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Single-cell transcriptional responses to cocaine exposure in the Drosophila brain

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Abstract

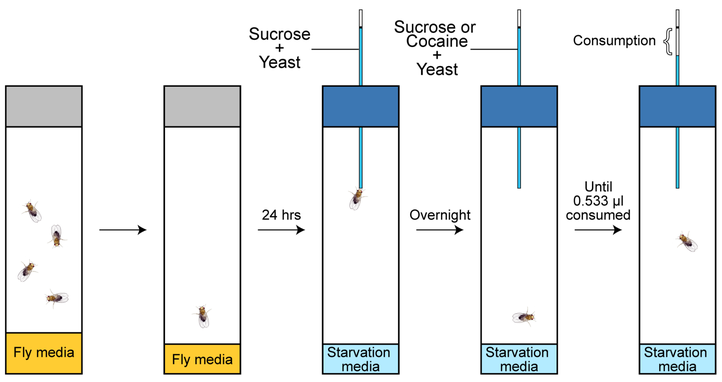
Introduction: Previous studies identified polymorphisms in candidate genes associated with variation in consumption of cocaine among lines of the Drosophila Genetic Reference Panel, and RNAi-mediated targeted gene disruption implicated dopaminergic projections to the mushroom bodies. To identify specific cell populations that respond to acute cocaine exposure, we analyzed single cell transcriptional responses in duplicate samples of flies that consumed fixed amounts of sucrose or sucrose supplemented with cocaine, sexes separately.

Methods: After exposure, 20 brains for each sample were dissected, pooled and dissociated. Cells were separated and lysed, and cDNA was synthesized using Chromium 10x microfluidics followed by sequencing on an Illumina Novaseq with an S1 chip.

Results: Integration of all eight samples distributed across sexes, conditions and replicates resulted in a dataset of 86,224 cells. Unsupervised clustering of this population yielded 36 distinct clusters. Annotation of clusters based on their gene markers revealed that all major cell types (neuronal and glial) as well as neurotransmitter types from most brain regions were represented (including the optic lobe and the mushroom body). Differential expression analysis within individual clusters indicated cluster-specific responses to cocaine. Specifically, clusters corresponding to glia, T1 and T4/T5 neurons of the optic lobe, Kenyon cells and photoreceptor cells showed dramatic transcriptional responses following cocaine exposure. Some clusters also showed significantly divergent responses across the sexes. Additionally, transcriptional responses to cocaine in most clusters were considerably more pronounced in male than in female brains.

Conclusion: Cocaine exposure elicits sexually dimorphic transcriptional responses in both glia and neurons in multiple compartments of the Drosophila brain.

Experimental design for acute exposure to cocaine

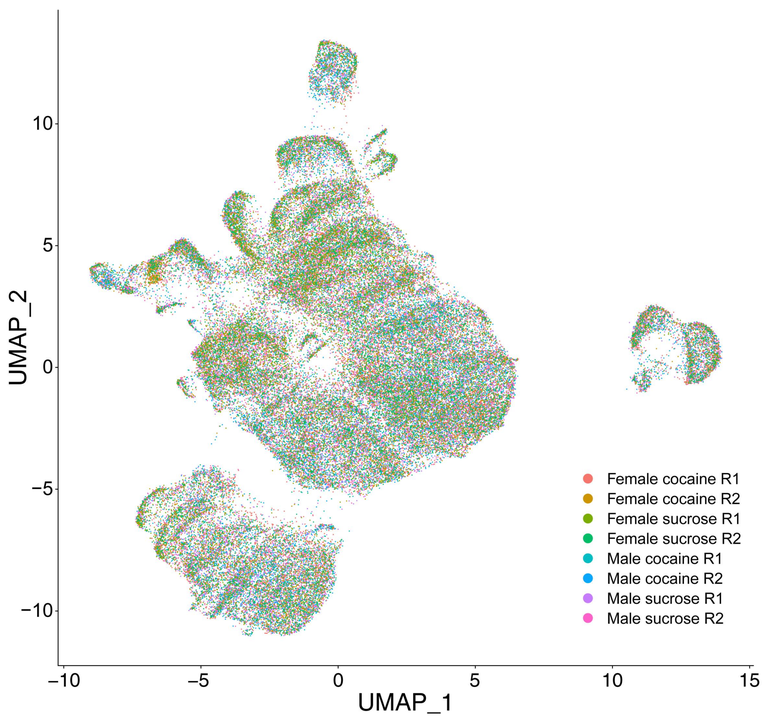


Results from sequencing and alignment to *D. melanogaster* reference genome (Release 6.22)

