

SUPPLEMENTAL METHODS

Cell collection.

A Vaseline well was constructed around the ganglion, in which ~2.5 mg/ml protease (Sigma – P6911, St. Louis, MO) in chilled physiological saline was added to disrupt connective tissue and loosen adherent support cells during a 10-15 minute incubation. The well was then thoroughly washed with fresh physiological saline to halt further enzymatic activity and remove any loose support or connective cells. Chilled ethylene glycol was gradually added in increasing concentration until a 70% solution of chilled ethylene glycol in saline was present to the well. During the process of protease digestion, normal activity continues in the STG (1), and this activity maintains patterns of gene expression in STG neurons for at least some classes of gene products (2, 3). Furthermore, the time from the initiation of protease digestion to suspension in cold ethylene glycol (which represents a stable end point) is < 45 minutes. Normal STG output can be maintained for many days (4, 5), and steady state mRNA levels remain relatively stable for at least 72 hours as long as activity is maintained (3). When activity is disrupted, significant changes in channel mRNAs can be detected only on the order of 8 hours (2), and our preliminary data show no changes within 2 hours in cardiac motor neurons (LCs; unpublished observation). While we cannot determine whether the collection process does not alter steady state mRNA levels for all gene products, present evidence suggests that the time frame over which the neurons are harvested is not likely to substantially change the state of these neurons relative to those in intact ganglia. Once the ganglia were suspended in cold 70% ethylene glycol solution, the saline outside the well was replaced with distilled water, and the entire dish was frozen at -20°C for 30 minutes. This constitutes a freeze substitution that allows the easy hand-dissection and removal of single, intact neurons for biochemical and molecular analyses (6). Due to the large size of *C. borealis* STG neuronal somata (50-150 μM in diameter) (7), fine forceps were used to manually remove each neuron.

Statistical Analysis.

All statistical analyses were performed using R version 3.5.3 (2019-03-11) -- "Great Truth" (8). We used single cell RNA-seq data to evaluate our methods under expected and near best case scenarios. To this end, we reduced the dimensionality of the data (28,695 contigs) by selecting the 2000 most variable contig and by selecting 922 highly variable contigs selected using the M3Drop implementation of the Brennecke method (9) (i.e. M3Drop::BrenneckeGetVariableGenes() function (10)) assuming a 0.2 false discovery rate. To test performance under ideal conditions we selected those contigs differentially expressed at an alpha of 0.2 or 0.05. We centered and scaled the aforementioned datasets and their progenitors via the caret::predict() and caret::preprocess() functions (11). We also tested dimensionality reduction via PCA. We further used PCA in exploratory data analysis to determine if any of the cell types were visually separable across four subsets of the data (Seq H2K, Seq HVG, Seq DE0.2, and Seq DE0.05).

Next, we performed cluster estimation using the optClust() function of the optCluster package (12). The algorithms used on each dataset varied by whether the data were counts or continuous. Allowed k values ranged from 2-10 (i.e. cells in dataset / 4, rounding up). We selected the top three predicted k values from each algorithm for visualization of the spread of predicted ks.

To assess the performance of unsupervised machine learning methods we tested several clustering algorithms – k-means clustering, hierarchical clustering (using a variety of distance metrics, (euclidean, maximum, manhattan, canberra, binary, minkowski, correlation, uncentered) and clustering methods (ward.D, ward.D2, single, complete, average, mcquitty, median, centroid, ward.D2)), and SNN-Cliq clustering (13). We then selected high performing clustering methods based on the Jaccard index calculated against cell identity. We selected one of the best performing combinations (Ward's method with correlation as the distance metric) for visualization.

We applied several supervised machine learning methods to evaluate predictive power of expression data in ideal circumstances (i.e. prior knowledge of a given cell type's molecular identity). Specifically, we tested elastic regression, k-nearest neighbors, linear discriminant analysis, neural network, multinomial neural network, random forest, support vector machine with a radial kernel, and support vector machine with a linear kernel. For each of these models we tested a variety of tuning parameters and selected the most effective parameter set before comparison with other methods. Methods were evaluated by using cross validation (with five folds) to produce the expected accuracy on new data. The same approaches were applied to the single cell RT-qPCR data set, with a few caveats. Given its relatively smaller size, dimensionality reduction was not necessary to overcome technical or practical hurdles. Thus, we tested both the raw and centered and scaled dataset in addition to PCA transformations of the same. We also increased the maximum k allowed in cluster estimation to 32.

