CellTypesProject_template

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1 Cell Types Project

Exploring biomarkers in depression: Comparison of gene expression profiles and electrophysiological features in excitatory and inhibitory neurons.

1.1 Team Member Names & Contributions

Group 13

Joanna, A16980082: Abstract, Research Question, Setup, Data Wrangling, Data Analysis and Results,

Lorin, A16982359: Background & Prior Work, Conclusion & Discussion

Abstract

Depression is a complex neurological disorder that significantly impacts mood, cognition, and behavior. The condition is often associated with disruptions in neurotransmitter systems, particularly the balance between excitatory (glutamate) and inhibitory (GABA) signaling. We compare inhibitory and excitatory neurons through several modalities including gene expression profiles and eletrophysiological features. We find that between inhibitory and excitatory cells the brain regions of expression, genes expressed within these pathways, as well as electrophysiological features significantly differ. This is key in understanding inhibitory and excitatory pathways in the brain and can be used to develop clinical biomarkers in the case of disease states.

1.2 Research Question

We aim to compare inhibitory and excitatory neurons through several modalities including gene expression profiles and eletrophysiological features. This is key in understanding inhibitory and excitatory pathways in the brain and can be used to develop clinical biomarkers in the case of disease states.

For gene expression data we focus on cells highly expressing GABA and Glutamate as the framework for inhibitory and excitatory cells. Here we: 1) Compare the brain regions where these two neurotransmitters are expressed most through finding cells which express related genes; 2) Find what other genes are most expressed in the cells connected to GABA/Glutamate to establish the differences in gene expression for inhibitory and excitatory cells.

For electrophysiology data we use spiny neurons as a proxy for excitatory cells while aspiny neurons are used as inhibitory cells and compare several key features to compare the spiking properties of these groups.

2 Background and Prior Work

Depression is a complex neurological disorder that significantly impacts mood, cognition, and behavior. The condition is often associated with disruptions in neurotransmitter systems, particularly the balance between excitatory (glutamate) and inhibitory (GABA) signaling. Investigating the molecular and electrophysiological profiles of neurons and glial cells can provide insights into the mechanisms underlying depression and identify potential therapeutic targets.

Glutamate and GABA are the brain's primary excitatory and inhibitory neurotransmitters, respectively. The glutamate-GABA balance is essential for maintaining proper neural network activity. In depression, dysregulation of glutamate receptors, such as NMDA and AMPA receptors, can lead to excitotoxicity and impair synaptic plasticity. Reduced GABAergic signaling has been linked to increased stress responses and impaired inhibitory control in neural circuits.

These disruptions are particularly evident in brain regions such as the hippocampus and prefrontal cortex, which are critical for emotion regulation and cognitive function.

Electrophysiological properties, such as membrane potential and firing rates, provide functional insights into the activity of neurons in depressive states. By analyzing these markers in conjunction with gene expression data, we can identify potential molecular pathways that mediate depression-related changes in brain function.

Datasets:

Brain Region-Specific Gene Expression Data:
- Source: brainarea_vs_genes_exp_w_reannotations.tsv
- Description: This dataset contains gene expression profiles across multiple brain regions, allowing for the identification of region-specific patterns of glutamate and GABA pathway-related genes.

Electrophysiology Data:
- Source: Allen Institute Cell Types Data
- Description: Provides electrophysiological properties of human brain cells, including features like firing rates, membrane potentials, and upstroke-to-downstroke ratios. These features can be linked to functional changes in excitatory and inhibitory signaling.

Prior studies have shown that increased glutamate activity and decreased GABAergic signaling are hallmarks of depression. For example, ketamine's antidepressant effects are thought to involve modulation of NMDA receptor activity. In addition, neuroinflammation has been implicated in the dysregulation of glutamate and GABA systems, with cytokines like IL-6 and TNF-alpha affecting synaptic transmission.

2.0.1 References (include links):

(1)Hu, YT., Tan, ZL., Hirjak, D. et al. Brain-wide changes in excitation-inhibition balance of major depressive disorder: a systematic review of topographic patterns of GABA- and glutamatergic alterations. Mol Psychiatry 28, 3257–3266 (2023). https://doi.org/10.1038/s41380-023-02193-x

(2)Marc S. Lener, et al. "Glutamate and Gamma-Aminobutyric Acid Systems in the Pathophysiology of Major Depression and Antidepressant Response to Ketamine." Biological Psychiatry, Elsevier, 12 May 2016. https://doi.org/10.1016/j.biopsych.2016.05.005

2.1 Hypothesis

Inhibitory (GABAergic) and excitatory (glutamatergic) neurons exhibit differential gene expression patterns that vary significantly by brain region, reflecting functional specialization.