Sequencing Sample ID	Number of Reads	Estimated Number of Cells	Mean Reads per Cell	Median Genes per Cell	Sequencing Saturation	Fraction Reads in Cells	Total Genes Detected	Median UMI Counts per Cell
FS_1	87,765,954	9,072	9,674	772	41.60%	71.50%	11,958	1,347
FS_2	72,264,746	11,693	6,180	807	29.70%	80.90%	12,049	1,476
MS_1	123,911,975	13,193	9,392	769	29.20%	79.50%	12,523	1,378
MS_2	114,946,639	11,033	10,418	799	31.10%	72.00%	12,354	1,440
FC_1	120,400,905	13,072	9,211	733	22.40%	69.70%	12,203	1,304
FC_2	142,640,818	9,367	15,228	1,080	52.80%	83.30%	12,618	2,286
MC_1	78,416,962	10,437	7,513	680	22.00%	68.40%	11,631	1,190
MC_2	162,182,108	27,935	5,806	747	21.70%	82.90%	12,856	1,417

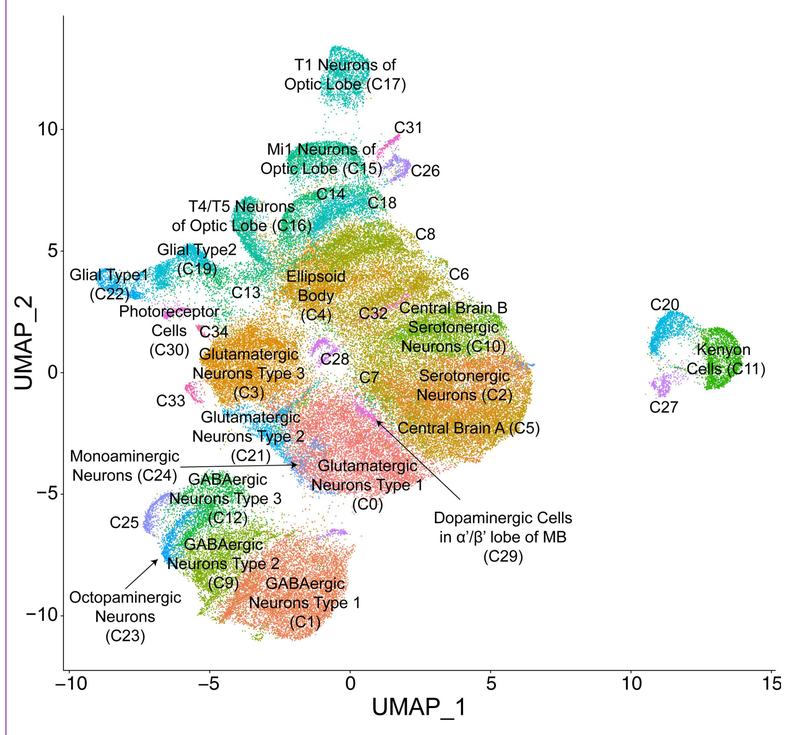
Output derived from CellRanger 3.1 mkfastq and counts pipelines

Non-linear ordination of gene expression profiles from all cells



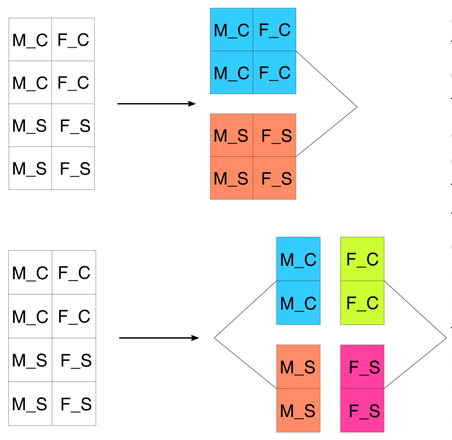
Raw counts data were filtered, normalized and scaled using the SCTransform algorithm within the Seurat pipeline. The integration and alignment of all eight samples using anchor genes has been visualized in the above ordination plot. In the plot, no single cluster or group of cells appears to be dominated by cells from a specific sample. This even distribution of cells across the entire plot indicates that SCTransform successfully aligned all eight samples to each other to create a common reference.

