Investigating the function of the mouse hypothalamic preoptic region using scRNA-seq and MERFISH data produced by Moffit et al. (2018)

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INTRODUCTION

The hypothalamus is a region of the brain that comprises a collection of small nuclei with diverse functions. One of its main functions is to produce and release hormones which contribute to the regulation of several endocrine glands and organs. In addition to its neuroendocrine role, the hypothalamus is also responsible for regulating physiological and behavioral processes such as body temperature, emotions, sleep, thirst, and hunger. The preoptic region, which is a set of nuclei in the hypothalamus, is known to be responsible for thermoregulation, sleep, and social behaviors such as parenting, aggression, and mating. Structural understanding of this region is limited given the intrinsic spatial complexity of the brain. Therefore, we aimed to investigate the composition, spatial organization, and gene expression profiles of cells in the hypothalamic preoptic region by analyzing single-cell RNA sequencing (scRNA-seq) and Multiplexed Error Robust Fluorescence In Situ Hybridization (MERFISH) data obtained from mice by Moffit et al. (2018).

RESULTS AND DISCUSSION

scRNA-seq profile of the hypothalamic preoptic region

scRNA-seq profiles of 31,299 cells were obtained from the medial preoptic area (MPOA) and surrounding nuclei (-2.5 by 1.5 by 1.1mm, Bregma +0.5 to -0.6) of the hypothalamus of male (n =3) and female mice (n = 3) by Moffit et al. (2018) using droplet based scRNA-seq. Our quality control measures involved removing outliers including cells with unusually high counts and cells with more than 5% expression of mitochondrial genes as recommended by the literature. This left us with 27,998 scRNA-seq profiles to use for our subsequent analysis.

Neuronal clusters enumerated and identified with scRNA-seq

To determine how many neuronal cell populations are present in the mouse hypothalamic preoptic region, we used t-distributed stochastic neighbor embedding (tSNE) clustering analysis to preserve small pairwise distances between cells. A total of 12 clusters were identified. We then distinguished cell types by finding markers that define neuronal cell types via differential expression (Table 1). These results are visualized in Figure 1.

Only two of our 12 clusters were categorized as Mixed Populations due to the diversity of cell populations identified within the cluster. Note that the Mixed Population cluster at the periphery is of negligible size compared to the rest of the clusters and and appears to be equidistant to its two nearest clusters (See Figure 1), indicating that it might have been incorrectly grouped. Our results otherwise indicate that the hypothalamic preoptic region is enriched with excitatory and inhibitory neurons and mature oligodendrocytes. The presence of inhibitory neurons is consistent with what we know about the hypothalamic preoptic region's role in modulating sleep and hunger. The activation of these cells could inhibit excitatory networks that keep us awake or make us hungry. The reciprocal logic could be used to explain the role of excitatory neurons. Excitatory and inhibitory neurons may also be involved in thermoregulation, where heat-sensitive neurons induce hypothermia after GABAergic stimulation. Moreover, the dominance of of mature oligodendrocytes and oligodendrocytes precursor cells (OPC) in the hypothalamic preoptic region corroborates the preoptic region's known role in producing cells that can migrate to the nearby optic chiasm region to innervate optic nerves and contribute to faster neuronal transmission.

Gene ontologies of scRNA cell-type clusters show functional agreement

Gene ontology (GO) enrichment analysis was performed using the lists of differentially expressed genes found in each cell-type cluster identified in the scRNA-seq analysis to define protein functions. The GO enrichment analysis package `gprofiler2` was used within R and gene lists were limited to the top 500 most significantly differentially expressed genes, except for the population of immature oligodendrocytes, of which 1,000 genes were needed to observe significant GO term enrichment. There was strong correlation between the significantly enriched GO terms and the identified predominant cell-type within each cluster (Table 2,Figures S1 - S11). The cluster microglia had the lowest p-values ($3.1x10^{-81}$- $1.8x10^{-51}$) of enriched GO terms, associated immune system function and defense (Figure S9). While the lowest enrichment of biological process GO terms was found in the immature oligodendrocyte cluster, there was a significant enrichment of associated transcription factors related to cell differentiation likely due to the immature nature of the cells within the cluster (Figure S6). Another cluster of note is the mixed cell population (Figure S10) which had significant enrichment of GO terms related to the general cell function of protein synthesis, further demonstrating that the cluster was not predominated by any particular cell type. A direct comparison of the co-enriched GO terms of excitatory and inhibitory neurons (Figures S4 & S7) showed a more significant enrichment of synaptic signaling in the excitatory neuronal cluster. This could suggest that the preoptic region analyzed in this study has greater signaling participation from the excitatory cell population despite its size, or that the gprofiler2 analysis can achieve higher significance measured by p-value despite a lower population size.

MERFISH cell atlas of the mouse hypothalamic preoptic region

A cell atlas of the mouse hypothalamic preoptic region was generated using a MERFISH-based imaging and analysis platform developed by Moffit et al. (2018). MERFISH measurements of the preoptic region were 1.8 by 1.8 by 0.6 mm, Bregma +0.26 to 0.34 within the area characterized with scRNA-seq.

Spatially varying genes identified with MERFISH elucidates cellular function

To investigate the spatial organization of gene expression and cellular function of the mouse hypothalamic preoptic region, we analyzed the cell atlas produced by the MERFISH-based imaging analysis platform developed by Moffitt et al. (2018). This provided greater insight into how cells organize to achieve some overarching biological function.

In particular, we analyzed three distinct sections of the preoptic region (Bregma = 0.11 mm, 0.16 mm, 0.26 mm) for mice evincing different behaviors to observe differences in the localization of cell populations among behaviors and sexes. The behaviors include naïve (i.e. has not reproduced), mating, and parenting. Figure 2 illustrates a sagittal section of a mouse brain, with lines marking the coronal slices imaged and analyzed using MERFISH.

Unsupervised clustering analysis and nearest-neighbor Gaussian processes were performed to determine the most spatially variable genes (SVGs) for each previously mentioned Bregma value, sex, and behavior. The positions of the forty most spatially varying genes in the tissue section were plotted and compared to the known positions of the centroids of all cell types for that section. An example of the results of this process is shown in Figure 3, a tissue section taken from a naive female organism at a Bregma value 0.16 mm. The left plot of Figure 3 illustrates the spatial arrangement of the different cells in this tissue section. The right plot demonstrates that the gene *Cd24a* exhibits a statistically significant spatial expression pattern. It is evident that the *Cd24a* gene is strongly expressed in the ependymal cells. The CD24 protein is known to be a surface protein which acts as a cell-adhesion molecule. Furthermore, this protein contributes to downstream signaling networks crucial for neuronal development. Hence, it is reasonable to assert that ependymal cells are responsible for cell adhesion, neuronal generation, and growth in the mouse brain.

This technique can be applied to all significantly spatially varying genes, from which the function of the cells in the mouse brain can be determined. This could conceivably sharpen understanding of the overall function of the mouse hypothalamic preoptic region. Intriguingly, the application of this method to the neuronal cells of the brain could help establish a molecular basis for organism behavior.

To this end, the formation of a “Platonic ideal” for each gender-behavior pair was attempted by running the unsupervised clustering algorithm on all organisms of a certain gender and behavior. It was found that the histological variation between organisms of a gender-behavior pair at a certain Bregma value caused the clustering algorithm to return a badly distorted picture that was felt to be unrepresentative of a typical organism with gender-behavior characteristics. Thus, the data analysis pipeline was applied to a single individual from each gender-behavior pair, and the SVGs between individuals were compared. The top ten most SVGs for each (gender, behavior, Bregma) triplet are detailed in Table 3. Using a table like this one, the highly significant SVGs common or unique to each behavior could be determined. Figure 4 and the accompanying text illustrate the number and types of genes common or unique to each behavioral type.

From Figure 4, we conclude that the *Sln* gene is ubiquitously expressed in the mouse hypothalamic preoptic area regardless of the behaviors assessed. Figure 5 shows where the *Sln* gene is being expressed for three mice: a naive female, a naive male, and a mating female. Evidently, in all three cases, this gene is primarily expressed in excitatory neurons. It is known that the *Sln* gene encodes the protein Sarcolipin, which is important in the maintenance of a warm body temperature. Thus, it is reasonable to suggest that the excitatory neurons in the mouse hypothalamic preoptic region are responsible for mediating brain-induced thermogenesis by eliciting shivering-like behaviors.