Sub-hypotheses:

Specific brain regions show higher GABAergic or glutamatergic activity due to differential expression of receptor-related genes (e.g., GABRA1, GRIN1). The top-expressed genes in cells highly expressing GABAergic or glutamatergic markers will reveal key functional pathways associated with neurotransmitter signaling.

2.2 Setup

This project uses two datasets from the Allen Institute and several Python packages, which provide key functionality for computational analysis and visualization.

[98]:
```
# Python packages that need to be imported
import pandas as pd
from scipy import stats
import matplotlib as mpl
import matplotlib.pyplot as plt
import numpy as np
```

[99]:
```
# For comparing gene expression profiles we use the the␣
 ↪brainarea_vs_genes_exp_w_reannotations.tsv
# data file found in homework a1-ConditionallyExpressed.
gene_df = pd.read_csv('brainarea_vs_genes_exp_w_reannotations.tsv', delimiter =␣
 ↪'\t')
gene_df = gene_df.set_index('gene_symbol')
gene_df.head()
```

[99]:
```
             CA1 field  CA2 field  CA3 field  CA4 field  \
gene_symbol
A1BG          0.856487  -1.773695  -0.678679  -0.986914
A1BG-AS1      0.257664  -1.373085  -0.619923  -0.636275
A1CF         -0.089614  -0.546903   0.282914  -0.528926
A2M           0.552415  -0.635485  -0.954995  -0.259745
A2ML1         0.758031   1.549857   1.262225   1.338780

             Crus I, lateral hemisphere  Crus I, paravermis  \
gene_symbol
A1BG                           0.826986            0.948039
A1BG-AS1                       0.362799            0.353296
A1CF                           0.507916            0.577696
A2M                           -1.687391           -1.756847
A2ML1                         -0.289888           -0.407026

             Crus II, lateral hemisphere  Crus II, paravermis  \
```

gene_symbol		
A1BG	0.935427	1.120774
A1BG-AS1	0.422766	0.346853
A1CF	0.647671	0.306824
A2M	-1.640242	-1.733110
A2ML1	-0.358798	-0.589988

gene_symbol	Edinger-Westphal nucleus	Heschl's gyrus	…
A1BG	-1.018554	0.170282	…
A1BG-AS1	-0.812015	0.903358	…
A1CF	0.089958	0.149820	…
A2M	-0.091695	0.003428	…
A2ML1	0.944684	-0.466327	…

gene_symbol	temporal pole, inferior aspect	temporal pole, medial aspect
A1BG	0.277830	0.514923
A1BG-AS1	1.074116	0.821031
A1CF	-0.030265	-0.187367
A2M	-0.058505	0.207109
A2ML1	-0.472908	-0.598317

gene_symbol	temporal pole, superior aspect	transverse gyri
A1BG	0.733368	-0.104286
A1BG-AS1	1.219272	0.901213
A1CF	-0.428358	-0.465863
A2M	-0.161808	0.183630
A2ML1	-0.247797	-0.282673

gene_symbol	trochlear nucleus	tuberomammillary nucleus
A1BG	-0.910245	1.039610
A1BG-AS1	-1.522431	0.598719
A1CF	-0.136936	1.229487
A2M	0.948098	-0.977692
A2ML1	1.396365	0.945043

gene_symbol	ventral tegmental area	ventromedial hypothalamic nucleus
A1BG	-0.155167	-0.444398
A1BG-AS1	-1.709745	-0.054156
A1CF	-0.110680	-0.118175
A2M	0.911896	-0.499357
A2ML1	0.158202	0.572771

```
             vestibular nuclei  zona incerta
gene_symbol
A1BG                 -0.901361     -0.236790
A1BG-AS1             -1.695843     -1.155961
A1CF                 -0.139776      0.123829
A2M                   1.469386      0.557998
A2ML1                 0.073088     -0.886780

[5 rows x 232 columns]
```

