calculate the rate ratio as $\exp(\beta_1)$ and derive the 95% confidence intervals using the standard error of β_1:

$$\text{Rate Ratio} = \exp(\beta_1) \tag{7}$$

$$\text{CI}_{\text{lower}} = \exp(\beta_1 - 1.96 \times \text{SE}(\beta_1)) \tag{8}$$

$$\text{CI}_{\text{upper}} = \exp(\beta_1 + 1.96 \times \text{SE}(\beta_1)) \tag{9}$$

7. **Fit the Null Model**
   Fit a Null Negative Binomial model without the condition effect to serve as a baseline for comparison:

$$Y_{ij} \sim \text{Negative Binomial}(\mu_{ij}, \theta) \tag{10}$$

$$\log(\mu_{ij}) = \beta_0 + u_i \quad \text{(if using random effects)} \tag{11}$$

8. **Perform Likelihood Ratio Test (LRT)**
   Compare the full model and the null model using LRT to assess the significance of the condition effect:

$$\text{LRT} = \text{anova}(null\_model, nb\_model) \tag{12}$$

9. **Adjust for Multiple Testing**
   Apply the Benjamini-Hochberg method to adjust p-values obtained from the LRT for multiple comparisons:

$$p_{\text{adj}} = \text{p.adjust}(p\_value, \text{method} =' BH') \tag{13}$$

10. **Visualize Results with Heatmaps**
    Create heatmaps of the log2 rate ratios for all cell type pairs, highlighting statistically significant interactions based on the adjusted p-values.
11. **Conduct Sensitivity Analysis on (n)**
    Evaluate the robustness of the results by repeating the analysis with different values of the hyperparameter (n) and comparing the outcomes.
12. **Interpret Biological Implications**
    Analyze the significant rate ratios and their confidence intervals to draw conclusions about how experimental conditions affect the spatial relationships between different cell types.

Question 4 Missing Data

a. Type of missingness

The source of data is MERFISH (Multiplexed Error-Robust Fluorescence In Situ Hybridization), which is highly effective at mitigating issues of missing data in the gene expression matrix. MERFISH is designed with robust error-correction capabilities that correct barcode errors by assigning ambiguous barcodes to their closest valid match. This approach significantly reduces the likelihood of missing gene expression data. Thus, our dataset does not exhibit missing data in the gene expression matrix.

MERFISH data also provides spatial coordinates for each cell, enabling us to conduct spatial analyses without concerns about missing values in either expression or location data. Because of this error robustness and complete spatial mapping, our dataset does not contain missing data in any critical variables for our analysis.

Conclusion:
Due to the inherent strengths of the MERFISH technology in error correction and spatial localization, there is no need to handle missing data in this project. We can proceed with our analysis with confidence that the dataset is complete and reliable for both gene expression and spatial location information.

b. Steps to Address Missing Data

Given that our dataset is generated from MERFISH, which includes robust error-correction capabilities that virtually eliminate missing gene expression data and provide complete spatial coordinates, no missing data imputation or exclusion is necessary. Consequently, we have not implemented any missing data handling procedures in our analysis.

Question 5 Modeling

a. Linear, flexible/additive, or other methods (LASSO, ridge)

Our analysis plan does not include this type of model, therefore we figure out a way apply these models.

For one cell type pair (cell type A and cell type B), we can fit a model as follows:

$$\text{Distance}_i = \beta_0 + \beta_1 \cdot \text{Sex}_i + \beta_2 \cdot \text{Condition}_i + \beta_3 \cdot \text{Sex}_i \cdot \text{Condition}_i + \epsilon_i \tag{14}$$

Where:

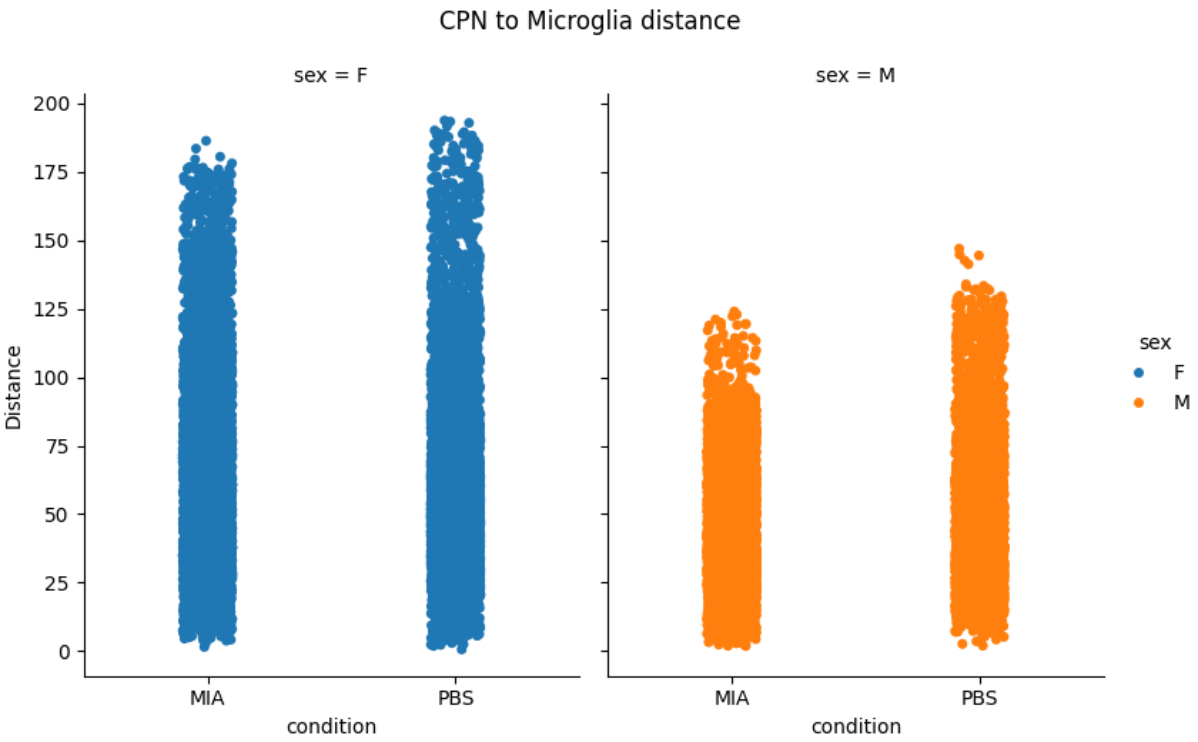
- Y_i is the distance from cell type A to cell type B in sample (i).
- Sex_i is the biological sex of the sample, $Sex_i = 1$ if male, and $sex_i = 1$ if female.
- Condition_i indicates whether the sample is under control or diseased conditions. $condition_i = 1$ if condition is MIA, else 0.

This model can be used in the EDA part to help us understand how distances between cell types change in different experimental condition and biological sex. There are 25 different cell types in our dataset, so we will need to fit 25×24 linear regression models to understand how would conditions and sex affect cell-cell distance.

Here, we demonstrate this idea using CPN (neocortical projection neurons) and microglia. We first calculated the distance between two cell types on all samples, and organized it into a dataframe. Then, we fitted the model, where fitted coefficients are $\beta_0 = 67.69, \beta_1 = -21.93, \beta_2 = -6.13, \beta_3 = 10.56$.

