Supporting Online Material for

Behavior-Specific Changes in Transcriptional Modules Lead to Distinct and Predictable Neurogenomic States

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This file contains Materials and Methods, Supplementary Tables S1-S12 and Supplementary Figures S1-S12. Supplementary Table S11 is available online at www.igb.uiuc.edu/labs/price/downloads/

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Materials and Methods

BeeSpace project

The experiments upon which this study is based arose from the BeeSpace Project (www.beespace.uiuc.edu/), an initiative funded by the US National Science Foundation Frontiers in Biological Research Program to study hereditary and environmental influences on brain gene

expression and social behavior by conducting large-scale genomic analysis and developing new informatics tools. This paper represents a meta-analysis of the large number of studies conducted under the auspices of this initiative; detailed analyses of some experiments have been published previously (1-3) or will be described in forthcoming publications. Each experiment was replicated with ca. 10-20 bees per group for a total of 853 bees and 1305 microarrays. See Supplementary Table 1 legend for a summary of the number of bees, experiments and microarrays that comprised the BeeSpace Project.

Sample processing and microarrays

We used microarrays to profile gene expression in the whole brains of individual bees. We used a 70-mer oligo spotted microarray, with probes designed based on gene predictions from the honey bee genome sequencing project; the microarray has been characterized and described previously (2).

Each study was replicated with ca. 10-20 bees per group for a total of 853 bees and 1305 microarrays across all of the experiments. Each experiment was implemented as a separate loop design involving 30-200 microarrays. Sample processing and microarray procedures for all experiments were performed at the Institute for Genomic Biology at the University of Illinois and were performed as previously described[2]. Briefly, partially lyophilized, frozen brains were dissected out of head capsules. RNA extracted from individual brains (Qiagen, RNeasy) was subjected to one round of linear amplification and labeled with fluorescent dye (Cy3 or Cy5;) using the Amino-Allyl Message AmpII kit (Ambion) or the Message AmpII kit in combination with a Universal Labeling System (Kreatech). Labeled aRNA was hybridized to a custom, oligonucleotide microarray containing 28,800 oligos, including 13,440 duplicately spotted experimental probes, primarily based on gene models from the Honey bee Genome Sequencing Project[6]. Slides were scanned with an Axon 4000B scanner, and images were analyzed with GENEPIX software (Agilent Technologies).

Differentially expressed genes

Statistical analyses to identify differentially expressed genes (DEGs) were performed separately for each experiment using consistent and standard methods, as in (2). Genes abundantly expressed in hypopharyngeal glands (a potential source of tissue contamination in brain samples) were filtered prior to analysis. A Loess transformation was performed using the software program *Beehive* (http://stagbeetle.animal.uiuc.edu/Beehive) to normalize expression intensities. A linear mixed-effects model implemented by using restricted maximum likelihood was used to describe the normalized log2 transformed gene intensities values, including the effects of experimental variables, dye, bee, and microarray. Effects were evaluated with an F-test statistic

and the P-values were adjusted for multiple hypothesis testing by using a FDR criterion. The resulting lists of DEGs below a given cutoff (usually FDR < 0.05) were used to characterize each of the TF-target modules.

We used a different method to characterize the strongest DEGs in our analysis of genes that were or were not accurately modeled in the Transcriptional Regulatory Network (TRN). For this analysis, we used Mann-Whitney rank-sum tests to characterize differentially expressed genes ($P < 0.05$) in each of the 48 states. **This initial test showed that every gene in the dataset was differentially expressed in at least one state**. We therefore focused on the 100 genes with the lowest P-values in each state, resulting in a list of 2770 genes. As described in the main text, the 2176 genes in the TRN were strongly enriched for this gene set. Similar P-values for enrichment were obtained using the top 50 or 200 genes in each state, and also when we used the genes with the largest fold changes instead of those with the lowest P-values. The same list of strong DEGs was also used for Gene Ontology enrichment analysis to characterize strong DEGs that were or were not accurately modeled in the TRN.

Data normalization for network inference

We performed additional normalization and filtering to generate a uniform dataset for network analyses. Expression profiles for individual bees were generated using a linear mixed-effects model with the effects of dye, bee, and microarray (but excluding biological variables). Microarray data from various studies were assembled into one dataset and then quantile normalized. Genes with more than 10% missing values were removed, as were individual bees for which there were more than 30% missing values or those that were not performed on whole brain tissue. These filters resulted in a final data set of 9544 probes and 631 experiments (bees) for network inference.