We also found that the gene *Ar* exhibits significant spatially variable expression for mice that are either mating or parenting. The *Ar* gene encodes for androgenic receptors which are critical for sexual development and regulating sex drive. Figure 6 illustrates that in males the *Ar* gene is expressed in both excitatory and inhibitory neurons, but particularly the latter. It has been shown that AR knockout mice experience higher than average testosterone and luteinizing hormone due to impaired negative feedback mechanisms that are likely mediated by these inhibitory neurons. It appears that the *Ar* gene expressed in the inhibitory neurons of the mouse hypothalamic preoptic region are tightly linked to the endocrine system of male mice and contribute to normal sexual development and behavior. Finally, we observed that the gene *Omp*, which encodes for the olfactory marker protein found in mature olfactory neurons, is uniquely present in

parenting mice. These highly active neurons aid parental mice in recognizing their offspring based on their unique odor.

CONCLUSION

Through our various routes of inquiry, we determined that the mouse hypothalamic preoptic region is responsible for at least three functions: thermoregulation, offspring odor-mediated recognition, and regulation of the endocrine system in achieving normal sexual development in mice. These findings are consistent with the published literature, and broader application of these analyses could further our understanding of the mouse hypothalamic preoptic region.

DIVISION OF LABOR

Chandler Nielsen - MERFISH spatially varying gene analysis and data analysis; Behavior associated gene analysis
Evan London - GO enrichment analysis
Nicole Pietrunti - Reporting, editing, flow of inquiry, scientific reasoning
Simon Lizarazo - scRNA-seq clustering and DEG analysis; MERFISH spatially varying gene analysis; Behavior-associated gene analysis

APPENDIX

Cell Type	Gene Marker
Astrocyte	Cpe, Clu, Aldoc, Slc1a3, Slc1a2, Gfap
Endothelial Cells	Itm2a, Bsg, Ifitm1, Apold1, Rsg5
Ependymal Cells	Tabl2, Rpcp4l1, Riiad1, Tm4sf1
Microglia	Ccl4, Ccl3, Ctss, Tyrobp, Cd83
Excitatory Neurons	Glut, Shox2, Meis2, Foxp2, Slc17a6, Nrgn
Inhibitory Neurons	Gabarapl2, Gal, Gabarap, Gabarapl1, Gad1, Pax2, Gad2, Pvalb
Mature Oligodendrocytes	Cnp, Mal, Mog, Cryab, Mag
Immature Oligodendrocytes	Plp1, Cnp, Bcas1, Ennp6
Oligodendrocytes Precursor Cells (OPC)	Pdgfra, Cnn1, Tnr, Olig1, Epn2, Sox10, Neu4

Table 1: Gene markers associated with cell populations generated by tSNE clustering of scRNA-seq data obtained from the mouse hypothalamic preoptic region (n = 3 males, n = 3 females) by Moffit et al. (2018).

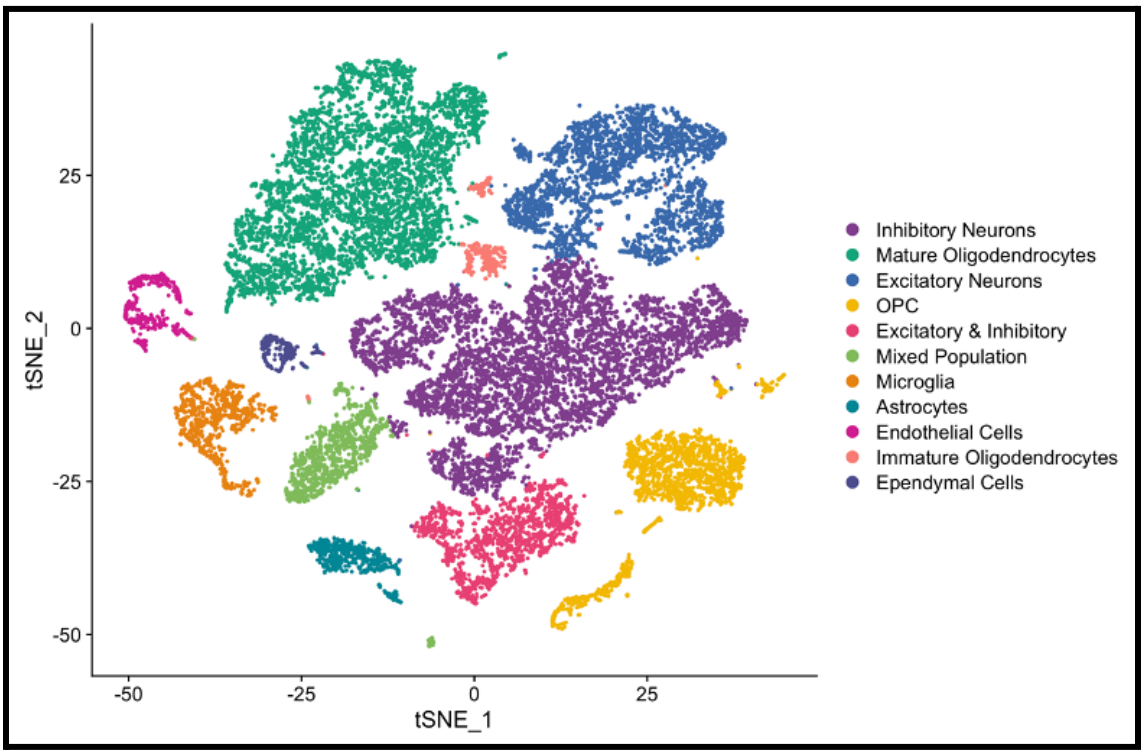


Figure 1. tSNE plot visualizing cell clusters by cell type using scRNA-seq data produced by Moffit et al. (2018).

Cell-type Cluster	Cell count	Top 3 gene ontology biological processes	Cell-type function in literature
Inhibitory Neurons	6,129	Synaptic signaling, chemical synaptic transmission, anterograde trans-synaptic signaling.	Produces inhibitory function through synaptic signaling, and can produce a positive response when inhibited.↟
Mature Oligodendrocytes	4,821	Nervous system development, ensheathment of neurons, axon ensheathment.	Glial cell line participating in myelination and repair of neuronal axons, enhancing signal transmission, and reducing degradation.
Excitatory Neurons	2,936	Synaptic signaling, regulation of biological quality, cell-cell signaling.	Activating through synaptic signaling, and can produce a negative response when inhibited.*
Oligodendrocyte progenitor Cells	1,788	System development, anatomical structure development, multicellular organism development.	Stem-cell line with that will mature into oligodendrocytes, myelinates the central nervous system during development with high mobility
Excitatory & Inhibitory	1,469	Nervous system development, neurogenesis, system development	↟*
Mixed Population	1,020	Cytoplasmic translation, translation, peptide biosynthetic process.	N/A
Microglia	856	Immune system process, immune response, inflammatory response.	Glial immune cell line within the brain, having macrophage-like function of the innate immune system.
Astrocytes	568	Organic substance transport, small molecule metabolic process, carboxylic acid metabolic process.	Synaptic linking glial cell line participating in tripartite synapses with many neuron pairs.
Endothelial Cells	471	Blood cell morphogenesis, vasculature development, blood vessel development	Blood cells associated with vasculature of the brain
Immature Oligodendrocytes	346	Protein modification process, macromolecule modification, chromatin organization	Pre-myelinating oligodendrocytes
Ependymal Cells	269	Anatomical structure development, developmental process, animal organ development	Structural cells that begin development in the early neural tube.

Table 2. Significantly enriched gene ontology terms determined using gprofiler2 using default settings. The full gene ontology plots are within the supplement with the top 10 most significant biological processes highlighted.