SUPPLEMENTAL REFERENCES

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SUPPLEMENTAL FIGURES

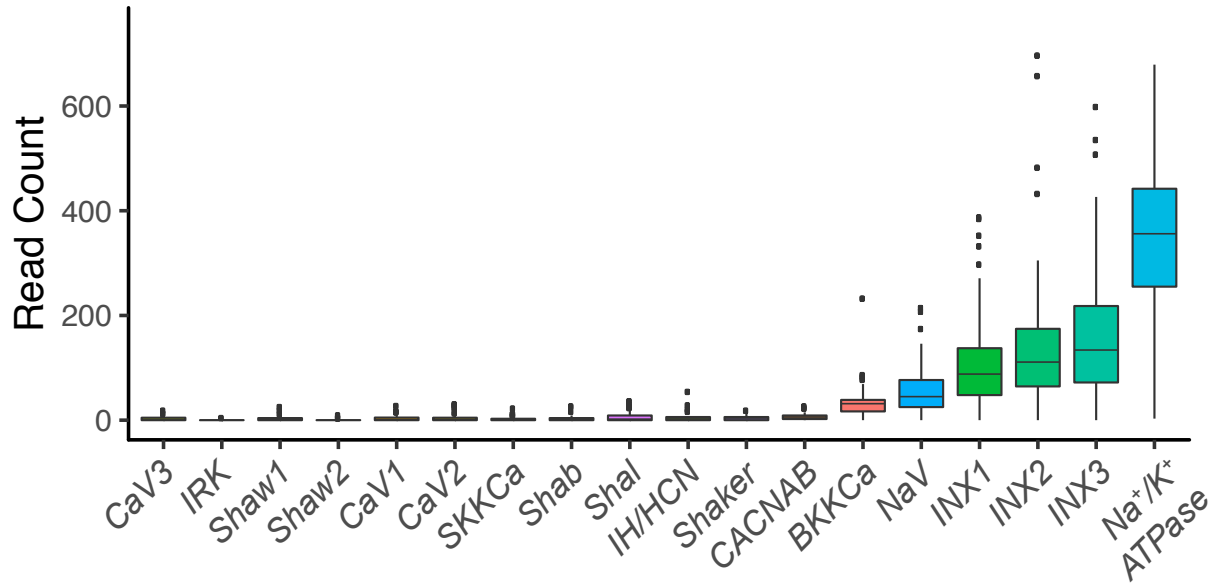


Figure S1. Count numbers for selected voltage-gated ion channels from the RNA-seq data. The median counts for each of the voltage-gated channels used in the RT-PCR analysis was generated by pooling cell type. Innexins and the Na⁺/K⁺ ATPase are used as a reference of more highly abundant gene products.

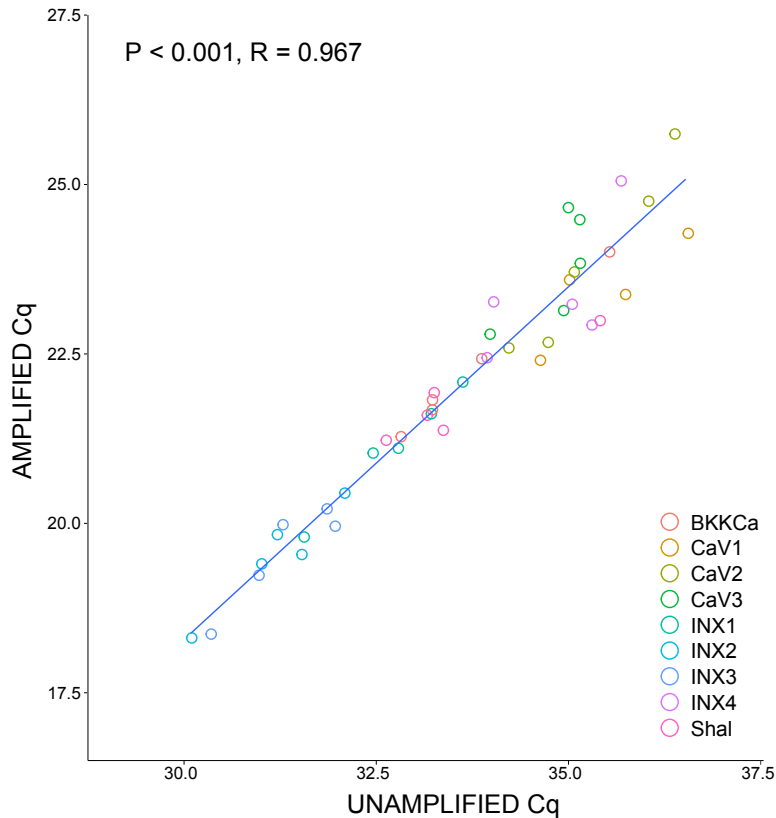


Figure S2. Comparison of expression levels of the same single cell samples (N = 5) before (Unamplified) and after (Amplified) 14 cycles of preamplification of cDNA. Each sample represents a cDNA pool from a single identified neuron, half of which was preamplified and half remained unamplified. Data are shown as quantitation cycle (Cq) values. Statistics shown report values for Pearson's Correlation test.

SUPPLEMENTAL TABLES

Table S1. Top ten contributing genes or contigs to PCs1-3 for each dataset.