Approach overview

There are several advantages of our approach for network inference over using ARACNE or LARS alone. First, whereas ARACNE gives only the topology of the network, this combination outputs both the topology and also whether each interaction is activating or inhibitory. Second, we can estimate the percentage of the variance in a target gene's expression that is explained by each TF, and predict the expression of a target gene in new conditions. Third, by performing variable reduction and removing indirect interactions in ARACNE, we avoid problems faced by LARS with large numbers of interactions and highly correlated variables (4). Information-theoretic approaches are comparatively effective for studying large networks where putative gene-gene interactions are learned from a relatively small amount of expression data (5). Furthermore, our approach gives a quantitative predictive model for regulation for individual genes, which most network inference algorithms based on mutual information do not provide. A

similar network inference tool, the Inferelator (6) also provides a quantitative model of the regulatory network, but it has been used to model groups of genes (biclusters) rather than individual genes. Generally, coexpression networks tend to identify entire subpathways rather than individual interactions (7). Comparison with Bayesian and relevance networks is discussed elsewhere (8).

Enrichment analysis

All enrichment analyses were performed with hypergeometric tests. We calculated a P-value for each test by summing over probabilities for all values of overlap (>= l, the length of the overlap). We corrected for multiple hypothesis testing by using a Monte-Carlo simulation approach to estimate the False Discovery Rate (FDR) for each test. For each gene list, we took 1000 random samples of the same size as the original gene list and estimated the fraction of enrichments that were of similar or greater significance than the original P-value. This approach was applied to study enrichment for Biological Processes annotated in Gene Ontology (GO), pathways annotated by the Kyoto Encyclopedia of Genes and Genomes (KEGG), *cis*-regulatory DNA sequences within promoter regions, and differentially expressed genes in each of the 27 behavior-related comparisons (Table S1). We also used enrichment analyses to characterize the dominant biological themes represented by the genes accurately modeled in the TRN (compared to differentially expressed genes that were not accurately modeled) and to functionally characterize each of the TF-target modules within the TRN. Specific methods for each of these analyses are described below.

Gene Ontology

We used *Drosophila melanogaster* orthologs of honey bee genes (1) and version 1.1283, downloaded on 03/06/2010.

KEGG

We used KEGG annotations of honey bee genes publicly available on the KEGG website (www.genome.jp/kegg)

cis-regulatory motifs

cis-regulatory motif module predictions were generously provided by Professor Saurabh Sinha (University of Illinois at Urbana-Champaign). A motif module is defined as the set of genes that have a significant presence of the motif in their promoters; it was determined as described in (1). Every 500 bp segment in the 5 Kbp region upstream of a gene was scanned for a motif's

presence using the SWAN program, which produces a likelihood ratio statistic that captures the presence of one or more strong or weak matches to the motif in the segment. This score was then converted to an empirical P-value by comparing it to scores obtained from all segments of similar G/C content as the original segment. An empirical P-value of <= 0.01 for any segment in a gene's 5 KBp upstream region was used to designate that gene as belonging to the motif module.

Distribution of global and broadly acting vs. behavior-specific TFs

The statistical significance of the distribution of P-values for each of the three behavioral categories was estimated using random sampling. We shuffled the P-values for enrichment in the network to generate a background distribution for global regulators and the three sub-categories: aggression, foraging, and maturation. We then estimated p-values using t-test comparison with the background random distribution. We obtained a P-value < 2E-6 for global regulators, 2E-8 for maturation and ~0.3 for both aggression and foraging; the overall P-value for the distribution of enrichments in the entire table (Table S7) was < 2E -10.

Differential rank conservation (DIRAC) analysis of the network

Differential Rank Conservation (DIRAC) (9) was used to investigate changes in relative gene expression within each module, either across individual bees within states, or between states. Specifically, DIRAC quantifies and assesses expression consistency in the context of the rankings of target genes within a selected module: for each microarray, the expression values of the module genes are ordered from highest expression (ranked first) to lowest expression (ranked last). For any state or comparison in which genes of a particular module were not expressed, this module was omitted from DIRAC calculations.

We first used DIRAC to characterize the consistency of rank ordering within each module for the individual bees within each of the 48 states, as detailed in (9). A network is considered tightly regulated within a state if the relative expression of module genes is mostly consistent across individuals; a network is considered loosely regulated if the relative expression of genes is greatly varied between individuals of a state. Consistency of relative expression for a module in a selected state can range between 0.5 (relative expression of module genes is completely different in each individual) and 1.0 (relative expression of modules genes is identical in all individuals); the average consistency of all network modules across all states was 0.89±0.06, indicating that modules are tightly regulated in general within states (Figs. S7, S8).

We next used DIRAC to detect changes in relative expression of genes between states for each module. DIRAC identifies variably expressed or 'shuffled' modules that, for each comparison, enable statistically significant classification of expression profiles between states (9). We

estimated P-value and FDR for classification accuracies by repeatedly performing calculations for each comparison with 10,000 sets of randomly permuted individuals.