[100]:
```
# For electrophysiology data we use the Allen Institute Cell Types data.
from allensdk.core.cell_types_cache import CellTypesCache
from allensdk.api.queries.cell_types_api import CellTypesApi
ctc = CellTypesCache(manifest_file='cell_types/manifest.json')
human_cells = ctc.get_cells(species=[CellTypesApi.HUMAN])
human_df = pd.DataFrame(human_cells)
human_df = human_df.set_index('id')

ephys_features = pd.DataFrame(ctc.get_ephys_features()).set_index('specimen_id')
human_ephys_df = human_df.join(ephys_features)
human_ephys_df.head()
```

[100]:
```
           reporter_status     cell_soma_location       species  \
id
525011903            None  [273.0, 354.0, 216.0]  Homo Sapiens
528642047            None    [69.0, 254.0, 96.0]  Homo Sapiens
537256313            None   [322.0, 255.0, 92.0]  Homo Sapiens
519832676            None    [79.0, 273.0, 91.0]  Homo Sapiens
596020931            None   [66.0, 220.0, 105.0]  Homo Sapiens

                             name structure_layer_name  structure_area_id  \
id
525011903     H16.03.003.01.14.02                    3              12113
528642047  H16.06.009.01.02.06.05                    5              12141
537256313     H16.03.006.01.05.02                    4              12141
519832676     H16.03.001.01.09.01                    3              12141
596020931     H17.06.009.11.04.02                    4              12141

           structure_area_abbrev transgenic_line dendrite_type     apical  …  \
id                                                                         …
525011903                 FroL                         spiny     intact  …
528642047                  MTG                        aspiny         NA  …
537256313                  MTG                         spiny  truncated  …
519832676                  MTG                         spiny  truncated  …
596020931                  MTG                        aspiny         NA  …

           trough_t_ramp trough_t_short_square  trough_v_long_square  \
```

```
id
525011903      4.134987              1.375253           -53.968754
528642047           NaN              1.051160           -67.468758
537256313      5.694547              1.389900           -52.125004
519832676      9.962780              1.211020           -53.875004
596020931     14.667340              1.336668           -63.593754

          trough_v_ramp  trough_v_short_square  \
id
525011903    -59.510420             -71.197919
528642047           NaN             -70.875002
537256313    -51.520836             -72.900002
519832676    -52.416668             -73.693753
596020931    -63.239583             -75.518753

           upstroke_downstroke_ratio_long_square  \
id
525011903                               2.895461
528642047                               1.891881
537256313                               3.121182
519832676                               4.574865
596020931                               1.452890

           upstroke_downstroke_ratio_ramp  \
id
525011903                        2.559876
528642047                             NaN
537256313                        3.464528
519832676                        3.817988
596020931                        1.441754

           upstroke_downstroke_ratio_short_square  vm_for_sag      vrest
id
525011903                                3.099787  -88.843758 -70.561035
528642047                                1.989616 -101.000000 -69.209610
537256313                                3.054681  -87.531250 -72.628105
519832676                                4.980603  -84.218758 -72.547661
596020931                                1.556087  -82.531250 -74.260269

[5 rows x 70 columns]
```

2.3 Data Wrangling

We first conduct gene expression analysis, where we isolate cells which express GABA and Glutamate-related genes the most. GABA being the most prominent inhibitory neurotransmitter and Glutamate the most common excitatory neurotransmitter are good markers for inhibitory/excitatory cells. We successfully isolate related genes, mainly coding for the receptors of

these neurotransmitters. Thus, we use cells expressing these related genes as proxies for inhibitory and excitatory cells.

We take two approaches to comparing inhibitory and excitatory cells using gene expression profiles. Firstly, we determine the brain regions where the GABA/Glutamate-related genes are expressed the most and compare the physiological viability of this. Secondly, we find genes that are expressed most within the excitatory and inhibitory cells. This gives us insight into the gene expression profile differences between excitatory and inhibitory cells.

As for electrophysiological features between inhibitory and excitatory cells, we use the aspiny and spiny marker in the Allen Institute cell types dataset as a proxy. In literature, it is considered that spiny neurons are excitatory, while aspiny neurons are more often inhibitory. We compare resting membrane potential, input resistance and sag between aspiny and spiny neurons to validate the electrophysiological differences between inhibitory and excitatory neurons.