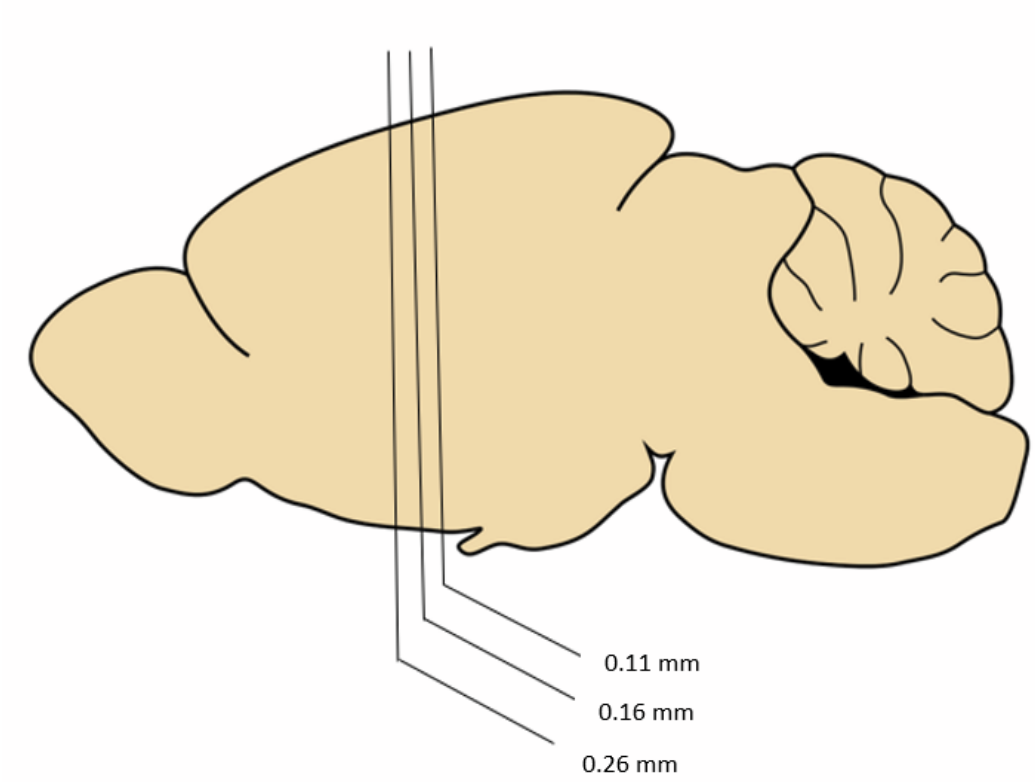


Figure 2. Coronal slices of mouse hypothalamic preoptic region imaged using MERFISH. Distances are measured with respect to the Bregma region of the skull. Figure is not to scale.

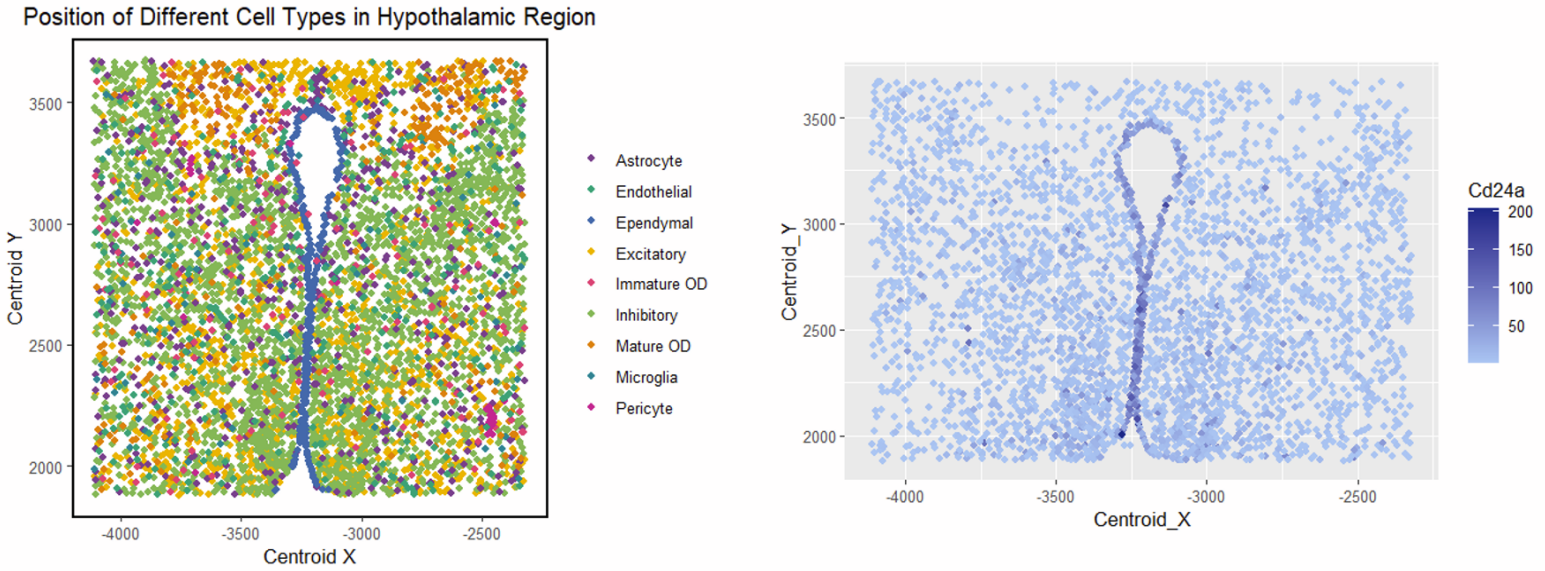
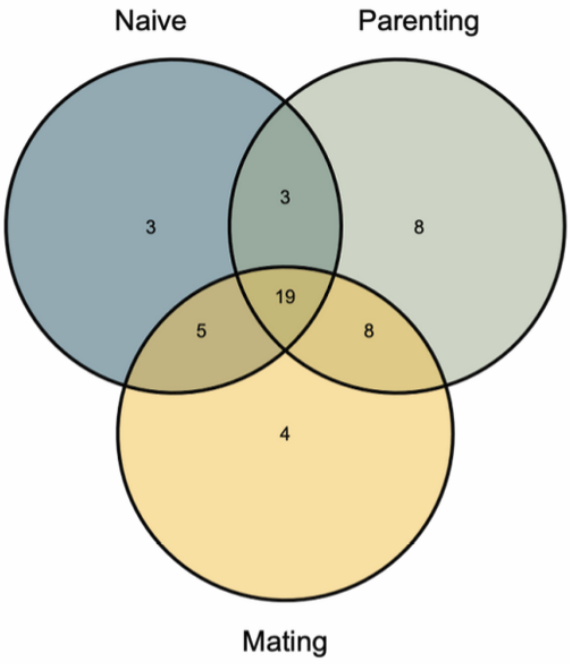


Figure 3: The left plot shows a map of the relative positions of all cell types identified by Moffitt et al. (2018) in a tissue sample taken from the hypothalamic preoptic region of a naive female mouse at Bregma value 0.16 mm. The right plot illustrates the expression level of gene *Cd24a*. Evidently, this gene is expressed primarily in ependymal cells.

10 Most Spatially Varying Genes for (Behavior, Gender, Bregma) tuples

SVGs	Naïve						Mating						Parenting					
	Male			Female			Male			Female			Male			Female		
	0.11	0.16	0.26	0.11	0.16	0.26	0.11	0.16	0.26	0.11	0.16	0.26	0.11	0.16	0.26	0.11	0.16	0.26
1	Mbp	Mbp	Gal	Mbp	Mbp	Mbp	Nnat	Cck	Tac1	Adcyap1	Gnrh1	Sln	Nnat	Sln	Mbp	Nnat	Mbp	Mbp
2	Nnat	Sln	Mbp	Nnat	Gal	Adcyap1	Sln	Mbp	Mbp	Tac1	Tac1	Gnrh1	Cd24a	Mbp	Sln	Sln	Nnat	Tac1
3	Sln	Gal	Th	Sln	Sln	Gal	Mbp	Ucn3	Oxt	Nts	Adcyap1	Cck	Mbp	Nnat	Myh11	Necab1	Sln	Tac2
4	Tac1	Th	Sln	Tac2	Tac1	Nnat	Oxt	Sst	Gal	Sst	Nnat	Gal	Omp	Omp	Nnat	Cd24a	Cd24a	Nnat
5	Nts	Tac2	Tac1	Tac1	Nnat	Tac1	Cck	Ebf3	Trh	Trh	Tac2	Adcyap1	Sln	Ebf3	Ermn	Slc17a6	Tac1	Ermn
6	Ebf3	Crh	Tac2	Ermn	Ermn	Ucn3	Gnrh1	Trh	Cck	Cartpt	Trh	Tac2	Pak3	Tac1	Tac1	Ntng1	Necab1	Sln
7	Th	Adcyap1	Adcyap1	Lpar1	Th	Sln	Irs4	Nnat	Ermn	Nnat	Mbp	Mbp	Ar	Myh11	Sgk1	Tac1	Myh11	Lpar1
8	Adcyap1	Nnat	Cartpt	Nts	Myh11	Ermn	Cd24a	Nts	Nnat	Gal	Sln	Trh	Mlc1	Ermn	Lmod1	Mbp	Ebf3	Ndrg1
9	Gal	Cck	Ermn	Opalin	Lpar1	Lpar1	Th	Tac2	Adcyap1	Sln	Sst	Oxt	Tac1	Ndrg1	Ndrg1	Myh11	Prlr	Gda
10	Tac2	Ermn	Lpar1	Cartpt	Tac2	Cck	Scg2	Gnrh1	Sst	Mbp	Nts	Crh	Myh11	Cd24a	Lpar1	Mlc1	Lmod1	Gjc3

Table 3: This table describes the ten most spatially varying genes for each (behavior, gender, Bregma) triplet. We sought to determine the genes that are differentially expressed for all such triplets (such as *Mbp*) and genes that are unique to certain triplets (such as *Oxt*, which has significant spatial variation only for mating males and females).