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Supplementary Tables

Table S1. Honey bee behavioral states and behavioral comparisons used for gene expression profiling and reconstruction of brain TRN

The TRN is based on the results of a set of experiments that used microarrays to profile gene expression in the whole brains of individual bees in experiments that analyzed 48 distinct behavioral states (1st column) and 27 direct comparisons between states, with ca. 10-20 bees/state. The behavioral states fall into three ecologically important categories: aggression, maturation, and foraging. For each of these three categories, some comparisons assessed hereditary differences (between sub-species or strains) and/or environmentally induced changes in behavior. TRN inference was performed using gene expression data from the 48 behavioral states, and TRN dynamics and regulation were studied with the results from the 27 comparisons. Twenty-four out of the 27 comparisons involved states used in TRN inference. We excluded two (26: Foraging Experience and 27: Distance Perception) because they involved measurements of gene expression in sub-regions of the brain, rather than the whole brain (comparison 26 was used in a subsequent test to determine the performance of the whole brain TRN model on brain region data). We excluded Comparison 16 (Queen Mandibular Pheromone) from TRN inference because of a high rate of 'missing' (poorly hybridizing) probes on the arrays for this study. Comparison 28, which involved States 45-48 (related to comparisons of drones vs. workers) were used only during TRN inference but were excluded from subsequent analyses because they relate less directly to worker bee behavior. For each comparison, we provide the following information: a description of the states and their relevance to social behavior; the total number of differentially expressed genes (DEGs; ANOVA, False Discovery Rate [FDR] < 0.05 across all probes on the microarray unless otherwise noted); the number of DEGs that were accurately modeled as targets in the TRN; the number of transcription factors (TFs) that were predicted to regulate these target genes (the TFs themselves were not necessarily differentially expressed); the total number of TF–target interactions involving DEGs in each experiment; 'Major Regulators' are TFs whose targets were significantly enriched for DEGs in each comparison; they were named according to the symbol of the orthologous *Drosophila* TF. In addition, for each state/comparison we list citations for those experiments that are already published or the names of lead contributors for those that have not yet been published.

AGGRESSION

Comparison (States)	*Description*	*#DEGs (Total)*	*TRN Target*	*TRN TFs*	*Inter-actions*	*Major Regulators*	*Citation/ Contributors*
Hereditary influences							
1 *(States 1-4)*	**Africanized (AHB) vs. European (EHB) Honey bee Guards: Individual Genotype** Bees in colonies of Africanized sub-species are more aggressive than bees in colonies of European sub-species due to a combination of individual genetics and colony-level environmental factors. AHB and EHB individuals were co-fostered in AHB and EHB colonies. Guards are bees at the hive entrance that alert colony members to a potential intruder by releasing alarm pheromone. Comparison 1 identified genes that differed according to a guard's individual genotype, independent of colony genotype.	250	29	43	86		*(1)*
2 *(States 5-8)*	**AHB vs EHB Soldiers: Individual Genotype** As above, but soldiers: bees that respond aggressively to intruders, seeking them out to sting.	545	72	92	224	*dl, lilli*	*(1)*
Environmental influences (long-term)							
3 *(States 1-4)*	**AHB vs EHB Guards: Colony Genotype** Effects of colony genotype on gene expression in guards, independent of individual genotype (cross-fostering design).	502	62	80	196	*CG34422*	*(1)*
4 *(States 5-8)*	**AHB vs EHB Soldiers: Colony Genotype** Effects of colony genotype on gene expression in soldiers, independent of individual genotype (cross-fostering design).	840	137	106	413	*YL-1, CG14711, Pbp95, CG34422, CG9215, CTCF, Ets97D, Su(var)2-10*	*(1)*
Environmental influences (short-term)							
5 *(*States *9, 10)*	**Alarm Pheromone** The chemical iso-pentyl acetate (IPA) acts as alarm pheromone to induce aggressive behavior. Caged, foraging-age bees were exposed to alarm pheromone and compared to a control condition.	442	80	94	252	*Deaf1, E2f2, Ets97D, Mio, Su(var)2-10, br, kin17, pan*	*(1)*