Gene Expression Analysis - Find genes related to GABA and Glutamate

[101]:
```
## YOUR CODE HERE
## FEEL FREE TO ADD MULTIPLE CELLS PER SECTION
```

[102]:
```
# Create a list of genes included in the dataset
genes = [item for item in gene_df.index]

# Find all GABA-related genes, informed by https://www.genenames.org/data/
 ↪genegroup/#!/group/562
gaba_genes = []
for item in genes:
    if "GABR" in item or "GABBR" in item:
        gaba_genes.append(item)
print("GABA gene list:")
print(gaba_genes)
print("")

# Find all Glutamate-related genes, informed by https://www.genenames.org/data/
 ↪genegroup/#!/group/282
glutamate_genes = []
for item in genes:
    if "GRM" in item or "GRI" in item:
        glutamate_genes.append(item)
print("Glutamate gene list:")
print(glutamate_genes)
```

```
GABA gene list:
['GABBR1', 'GABBR2', 'GABRA1', 'GABRA2', 'GABRA3', 'GABRA4', 'GABRA5', 'GABRA6',
'GABRB1', 'GABRB2', 'GABRB3', 'GABRD', 'GABRE', 'GABRG1', 'GABRG2', 'GABRG3',
'GABRP', 'GABRQ', 'GABRR1', 'GABRR2', 'GABRR3']

Glutamate gene list:
['GRIA1', 'GRIA2', 'GRIA3', 'GRIA4', 'GRID1', 'GRID2', 'GRID2IP', 'GRIK1',
```

```
'GRIK1-AS2', 'GRIK2', 'GRIK3', 'GRIK4', 'GRIK5', 'GRIN1', 'GRIN2A', 'GRIN2B',
'GRIN2C', 'GRIN2D', 'GRIN3A', 'GRIN3B', 'GRINA', 'GRIP1', 'GRIP2', 'GRIPAP1',
'GRM1', 'GRM2', 'GRM3', 'GRM4', 'GRM5', 'GRM6', 'GRM7', 'GRM8', 'PGRMC1',
'PGRMC2', 'RPGRIP1', 'RPGRIP1L']
```

Gene Expression Analysis - Find brain regions where GABA and Glutamate genes are expressed the most.

[103]:
```
# We sum the relative gene expression of these genes to find where they are␣
 ↪expressed the most.
# Find brain regions where GABA receptors are expressed the most
gaba_gene_df = gene_df.loc[gaba_genes]

# Sum expression levels across all GABA receptor genes for each brain area
gaba_region_expression = gaba_gene_df.sum(axis=0).sort_values(ascending=False)
top_gaba_regions = gaba_region_expression.head(10).index

# Display the top 10 brain areas with highest GABA receptor expression
print("Top Brain Regions with Highest GABA Receptor Gene Expression:")
print(gaba_region_expression.head(10))
print("")

# Find brain regions where Glutamate receptors are expressed the most
glutamate_gene_df = gene_df.loc[glutamate_genes]

# Sum expression levels across all GABA receptor genes for each brain area
glutamate_region_expression = glutamate_gene_df.sum(axis=0).
 ↪sort_values(ascending=False)
top_glutamate_regions = glutamate_region_expression.head(10).index

# Display the top 10 brain areas with highest GABA receptor expression
print("Top Brain Regions with Highest Glutamate Receptor Gene Expression:")
print(glutamate_region_expression.head(10))
```

```
Top Brain Regions with Highest GABA Receptor Gene Expression:
cortico-medial group                 13.904768
ventromedial hypothalamic nucleus    12.649852
frontal pole, medial aspect          11.393687
preoptic region                      11.343850
basomedial nucleus                   11.219342
piriform cortex                      10.642210
CA2 field                             9.945500
dentate gyrus                         9.773178
central nucleus                       9.608634
subiculum                             8.666789
dtype: float64

Top Brain Regions with Highest Glutamate Receptor Gene Expression:
```

```
frontal pole, medial aspect                                          16.366552
dentate gyrus                                                        16.012610
CA2 field                                                            15.687292
subiculum                                                            15.522064
frontal pole, inferior aspect                                        13.716218
CA4 field                                                            12.919245
paracentral lobule, posterior part, bank of cingulate sulcus         12.836223
pineal gland                                                         12.226804
CA3 field                                                            11.957748
medial group of nuclei                                               11.917814
dtype: float64
```

Gene Expression Analysis - Find other most expressed genes in the cells that express GABA/Glutamate- related genes most.