| Dataset | Rank | PC1 | PC1 value | PC2 | PC2 value | PC3 | PC3 value |
|-------------------|-------------|------------|------------------|------------|------------------|------------|------------------|
| <i>qRTPCR</i> | 1 | LCCH3r | 3.54 | NaV | 6.30 | GluCl | 9.12 |
| <i>qRTPCR</i> | 2 | mGABA3 | 3.10 | Shal | 5.65 | GABAB R1 | 8.04 |
| <i>qRTPCR</i> | 3 | mGluR5 | 3.06 | NMDA 1A | 5.16 | vAChT | 7.56 |
| <i>qRTPCR</i> | 4 | CaV2 | 3.05 | KCNK1 | 4.95 | HisCL | 6.74 |
| <i>qRTPCR</i> | 5 | Shab | 3.02 | Shaker | 4.54 | ChAT | 6.57 |
| <i>qRTPCR</i> | 6 | KCNH3 | 2.97 | IH | 3.47 | IH | 6.16 |
| <i>qRTPCR</i> | 7 | DAR1A | 2.91 | KCNK2 | 3.45 | INX4 | 6.13 |
| <i>qRTPCR</i> | 8 | SKKCa | 2.87 | BKKCa | 3.10 | vGluT | 5.29 |
| <i>qRTPCR</i> | 9 | His 3r | 2.86 | Dopa 1Br | 3.06 | RDLr | 4.16 |
| <i>qRTPCR</i> | 10 | TRP A like | 2.82 | RDLr | 2.93 | CCAPr | 3.11 |
| <i>seq_h2k</i> | 1 | c1318 | 0.10 | c4636 | 0.50 | c4191 | 0.59 |
| <i>seq_h2k</i> | 2 | c724 | 0.10 | c8463 | 0.50 | c751 | 0.58 |
| <i>seq_h2k</i> | 3 | c2022 | 0.10 | c28755 | 0.50 | c8533 | 0.49 |
| <i>seq_h2k</i> | 4 | c1834 | 0.10 | c17319 | 0.50 | c953 | 0.46 |
| <i>seq_h2k</i> | 5 | c718 | 0.10 | c10220 | 0.50 | c2665 | 0.46 |
| <i>seq_h2k</i> | 6 | c2357 | 0.10 | c27163 | 0.49 | c2126 | 0.45 |
| <i>seq_h2k</i> | 7 | c196 | 0.10 | c5528 | 0.49 | c2981 | 0.45 |
| <i>seq_h2k</i> | 8 | c739 | 0.10 | c10716 | 0.49 | c3881 | 0.42 |
| <i>seq_h2k</i> | 9 | c2301 | 0.10 | c9333 | 0.49 | c23433 | 0.41 |
| <i>seq_h2k</i> | 10 | c1048 | 0.10 | c13463 | 0.49 | c1647 | 0.40 |
| <i>seq_hvg</i> | 1 | c38450 | 0.50 | c18443 | 0.63 | c29394 | 0.80 |
| <i>seq_hvg</i> | 2 | c5595 | 0.50 | c17911 | 0.63 | c23916 | 0.80 |
| <i>seq_hvg</i> | 3 | c28755 | 0.50 | c13615 | 0.63 | c39794 | 0.80 |
| <i>seq_hvg</i> | 4 | c11256 | 0.49 | c16416 | 0.63 | c24360 | 0.80 |
| <i>seq_hvg</i> | 5 | c20433 | 0.49 | c17569 | 0.63 | c18403 | 0.79 |
| <i>seq_hvg</i> | 6 | c39762 | 0.49 | c17622 | 0.63 | c7694 | 0.79 |
| <i>seq_hvg</i> | 7 | c13489 | 0.49 | c18306 | 0.63 | c11984 | 0.79 |
| <i>seq_hvg</i> | 8 | c19224 | 0.49 | c19165 | 0.63 | c16991 | 0.79 |
| <i>seq_hvg</i> | 9 | c30088 | 0.49 | c19999 | 0.63 | c18899 | 0.79 |
| <i>seq_hvg</i> | 10 | c4923 | 0.49 | c22142 | 0.63 | c25542 | 0.79 |
| <i>seq_DE0.2</i> | 1 | c5749 | 1.62 | c4517 | 3.20 | c13441 | 5.94 |
| <i>seq_DE0.2</i> | 2 | c1898 | 1.44 | c398 | 2.92 | c1058 | 4.27 |
| <i>seq_DE0.2</i> | 3 | c23967 | 1.43 | c878 | 2.92 | c31757 | 3.14 |
| <i>seq_DE0.2</i> | 4 | c5120 | 1.41 | c4945 | 2.87 | c9248 | 3.05 |
| <i>seq_DE0.2</i> | 5 | c1219 | 1.39 | c3559 | 2.69 | c2212 | 3.02 |
| <i>seq_DE0.2</i> | 6 | c8871 | 1.37 | c15559 | 2.64 | c4534 | 2.63 |
| <i>seq_DE0.2</i> | 7 | c972 | 1.35 | c8507 | 2.50 | c8114 | 2.61 |
| <i>seq_DE0.2</i> | 8 | c973 | 1.33 | c1151 | 2.15 | c10145 | 2.57 |
| <i>seq_DE0.2</i> | 9 | c12663 | 1.32 | c8323 | 2.06 | c2981 | 2.47 |
| <i>seq_DE0.2</i> | 10 | c910 | 1.30 | c1800 | 2.00 | c14660 | 2.35 |
| <i>seq_DE0.05</i> | 1 | c21272 | 4.69 | c4517 | 8.85 | c1058 | 13.16 |
| <i>seq_DE0.05</i> | 2 | c5716 | 4.21 | c16963 | 7.16 | c13441 | 11.60 |
| <i>seq_DE0.05</i> | 3 | c5120 | 4.14 | c3348 | 6.68 | c2212 | 10.64 |
| <i>seq_DE0.05</i> | 4 | c3737 | 3.77 | c1151 | 6.05 | c2586 | 7.17 |
| <i>seq_DE0.05</i> | 5 | c8114 | 3.72 | c14320 | 5.14 | c5845 | 5.83 |
| <i>seq_DE0.05</i> | 6 | c16240 | 3.59 | c5222 | 4.93 | c14320 | 4.87 |
| <i>seq_DE0.05</i> | 7 | c2796 | 3.56 | c8323 | 4.57 | c4997 | 4.10 |
| <i>seq_DE0.05</i> | 8 | c1713 | 3.43 | c5067 | 4.35 | c14660 | 4.06 |
| <i>seq_DE0.05</i> | 9 | c49 | 3.38 | c1324 | 4.12 | c3348 | 4.00 |
| <i>seq_DE0.05</i> | 10 | c3716 | 3.23 | c24846 | 4.08 | c4534 | 3.86 |

Table S2. Gene Ontology Enrichment analysis of Molecular Function for H2K RNAseq data.

| GO term: molecular function | Fold Enrichment | FDR p-value |
|--|------------------------|--------------------|
| proton-transporting ATP synthase activity, rotational mechanism (GO:0046933) | 8.08+ | 2.88E-04 |
| clathrin binding (GO:0030276) | 6.82+ | 4.51E-03 |
| ubiquitin conjugating enzyme activity (GO:0061631) | 4.6+ | 1.59E-02 |
| intramolecular oxidoreductase activity (GO:0016860) | 4.32+ | 2.22E-02 |
| structural constituent of ribosome (GO:0003735) | 4.02+ | 4.61E-11 |
| proton-transporting ATPase activity, rotational mechanism (GO:0046961) | 3.84+ | 4.35E-02 |
| structural constituent of cytoskeleton (GO:0005200) | 3.84+ | 4.30E-02 |
| unfolded protein binding (GO:0051082) | 3.8+ | 4.00E-05 |
| heat shock protein binding (GO:0031072) | 3.73+ | 5.01E-02 |
| mRNA 3'-UTR binding (GO:0003730) | 3.73+ | 4.96E-02 |
| cell adhesion molecule binding (GO:0050839) | 3.57+ | 3.89E-02 |
| translation factor activity, RNA binding (GO:0008135) | 3.27+ | 4.57E-03 |
| electron transfer activity (GO:0009055) | 3.07+ | 1.79E-02 |
| GTPase activity (GO:0003924) | 3.07+ | 6.09E-05 |
| GTP binding (GO:0005525) | 2.97+ | 6.78E-05 |
| actin binding (GO:0003779) | 2.95+ | 4.42E-04 |
| kinase binding (GO:0019900) | 2.85+ | 7.78E-03 |
| microtubule binding (GO:0008017) | 2.75+ | 3.98E-03 |
| phospholipid binding (GO:0005543) | 2.56+ | 3.16E-02 |
| calcium ion binding (GO:0005509) | 2.4+ | 1.60E-03 |
| protein-containing complex binding (GO:0044877) | 2.39+ | 7.29E-04 |
| ATP binding (GO:0005524) | 2.31+ | 1.36E-10 |
| protein serine/threonine kinase activity (GO:0004674) | 2.17+ | 1.31E-02 |
| enzyme regulator activity (GO:0030234) | 1.83+ | 1.40E-02 |
| DNA-binding transcription factor activity (GO:0003700) | 0.41- | 1.79E-02 |
| serine-type endopeptidase activity (GO:0004252) | 0.1- | 1.90E-03 |