Unique to Naive - "Opalin" "Syt4" "Penk"

Unique to Parenting - "Ntng1" "Rxfp1" "Prlr" "Ccnd2" "Baiap2" "Ano3" "Dgkk" "Omp"

Unique to Mating - "Sst" "Trh" "Scg2" "Greb1"

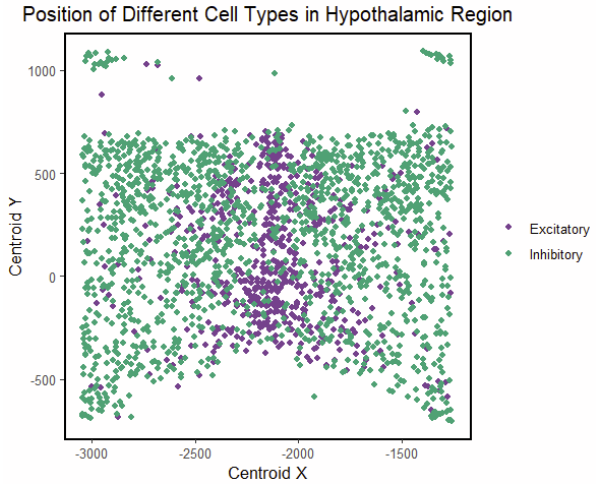
Mating and Parenting - "Necab1" "Cd24a" "Slc17a6" "Mlc1" "Pak3" "Esr1" "Cbln2" "Ar"

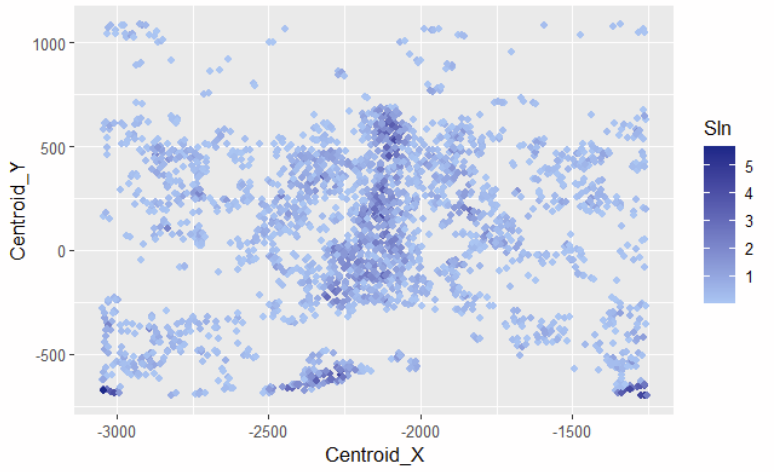
Mating and Naive - "Nts" "Cartpt" "Th" "Gal" "Crh"

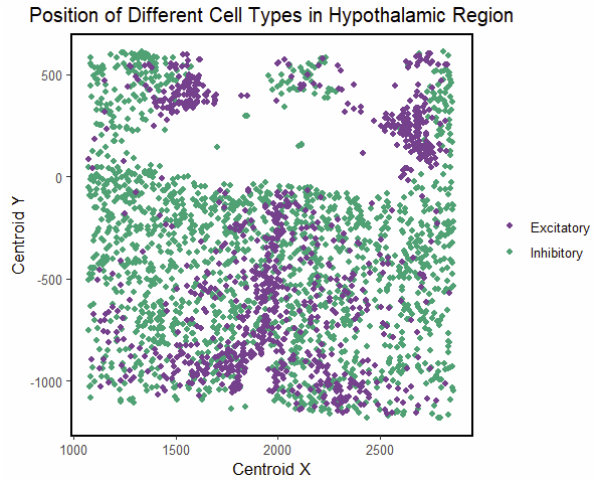
Naive and Parenting - "Myh11" "Lmod1" "Ttyh2"

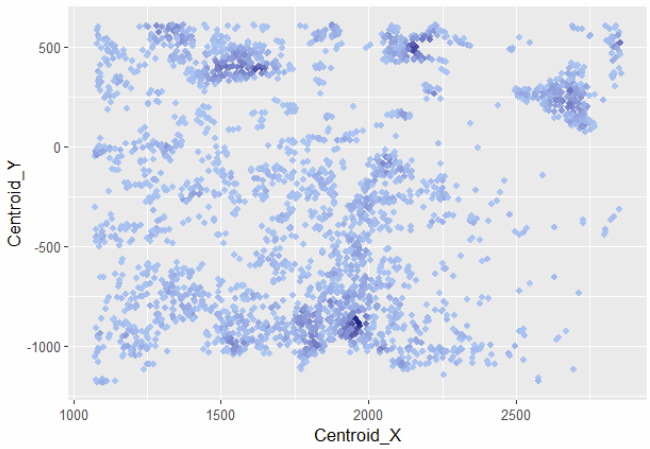
Mating and Naive and Parenting - "Mbp" "Nnat" "Sln" "Tac2" "Tac1" "Ermn" "Lpar1" "Ucn3" "Gjc3" "Gad1" "Sgk1" "Ndrg1" "Cck" "Adcyap1" "Irs4" "Gnrh1" "Oxt" "Ebf3" "Gda"

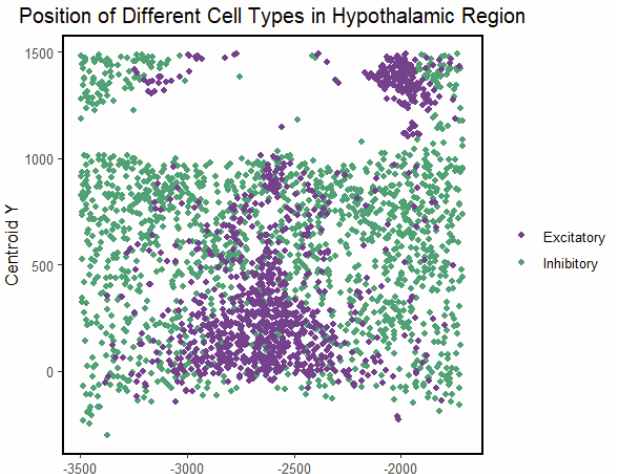
Figure 4: A Venn diagram illustrating the number of highly significant SVGs for each behavior. Listed are the specific genes for each area of the Venn diagram.