[104]:
```
# Find the cells with the highest GABA-related gene expression
gaba_expression_df = gene_df.loc[GABA_genes]
gaba_cell_expression = gaba_expression_df.sum(axis=0)

# Get the top 10% of cells with the highest GABA expression
top_gaba_cells = gaba_cell_expression[gaba_cell_expression >␣
 ↪gaba_cell_expression.quantile(0.9)].index

# Find the most expressed genes in these top GABA-expressing cells
top_gaba_cell_df = gene_df[top_gaba_cells]
top_gaba_gene_expression = top_gaba_cell_df.sum(axis=1).
 ↪sort_values(ascending=False)

# Display the top 10 most expressed genes in these cells
print("Top Genes Most Expressed in the GABA-Expressing Cells:")
print(top_gaba_gene_expression.head(10))
print("")

# Find the cells with the highest Glutamate-related gene expression
glutamate_expression_df = gene_df.loc[glutamate_genes]
glutamate_cell_expression = glutamate_expression_df.sum(axis=0)

# Get the top 10% of cells with the highest Glutamate expression
top_glutamate_cells = glutamate_cell_expression[glutamate_cell_expression >␣
 ↪glutamate_cell_expression.quantile(0.9)].index

# Find the most expressed genes in these top Glutamate-expressing cells
top_glutamate_cell_df = gene_df[top_glutamate_cells]
top_glutamate_gene_expression = top_glutamate_cell_df.sum(axis=1).
 ↪sort_values(ascending=False)

# Display the top 10 most expressed genes in these cells
print("Top Genes Most Expressed in the Glutamate-Expressing Cells:")
```

```
print(top_glutamate_gene_expression.head(10))
```

```
Top Genes Most Expressed in the GABA-Expressing Cells:
gene_symbol
TRPC4        30.835731
ACVR2A       30.805561
TDRD9        30.037315
ZNF667       29.442628
LPPR2        28.404406
LINC00158    28.145336
SYNGAP1      27.794682
RAP2B        27.730145
PPP4R4       27.669746
SUSD4        27.415054
dtype: float64

Top Genes Most Expressed in the Glutamate-Expressing Cells:
gene_symbol
SLC16A2      30.888883
NEURL1B      29.080687
PRKCG        28.001237
ARHGAP28     27.811188
ACVR2A       27.617610
TNFAIP8L3    27.448417
PCDHGC5      27.397415
TENM3        27.110805
PGA3         27.077198
PGA4         27.077198
dtype: float64
```

Electrophysiological feature analysis - Split spiny and aspiny neuron data with features of interest: resting membrane potential, input resistance and sag.

[105]:
```
# For this dataset we use spiny neurons as a proxy for excitatory cells while␣
 ↪aspiny neurons are used as inhibitory cells.
# Split the data by dendrite type
spiny_neurons = human_ephys_df[human_ephys_df['dendrite_type'] == 'spiny']
aspiny_neurons = human_ephys_df[human_ephys_df['dendrite_type'] == 'aspiny']

# We choose to compare resting membrane potential, input resistance and sag
features = ['vrest', 'input_resistance_mohm', 'sag']

# Create dictionaries for each feature's data for spiny and aspiny neurons
spiny_data = {}
aspiny_data = {}

for feature in features:
```

```
    spiny_data[feature] = human_ephys_df[human_ephys_df['dendrite_type'] ==␣
↪'spiny'][feature]
    aspiny_data[feature] = human_ephys_df[human_ephys_df['dendrite_type'] ==␣
↪'aspiny'][feature]
```

2.4 Data Analysis & Results

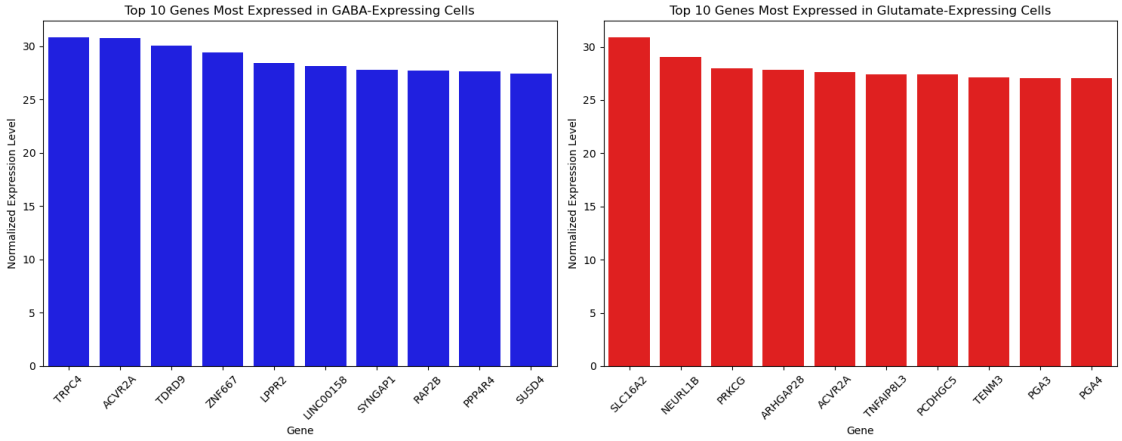
Our analysis revealed significant variation in the expression of GABAergic and glutamatergic genes across brain regions. These findings align with known functional specializations of these regions, supporting the hypothesis that gene expression reflects region-specific roles in inhibitory and excitatory signaling.