Interpretation:

- According to this sample, on average, being in the MIA condition decreases the distance between OPC and microglia by 6.13 μm for females, compared to females in the PBS condition.
- On average, being in the MIA condition decreases the distance between OPC and microglia for males by 6.13 - 10.56 = -16.69 μm compared to males in the PBS condition. This suggests a larger reduction in distance for males than for females when in the MIA condition.
- The positive interaction term $\beta_3 = 10.56\ \mu m$ indicates that the effect of the MIA condition on the distance between OPC and microglia differs by sex. Specifically, males in the MIA condition have an additional 10.56 μm increase in distance relative to the main effect of condition, partially offsetting the reduction observed in females. This suggests that the MIA condition reduces the distance more for males than for females, with sex modifying the impact of condition on distance.



sex	F	M
condition		
MIA	393917	346496
PBS	423252	308921

```
Call:
lm(formula = Distance ~ condition + sex + condition * sex, data = model_data)

Residuals:
     Min      1Q  Median      3Q     Max
-66.177 -20.929 -5.181 15.508 132.185

Coefficients:
                            Estimate  Std. Error     t value      Pr(>|t|)
Intercept                  67.694086    0.414629  163.264296  0.000000e+00  ***
sex[T.M]                  -21.933076    0.559778  -39.181773  0.000000e+00  ***
condition[T.PBS]           -6.132829    0.559699  -10.957372  7.201064e-28  ***
sex[T.M]:condition[T.PBS]  10.564857    0.811732   13.015206  1.381033e-38  ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 30.13 on 22561 degrees of freedom
Multiple R-squared:  0.07839,   Adjusted R-squared:  0.07826
F-statistic: 639.6 on 3 and 22561 DF,  p-value: 0.00e+00
```

b. Logistic, multinomial, ordinal, generalized ordinal

Our primary analysis does not include this type of model. We can use brain region as Y and gene expression as X to fit a multinomial regression.

The model can be written as following:

$$\log\left(\frac{P(Y=j)}{P(Y=1)}\right) = \beta_0 + \beta_1 \cdot gene_1 + \cdots + \beta_p \cdot gene_p \tag{15}$$

where $j = 1, \ldots, k$, k is the number of brain regions, and p is the number of genes.

Here, we demonstrate this idea by fitting the **multinomial** regression using cells in one sample. Since the original dimensionality is too high, fitting the multinomial regression becomes impossible. To address this, we first selected 50 highly variable genes, and we limited our analysis to 3 brain regions *(CP: Cortical Plate, IZ: Intermediate Zone, Basal_tel: basal telencephalon)*. We set basal telencephalon as the reference group, and fitted two multinomial regression. The response level, fitted coefficient, p-value and adjusted p value are shown as follows. Positive coefficients mean that the expression of that gene increases the probability of belonging to certain regions. According to our domain knowledge in neuroscience, the results make sense. **Pdgfra** is a marker for oligodendrocyte precursor cells (OPCs). Positive associations suggest increased OPC presence in the CP and IZ, regions where myelination processes begin to occur during development. Somatostatin is a neuropeptide expressed by a subset of inhibitory interneurons in the brain. The negative coefficients indicate that higher expression of **Sst** is associated with a decreased likelihood of cells being in the CP and IZ regions. This suggests that somatostatin-expressing interneurons may be less prevalent or less active in these regions during the developmental stage studied.

	Gene	ResponseLevel	Coefficient	p_value	adjusted_p_value	Significance
1	(Intercept)	CP	0.0520183225	5.045064e-06	6.683072e-06	***
2	(Intercept)	IZ	0.4766761600	0.000000e+00	0.000000e+00	***
3	Sst	CP	-0.0484096875	0.000000e+00	0.000000e+00	***
4	Sst	IZ	-0.0680954515	0.000000e+00	0.000000e+00	***
5	Nkx2.1	CP	-0.1480248566	0.000000e+00	0.000000e+00	***
6	Nkx2.1	IZ	-0.1568699807	0.000000e+00	0.000000e+00	***
7	Igf2	CP	0.0097422371	6.883383e-15	1.097039e-14	***
8	Igf2	IZ	-0.0291514726	0.000000e+00	0.000000e+00	***
9	Vtn	CP	0.0416030605	0.000000e+00	0.000000e+00	***
10	Vtn	IZ	0.0606434320	0.000000e+00	0.000000e+00	***
11	Lhx6	CP	0.0214464737	0.000000e+00	0.000000e+00	***
12	Lhx6	IZ	-0.0061328810	1.136666e-10	1.680289e-10	***
13	Pdgfra	CP	0.0544659969	0.000000e+00	0.000000e+00	***
14	Pdgfra	IZ	0.0353647109	0.000000e+00	0.000000e+00	***
15	Egfl7	CP	-0.0003785051	8.465625e-01	8.811160e-01	
16	Egfl7	IZ	-0.0108506552	4.260522e-06	5.718069e-06	***
17	Trp73	CP	-0.0151682944	0.000000e+00	0.000000e+00	***
18	Trp73	IZ	-0.1205096125	0.000000e+00	0.000000e+00	***
19	Pecam1	CP	-0.0004785970	8.455082e-01	8.811160e-01	
20	Pecam1	IZ	0.0001348384	9.606751e-01	9.606751e-01	