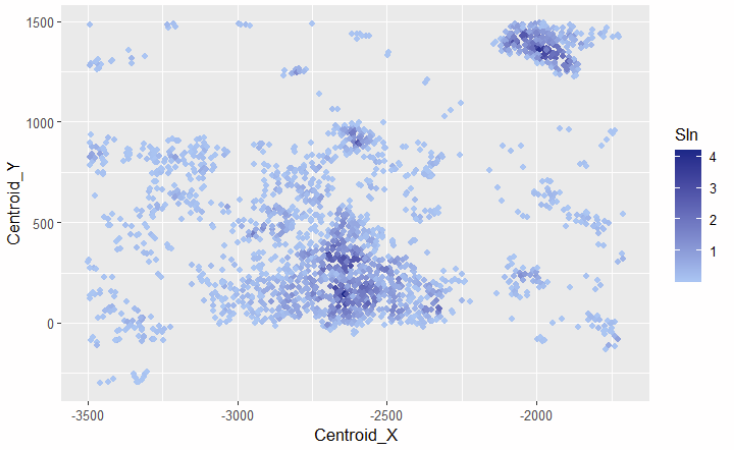
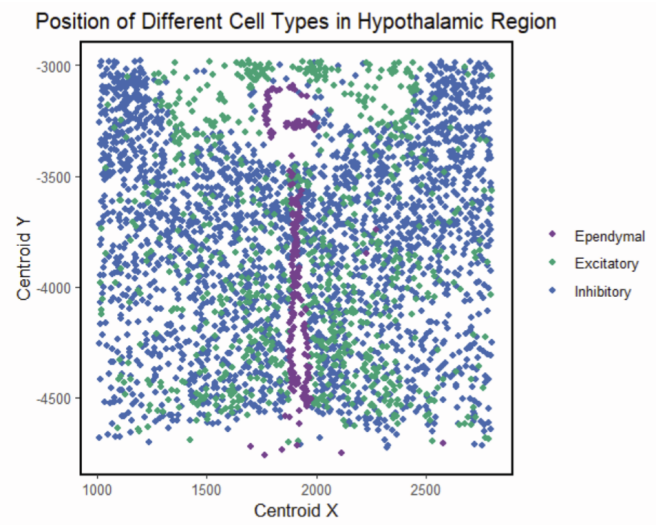
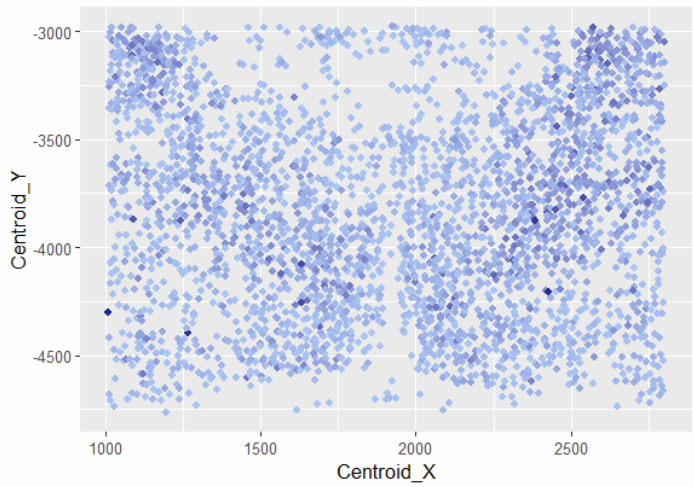
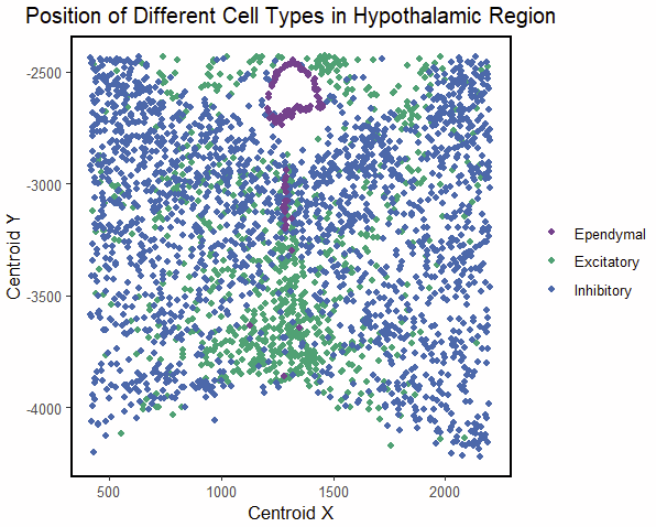


Figure 5: *Sln* expression in three mice (top: Naive Female Bregma=0.11 mm, middle: Naive Male Bregma = 0.16 mm, bottom: Mating Female Bregma = 0.16 mm). Evidently, the *Sln* gene is primarily expressed in excitatory neurons. Sarcolipin, the protein encoded by the *Sln* gene, helps mediate heat production; the protein is critical for many organisms to maintain a warm body.







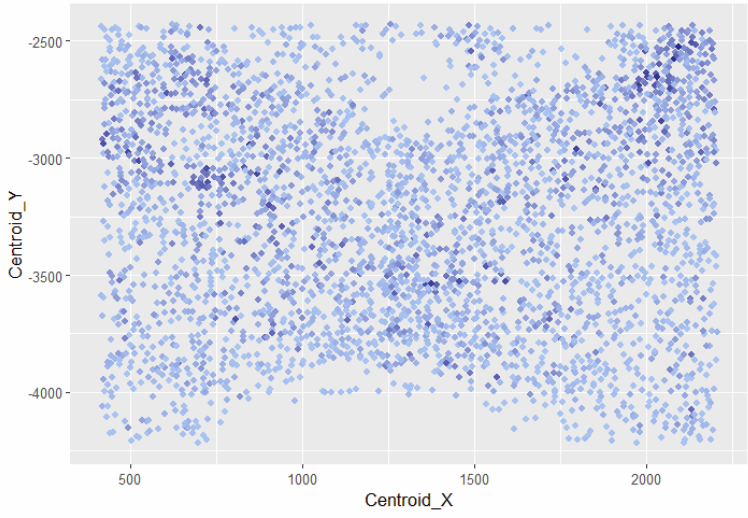
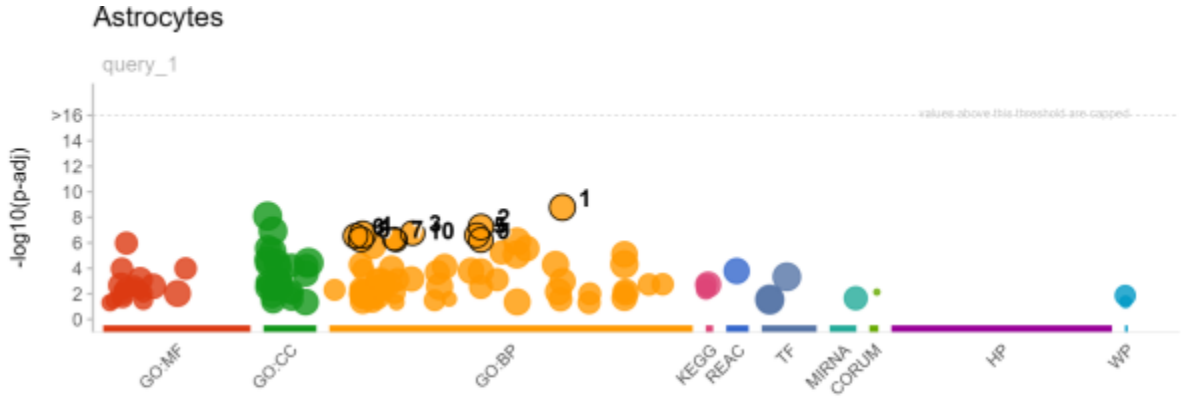


Figure 6: Expression of *Ar* gene in male mice (top: Mating Male Bregma = 0.11 mm, bottom: Parenting Male Bregma = 0.11 mm). Note that the spatial variation in the *Ar* gene closely matches the arrangement

of excitatory and inhibitory neurons. The *Ar* encodes for androgenic receptors, which have been identified as being crucial for both male and female sexual development.

Supplementary Figures

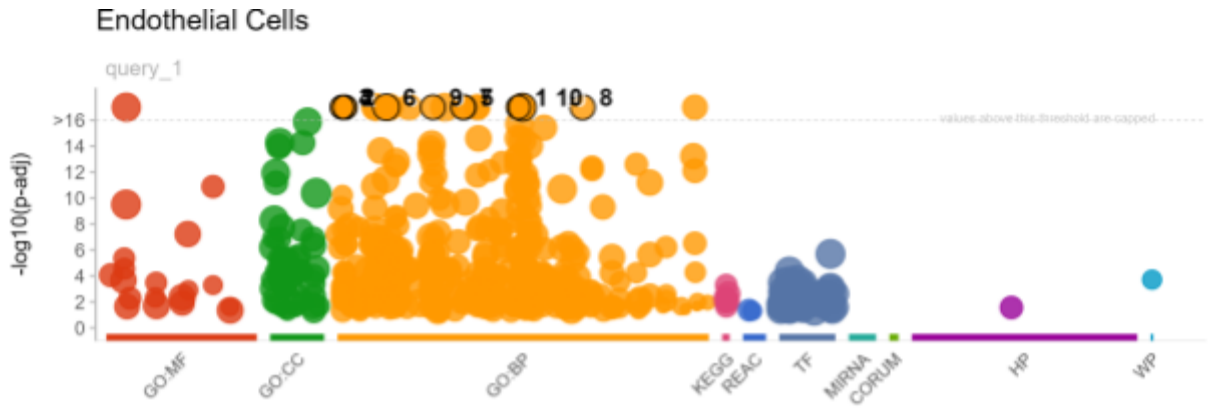
Figure S1 - S11: Top 10 most significantly enriched gene ontology biological processes for each cell-type cluster identified from the single-cell RNA sequencing dataset.