[1]:
```
## Plot brain regions where GABA and Glutamate-related genes are expressed most
fig, axes = plt.subplots(1, 2, figsize=(15, 6))

# Plot the top 10 brain regions with highest GABA receptor gene expression
sns.barplot(x=gaba_region_expression.head(10).index, y=gaba_region_expression.
↪head(10).values, color='blue', ax=axes[0])
axes[0].set_title("Top 10 Brain Regions with Highest GABA Receptor Expression")
axes[0].set_xlabel("Brain Region")
axes[0].set_ylabel("Expression Level")
axes[0].tick_params(axis='x', rotation=45)

# Plot the top 10 brain regions with highest Glutamate receptor gene expression
sns.barplot(x=glutamate_region_expression.head(10).index,␣
↪y=glutamate_region_expression.head(10).values, color='red', ax=axes[1])
axes[1].set_title("Top 10 Brain Regions with Highest Glutamate Receptor␣
↪Expression")
axes[1].set_xlabel("Brain Region")
axes[1].set_ylabel("Expression Level")
axes[1].tick_params(axis='x', rotation=45)

plt.tight_layout()
plt.show()
```

```
---------------------------------------------------------------------------
NameError                                 Traceback (most recent call last)
Cell In[1], line 2
      1 ## Plot brain regions where GABA and Glutamate-related genes are␣
↪expressed most
----> 2 fig, axes = plt.subplots(1, 2, figsize=(15, 6))
      4 # Plot the top 10 brain regions with highest GABA receptor gene␣
↪expression
      5 sns.barplot(x=gaba_region_expression.head(10).index,␣
↪y=gaba_region_expression.head(10).values, color='blue', ax=axes[0])
```

```
NameError: name 'plt' is not defined
```

[107]:
```
## Plot most expressed genes in the cells that express GABA and Glutamate most
fig, axes = plt.subplots(1, 2, figsize=(15, 6))

# Plot the top 10 most expressed genes in the GABA-related cells
top_gaba_gene_expression_top_10 = top_gaba_gene_expression.head(10)
sns.barplot(x=top_gaba_gene_expression_top_10.index,␣
 ↪y=top_gaba_gene_expression_top_10.values, color='blue', ax=axes[0])
axes[0].set_title("Top 10 Genes Most Expressed in GABA-Expressing Cells")
axes[0].set_xlabel("Gene")
axes[0].set_ylabel("Normalized Expression Level")
axes[0].tick_params(axis='x', rotation=45)

# Plot the top 10 most expressed genes in the Glutamate-realted cells
top_glutamate_gene_expression_top_10 = top_glutamate_gene_expression.head(10)
sns.barplot(x=top_glutamate_gene_expression_top_10.index,␣
 ↪y=top_glutamate_gene_expression_top_10.values, color='red', ax=axes[1])
axes[1].set_title("Top 10 Genes Most Expressed in Glutamate-Expressing Cells")
axes[1].set_xlabel("Gene")
axes[1].set_ylabel("Normalized Expression Level")
axes[1].tick_params(axis='x', rotation=45)

plt.tight_layout()
plt.show()
```