MATURATION

Comparison (States)	*Description*	*DEGs (Total)*	*TRN Target*	*TRN TFs*	*Inter-actions*	*Major Regulators*	*Citation/ Contributors*
Hereditary influences							
6 *(States 11-14)*	**AHB vs. EHB 4-day-old Adult Hive Bees: Individual/Colony Genotype** AHB show faster maturation (initiate foraging at a younger age) than EHB (10). Pre-foraging-age bees (4-day-old and 14-day-old adults) were collected from inside AHB and EHB hives and gene expression was measured in a 2x2 factorial with genotype and age. Unlike the experiments with guards and soldiers, the effects of colony and individual genotype were considered as a single variable in this experiment. Comparison 6 describes genotypic differences in 4-day-old bees.	3755	804	183	2566	*fd85E, blos1, CG33695, Mtp, CG9890, Camta, Dp, Myb, Ssb-c31a, Stat92E, aop, bs, crp, fru, ftz-f1, lilli, nej, onecut, pnt, rn, sima, slp2, vnd*	*C. Alaux, S.L. Rodriguez-Zas & G.E. Robinson*
7 *(States 11-14)*	**AHB vs. EHB 14-day-old Adult Hive Bees: Individual/Colony Genotype** Genotypic effects in 14-day-olds from experiment above.	3922	834	184	2661	*fd85E, blos1, CG33695, Mtp, CG9890, Camta, Dp, Myb, SoxN, Ssb-c31a, Stat92E, bs, crp, fru, ftz-f1, lilli, nej, pnt, rn, sima, vnd*	*C. Alaux, S.L. Rodriguez-Zas & G.E. Robinson*
8 *(States 15-18)*	***A. mellifera ligustica* vs. *A. mellifera mellifera* 10-day-old Adult Hive Bees: Individual/Colony Genotype** Sub-species from Northern (*A. mellifera mellifera*) and Southern (*A. melliefera ligustica*) Europe differ in maturation; *ligustica* are faster (11). Pre-foraging-age bees (10-days old) from colonies of *mellifera* and *ligustica* were measured in a 2x2 factorial with genotype and age.	3136	582	173	1827	*CG42748, blos1,Ssb-c31a br, dac, maf-S, tgo*	*C. Alaux, Y. LeConte, S.L. Rodriguez-Zas & G.E. Robinson*
9 *(States 15-18)*	***ligustica* vs. *mellifera* 15-day-old Adult Hive Bees: Individual/Colony Genotype** Same as Experiment 8, but for 15-day-old bees.	3218	593	171	1868	*CG42748, blos1,Ssb-c31a br, dac, maf-S*	*C. Alaux, Y. LeConte, S.L. Rodriguez-Zas & G.E. Robinson*
10 *(States 19, 20)*	**High vs. Low Pollen Hoarding** Artificial selection on the amount of pollen stored in the hive ('pollen hoarding') has led to changes in a suite of individual behavioral characteristics, including precocious maturation in high-pollen hoarding line (12). 7-day-old hive bees from high- and low-hoarding lines were compared.	460	103	103	332	*CG17912, CG32532, crc*	*A.L. Toth, R.E. Page, G.V. Amdam, S.L. Rodriguez-Zas & G.E. Robinson*

Environmental influences (long-term)							
11 *(States 21, 22)*	**Young Nurses vs. Old Foragers (Typical colony)** One-week-old bees were collected performing brood care ('nursing'), the in-hive task performed by most 1-2-week-old workers; One-month-old bees were collected foraging outside the hive for nectar or pollen.	1394	231	129	727	*CG33695, Xbp1*	*(2)*
12 *(States 23, 24)*	**Young Nurses vs. Young Foragers (Single-cohort colony)** The effects of behavior and age can be separated by creating 'single-cohort' colonies in which all bees are the same age but nonetheless form a division of labor between nurses and foragers. A 2x2 factorial design was used, with behavior (nurse or forager) and age (7- or 30-days-old). Comparison 12 contrasts age-matched 7-day-old bees at different stages of maturation.	183	21	47	67	*kay*	*(2)*
13 *(States 25, 26)*	**Old Nurses vs. Old Foragers (Single-cohort colony)** Same as Experiment 12 but with age-matched 30-day-old bees at different stages of maturation.	460	73	94	211	*CG14711, CG3815*	*(2)*
14 *(States 11-14)*	**4-day-old vs. 14-day-old Adult Hive Bees** A bee's age does not directly cause a shift from hive work to foraging; older bees are just more likely to make this transition (13). 14-day-old vs. 4-day-old hive bees from AHB and EHB were compared; genes with a significant age x genotype interaction excluded.	2985	492	168	1508	*CG11294, MEP-1, CG7786, CG9139,CG9932, D, E2f, Optix, al, bab1, cg, oc, trh, unpg*	*C. Alaux, S.L. Rodriguez-Zas & G.E. Robinson*
15 *(States 15-18)*	**10-day-old vs. 15-day-old Adult Hive Bees** 15-day-old bees vs. 10-day-old bees from *A. mellifera ligustica* and *A. mellifera mellifera.* Genes with a significant age x genotype interaction excluded.	691	130	118	430	*CG17912, CG3407, CG7786, CrebB-17A, dl*	*C. Alaux, S.L. Rodriguez-Zas & G.E. Robinson*
Environmental influences (short-term)							
16 *(States N.A.)*	**Queen Mandibular Pheromone** The pace of worker maturation is influenced by the social environment, signaled in part by the presence of pheromones. Queen Mandibular Pheromone (QMP)—a chemical blend produced by the queen—delays maturation[7]. The response of caged bees exposed chronically to QMP for 4 days was	753 (FDR < 0.2)	209	126	604	*Bgb, CG15715, CG17912, blos1, CG32121, Mtp, CG9776,Dref, Ets65A, HLHm&bgr, Jra, MTA1-like, Mio, Oli, gem, l(2)k10201, pnt*	*C. Alaux, S.L. Rodriguez-Zas & G.E. Robinson*