id	source	term_id	term_name	term_size	p_value
1	GO:BP	GO:0071702	organic substance transport	2837	1.7e-09
2	GO:BP	GO:0044281	small molecule metabolic process	1874	5.7e-08
3	GO:BP	GO:0019752	carboxylic acid metabolic process	990	1.7e-07
4	GO:BP	GO:0006810	transport	4664	2.2e-07
5	GO:BP	GO:0043436	oxoacid metabolic process	1010	2.6e-07
6	GO:BP	GO:0006082	organic acid metabolic process	1017	3.0e-07
7	GO:BP	GO:0015711	organic anion transport	453	4.9e-07
8	GO:BP	GO:0006629	lipid metabolic process	1462	5.3e-07
9	GO:BP	GO:0044255	cellular lipid metabolic process	1055	6.1e-07
10	GO:BP	GO:0015849	organic acid transport	392	6.2e-07

g:Profiler (biit.cs.ut.ee/gprofiler)

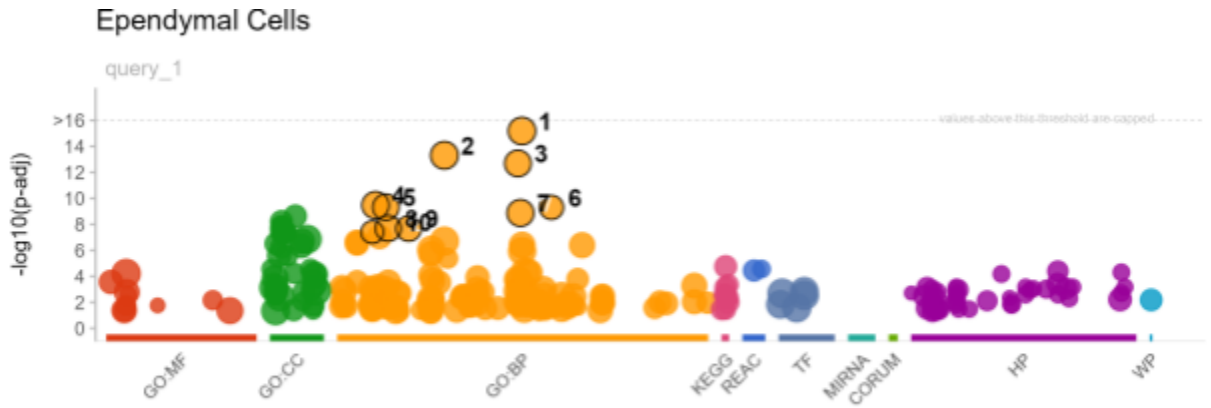
Figure S1



id	source	term_id	term_name	term_size	p_value
1	GO:BP	GO:0048514	blood vessel morphogenesis	650	1.9e-29
2	GO:BP	GO:0001944	vasculature development	779	6.7e-29
3	GO:BP	GO:0001568	blood vessel development	747	9.8e-29
4	GO:BP	GO:0001525	angiogenesis	553	4.6e-27
5	GO:BP	GO:0035295	tube development	1173	1.1e-26
6	GO:BP	GO:0009653	anatomical structure morphogenesis	2787	1.6e-26
7	GO:BP	GO:0035239	tube morphogenesis	921	2.1e-25
8	GO:BP	GO:0072359	circulatory system development	1203	3.3e-25
9	GO:BP	GO:0030334	regulation of cell migration	971	1.5e-21
10	GO:BP	GO:0048856	anatomical structure development	6228	8.4e-21

g:Profiler (biit.cs.ut.ee/gprofiler)

Figure S2



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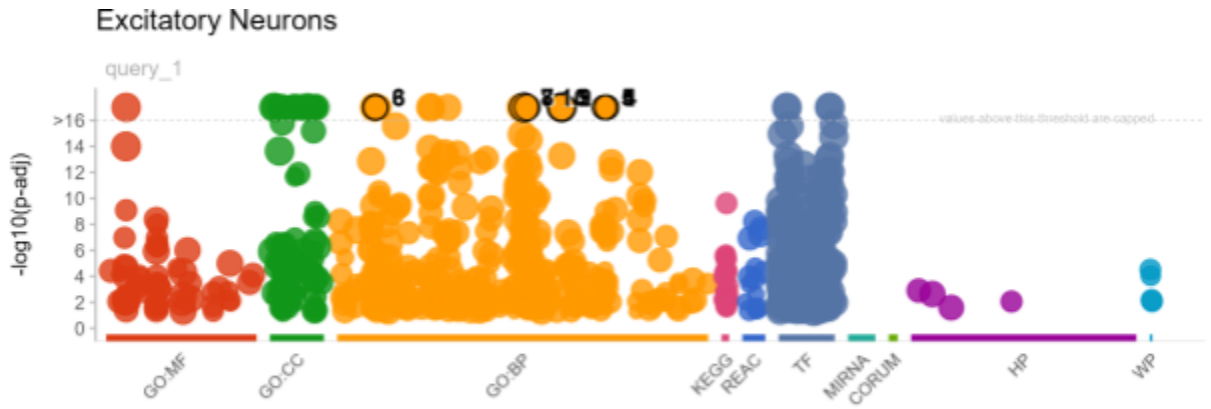
2	GO:BP	GO:0032502	developmental process	6865	4.9e-14
3	GO:BP	GO:0048513	animal organ development	3326	2.1e-13
4	GO:BP	GO:0007275	multicellular organism development	4873	3.3e-10
5	GO:BP	GO:0009653	anatomical structure morphogenesis	2787	4.8e-10
6	GO:BP	GO:0061061	muscle structure development	724	5.0e-10
7	GO:BP	GO:0048731	system development	4115	1.3e-09
8	GO:BP	GO:0009888	tissue development	2099	2.0e-08
9	GO:BP	GO:0016477	cell migration	1542	2.1e-08
10	GO:BP	GO:0006936	muscle contraction	315	3.9e-08

Figure S3

g:Profiler (biit.cs.ut.ee/gprofiler)



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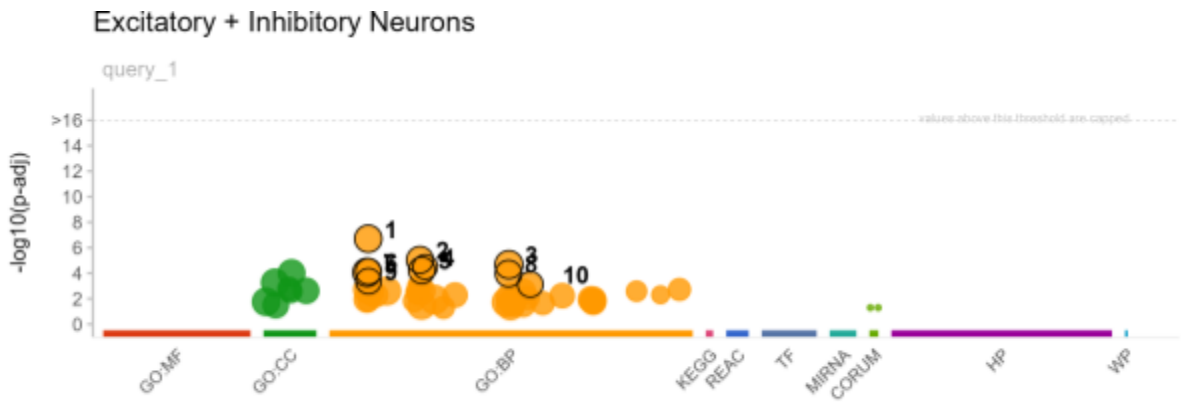
1	GO:BP	GO:0099536	synaptic signaling	941	2.0e-31
2	GO:BP	GO:0065008	regulation of biological quality	3244	2.0e-28
3	GO:BP	GO:0007267	cell-cell signaling	1748	7.4e-28
4	GO:BP	GO:0099537	trans-synaptic signaling	902	1.2e-27
5	GO:BP	GO:0098916	anterograde trans-synaptic signaling	895	7.0e-27
6	GO:BP	GO:0007268	chemical synaptic transmission	895	7.0e-27
7	GO:BP	GO:0050794	regulation of cellular process	12308	1.7e-26
8	GO:BP	GO:0050789	regulation of biological process	13424	1.7e-23
9	GO:BP	GO:0065007	biological regulation	13838	1.4e-22
10	GO:BP	GO:0051049	regulation of transport	1930	2.6e-21

g:Profiler (biit.cs.ut.ee/gprofiler)