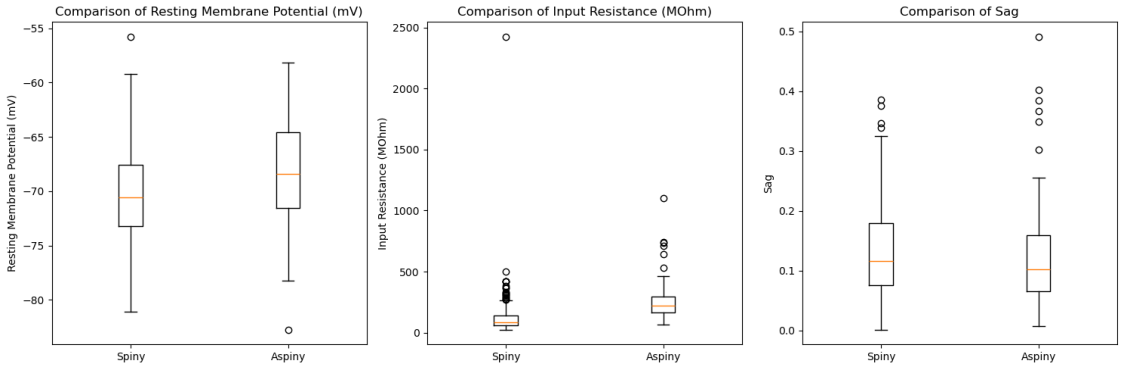


[108]:
```
## Perform Mann-Whitney U tests for each feature to determine statistical␣
 ↪significance between aspiny and spiny cells.
test_results = {}

for feature in features:
```

```
    spiny_values = spiny_data[feature]
    aspiny_values = aspiny_data[feature]
    u_stat, p_val = stats.mannwhitneyu(spiny_values, aspiny_values)
    test_results[feature] = {'U-statistic': u_stat, 'p-value': p_val}

# Display the test results
for feature, result in test_results.items():
    print(f"Feature: {feature}")
    print(f"  U-statistic: {result['U-statistic']}")
    print(f"  p-value: {result['p-value']}")
    if result['p-value'] < 0.05:
        print("  Result is statistically significant!")
    else:
        print("  Result is not statistically significant.")
    print("")
```

```
Feature: vrest
  U-statistic: 9170.0
  p-value: 0.00013793086507725568
  Result is statistically significant!

Feature: input_resistance_mohm
  U-statistic: 3912.0
  p-value: 1.6720249665943428e-21
  Result is statistically significant!

Feature: sag
  U-statistic: 13743.0
  p-value: 0.24819535713115315
  Result is not statistically significant.
```

[109]:
```
## Plot electrophysiological feature comparison for spiny and aspiny cells on␣
 ↪boxplots
fig, ax = plt.subplots(1, 3, figsize=(15, 5))

labels = ['Resting Membrane Potential (mV)', 'Input Resistance (MOhm)', 'Sag']
for i, feature in enumerate(features):
    data = [spiny_data[feature], aspiny_data[feature]]  # Prepare data for␣
 ↪boxplot
    ax[i].boxplot(data)
    ax[i].set_xticklabels(['Spiny', 'Aspiny'])
    ax[i].set_ylabel(labels[i])
    ax[i].set_title(f'Comparison of {labels[i]}')

plt.tight_layout()
plt.show()
```



2.5 Conclusion & Discussion

Conclusion & Discussion

Differential Gene Expression Across Brain Regions

Our analysis revealed significant variation in the expression of GABAergic and glutamatergic genes across brain regions. These findings align with known functional specializations of these regions, supporting the hypothesis that gene expression reflects region-specific roles in inhibitory and excitatory signaling.

GABA-Expressing Genes

- Ventromedial Hypothalamic Nucleus (VMH): The VMH exhibited high expression of GABA-related genes such as GAD1 and GAD2, which are responsible for GABA synthesis. This aligns with the VMH's established role in regulating stress responses and energy homeostasis, where inhibitory control is crucial for suppressing overactivity in stress-related pathways.
- Frontal Pole (Medial Aspect): High GABA expression in the frontal pole suggests the region's reliance on inhibitory signaling to maintain balance in decision-making and emotional regulation. This region's involvement in executive functions necessitates precise control over neural excitability, provided by GABAergic neurons.

Glutamate-Expressing Genes

- Dentate Gyrus (Hippocampus): The dentate gyrus displayed elevated expression of glutamate-related genes, such as GRIN1 (an NMDA receptor subunit). This supports its critical role in synaptic plasticity and neurogenesis, processes fundamental to learning and memory formation.
- CA2 Field (Hippocampus): The CA2 region showed prominent glutamate-related activity, reflecting its involvement in social memory and synaptic connectivity. Genes like SYNGAP1, essential for synaptic development, were highly expressed, highlighting the region's excitatory nature.

These region-specific patterns demonstrate the functional specialization of inhibitory and excitatory signaling across different brain areas. They also emphasize how variations in neurotransmitter-related gene expression can contribute to the distinct roles of these regions in cognition, memory, and emotional regulation.