c. Poisson and Extensions

In this analysis, we examine whether experimental conditions (control vs. diseased) impact the spatial proximity between one example cell pairs: microglia cells (cell type A) and immature excitatory neurons (cell type B). Specifically, we model the counts of microglia cells within a certain radius of each immature excitatory neuron using Poisson and Negative Binomial regression.

Data overview:

- **Dependent Variable**: The count of cell type A (microglia) within a specified radius of each cell type B (immature excitatory neuron).
- **Independent Variable**: Experimental condition (`condition`), with levels: PBS (control) and MIA (diseased).

We calculated the counts based on the mean nearest-neighbor distance, adjusted by a factor of 5 to determine the radius for proximity.

To analyze the data, we fit both a Poisson regression model and a Negative Binomial regression model to address potential overdispersion.

Model Formula:

$$\mathrm{Y}(\text{Count of Cell Type A}) \sim \text{Condition (PBS vs. MIA)} \tag{16}$$

$$E(\text{Count\_of\_cell\_type\_A}_i|X) = \beta_0 + \beta_1 \cdot \text{Condition}_i \tag{17}$$

Where $Conditon_i = 1$, when conditon = MIA, else 0.

1. **Poisson Model**:
    - Fit using the `glm()` function in R.
    - We first tested this model for overdispersion, which would indicate if the Negative Binomial model is necessary.
2. **Negative Binomial Model**:
    - Used if overdispersion is detected in the Poisson model.
    - Fit using `glm.nb()` from the MASS package.

Results from Poisson Model

Summary Statistics

Counts of Microglia near Immature ExN cells:

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
0.00000 0.00000 0.00000 0.03575 0.00000 6.00000
```

- **Minimum Count:** 0
- **Maximum Count:** 6
- **Median and Quartiles:** All zeros, indicating that more than 75% of the observations have zero counts.
- **Mean Count:** Approximately 0.036
- **Interpretation:** The majority of Immature ExN cells have **no neighboring Microglia** within the specified radius.

Frequency of Counts:

```
     0      1      2      3      4      5      6
246,792  6,122    850    237     91     21     14
```

- **Total Observations:** 254,127
- **Zero Counts:** 246,792 (97.11%)
- **Non-Zero Counts:** 7,335 (2.89%)

- **Interpretation:** The data is **highly zero-inflated**, with a small proportion of cells having any neighboring Microglia.

Mean-Variance Comparison

```
Mean of Counts: 0.03575378
Variance of Counts: 0.05436335
```

- **Mean:** ~0.036
- **Variance:** ~0.054
- **Interpretation:** The variance is slightly higher than the mean, suggesting **overdispersion**. In a Poisson distribution, the mean and variance are equal. Overdispersion indicates that the data may not fit a Poisson model well.

Goodness-of-Fit Test for Negative Binomial Distribution

```
Goodness-of-fit test for nbinomial distribution

                  X^2 df     P(> X^2)
Likelihood Ratio 75.89457  4 1.288787e-15
```

- **Chi-Squared Statistic:** 75.89457
- **Degrees of Freedom:** 4
- **P-value:** 1.288787×10^{-15}
- **Interpretation:** The **significant p-value** indicates that the Negative Binomial distribution does **not** fit the data well. This suggests that even the Negative Binomial model may not be adequate due to the extreme zero-inflation.

Poisson Regression Model

Model Output