Table S3. Gene Ontology Enrichment analysis of Biological Process for H2K RNAseq data.

| GO term: biological process | Fold Enrichment | FDR p-value |
|--|------------------------|--------------------|
| regulation of short-term neuronal synaptic plasticity (GO:0048172) | 10.96+ | 6.64E-03 |
| positive regulation of neuron remodeling (GO:1904801) | 10.63+ | 7.39E-05 |
| substrate adhesion-dependent cell spreading (GO:0034446) | 10.23+ | 2.68E-03 |
| actin filament polymerization (GO:0030041) | 9.59+ | 9.52E-03 |
| clathrin-dependent synaptic vesicle endocytosis (GO:0150007) | 8.77+ | 3.43E-02 |
| protein N-linked glycosylation via asparagine (GO:0018279) | 8.77+ | 3.42E-02 |
| gluconeogenesis (GO:0006094) | 8.53+ | 1.31E-02 |
| dorsal closure, spreading of leading edge cells (GO:0007395) | 8.53+ | 1.31E-02 |
| morphogenesis of larval imaginal disc epithelium (GO:0016335) | 7.67+ | 4.63E-02 |
| retrograde axonal transport (GO:0008090) | 7.67+ | 1.79E-02 |
| ATP synthesis coupled proton transport (GO:0015986) | 7.03+ | 1.14E-04 |
| vesicle transport along microtubule (GO:0047496) | 6.98+ | 2.40E-02 |
| axonal transport of mitochondrion (GO:0019896) | 6.98+ | 2.39E-02 |
| anterograde axonal transport (GO:0008089) | 6.98+ | 2.39E-02 |
| cellular response to metal ion (GO:0071248) | 6.39+ | 3.07E-02 |
| axonal fasciculation (GO:0007413) | 6.14+ | 3.23E-03 |
| female germ-line stem cell asymmetric division (GO:0048132) | 5.97+ | 7.80E-03 |
| regulation of reactive oxygen species metabolic process (GO:2000377) | 5.9+ | 3.80E-02 |
| actin nucleation (GO:0045010) | 5.48+ | 4.75E-02 |
| positive regulation of photoreceptor cell differentiation (GO:0046534) | 5.48+ | 4.74E-02 |
| sevenless signaling pathway (GO:0045500) | 5.42+ | 2.40E-02 |
| ovarian follicle cell stalk formation (GO:0030713) | 5.42+ | 2.39E-02 |
| synaptic vesicle priming (GO:0016082) | 5.42+ | 2.39E-02 |
| flight behavior (GO:0007629) | 5.42+ | 2.39E-02 |
| positive regulation of endocytosis (GO:0045807) | 5.42+ | 3.15E-04 |
| positive regulation of lipid localization (GO:1905954) | 5.42+ | 2.38E-02 |
| positive regulation of canonical Wnt signaling pathway (GO:0090263) | 5.39+ | 1.58E-04 |
| ribosomal large subunit assembly (GO:0000027) | 5.37+ | 1.19E-02 |
| positive regulation of smoothed signaling pathway (GO:0045880) | 5.31+ | 3.15E-03 |
| positive regulation of protein modification by small protein conjugation or removal (GO:1903322) | 5.12+ | 2.94E-02 |
| glucose homeostasis (GO:0042593) | 5.12+ | 2.40E-04 |
| lumen formation, open tracheal system (GO:0035149) | 5.12+ | 3.83E-03 |
| cellular protein complex disassembly (GO:0043624) | 5.12+ | 7.47E-03 |
| glycolytic process (GO:0006096) | 4.91+ | 8.93E-03 |
| oocyte microtubule cytoskeleton polarization (GO:0008103) | 4.85+ | 3.49E-02 |
| hemocyte migration (GO:0035099) | 4.6+ | 4.17E-02 |
| negative regulation of supramolecular fiber organization (GO:1902904) | 4.55+ | 1.26E-02 |
| maintenance of protein location in cell (GO:0032507) | 4.51+ | 4.05E-03 |
| regulation of R7 cell differentiation (GO:0045676) | 4.39+ | 4.95E-02 |
| cell adhesion mediated by integrin (GO:0033627) | 4.39+ | 4.94E-02 |
| positive regulation of neuromuscular junction development (GO:1904398) | 4.39+ | 4.76E-03 |
| terminal branching, open tracheal system (GO:0007430) | 4.39+ | 1.49E-02 |
| pole plasm oskar mRNA localization (GO:0045451) | 4.32+ | 9.07E-03 |
| positive regulation of organ growth (GO:0046622) | 4.3+ | 3.01E-02 |
| antibiotic metabolic process (GO:0016999) | 4.3+ | 3.00E-02 |
| adherens junction organization (GO:0034332) | 4.26+ | 5.62E-03 |
| epithelial cell migration, open tracheal system (GO:0007427) | 4.26+ | 5.61E-03 |
| regulation of cell-cell adhesion (GO:0022407) | 4.23+ | 1.77E-02 |
| intestinal stem cell homeostasis (GO:0036335) | 4.23+ | 1.77E-02 |
| regulation of filopodium assembly (GO:0051489) | 4.19+ | 1.06E-02 |
| heart morphogenesis (GO:0003007) | 4.15+ | 6.50E-03 |
| negative regulation of autophagy (GO:0010507) | 4.13+ | 3.47E-02 |
| reactive oxygen species metabolic process (GO:0072593) | 4.13+ | 3.45E-02 |
| plasma membrane invagination (GO:0099024) | 4.13+ | 3.45E-02 |
| ATP hydrolysis coupled proton transport (GO:0015991) | 4.04+ | 7.57E-03 |
| olfactory learning (GO:0008355) | 4.04+ | 7.54E-03 |
| positive regulation of translation (GO:0045727) | 4.04+ | 7.50E-03 |
| rhabdomere development (GO:0042052) | 4.02+ | 4.61E-03 |
| synaptic growth at neuromuscular junction (GO:0051124) | 3.95+ | 1.43E-02 |
| protein folding (GO:0006457) | 3.93+ | 6.74E-08 |
| establishment of mitotic spindle localization (GO:0040001) | 3.84+ | 4.66E-02 |
| salivary gland cell autophagic cell death (GO:0035071) | 3.84+ | 4.65E-02 |
| insulin receptor signaling pathway (GO:0008286) | 3.84+ | 4.64E-02 |