	compared to controls. Note: Bees from the QMP study were not included in the initial reconstruction of the TRN, but the gene list was used for subsequent analyses of TRN function.						
17 *(States 27-29)*	**Rich vs. Poor Diet** Nutritional status is another factor that influences maturation (14). Bees were caged and fed either a rich diet (pollen, honey, and sugar syrup) or a poor diet (sugar syrup only). These bees also were compared to age-matched controls reared in a colony. Comparison 17 contrasts brain gene expression in bees fed rich vs. poor diet.	372	66	81	187	*CG11085, Ets65A, HLHm&bgr, aop, cnc, fru, ftz-f1*	*S.A. Ament, M.M. Wheeler, S.L. Rodriguez-Zas & G.E. Robinson*
18 *(States 30-32)*	**Vitellogenin RNAi** Titres of the yolk protein vitellogenin decrease prior to the onset of foraging, and *vg* has causal effects on maturation; *vg* dsRNA treatment causes precocious foraging (15). Bees were injected intra-abdominally with *vg*dsRNA, saline buffer, or sham manipulated. Comparison 18 contrasts brain gene expression in *vg* RNAi vs. saline injected bees.	3138	690	178	2073	*Ada2b, CG12071, MEP-1, CG32532, Nf-YA, CG6854, CG34422, Vps45, Ets65A, HLH106, NC2&bgr, adp, crc, fru, nej*	*S.A. Ament, M.M. Wheeler, S.L. Rodriguez-Zas & G.E. Robinson*

FORAGING

Comparison (States)	***Description***	***#DEGs (Total)***	***TRN Target***	***TRN TFs***	***Inter-actions***	***Major Regulators***	***Citation/ Contributors***
Hereditary influences							
19 *(States 33-36)*	**AHB vs EHB Foragers: Individual Genotype** See Comparisons 1 and 4 for experimental details. Relative to EHB, AHB foragers have a preference for pollen and lower sucrose response thresholds during nectar foraging. Comparison 19 describes the effects of individual genotype.	59	5	17	18	*Vps45*	*(1)*
Environmental influences (long-term)							
20 *(States 33-36)*	**AHB vs EHB Foragers: Colony Genotype** Same as Experiment 19 but this comparison describes the effects of colony genotype (cross-fostering design).	351	48	80	146	*crc*	*(1)*
Environmental influences (short-term)							
21 *(States 37, 38)*	**Scout vs. Recruit** Individual foragers differ in how they find the	1126	226	138	681	*BtbVII, blos1, Mtp, Ets65A, MBD-like,*	*Z. Liang, S.L. Rodriguez-Zas*

	flowers upon which they forage. Scout bees seek out novel sites independently and assess their quality. They then recruit other foragers ('recruits') to these sites (16) with the dance language. Scouts were collected after foraging at novel sites on multiple days; recruits were collected that repeatedly foraged at a known site but did not visit novel sites.					*bun, ey, mip120, onecut, p53, pros, vnd*	*& G.E. Robinson*
22 *(States 39, 40)*	**Vibration Signalers** Specialist foragers perform a stereotyped vibratory signal that modulates the activity of other bees in the colony. Vibration signal specialists were compared to age-matched bees that did not perform this behavior.	763	155	116	437	*Su(H), Xbp1, fru, ftz-f1, gem, mbf1, pnt, vnd*	*(3)*
23 *(States 41-44)*	**Time-trained: Spatiotemporal Memories** Foragers form spatiotemporal memories for particular flowers and forage at those sites only at the appropriate time of day. Different groups of foragers from the same hive were trained to forage either in the morning or the afternoon. To determine the imprint of these spatiotemporal memories on brain gene expression, bees from both training groups were collected in the morning and afternoon (a 2x2 factorial with training time and collection time). Comparison 23 contrasts bees trained in the morning to bees trained in the afternoon (independent of collection time).	229 (FDR < 0.01)	60	76	177	*CG15011, CG9776, Lag1, NC2&bgr, dl, exd, kay*	*(17)*
24 *(States 41-44)*	**Time-trained: Morning vs. Afternoon** Same as Comparison 23, except this comparison describes brain gene expression differences associated with collection time.	798 (FDR < 0.01)	146	116	453	*fru*	*(17)*
25 *(States 41-44)*	**Time-trained: Anticipating vs. Inactive** Same as Comparison 23, except this comparison contrasts bees based on their activity level by comparing bees collected at the time they had been trained to forage ('anticipating' because bees were collected prior to their leaving the hive, but already showing stereotyped anticipatory behavior) to bees that were inactive at that time because they had been trained to forage in other parts of the day.	3439	804	184	2456	*A3-3, Alh, Bgb, CG15011, CG42748, CG32121, CG33695, Mtp, Dp, Dsp1, Eip74EF, Ets65A, Fer3, HLHm&bgr, MTA1-like, NC2&bgr, SoxN, Ssb-c31a, Xbp1, aop, ap, bigmax, br, bun, crc, dac, dl, exd, fru, ftz-f1, gem, kay, l(2)k10201, lilli, mbf1, nej, pan, pnt, sd*	*(17)*