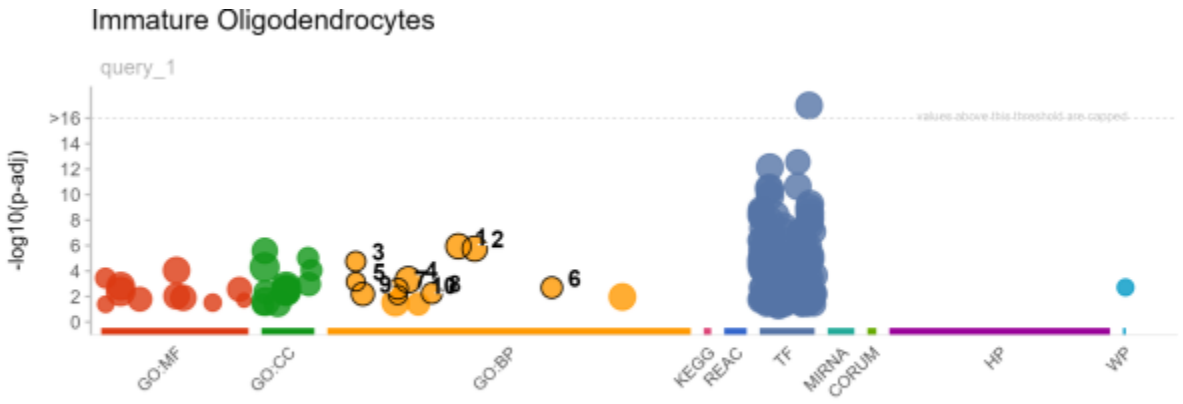
Figure S4



id	source	term_id	term_name	term_size		p_value
1	GO:BP	GO:0007399	nervous system development	2555		2.0e-07
2	GO:BP	GO:0022008	neurogenesis	1871		8.9e-06
3	GO:BP	GO:0048731	system development	4115		2.1e-05
4	GO:BP	GO:0030900	forebrain development	444		3.2e-05
5	GO:BP	GO:0030182	neuron differentiation	1555		5.9e-05
6	GO:BP	GO:0007417	central nervous system development	1077		7.3e-05
7	GO:BP	GO:0007275	multicellular organism development	4873		8.6e-05
8	GO:BP	GO:0048699	generation of neurons	1631		1.1e-04
9	GO:BP	GO:0007420	brain development	797		4.2e-04
10	GO:BP	GO:0060322	head development	850		7.5e-04

g:Profiler (biit.cs.ut.ee/gprofiler)

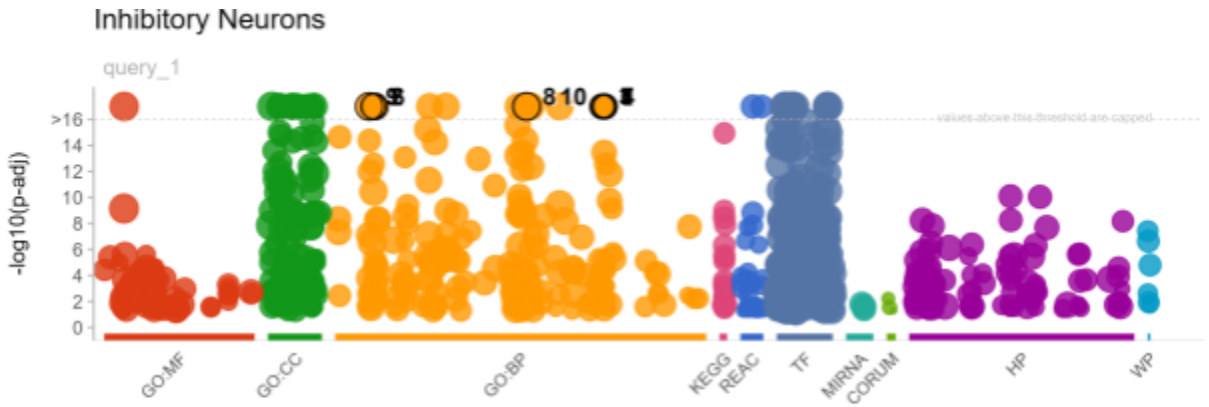
Figure S5



id	source	term_id	term_name	term_size	p_value
1	GO:BP	GO:0036211	protein modification process	3610	1.1e-06
2	GO:BP	GO:0043412	macromolecule modification	3805	1.7e-06
3	GO:BP	GO:0006325	chromatin organization	643	1.7e-05
4	GO:BP	GO:0019538	protein metabolic process	5554	4.8e-04
5	GO:BP	GO:0006338	chromatin remodeling	470	6.8e-04
6	GO:BP	GO:0070647	protein modification by small protein conjugation or removal	1002	2.0e-03
7	GO:BP	GO:0016567	protein ubiquitination	809	2.5e-03
8	GO:BP	GO:0032446	protein modification by small protein conjugation	860	5.5e-03
9	GO:BP	GO:0007049	cell cycle	1817	6.4e-03
10	GO:BP	GO:0016570	histone modification	493	7.5e-03

g:Profiler (biit.cs.ut.ee/gprofiler)

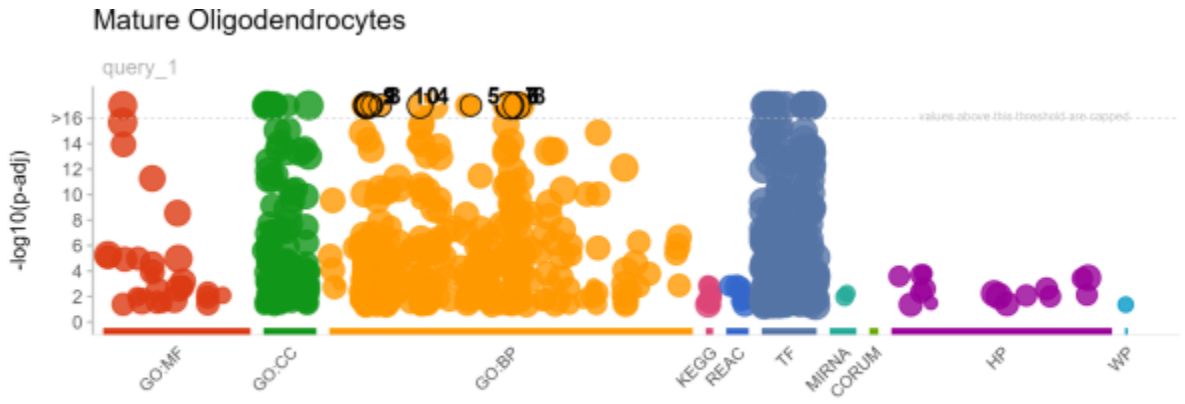
Figure S6



id	source	term_id	term_name	term_size	p_value
1	GO:BP	GO:0099536	synaptic signaling	941	8.5e-27
2	GO:BP	GO:0007268	chemical synaptic transmission	895	7.9e-26
3	GO:BP	GO:0098916	anterograde trans-synaptic signaling	895	7.9e-26
4	GO:BP	GO:0099537	trans-synaptic signaling	902	1.3e-25
5	GO:BP	GO:0099003	vesicle-mediated transport in synapse	256	6.8e-25
6	GO:BP	GO:0007399	nervous system development	2555	2.8e-22
7	GO:BP	GO:0099504	synaptic vesicle cycle	218	4.9e-22
8	GO:BP	GO:0051179	localization	5491	6.8e-22
9	GO:BP	GO:0006810	transport	4664	5.5e-20
10	GO:BP	GO:0051234	establishment of localization	4823	9.1e-20

g:Profiler (biit.cs.ut.ee/gprofiler)