| | | |
|--|-------|----------|
| regulation of lipid storage (GO:0010883) | 3.74+ | 1.15E-02 |
| negative regulation of cytoskeleton organization (GO:0051494) | 3.72+ | 3.20E-02 |
| negative regulation of smoothened signaling pathway (GO:0045879) | 3.72+ | 3.20E-02 |
| mitochondrial ATP synthesis coupled electron transport (GO:0042775) | 3.65+ | 1.75E-04 |
| regulation of peptide secretion (GO:0002791) | 3.64+ | 2.21E-02 |
| behavioral response to ethanol (GO:0048149) | 3.63+ | 3.40E-03 |
| regulation of chemotaxis (GO:0050920) | 3.61+ | 3.63E-02 |
| response to unfolded protein (GO:0006986) | 3.61+ | 3.62E-02 |
| positive regulation of cell size (GO:0045793) | 3.57+ | 1.48E-02 |
| tight junction organization (GO:0120193) | 3.54+ | 2.50E-02 |
| apical junction assembly (GO:0043297) | 3.52+ | 1.02E-02 |
| cytosolic transport (GO:0016482) | 3.5+ | 4.39E-03 |
| cytokinetic process (GO:0032506) | 3.49+ | 1.69E-02 |
| regulation of axonogenesis (GO:0050770) | 3.47+ | 3.00E-03 |
| negative regulation of protein phosphorylation (GO:0001933) | 3.44+ | 2.05E-03 |
| positive regulation of cell morphogenesis involved in differentiation (GO:0010770) | 3.41+ | 4.69E-02 |
| establishment or maintenance of apical/basal cell polarity (GO:0035088) | 3.37+ | 3.20E-02 |
| regulation of multicellular organism growth (GO:0040014) | 3.34+ | 2.20E-02 |
| morphogenesis of follicular epithelium (GO:0016333) | 3.31+ | 1.47E-02 |
| translational initiation (GO:0006413) | 3.25+ | 1.65E-02 |
| regulation of protein stability (GO:0031647) | 3.25+ | 1.65E-02 |
| neuromuscular synaptic transmission (GO:0007274) | 3.23+ | 1.13E-02 |
| autophagy (GO:0006914) | 3.23+ | 4.15E-04 |
| imaginal disc-derived wing margin morphogenesis (GO:0008587) | 3.19+ | 1.86E-02 |
| mitotic cytokinesis (GO:0000281) | 3.16+ | 6.07E-03 |
| long-term memory (GO:0007616) | 3.15+ | 4.15E-03 |
| germ-line stem cell population maintenance (GO:0030718) | 3.15+ | 2.84E-03 |
| asymmetric neuroblast division (GO:0055059) | 3.14+ | 4.50E-02 |
| cell redox homeostasis (GO:0045454) | 3.13+ | 3.09E-02 |
| response to growth factor (GO:0070848) | 3.13+ | 3.08E-02 |
| synaptic target recognition (GO:0008039) | 3.13+ | 3.07E-02 |
| amino acid transport (GO:0006865) | 3.01+ | 3.76E-02 |
| axon guidance (GO:0007411) | 2.94+ | 1.77E-08 |
| positive regulation of locomotion (GO:0040017) | 2.63+ | 4.08E-02 |
| negative regulation of neurogenesis (GO:0050768) | 2.63+ | 3.09E-02 |

Table S4. Gene Ontology Enrichment analysis of Molecular Function for HVG RNAseq data.

| GO term: molecular function | Fold Enrichment | FDR p-value |
|------------------------------------|------------------------|--------------------|
| ATP binding (GO:0005524) | 3.1+ | 1.72E-03 |
| transferase activity (GO:0016740) | 2+ | 2.38E-02 |

Table S5. Gene Ontology Enrichment analysis of Biological Process for HVG RNAseq data.

| GO term: molecular function | Fold Enrichment | FDR p-value |
|--|------------------------|--------------------|
| regulation of protein localization to plasma membrane (GO:1903076) | 35.33+ | 4.79E-02 |
| chromatin silencing (GO:0006342) | 7.69+ | 3.12E-02 |
| nucleic acid metabolic process (GO:0090304) | 2.17+ | 3.74E-02 |
| macromolecule modification (GO:0043412) | 2.08+ | 3.91E-02 |
| cellular macromolecule metabolic process (GO:0044260) | 1.84+ | 2.22E-02 |

Table S6. Gene Ontology Enrichment analysis of Molecular Function for DE0.2 RNAseq data.