Most Expressed Genes in GABA and Glutamate Cells

In addition to regional differences, we identified the top-expressed genes in cells associated with GABAergic and glutamatergic signaling. These genes underscore the molecular basis for the functional differences between inhibitory and excitatory neurons.

GABA-Expressing Cells

• TRPC4: A calcium-signaling gene, TRPC4 supports the regulation of GABAergic transmission. Calcium signaling is essential for neurotransmitter release, emphasizing its importance in GABA-mediated inhibition.
• ACVR2A: Activin A receptor type 2A, involved in the TGF-beta signaling pathway, which is essential for synaptic plasticity and neuronal remodeling. ACVR2A likely plays a role in maintaining inhibitory circuit stability and responding to stress-related changes in excitatory input.
• ZNF667: A transcription factor highly expressed in GABAergic cells, potentially involved in maintaining neuronal identity and regulating inhibitory-specific gene networks.

Glutamate-Expressing Cells

• SYNGAP1: This gene, a critical regulator of synaptic plasticity, was highly expressed in glutamate-expressing cells. Its role in strengthening synaptic connections aligns with the excitatory functions of glutamatergic neurons in learning and memory.
• GRIN1: Encoding a subunit of the NMDA receptor, GRIN1 facilitates excitatory neurotransmission. Its high expression highlights its importance in glutamate-mediated synaptic signaling and long-term potentiation.
• PPP4R4: A gene involved in regulatory pathways affecting excitatory transmission. Its expression may reflect the role of glutamate cells in modulating synaptic strength and excitability.

The distinct expression patterns of these genes in GABAergic and glutamatergic cells underline their specialized roles in inhibitory and excitatory neurotransmission. These findings suggest that molecular mechanisms governing neurotransmitter synthesis, release, and receptor activity are tailored to meet the functional demands of specific cell types.

Gene Expression Analysis:

Top-Expressed Genes in GABAergic and Glutamatergic Cells:
These gene expression patterns reflect the functional roles of inhibitory and excitatory neurons:
• GABAergic neurons maintain inhibitory control, synaptic stability, and neuronal differentiation through their unique gene sets.
• Glutamatergic neurons drive excitatory signaling and plasticity, critical for learning and memory.

Brain Region-Specific Differences:

Significant differences in gene expression were observed across brain regions.
These findings suggest that brain regions are functionally specialized based on the proportion of excitatory and inhibitory neurons and their gene expression profiles.

Electrophysiological Analysis:
Spiny (excitatory) and aspiny (inhibitory) cells exhibit distinct electrophysiological properties:
• Resting Membrane Potential (vrest):
• Spiny cells had significantly less hyperpolarized resting membrane potentials compared to aspiny cells ($p = 0.0001$).
• This reflects the higher excitability of excitatory neurons.
• Input Resistance (input_resistance_mohm):
• Aspiny cells showed significantly higher input resistance ($p < 0.00001$), consistent with their

slower and more regulated firing patterns.
- Sag Potential (sag):
- No significant difference was observed for sag potential between spiny and aspiny cells.

Interpretation:

- The electrophysiological properties align with the roles of spiny (glutamatergic) and aspiny (GABAergic) neurons:

- Spiny neurons: Designed for rapid signal transmission and excitatory function.

- Aspiny neurons: Optimized for inhibitory control and stability within neural circuits.

Integration of Gene Expression and Electrophysiology:

The gene expression profiles and electrophysiological characteristics are consistent with the known functional roles of GABAergic and glutamatergic neurons.

- TRPC4 and ACVR2A in GABAergic neurons may regulate inhibitory signaling and plasticity to prevent overexcitation.

- SYNGAP1 and GRIN1 in Glutamatergic neurons drive synaptic plasticity and excitatory neurotransmission, enabling memory and learning functions.

Functional Specialization of Brain Regions:

- Gene expression and electrophysiological data confirm that brain regions are highly specialized based on their excitatory-inhibitory balance.

- GABA-related genes dominate regions associated with stress and control, while glutamate-related genes are enriched in regions involved in learning and memory.

Clear Cell-Type Differences: • Spiny (excitatory) and aspiny (inhibitory) cells exhibit distinct molecular and functional signatures, underscoring the importance of these neurons in maintaining neural circuit balance.

Insights into Depression Mechanisms: • Findings suggest potential targets for studying depression-related phenotypes, including genes like ACVR2A (linked to stress response) and GRIN1 (associated with synaptic dysfunction).