Environmental influences (brain sub-region data)							
26 *(States N.A.)*	**Foraging Experience** Extensive foraging experience (> 1 week of foraging) causes an enlargement of the mushroom bodies -- a brain region involved in associative learning – and behavioral differences in how foragers learn and remember food sources (18, 19). Mushroom body gene expression was measured in bees with 4, 8, 12, or 16 days of foraging experience. The most extensive differences were found between bees with 8 vs. 12 days of foraging experience, corresponding to the period of mushroom body expansion, and these differences are measured in Comparison 26. Because this experiment measured gene expression only in one brain region (corresponding to ca. 40% of the brain by volume), bees from this experiment were not included in the initial reconstruction of the TRN model but were used as a test set to determine the predictive ability of the TRN model for a sub-region of the brain. Foraging experience was considered a long-term difference in behavior.	371	45	72	137	*CG9139, Deaf1, Ets97D, Su(var)2-10*	Lutz, C.C., Rodriguez-Zas, S., Fahrbach, S.E., G.E. Robinson
27 *(States N.A.)*	**Distance Perception** Foraging honey bees remember the locations of floral resources (distance and direction relative to the hive) and communicate this information socially to other workers via the dance language. Distance-responsive genes were identified in two regions of the bee brain, the mushroom bodies and optic lobes. Bees were trained to fly to a feeding station through a narrow tunnel with interchangeable visual patterns on the walls. Because bees measure distance from optic flow, they can be tricked into perceiving a long distance when flying past a vertical striped pattern (generating high amounts of image motion, similar to a visually dense landscape), and a short distance when flying past a horizontal striped pattern (similar to a visually sparse landscape). Comparison 27 contrasts bees that had foraged at a perceived long vs. short distance but had otherwise identical experience. We used genes for which there was a significant main effect of distance perception, independent of brain region. Distance perception is a short-term	52	8	0	0		*(20)*

	difference in behavior.						

ADDITIONAL STATES

28 *(States 45-48)*	**Drone vs. Worker** Drones (male bees) and worker bees (sterile females) both mature from hive-restricted behavior to flying outside the hive, but the two castes perform different behaviors (drones mate, workers forage). We compared one-day-old (in-hive) and 21-day-old (flying) drones and workers in a 2x2 factorial with caste and age. Bees from this experiment were included in TRN reconstruction but not for subsequent analyses.	*N.A.*					*A. Zayed, N.L. Naeger, S.L. Rodriguez-Zas & G.E. Robinson*

Table S2. Model Summary Statistics

Total Microarrays	1305
Total Individual bees studied	853
Individual bees used for Network Inference (NI)	631*
Individual bees used for Clustering	751*
Individual bees for mushroom body (MB) expt.	120
Total Bees used for NI, MB and generating gene lists	853*
Total genes in the network	2176
TFs in the data set	236
TFs in the network	190
Interactions in the network	6757
Mean Indegree	3
Standard Deviation Indegree	1
Mean Out degree	35
Standard Deviation Out degree	50
P-value threshold for Mutual information	1.00E-6
DPI threshold	0.1
Total modules	190
Modules enriched for GO/Kegg	166
Modules enriched for an upstream motif	109
Modules with enrichment for differentially expressed genes (DEG)	108
Total genes in the data set	9544
Global regulators	4
Correlation threshold	0.8
Correlation Ten-fold CV (train & test)	0.87
Correlation background	0

* Some of the 853 bees were excluded for inferring the TRN if their expression profiles had many missing values, or if they were analyzed for brain region, rather than whole brain, expression, resulting in 631 bees being used for NI out of the 853 bees. For clustering, only data sets with many missing values were excluded, resulting in 751 bee profiles.

Table S3. Global analysis of bee brain gene expression and social behavior – Controls and Statistical Significance

To check the robustness of these clusters with respect to changes in the transcriptome, we took random subsets of the transcriptome to see if the phenotype—expression relationship still holds true. We found that with as little as 10% of the genes, the phenotypes still grouped into clusters specific to Aggression (Genetic influence subcluster: AHB vs. EHB Guards (Indiv.); AHB vs. EHB Soldiers (Indiv.); lig vs. mel (10-d-old); lig vs. mel (15-d-old); AHB vs. EHB (4-d-old); AHB vs. EHB (14-d-old); AHB vs. EHB Forager (Indiv.). Environmental influence subcluster: AHB vs. EHB Guards (Colony); AHB vs. EHB Soldiers (Colony); AHB vs. EHB Forager (Colony)) and foraging (Morning vs. Afternoon; Training time; Anticipating vs. Inactive; Scout vs. Recruit; Vibratory Signal). Table below shows the fraction of times these clusters were present in among 100 random samples from a subset of the transcriptome. The fraction % for each subset is given at the top of the table. Since the maturation phenotype was spread across multiple clusters we weren't able to quantify it computationally.

Additional controls are discussed in the Methods section and Figure S1.