| GO term: molecular function | Fold Enrichment | raw p-value |
|--|------------------------|--------------------|
| choline:sodium symporter activity (GO:0005307) | > 100+ | 7.51E-03 |
| acetylcholine transmembrane transporter activity (GO:0005277) | > 100+ | 7.51E-03 |
| dihydroorotase activity (GO:0004151) | > 100+ | 7.51E-03 |
| choline O-acetyltransferase activity (GO:0004102) | > 100+ | 7.51E-03 |
| carboxyl- or carbamoyltransferase activity (GO:0016743) | > 100+ | 7.51E-03 |
| carbamoyl-phosphate synthase (glutamine-hydrolyzing) activity (GO:0004088) | > 100+ | 7.51E-03 |
| aspartate carbamoyltransferase activity (GO:0004070) | > 100+ | 7.51E-03 |
| very-long-chain-acyl-CoA dehydrogenase activity (GO:0017099) | > 100+ | 7.51E-03 |
| latrotoxin receptor activity (GO:0016524) | > 100+ | 7.51E-03 |
| glutamine binding (GO:0070406) | > 100+ | 7.51E-03 |
| L-iduronidase activity (GO:0003940) | > 100+ | 7.51E-03 |
| choline binding (GO:0033265) | > 100+ | 7.51E-03 |
| myosin II light chain binding (GO:0032033) | > 100+ | 1.12E-02 |
| kinetochore binding (GO:0043515) | > 100+ | 1.12E-02 |
| GABA-gated chloride ion channel activity (GO:0022851) | 88.25+ | 1.50E-02 |
| receptor antagonist activity (GO:0048019) | 88.25+ | 1.50E-02 |
| GABA-A receptor activity (GO:0004890) | 66.19+ | 1.87E-02 |
| smoothened binding (GO:0005119) | 66.19+ | 1.87E-02 |
| patched binding (GO:0005113) | 66.19+ | 1.87E-02 |
| histone demethylase activity (H3-K4 specific) (GO:0032453) | 66.19+ | 1.87E-02 |
| kinesin binding (GO:0019894) | 58.83+ | 7.47E-04 |
| histone demethylase activity (H3-K36 specific) (GO:0051864) | 52.95+ | 2.24E-02 |
| morphogen activity (GO:0016015) | 52.95+ | 2.24E-02 |
| MAP-kinase scaffold activity (GO:0005078) | 52.95+ | 2.24E-02 |
| extracellular matrix binding (GO:0050840) | 52.95+ | 2.24E-02 |
| axon guidance receptor activity (GO:0008046) | 44.12+ | 2.61E-02 |
| protein kinase C binding (GO:0005080) | 44.12+ | 2.61E-02 |
| RNA polymerase II activity (GO:0001055) | 29.42+ | 3.70E-02 |
| epidermal growth factor receptor binding (GO:0005154) | 29.42+ | 3.70E-02 |
| phosphatidylserine binding (GO:0001786) | 29.42+ | 3.70E-02 |
| cell-cell adhesion mediator activity (GO:0098632) | 24.07+ | 4.42E-02 |
| fatty-acyl-CoA binding (GO:0000062) | 24.07+ | 4.42E-02 |
| microtubule plus-end binding (GO:0051010) | 22.06+ | 4.78E-02 |
| calcium-dependent phospholipid binding (GO:0005544) | 22.06+ | 4.78E-02 |
| amino acid transmembrane transporter activity (GO:0015171) | 9.46+ | 2.01E-02 |
| flavin adenine dinucleotide binding (GO:0050660) | 7.79+ | 2.85E-02 |
| GTP binding (GO:0005525) | 5.12+ | 2.15E-02 |

Table S7. Target primer and probe sequences for qRT-PCR Multiplex assays. Each box represents a group of four to five genes that were combined into a single multiplex reaction.