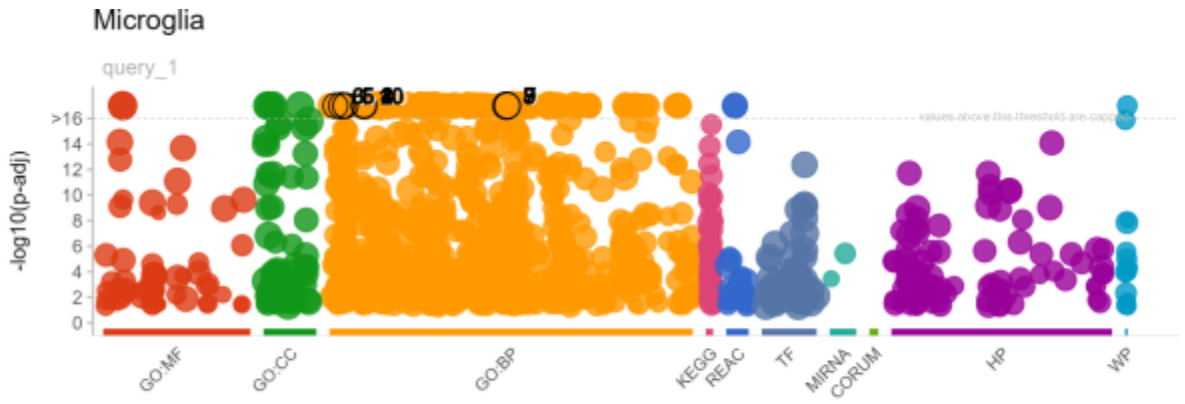
Figure S7



id	source	term_id	term_name	term_size		p_value
1	GO:BP	GO:0007399	nervous system development	2555		1.3e-28
2	GO:BP	GO:0007272	ensheathment of neurons	175		2.4e-26
3	GO:BP	GO:0008366	axon ensheathment	175		2.4e-26
4	GO:BP	GO:0022008	neurogenesis	1871		3.2e-26
5	GO:BP	GO:0042552	myelination	172		2.1e-25
6	GO:BP	GO:0048856	anatomical structure development	6228		5.9e-25
7	GO:BP	GO:0048731	system development	4115		4.4e-24
8	GO:BP	GO:0051179	localization	5491		1.9e-23
9	GO:BP	GO:0007275	multicellular organism development	4873		7.7e-23
10	GO:BP	GO:0010001	glial cell differentiation	257		3.3e-22

g:Profiler (biit.cs.ut.ee/gprofiler)

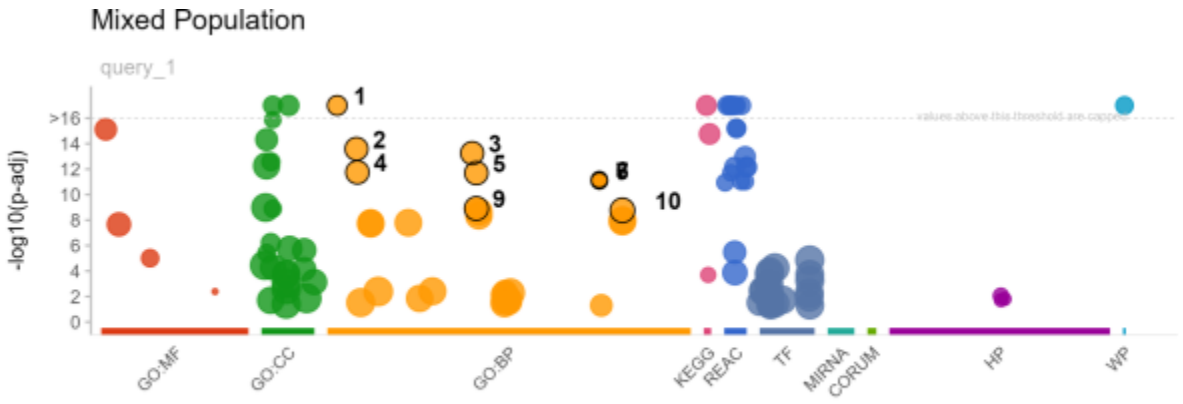
Figure S8



id	source	term_id	term_name	term_size		p_value
1	GO:BP	GO:0002376	immune system process	2720		3.1e-82
2	GO:BP	GO:0006955	immune response	1875		7.7e-65
3	GO:BP	GO:0006954	inflammatory response	769		2.7e-63
4	GO:BP	GO:0006952	defense response	1863		2.2e-59
5	GO:BP	GO:0002682	regulation of immune system process	1581		5.6e-58
6	GO:BP	GO:0001775	cell activation	1222		8.1e-55
7	GO:BP	GO:0048584	positive regulation of response to stimulus	2378		7.5e-54
8	GO:BP	GO:0048518	positive regulation of biological process	6732		2.0e-53
9	GO:BP	GO:0048583	regulation of response to stimulus	4056		2.7e-52
10	GO:BP	GO:0002684	positive regulation of immune system process	1145		1.8e-51

g:Profiler (biit.cs.ut.ee/gprofiler)

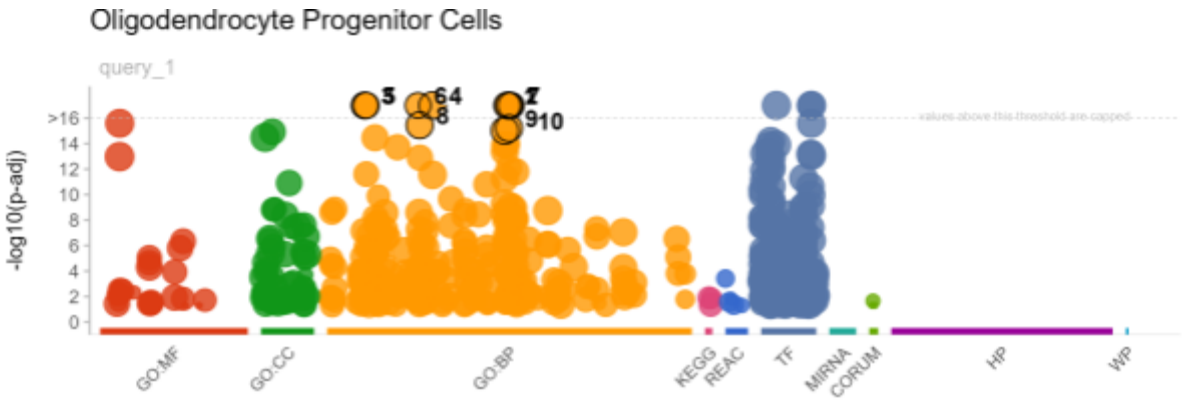
Figure S9



id	source	term_id	term_name	term_size	p_value
1	GO:BP	GO:0002181	cytoplasmic translation	157	4.4e-23
2	GO:BP	GO:0006412	translation	741	2.6e-14
3	GO:BP	GO:0043043	peptide biosynthetic process	765	5.5e-14
4	GO:BP	GO:0006518	peptide metabolic process	884	1.8e-12
5	GO:BP	GO:0043604	amide biosynthetic process	889	2.0e-12
6	GO:BP	GO:0140236	translation at presynapse	47	6.6e-12
7	GO:BP	GO:0140241	translation at synapse	48	8.3e-12
8	GO:BP	GO:0140242	translation at postsynapse	48	8.3e-12
9	GO:BP	GO:0043603	amide metabolic process	1171	1.2e-09
10	GO:BP	GO:1901566	organonitrogen compound biosynthetic process	1749	1.7e-09

g:Profiler (biit.cs.ut.ee/gprofiler)

Figure S10



id	source	term_id	term_name	term_size	p_value
1	GO:BP	GO:0048731	system development	4115	1.6e-26
2	GO:BP	GO:0048856	anatomical structure development	6228	7.3e-26
3	GO:BP	GO:0007275	multicellular organism development	4873	1.8e-25
4	GO:BP	GO:0032502	developmental process	6865	2.9e-24
5	GO:BP	GO:0007399	nervous system development	2555	4.9e-18
6	GO:BP	GO:0022008	neurogenesis	1871	7.8e-18
7	GO:BP	GO:0050793	regulation of developmental process	2702	8.3e-18
8	GO:BP	GO:0030154	cell differentiation	4715	3.6e-16
9	GO:BP	GO:0048869	cellular developmental process	4748	6.1e-16
10	GO:BP	GO:0048518	positive regulation of biological process	6732	9.3e-16

g:Profiler (biit.cs.ut.ee/gprofiler)

Figure S11