Clusters	10%	20%	30%	40%	50%	60%	70%	80%	90%	100 %
Aggression (Genetic)	0.86	0.95	0.99	1	1	1	1	1	1	1
Aggression (Environmental)	0.98	1	1	1	1	1	1	1	1	1
Foraging Behaviors	0.45	0.66	0.82	0.8	0.83	0.86	0.95	0.96	0.95	0.98

Table S4. Enriched biological processes in the bee brain TRN

The following Gene Ontology biological processes were over-represented (FDR < 0.05) among differentially expressed genes in the TRN, compared to differentially expressed genes that were not accurately modeled. Categories related to neuronal functions are highlighted (manual annotation). The P-value for enrichment is shown for each category.

GO category	P-value
very long-chain fatty acid metabolic process	0
HIR complex	0
DNA strand annealing activity	0
neurotransmitter uptake	0
sebaceous gland cell differentiation	0
immune response-regulating cell surface receptor signaling pathway	0.000243
mesodermal-endodermal cell signaling	0
endodermal-mesodermal cell signaling	0
cell-cell signaling involved in amphid sensory organ development	0
single-stranded DNA binding	0
actin binding	2.68E-08
actin monomer binding	2.33E-06
phosphoglycerate dehydrogenase activity	0
phosphorylase activity	0
nicotinic acetylcholine-activated cation-selective channel activity	0
G-protein coupled receptor activity	0.000115
calcitonin receptor activity	0.000269
guanyl-nucleotide exchange factor adaptor activity	0
GDP-dissociation inhibitor activity	0
epidermal growth factor receptor binding	0
structural constituent of cell wall	0.000102
structural constituent of cytoskeleton	1.79E-06
extracellular matrix structural constituent	0.000102
structural constituent of eye lens	2.26E-05
structural constituent of chorion	2.26E-05
ion channel activity	0
intracellular cyclic nucleotide activated cation channel activity	0
calcium activated cation channel activity	0
voltage-gated ion channel activity	0
voltage-gated potassium channel activity	3.31E-05
delayed rectifier K channel activity	0.000243
open rectifier potassium channel activity	2.26E-05
potassium channel activity	0
Ach transmembrane transporter activity	0
dopamine:sodium symporter activity	0
tropomyosin binding	7.45E-05
chaperonin-containing T-complex	0.000324
coated pit	0
gap junction	0
protein folding	0.000326
de novo protein folding	0.000125
folic acid and derivative metabolic	0

process		high-affinity potassium ion import	3.39E-06
receptor-mediated endocytosis	2.26E-05	ER body	0
positive regulation of antifungal peptide biosynthetic process	0	temperature compensation of the circadian clock	0
post-chaperonin tubulin folding pathway	0.000259	light-activated ion channel activity	0
		cell-cell signaling involved in quorum sensing	0
male meiosis chromosome segregation	0		
		amidinotransferase activity	0
synaptic transmission	1.17E-05	ion transmembrane transporter activity	0
neuromuscular synaptic transmission	0.000329		
axon target recognition	9.93E-06	calcium-activated potassium channel activity	0
haltere development	0		
visceral mesoderm-endoderm interaction involved in midgut development	0	outward rectifier potassium channel activity	0.000243
		ligand-gated ion channel activity	0
ectoderm and mesoderm interaction	0	polyspecific organic cation transmembrane transporter activity	0
structural constituent of bone	2.26E-05		
N-methyltransferase activity	2.04E-06	tubulin binding	4.65E-06
G-protein coupled amine receptor activity	8.25E-05	acetylcholine transport	0
		mannosidase activity	0
structural constituent of muscle	1.61E-08	determination of muscle attachment site	0
structural constituent of vitelline membrane	2.26E-05		
		selenocysteine methyltransferase activity	0.00033
gurken receptor binding	0		
female germline ring canal stabilization	0	GDP-dissociation stimulator activity	0
		apoptotic protease activator activity	0
mechanically-gated ion channel activity	0	long-chain-3-hydroxyacyl-CoA dehydrogenase activity	0
taste receptor activity	4.24E-05	transferase activity, transferring one-carbon groups	0.000118
photoreceptor cell morphogenesis	0		
caspase activator activity	0	transferase activity, transferring nitrogenous groups	0
cyclic nucleotide metabolic process	0		
folic acid and derivative biosynthetic process	0	oximinotransaminase activity	0
		G-protein coupled acetylcholine receptor activity	0
microtubule-based flagellum	0		
guard cell differentiation	0	DNA topoisomerase I binding	0
root epidermal cell differentiation	0	receptor mediated endocytosis of virus by host	0
maintenance of meristem identity	0		
potassium ion import	3.39E-06	cytokine-mediated signaling pathway	0.000243