| <i>Gene</i> | <i>Accession #</i> | <i>Forward / Reverse Primer 5'-3' sequence</i> | <i>Probe 5'-3' sequence</i> |
|--------------------|--------------------|--|-------------------------------|
| <i>HTR1A</i> | KU710381.1 | AACCGCTGTGGTAGTTTCCA / TGCTCGTTAACCCGGACTAAG | AGCGCCTTTATTGGCTGGAAGGA |
| <i>HTR2</i> | KU710380.1 | TCCGCCTCCATCAAGTTTGG / GCACGTTGGCGATGAAGAAC | TCATCGAAGAGACACGGGAGGACC |
| <i>HTR7</i> | KU710379.1 | ACGGCGATGGCTCCATCTGG / CGGTGAGCGGGATGAGAAG | TGAGGTTGCAACAACCTCTGGTACC |
| <i>DAR2</i> | KU710378.1 | GAAGCCGAAAGTGAGTGATACA / TCCGAATAAGCCGTGTTTC | TGTGATCGAGAATGTACACAGACGA |
| <i>DAR1A</i> | KU710377.1 | GGCGCCTGTCCATTCACT / CGCGGTAGATGCGGAAGTAAG | ACCTTGTGTCTCTCTACTACTGACCTTCT |
| <i>GABAB-R1</i> | KU986868.1 | TCGTCTCGTTTGCATCATC / GGTGCCGAACCTCAATGATC | TCTGTCTCTCTCTCCATGGCT |
| <i>LCCH3r</i> | KU986871.1 | TGACGGCTCCATCACTATGG / TTGGGTGTCGAGTGGATAGTAG | TTCACTACTAGCTTGGCTGCAT |
| <i>RDLr</i> | KU986872.1 | TGGTGTGGCTCGCTTCTAG / TCCGCTGTCTGCTAACTTC | AATACGCCCGGTGGGTACAT |
| <i>GluCl</i> | KX059698.1 | ACGGAGGATCTGGTGTCTTG / ACCCGTGTGGTCTTGCTGT | TACAGGTGACCAAGAACCCTCACC |
| <i>NALCN</i> | KU681457.1 | TCCGCTTCCACGGGTACATTC / GCGGTGCCTTTGTCTCAG | TCTCGTCTCTCTGGCTGCATGA |
| <i>CACNAB</i> | GEFB01006512 | GCAGCTGGCCAAAGACTTCTTT / AGACGCTGCAATACCTTAGGA | AGCGCCATCCTCGTGTACTTAAG |
| <i>IRK</i> | KU681451.1 | TACAGTGGCGTTGGACTCTAC / TCCACCACACCAAGGCAAATAG | TCGTGTTCGCTATGTCACTTATCAGC |
| <i>IH</i> | DQ103257.3 | TCGGTGCCACTAGACTACATC / GACCCGGTGGAGAATCTG | TCCTCATCTTCAACAGGACTTCAGC |
| <i>SKKCa</i> | KU710383.1 | GCATCGGAGCATTGAACAGAA / GCCCGACAGATAGTCATCAG | CAACTTCAACACTCCGGTTTGTCTCAA |
| <i>INX1</i> | JQ994479.1 | TGGAGCGTCATGATGCATTC / GAGCAGGATGCAAGGATCAC | TGCTGCTCTCAACATTTAAACGAA |
| <i>INX2</i> | JQ994480.1 | GGCTGTGGTGTCTGGTATG / GCGGAGAGCGTTCCTTAACAG | CTGCTGTACCGCCTGCCACTTT |
| <i>INX3</i> | JQ994481.1 | TGTCGGCCCTAGTGAAGAG / GGTACCGTGGGATGAGAACA | TGACGAGATGTGTACCACGGCTTAC |
| <i>INX4</i> | KJ642222.1 | CTGGCGTTACGCTCATTTG / CAGTCTCTGGGATCTCCTTAG | CACGCGTCAGTATGTCGGGAACC |
| <i>INX5</i> | KJ817410.1 | TGCCTTCCCTGCTGGATAA / GCGTCAACCATTGGTAGTAAC | AGGTGGCTATCCAGGCATCGGT |
| <i>Shaw1</i> | KU681456.1 | CGCGTCACTCTCAGGACTT / CCCAGCACAGGAAGAACAC | TGATACAGACTTCCGTGCATCCG |
| <i>Shaw2</i> | KU681455.1 | GAACGCCATCAAGCACTATC / ATGGCGCCGACAGCTTAG | TGGCTGAAGGACCGGCTCACA |
| <i>NaV</i> | EF089568.2 | TCAACGGGAGGTACCATAAGTG / TCGCTGTCAACCAAGAGTAG | CGGAGGGATGAAGCTCAACGCA |
| <i>Shaker</i> | FJ263946.1 | GAGGCTCAGAAGACCAGTCAAC / TGCGATATCAACCGAGCTCAT | CACCTGATGTCTCGCGGAGGAGAT |
| <i>Shab</i> | DQ103255.1 | GAGCCGGACAGACAGGAAC / TGGCCCTCTTCTGTAGTC | AAGAACCACCAACACCATGGGTC |
| <i>CaV1</i> | JN809809.1 | CCAGGCCTTCTACTGGCTCATT / GCTGGCGATAGTGTCACTG | TGTGCTCGTCTCTCAACACGG |
| <i>CaV2</i> | JN809808.1 | ATCCGGCGGACAGTAAAG / GTTCGGCAGCAACAACAAC | TGTTTCTACTGGTTCATCATATCTTGT |
| <i>CaV3</i> | JN809810.1 | TGGCTGCCACCGATACTTC / CAGCACAATGCCACAACCTG | CAGGACAGAGATGGAACAGTTGGA |
| <i>Shal</i> | DQ103254.1 | GACACCCTTCACTCCATTTC / GAACCATGTGCGCGTATCCTA | CGGGCTTTTGGTACACCTTGTAC |
| <i>BKKCa</i> | DQ103256.4 | GCTCAAACTCGGTTTCATTG / CTGGCTGTCTGGAGAAGTTT | AGAATCCCGGCGCTAAACATGACT |
| <i>mGluR1</i> | KU986879.1 | GCATCGTGTGGCTCATCTTTG / GGCCAACGTGACCACCTAAT | ACCTGTACGCGGGAGTCACTGATG |
| <i>mGluR2</i> | KU986880.1 | TCCCGAGGTGAGTTTCTTC / CCATGGCTTTCATTTGGTAATGG | CCACGTGCGCTGAACTGAGTAACA |
| <i>mGluR4</i> | KU986882.1 | GCGCGTTGATTCCGGTACT / CCATCATCGTCTCAACTTC | AAGTCTTCCCGTGGACTACGAAAC |
| <i>mGluR5</i> | KU986883.1 | GCCTGTCTTGGCATGATC / TGCGCATGTGATCTTCTTG | CGCTGTCAACAAGACCAACCCG |
| <i>mGluR7</i> | KU986884.1 | ACCGCGCTCGAGATTGTC / TGGCTGGCGTTTCCATATC | TCTGGACTGGTGGCTGTACGCT |
| <i>mACHrA</i> | KX021822.1 | GGTGTGATGCCTTTGTTTAC / GCCAGCCAGGTGTCACATAT | TGTACACGCTGATGGGATACTGGC |