```
Call:
glm(formula = count_A_near_B ~ condition, family = poisson, data = counts_df)

Coefficients:
             Estimate Std. Error  z value Pr(>|z|)
(Intercept)  -3.34717    0.01540 -217.386   <2e-16 ***
conditionMIA  0.03021    0.02104    1.436    0.151
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

- **Intercept β_0:** -3.34717
  - Corresponds to the log of the expected count for the **PBS** condition.
- **ConditionMIA β_1:** 0.03021
  - Represents the difference in the log count between **MIA** and **PBS** conditions.

- **P-value for ConditionMIA:** 0.151
    - **Not statistically significant** at the 0.05 level.

Model Deviance and AIC

```
Null deviance: 66100  on 254126  degrees of freedom
Residual deviance: 66098  on 254125  degrees of freedom
AIC: 81698
```

- **Null Deviance vs. Residual Deviance:** Minimal reduction, indicating that the predictor **does not significantly improve** the model.
- **AIC (Akaike Information Criterion):** 81,698

Goodness-of-Fit Test

```
Deviance: 66097.82 on 254125 degrees of freedom.
Goodness-of-Fit Test p-value: 1
```

- **P-value:** 1
- **Interpretation:** A p-value of 1 suggests that the model's deviance is **less than or equal to** what would be expected under the null hypothesis. However, given the overdispersion detected later, this result may be misleading.

Overdispersion Test

```
Overdispersion test

data:  poisson_model
z = 22.626, p-value < 2.2e-16
alternative hypothesis: true dispersion is greater than 1
sample estimates:
dispersion
  1.519514
```

- **Dispersion Parameter:** 1.519514 (greater than 1)
- **Z-value:** 22.626
- **P-value:** < 2.2e-16
- **Interpretation:** The **significant overdispersion** indicates that the Poisson model is **not appropriate** for this data. The variance exceeds the mean, violating the Poisson assumption.

Negative Binomial Regression Model

Model Output

```
Call:
glm.nb(formula = count_A_near_B ~ condition, data = counts_df,
    init.theta = 0.07545203964, link = log)

Coefficients:
             Estimate Std. Error  z value Pr(>|z|)
(Intercept)  -3.34717    0.01864 -179.523   <2e-16 ***
conditionMIA  0.03021    0.02553    1.183    0.237
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

- **Intercept (β_0):** -3.34717
- **ConditionMIA (β_1):** 0.03021
- **P-value for ConditionMIA:** 0.237
    - **Not statistically significant**.

Model Deviance and AIC

```
Null deviance: 31335  on 254126  degrees of freedom
Residual deviance: 31333  on 254125  degrees of freedom
AIC: 75269
```

- **Reduction in Deviance:** Minimal, suggesting that adding **condition** does not significantly improve the model.
- **AIC:** 75,269 (lower than the Poisson model's AIC, indicating a better fit).

Negative Binomial Parameter θ

```
Theta:  0.07545
Std. Err.:  0.00220
```

- **Interpretation:** A small value of θ indicates a high level of dispersion, which the Negative Binomial model accounts for.

Likelihood Ratio Test (Comparing Poisson and Negative Binomial Models)

