Brain Transcriptional Regulatory Network Quantitatively Predicts Behavior-Specific Gene Expression

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Behavior is a dynamic phenotype associated with multiple levels of cognitive processing. Given its dynamical nature, it's not known if behavior is influenced by the kind of Transcriptional Regulatory Networks(TRNs) known to regulate other phenotypes. We hence reconstructed a genome-scale TRN underlying behavior using 1300 microarrays from individual honey-bee brains sampled in 48 behavioral phenotypes. This TRN encompasses thousands of genes and accurately predicted their expression even in new behavioral states (average correlation - 0.87). We found transcription factors that are central actors in regulating behavior and demonstrate a remarkably close relationship between brain transcriptome and behavior.

***Keywords* — Systems Biology, Neuroscience, Transcriptional Regulatory Networks (TRNs), Behavior, Network Inference.**

Behavior is a dynamic phenotype influenced by both genotype and the environment. Molecular systems biology has been used to explore transcriptional regulation in the brain, but it is not known how behaviorally related neurogenomic states are defined and maintained. We hypothesized that behaviorally related brain gene expression could be used to reconstruct the type of transcriptional regulatory networks (TRNs) that operates for other phenotypes (*1*-*5*).

The natural behavior repertoire of the honey bee (*Apis mellifera*) is perhaps the best studied of any non-human animal (*6*); we sampled bees in one of 48 different states defined by behavior, genotype, and environment. Nearly all genes expressed in the brain were differentially expressed in $\geq$1 of the 48 states; this broad survey captured natural variation across most of the transcriptome, even without experimental genetic perturbation.

TRN reconstruction was performed with a combination of two well-known algorithms - *Network Inference Through Mutual Information And Regression* (NITMARE) approach generates a network of high-confidence TF-gene interactions using ARACNE (Accurate Reconstruction of Cellular Networks, (*2*)), and leverages these interactions to predict expression in new conditions with LARS (Least Angle Regression (*7*).

This TRN was able to model ca. 25% (2176) of the genes profiled (chosen based on strong fit in training set, Pearson correlation r > 0.8) and quantitatively predicted their expression with a remarkably high average correlation of 0.87 in the *test* sets. In comparison, a control experiment with TF expression permuted across phenotypes resulted in an average correlation of 0.22, and zero genes predicted with > 0.8 correlation even in the *training* set. The model captured many genes that appear relevant to behavior. It is enriched (P < 1e-10) for genes that were strongly differentially expressed between behavioral states. Further, the genes in the "modules" (sets of genes predicted to be regulated by a specific TF) have shared biological features: 166 of the 190 modules are enriched for specific biological processes or for evolutionarily conserved *cis*-regulatory motifs in upstream sequences (FDR < 0.1).

Although this TRN does not encompass all the layers of complexity inherent in brain function, our ability to accurately model a surprisingly high percentage of the transcriptome – absent information on nonlinear interactions between genes, physical interactions, time course, or brain subregion localization -- suggests that the relationship between brain gene expression and behavior is both stronger and more predictable than previously imagined. We consider this finding to be an important milestone in behavioral genomics.

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