| <i>mACHrB</i> | KX021821.1 | GCGGACCATCTCCATCTATTC / TGGTGCATGACAGAAAGCT | CCTTTGTGGCCTGTTGGACGCC |
| <i>mGABA2</i> | KU986869.1 | TGCTTACAAAGGTCTGCTAATGG / TGTTTCAAGGCGGGTATGTT | TGTTGTTTCTCGGCTGGGAAA |
| <i>mGABA3</i> | KU986870.1 | CGATCCATGACGAGACACAT / TCCACCTGTGGCTGATAGAC | CCTCACCTTAGAAGTGCAGCCGA |
| <i>mGluR3</i> | KU986881.1 | CACCGTGTATGCCGTCAAGAC / CCCGGTCCGAAGTAGATG | CCCCGAGAATTTCAACGAGAGCAAGT |
| <i>TRP-M3</i> | KX037433.1 | CCGCACCATCTACGAGAAC / TGCTGGCTGGAAAGATGT | TGCTCAAGTCTCTCGTCTTCAAC |
| <i>TRP-A-like</i> | KX037434.1 | TCGCGACCTTCCATAAATTC / CGGTACTCTGAGTCTCAACAC | CACGGTCTTCTTACTCTTCTCATCCG |
| <i>TRP-AI</i> | KX037435.1 | CTGCCAAGTACGGTCTGTAAAC / CCTCTGCATCTGCATCTGTA | ACGTCAGTCTGGGAGTCTCTGAA |
| <i>TRP-M1</i> | KX037436.1 | GAGGGCGGACCTCAAACTATC / TGTCGGCTGCTCTTCTGTT | CGTCAGGTGTGGAGTATGTCACTG |
| <i>TRP-M-like</i> | KX037437.1 | GACGGGACGACAGTCTCTTC / AGTGTCTGGGCTGTAGGTT | ACGGTATACGGTGTGCTATTTCCCA |
| <i>Dopa-1Br</i> | KU710376.1 | CGAAGATTGGCAACCTTCTC / CAGAGGGCAAAGCTCATCAC | TGCTCTGGTCTACTGCTACCTTCT |
| <i>5HTR-1Br</i> | KU710382.1 | TGACGCGAGTGGACTACATTC / GGAACGACACCACCAGATC | ACCGATCGCCGCTGCGATC |
| <i>His-1r</i> | KU716100.1 | TGCTGCCAGAGTAACTTATG / GACAGGTGGGAGGATTCTG | CATCACTTCTCACTGTCAACCCAAAC |
| <i>His-2r</i> | KU716101.1 | CCGCCACAGTCTCAAGTAACT / GCGTAGGTCGGGAACTCTCATC | ACGGTACTTCTTCCAGCTCACA |
| <i>His-3r</i> | KU716102.1 | ATCCGCGCAACAAAGCAT / GAGAGCGAAGGAGGTTGGAA | TGATGGTGGATCGAGTCTCAAGATATGAT |
| <i>kainate-1A</i> | KX016772.1 | CAGGTCCGAGTGCAGTAAAGAC / GCCACCAGTCCAGGATGTAGAAG | CGATGACCACCCAGACGAGTGC |
| <i>kainate-1B</i> | KX016773.1 | TGAGCAGAACGAGATCGAGTATG / CGCCACATGTTCTGATACGCT | AGGGCGGGTCCATGGCCCTT |
| <i>kainate-2A</i> | KX016774.1 | CGCATGGAGTCACTATTGAGA / AGGCCAAAGTGGTGCCAGTTG | TGAGGACCTTGTAAACCAGGACAA |
| <i>kainate-2B</i> | KX016775.1 | GCACGGCAAGTTTGACAAGAA / GTCTCCCTCTGTAAGTATG | AACGGCATGATTGGGCGCTGTT |
| <i>kainate-2C</i> | KX016776.1 | GGCTTGGTCAAGGAACTCAAG / GCTCTCCCTCCGCTAGTTG | TGATCTAGCGGTGGTCTATGACTA |
| <i>NMDA-1A</i> | KX016782.1 | GCCGTCAAATCAGGGAGGTT / ACCGGGGTTCACCAGTTCAC | AGGCGTTCATCTGGGACAGTTCACGT |
| <i>NMDA-1B</i> | KX016783.1 | ACAGCCAAGACGAAGAAGAC / CCGCTGTTCAAGATGACAGA | TGAGTTCATGGCCATCTCGGAGTC |
| <i>NMDA-2A</i> | KX016785.1 | TCGGGTTCTGTTCCCTTAC / TGATGCCGTCCTGTAGAGAAG | TGAGACCTCTTGGCAAGCACC |
| <i>NMDA-2B</i> | KX016786.1 | GCAAGGGTCAACCATCAGACA / CGCTGTGAGCATGATGTAGGTA | TGGAGAAACAACCTGAGGCCAATGGA |
| <i>NMDA-2-like</i> | KX016784.1 | GCGTTGGAGCATCTCATGTC / GCCACATACTGACGGGAAGTAC | CACGTTCTGCTGTTGGGTTGTTG |
| <i>KCNK2</i> | KU681437.1 | GACGCCTTCTACTACTGCTTATC / GAGGGCGTCTCTTCTGTAG | CCCTCACTACATTGGCTTCGGG |
| <i>KCNK1</i> | KU681438.1 | TGGCGAACGACTCAACAAAG / GTCCTGGCACTAAGGATCTTC | CTCTCCATCCGATCAGGCCAA |
| <i>KCNQ1</i> | KU681453.1 | GAGCCTCTTGGGAAACCTATC / CCGCTCCAGGAAGTTGTAGAC | CTCTCGAGGGACGTCGGCTAC |
| <i>KCNQ2</i> | KU681452.1 | GCTGCCATGTTGATCCAGTG / CCACGTTGCTGTAGAGTTGAAG | TGTGGCGTGTGATGCTGAGATAA |
| <i>KCNH2</i> | KU681459.1 | CACCGGAGATCCTTTCAC / CCTGATGTGGAGGCTGAGTAG | CATCTTCGAGACAGCTCGCAGG |
| <i>KCNH3</i> | KU681460.1 | GAGGCGACGTACTTACCTCTATG / AGTGGCGTACATGCAAGGATTC | ACTTCACTCAAGAGGCTCGCTAGA |
| <i>KCNH1</i> | KU681458.1 | GGTCACGTCACCACCATCATC / CCGCACGTTGTTGAGCATTC | ATGACCTCCGCGACCACCAAGT |
| <i>KCNT1</i> | KU681454.1 | CGTCCAGACCATGTTCAAGTTC / AAGCGCATGTTGGACGACTG | TCCCAACATCAAAATCATCACGGA |
| <i>CCAPr</i> | KM349850.1 | GCCCTTCTCTTCCAAATCAC / GTCGGTGAGAACGCTGATGAG | CCAGGACCAACTTCTCATCATGCATCT |
| <i>vGluT</i> | MK958905 | GCGTTCGTGGACCTTCTAC / TCAGCAACCTGTAAATGGAA | ATCACAGCCAACCTACTTCAGCGAG |
| <i>ChAT</i> | MK958903 | GGACCGCTGGCTAAGTAC / TCGCGGAGTCCATAAGG | AGGCGGCTCGACTCAAGTTCAGAC |
| <i>vAChT</i> | MK958904 | GCGTCAGCTGCTTCTCTCT / CAGCAGTGCCTGTCTATGAG | TTGCCAGCAACTCAAGGGTGT |
| <i>ACHE</i> | MK958902 | GGGCAACATGGGCATGTAC / GGTCAACCACCAAGAATTCATG | AGGCGTGGCCATCAAGTGGATAC |