```
Likelihood ratio test

Model 1: count_A_near_B ~ condition
Model 2: count_A_near_B ~ condition
  #Df LogLik Df  Chisq Pr(>Chisq)
1   2 -40847
2   3 -37631  1 6431.3  < 2.2e-16 ***
```

- **Degrees of Freedom (Df):** Difference of 1
- **Log-Likelihoods:** Improved from -40,847 (Poisson) to -37,631 (Negative Binomial)

- **Chi-Squared Statistic:** 6,431.3
- **P-value:** < 2.2e-16
- **Interpretation:** The Negative Binomial model provides a **significantly better fit** than the Poisson model, justifying its use due to overdispersion in the data.

Key Findings

1. **High Zero Counts:** Over 97% of the observations have zero counts, indicating a **highly zero-inflated dataset**.
2. **Overdispersion Detected:** Both the mean-variance comparison and the overdispersion test confirm that the data is **overdispersed**, violating Poisson model assumptions.
3. **Negative Binomial Model Fits Better:** The Negative Binomial model accounts for overdispersion and provides a **better fit** than the Poisson model, as evidenced by the lower AIC and significant likelihood ratio test.
4. **Condition is Not Significant:**
    - In both models, the effect of **condition (MIA vs. PBS)** on the counts of Microglia near Immature ExN cells is **not statistically significant**.
    - **P-values for conditionMIA:**
        - Poisson model: 0.151
        - Negative Binomial model: 0.237

Implications

- **Lack of Association:** There is **no significant evidence** to suggest that the **MIA condition** affects the number of Microglia near Immature ExN cells within the specified radius.
- **Model Appropriateness:** While the Negative Binomial model is more appropriate than the Poisson model for this data, the **extreme zero-inflation** may still pose challenges.
- **Model Limitations:** The significant goodness-of-fit test for the Negative Binomial distribution indicates that even this model may not fully capture the data's characteristics.

Conclusion

- **Statistical Analysis:**
    - The Negative Binomial regression model is preferred over the Poisson model due to overdispersion.
    - The condition (MIA vs. PBS) does not have a significant effect on the counts.
- **Biological Interpretation:**
    - There is no statistical evidence to suggest that the MIA condition influences the proximity of Microglia to Immature ExN cells within the specified radius.
- **Future Work:**
    - Consider alternative models that handle zero-inflation.
    - Investigate other potential factors or methods to better understand the data.

Note: The warnings about "NaNs produced" suggest potential computational issues, possibly due to the large number of zeros or model misspecification. It is advisable to check the data and model assumptions carefully.

d. Survival Analysis

Response:
We will not incorporate survival analysis into our project as our data don't warrant it. Our research questions do not involve time-to-event data, which is the focus of survival analysis methods. In survival analysis, the interest is typically in modeling the time until an event occurs (e.g., death, disease onset), with censoring of incomplete observations. However, our study centers around spatial transcriptomics data, focusing on cell-type co-localization and enrichment rather than any temporal component. Thus, survival analysis is not applicable to our data and research objectives.

Question 6 Abstract and Introduction

a. Abstract

Title: Spatial Characterization of Cell Pair Proximity Across Brain Regions Using Spatial Transcriptomics and Statistical Modeling

Background:
The spatial organization of cell types within brain tissue is critical for understanding cellular communication, development, and disease mechanisms. Shifts in proximity between specific cell types across different brain regions under pathological conditions can provide insights into neurodevelopmental or neurodegenerative processes.

Aim:
This study investigates spatial relationships between all potential cell type pairs across the entire brain region, analyzing whether these proximity patterns change between control and disease conditions.

Methods:
The Negative Binomial regression model with random effects was utilized to examine condition-specific differences in cell pair proximities. Incorporating random effects for samples accounted for variability across individual samples, ensuring the robustness of the results.

Spatial coordinates and cell type annotations across disease (maternal immune activation) and control conditions can be extracted from annotated spatial transcriptomic data. For each cell type pair, the measurement of cell proximity under both conditions can be obtained by calculating the number of cells of one type within a specidied radius around cells of the other type.

Statistical significance of the condition effect on cell pair proximities was assessed through likelihood ratio tests, with p-values adjusted for multiple comparisons using the Benjamini-Hochberg method. This approach ensures that the findings are statistically rigorous and interpretable.

Keywords:
Spatial Transcriptomics, Brain Regions, Cell Type Proximity, Poisson Distribution, Negative Binomial Distribution, Cellular Interactions, Neuroinflammation

b. Introduction

Background:
The arrangement and interactions of various cell types in the brain are essential for maintaining neural function and homeostasis. Spatial organization within brain regions affects everything from synaptic connectivity to immune responses. Alterations in the spatial proximity of cell types under different conditions, especially during disease states, could reveal important aspects of cellular behavior, intercellular communication, and tissue response to pathology.

Motivation:
Spatial transcriptomics allows for the examination of gene expression and cellular interactions within the anatomical context of the entire brain regions. This technology enables researchers to explore how cell types, such as neurons, astrocytes, and microglia are positioned relative to one another and whether these spatial relationships shift under pathological conditions. This study characterizes proximity relationships between all cell type pairs across the entire brain, aiming to uncover potential shifts that occur in response to disease.

Objectives:
The primary objectives of this study are:

1. To quantify proximity relationships between all cell type pairs across the entire brain region.
2. To determine if these proximity relationships differ significantly between disease and control conditions.

Data Description and Motivation

Data Source and Characteristics:
The spatial transcriptomic dataset includes data from both disease (maternal immune activation) and control (phosphate-buffered saline) conditions, covering the entire brain region. This dataset includes:

- **Cell-by-Gene Expression Matrix:** Captures expression data for individual cells.
- **Cell Metadata:** Provides cell type annotations, spatial coordinates, sample identifiers, condition labels, and brain region annotations.

With approximately 148,111 cells and several samples across the conditions, this dataset enables a robust analysis of intercellular spatial dynamics.

Motivation for Analysis:
Characterizing changes in cellular spatial relationships across brain regions is crucial for understanding neurodevelopment and disease mechanisms. Shifts in the proximity of specific cell pairs could indicate changes in cell communication or infiltration patterns, potentially highlighting processes like neuroinflammation or synaptic remodeling. This study aims to provide quantitative insights into these spatial dynamics using a comprehensive approach that examines all cell type pairs.

c. Research and Analysis Methods

Overview:
Our analytical approach consists of two primary components. First, we aim to quantify the proximity between all possible cell type pairs across different brain regions, that is, to measure how frequently cells of one type are located near cells of another type in each brain region. To do this, we calculate a biologically

meaningful radius based on the mean nearest-neighbor distance and then apply Negative Binomial regression model to assess if proximity counts differ significantly between disease and control conditions.

The second component involves testing for condition-specific proximity shifts across brain regions. Here, we seek to determine whether significant differences exist in cell type proximities between diseased and control states throughout the brain. To evaluate the condition effect for each cell type pair, we conduct likelihood ratio tests and apply multiple comparison corrections.

Detailed Methodology:

1. **Data Preprocessing**
   In the preprocessing stage, we subset cells by brain regions and obtain spatial coordinates for each cell type pair across the brain. To set the radius for proximity calculations, we compute the mean nearest-neighbor distance for each cell type and apply a scaling factor to define an appropriate radius.
2. **Proximity Counting and Modeling**
   For each cell type pair, we calculate the number of cells of one type that fall within the specified radius around cells of the other type, providing a measure of cell proximity. Using these counts, we fit a Negative Binomial regression model for each cell pair. By comparing the λs of two conditions, we can know whether proximity counts are dependent on the experimental condition. [Question]
3. **Multiple Testing Correction**
   Given the multiple comparisons involved in testing various cell type pairs, we apply the Benjamini-Hochberg method to adjust p-values to ensure statistical rigor in our findings.
4. **Visualization and Interpretation**
   To convey our findings, we generate heatmaps illustrating significant changes in cell type proximities. These visualizations aid in interpreting the biological implications of proximity shifts, particularly in relation to neuroinflammation and disease mechanisms.

Results/Findings and Discussion Sections (Planned):

- **Results:** This section will present statistical findings, highlighting significant cell type pair proximities and their variations across conditions.
- **Discussion:** Here, we will interpret our results in the context of existing literature, explore potential biological mechanisms underlying observed proximity shifts, and suggest directions for future research.

Question 7

YES

Our group aims to develop our findings into a publishable manuscript. We plan to create a comprehensive abstract, prepare detailed figures such as heatmaps and spatial plots, and draft sections including Introduction, Methods, Results, and Discussion. Additionally, we intend to prepare a poster presentation to share our findings at relevant academic conferences.