Clustering and characterization of cell types



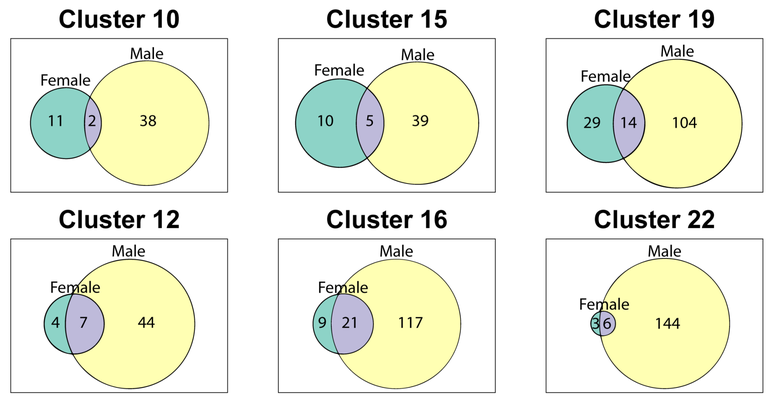
To identify the marker genes that define the expression profile of each cell cluster, differential expression analysis of each cluster against the rest of the cells in the dataset was performed. The top three genes based on positive expression (log_eFC > 0.5, adj p val < 0.05) for each cluster were used to characterize the cell type of each cluster.

Approaches to differential expression analysis



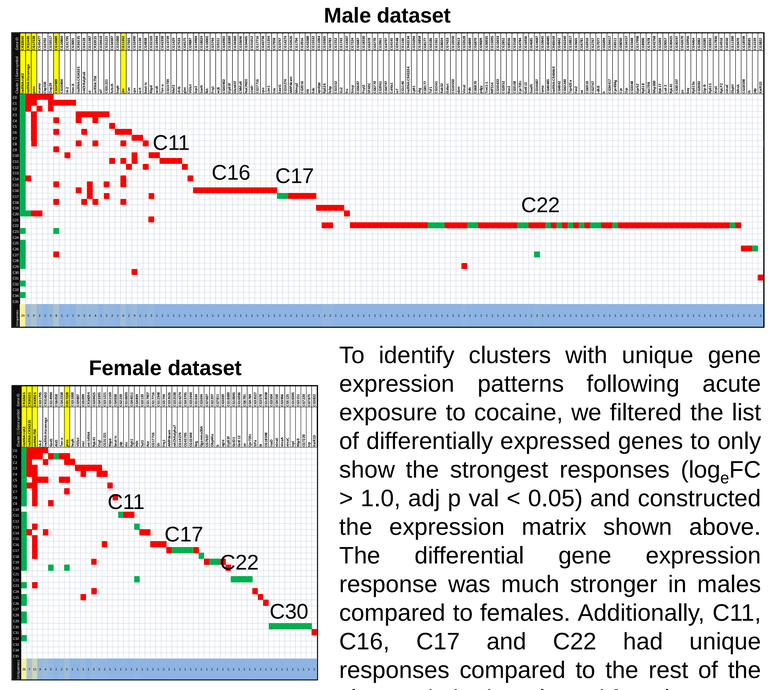
Differential expression analysis was performed in two different ways. (i) by combining the male and female samples together to assess differences in expression due to cocaine for each cluster. In addition, this approach was combined with TopKLists to identify gene expression response that is common to all clusters (simulation of bulk RNAseq global response). (ii) by assessing response to cocaine in males and females separately for each cluster.

Differential expression analysis
Sexually dimorphic responses



To assess the dissimilarities between the responses of male and female brains to cocaine, we compared the list of statistically significant differentially expressed genes (adj p val < 0.05) in each cluster between the sexes. Clusters with strong dissimilarities have been depicted as venn diagrams in the above figure. C22 and C16, which correspond to Glial Type 1 cells and T4/T5 neurons of the Optic Lobe, respectively seem to be strongly differentially expressed only in male brains with acute exposure to cocaine.

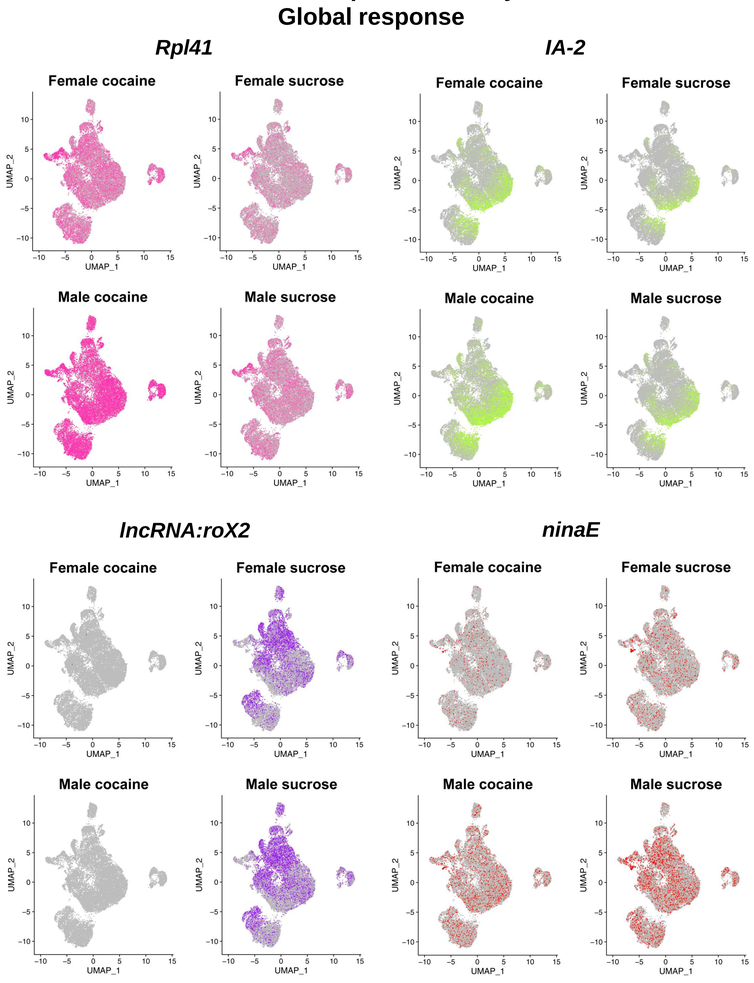
Distribution of differentially expressed genes across clusters



