Cell

Supplemental Information

Ig Superfamily Ligand and Receptor Pairs Expressed in Synaptic Partners in *Drosophila*

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Supplemental Experimental Procedures

Fly Stocks

The following stocks were used:

Genetic labeling of specific cell types for FACS: For experiments involving the isolation of R7 and R8 neurons, GMR-myr-tdtom, a general marker for retinal cells, was utilized in combination with cell-specific GFP markers for R7 and R8 neurons. The sens-F2Bshort-deltaE1-GFP transgene (Pepple et al., 2008) was used to specifically label R8 neurons in the retina. This marker also labels a subset of lamina neurons. To label R7 neurons we generated split GAL4 constructs as previous described (Luan et al., 2012; Pfeiffer et al., 2008, 2010). The R44F08 fragment was used to drive expression of GAL4DBD (Jenett et al., 2012), and the 465bp *pros* core-enhancer (Hayashi et al., 2008) was used to drive the expression of p65AD. Reconstituted GAL4 activity activated the expression of UAS-GFP-tagged RpL10 (McEwen, Zhang and Zipursky, unpublished). To isolate different lamina neurons, L1-L5 neurons were labeled using the pan-lamina driver R27G05-nlsLexAGADfl in su(Hw)attP2 (Janelia Research Campus; GAL4 pattern described in Riddiford et al., 2010) and LexAop-myr::tdTom, and particular subtypes were simultaneously labeled with cell-specific GFP reporters. L3, L4 and L5 neurons were labeled using 9-9-GAL4, *apterous*-GAL4, and 6-60-GAL4 (Nern et al., 2008) to drive expression of UAS-mCD8-GFP, respectively. For isolation of L3 neurons the nuclear marker UAS-H2AGFP (gifts from Barret Pfeiffer and Gerald Rubin (Janelia Research Campus)) was used in combination with UAS-mCD8-GFP. Svp-GAL4 (Kyoto: 103727) and Bab1-GAL4 (BDSC stock #47736. P{GMR73D08-GAL4}attP2) were used to label L1 and L2 neurons, respectively, through expression of UAS-mCD8-GFP.

Expression of Dpr, Beat, and Side proteins using MiMIC-derivatives: Cellular expression patterns were assessed in co-labeling experiments using MiMIC protein trap derivatives (GFP) and antibodies against nuclear proteins expressed specifically in R7, R8 or particular lamina neuron subtypes (L1-L5). The following MiMIC protein trap derivatives were used: MI03102 (*beatIIb*), MI03726 (*beatIIIc*), MI01052 (*CG34114*, *side* family), MI02201 (*dpr1*), MI02530 (*dpr2*), MI05963 (*dpr3*), MI01358 (*dpr6*), MI03557 (*dpr10*), MI01695 (*dpr12*), MI05577 (*dpr13*), MI01408 (*dpr15*), and MI08707 (*dpr17*). GFP expression from the original MiMIC line MI02231 was used to visualize the expression pattern for *dpr11*. The following antibodies were used to assess expression in specific cell types (see below for concentrations): anti-prospero (R7), anti-Senseless (R8), anti-Seven-up (L1), anti-Bab2 (L2), anti-erm (L3), anti-Bsh (L4, L5), anti-apterous (L4), anti-Pdm3 (L5).

Cellular expression of DIPs using MiMIC-derivatives: The following MiMIC protein trap derivatives (GFP) were used: MI02031 (DIP-α, CG32791), MI08287 (DIP-δ, CG34391), MI07948 (DIP-η, CG14010) and MI03191 (DIP-θ, CG31646). To visualize expression of DIPs β and γ MiMIC GAL4 derivatives of MI01971 (DIP-β, CG42343) and MI03222 (DIP-γ, CG14521) were used to drive expression of UAS-myr-GFP. GFP expression from the original MiMIC line MI03222 was also used to assess expression of DIP-γ. In these experiments Dm3 neurons were labeled using R25F07-LexAp65 (Janelia Research Campus) and LexAop-myr::tdTom. The expression of DIPs α, β, δ and θ in subsets of medulla neurons was assessed in co-labeling experiments using MiMIC protein trap lines (see above) and the following cell-type specific GAL4 drivers: Mi1 (R19F01), Mi4 (R72E01), Dm1 (R22D12), Dm4 (R23G11), Dm6 (R38H06), Dm12 (R47G08), Dm13 (R38A07), Dm14 (R47E05), Dm17 (VT43152), Dm18 (VT028450), Dm19 (VT024602), Tm1 (R74G01), Tm2 (R71F05), Tm3 (R59C10), Tm4

(R53C02), Tm9 (R24C08), and Tm20 (R33H10). All of these driver lines are from the Janelia Research Campus (R) (Jenett et al., 2012) and Vienna Drosophila Resource Center (VT) (Kvon et al., 2014) collections. Lines for individual cell types were identified and characterized as described for Dm neuron markers in (Nern et al., 2015). Each GAL4 line was crossed to pJFRC21-10XUAS-IVS-mCD8::RFP in attP18 (Pfeiffer et al., 2010) to label the cell bodies and processes of a specific medulla neuron type.

Bioinformatics

After the step of quality control, we first filtered out raw reads with low quality and containing sequencing adapters and then mapped reads (pair-end, 50bp in length) to the D. melanogaster reference genome (release FB2013_01) with the gapped aligner Tophat (Trapnell et al., 2009) with the default setting. The fly gene model was downloaded from the Ensembl database (version of Drosophila_melanogaster.BDGP5.73.gtf) and supplied to Tophat as the reference genome annotation. Only reads uniquely aligned were collected. In total for all libraries sequenced, 1,982,734,187 reads were uniquely mapped (corresponding to an overall mappability of 57%) and used for further analysis. The expression levels of genes were quantified using RPKM units (Reads Per Kilobase of exon per Million reads mapped) using customized scripts written in Perl after normalization based on the geometric means as described in DESeq (Anders and Huber, 2010). The accession number for the raw data reported in this paper is GEO: GSE68235.

Differential expression analysis was performed using the packages, DESeq (Anders and Huber, 2010) and edgeR (Robinson et al., 2010) in R (http://www.R-project.org). The original p-values

were corrected by the false discovery rate (FDR) for multiple testing errors. In addition to the FDR of <0.05, we considered differentially expressed genes with >5X differences. Thus, in summary, we considered genes as differentially expressed if: 1. the adjusted p-value was less than 0.05; 2. the expression ratio between two samples was >5X; 3. the maximal RPKM value for at least one group in the comparison was >5; and 4. there was agreement between DESeq and edgeR.

We performed a principal component analysis (PCA) on normalized read counts of all samples to compare gene expression under different developmental stages using the 'prcomp' function in R. In this analysis, we selected 500 top-ranked genes based on their variations across all samples using the function 'rowVars' in R matrixStats package. PCA revealed that our samples were clearly distinguishable by both the first and second principal component (PC1, 45.1% of the total variation; PC2, 28.7% of the total variation).

We performed weighted gene co-expression network analysis (WGCNA) (Langfelder and Horvath, 2008) to identify neuron-cell type specific modules. This identified co-expression modules by clustering transcripts that exhibit similar expression patterns as revealed through the analysis of all samples. To further understand the cell type specificity of the modules, we correlated the identified module eigengenes with traits/cell types represented as the theoretical expression patterns for all cell types in a binary fashion.

To identify cell surface and secreted membrane molecules (CSMs), we used a gene list established by Kai Zinn and co-workers (Kurusu et al., 2008). In brief, to define genes encoding

CSMs that might be relevant to cell recognition during neural development, the fly proteome was searched with sequences of every domain in the "extracellular" portion of the SMART domain database (http://smart.embl-heidelberg.de/browse.shtml). A total of more than 80 domain types had representatives in *Drosophila*. Several hundred proteins from this list were excluded. These included members of large groups containing proteins with almost identical structures, including small chitin-binding proteins, single-domain serine proteases, single-domain C-type lectins, protease inhibitors, and others thought to be unlikely to play important roles in cell-type specific recognition. In addition, the list did not include ion channels, pumps, transporters, secreted enzymes, and a variety of other classes of CSMs in the database. The final CSM cell-recognition database contains 976 proteins.

Immunohistochemistry

Pupal brains were dissected in PBS (137mM NaCl, 2.7mM KCl, 10mM Na_2HPO_4, 1.8mM KH_2PO_4) and fixed in PBL (4% paraformaldehide, 75mM lysine, and 37mM sodium phosphate buffer, pH 7.4) for 25 min at room temperature (RT). After several rinses with PBT (PBS 0.5% Triton-X10) at RT, samples were incubated in PBT containing 10% normal goat serum (blocking solution) for at least 1hr at RT. Brains were incubated overnight at 4^{o}C in primary and secondary antibodies for at least one day each with multiple blocking solution rinses at RT in between and afterwards. Incubations were extended for up to 5 days for some antibody combinations to increase the quality of the signal. Brains were mounted in EverBrite mounting medium (Biotium).

The following primary antibodies were used in this study: chicken-anti-GFP (1:1000, Abcam ab13970); rabbit-anti-DsRed (1:200, Clontech 632496); mouse-anti-Seven-up (Kanai et al.,

2005) (1:20, a gift from Yasushi Hiromi); rat-anti-Bab2 (1:500, a gift from Frank Laski); rabbit-anti-Erm (Janssens et al., 2014)(1:50, a gift from Cheng-yu Lee); rabbit-anti-Ap (1:5000, a gift from Claude Desplan); guinea pig-anti-Pdm3 (1:500, a gift from John Carlson); guinea pig-anti-Bsh (1:200, generated in the Zipursky lab); mouse-anti-Pros (1:20, MR1A from DSHB); guinea pig-anti-Sens (1:1000, a gift from Hugo Bellen); mouse-anti-24B10 (Zipursky et al., 1984)(1:20, DSHB), rat-anti-Elav (1:500, 7E810 from DSHB).

Secondary antibodies against chicken, rabbit, mouse, guinea pig and rat conjugated to Alexa Fluor -488, -555, -568, -647 or Cy5 with the following references and concentrations were used. From Life Technologies: A11039 (1:1000); A31572 (1:800); A11031 (1:500); A11011 (1:500); A11075 (1:500); A21235 (1:500) and A21450 (1:500). From Jackson ImmunoResearch: 112-175-143 (1:500) and 712-607-003 (1:200).

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