Red - upreg; green - downreg

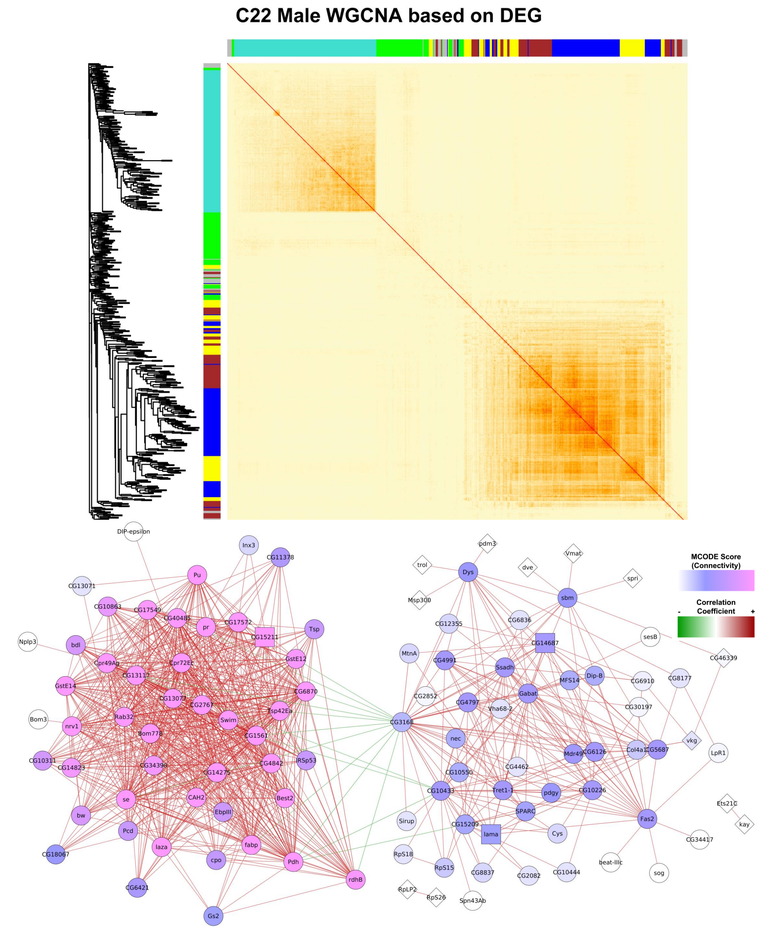
To identify clusters with unique gene expression patterns following acute exposure to cocaine, we filtered the list of differentially expressed genes to only show the strongest responses (log_eFC > 1.0, adj p val < 0.05) and constructed the expression matrix shown above. The differential gene expression response was much stronger in males compared to females. Additionally, C11, C16, C17 and C22 had unique responses compared to the rest of the clusters in both male and female sets.

Differential expression analysis
Global response



Assessment of global cocaine response that is common to both male and female brains indicates that there was a substantially greater number of genes upregulated than downregulated. Long-noncoding RNA roX2 was consistently downregulated in all clusters in both male and female as a response to cocaine.

Co-expression network analysis



Co-expression network analysis of C22 expression data in males using WGCNA revealed two large sub-networks of co-expresssed genes. The coefficient threshold determined by Random Matrix Theory was used to extract genes with strong co-expression. The MCODE algorithm in Cytoscape successfully identified several highly interconnected groups of genes within the larger network that correspond to specific biochemical pathways.

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