structural constituent of myelin sheath	2.26E-05	signaling pathway	
		asymmetric synapse	0
microneme	0	symmetric synapse	0
cerebellar Purkinje cell-granule cell precursor cell signaling involved in regulation of granule cell precursor cell proliferation	0	nucleomorph	0
		attachment organelle	0
		CAF-1 complex	0
		cell surface receptor linked signal transduction leading to integrin activation	0.000243
negative regulation of cell-cell adhesion	0		
protein maturation by protein folding	0.000259	pyridoxine 5-phosphate synthase activity	0
voltage-gated cation channel activity	1.34E-05		
CD40 signaling pathway	0.000243	Arp2/3 complex-mediated actin nucleation	0
actin filament-based movement	0		
clathrin vesicle coat	0	protein folding in endoplasmic reticulum	0.000259
clathrin coat of coated pit	0		
keratinocyte differentiation	0	adult chitin-based cuticle pattern formation	0
structural constituent of epidermis	2.26E-05		
structural constituent of chromatin	2.26E-05	histone acetyltransferase binding	0
pseudocleavage	0	regulation of tube diameter, open tracheal system	0
pseudocleavage during syncytial blastoderm formation	0		
		embryonic hemocyte differentiation	0
vitelline membrane formation	0	synaptic transmission, glutamatergic	0
regulation of actin filament polymerization	0	hair cell differentiation	0
		Roundabout signaling pathway	0.000243
negative regulation of actin filament polymerization	0	autocrine signaling	0
		protein refolding	0.000259
positive regulation of actin filament polymerization	0	5,10-methylenetetrahydrofolate-dependent methyltransferase activity	0.000102
platelet dense tubular network	0	ribosome assembly	0
rRNA methylation	0	error-prone translesion synthesis	0
neuromuscular junction	5.83E-08	structural constituent of cuticle	1.50E-05
receptor internalization	0	hormone biosynthetic process	7.45E-05
regulation of cyclin-dependent protein kinase activity involved by G1/S	0	ecdysis-triggering hormone receptor activity	0.000269
		positive regulation of circadian rhythm	0
regulation of cyclin-dependent protein kinase activity involved in G2/M	0	histone deacetylase binding	0
		single-stranded telomeric DNA binding	0
lipopolysaccharide-mediated	0.000243		

membrane-bounded organelle	0	cell-cell signaling involved in lung development	0
apical junction complex	0		
structural constituent of carboxysome	2.26E-05	mesenchymal-epithelial cell signaling	0
multi-organism biosynthetic process	0.000129	regulation of branching involved in mammary cord morphogenesis by fat precursor cell-epithelial cell signaling	0
multicellular organismal biosynthetic process	0.000129		
small molecule biosynthetic process	0.000129	regulation of mammary gland cord elongation by mammary fat precursor cell-epithelial cell signaling	0
exoneme	0		
plasma membrane part	7.12E-06		
negative regulation of cyclin-dependent protein kinase activity	0	cell-cell signaling involved in placenta development	0
regulation of protein kinase activity	0	epithelial-mesenchymal cell signaling	0
tetrahydrofolylpolyglutamate biosynthetic process	0	cell-cell signaling involved in mammary gland development	0
habituation	0	epiblast cell-extraembryonic ectoderm cell signaling involved in anterior/posterior axis specification	0
dATP(dGTP)-DNA purinetransferase activity	0		
regulation of neuronal synaptic plasticity	0	limb basal epidermal cell differentiation	0
cell morphogenesis involved in neuron differentiation	0	cell-cell signaling involved in kidney development	0
imaginal disc-derived appendage development	0	regulation of dendritic spine morphogenesis	0
presynaptic active zone membrane	0	peptidoglycan recognition protein signaling pathway	0.000243
antennal morphogenesis	0		
regulation of dendrite development	0	chaperone-mediated protein folding	0.000414
positive regulation of dendrite morphogenesis	0	mononeme	0
		actin-mediated cell contraction	0
sensory perception of bitter taste	0	oligosaccharide binding	0
sensory perception of sour taste	0	histone acetyl-lysine binding	0
sensory perception of umami taste	0	error-free translesion synthesis	0
actin filament severing	0	potassium ion export	3.39E-06
actin filament binding	4.65E-07	semaphorin-plexin signaling pathway	0.000243
excitatory synapse	0	regulation of pre-tubular aggregate formation by cell-cell signaling	0
inhibitory synapse	0		
synaptic transmission involved in micturition	9.93E-06	receptor-mediated endocytosis of low-density lipoprotein involved in cholesterol transport	0
oenocyte delamination	0		

Table S5. Annotation of TF-target modules in the bee brain TRN

For each TF (listed by the name of the orthologous *D. melanogaster* TF), we list the number of predicted target genes. For each set of target genes we describe over-represented Gene Ontology biological processes (GO; FDR < 0.05), pathways from the Kyoto Encyclopedia of Genes and Genomes (KEGG; FDR < 0.1), *cis*-regulatory DNA sequences in promoter regions (*cis*; the most significant motif, FDR < 0.1), enrichment for genes differentially expressed in each of 27 behavioral comparisons related to aggression, maturation, or foraging (DEG; FDR < 0.1), overlap with the regulatory network of their *Drosophila* counterpart based on physical binding data or enrichment for the binding motif of their corresponding ortholog in *Drosophila* (Dmel; P < 0.1; highlighted in yellow)

TF Name	No. of target genes	Enrichment	Enrichment Category	P-value
Lag1 / Longevity assurance gene 1	394			
		Time Trained: Spatiotemporal Memories	DEG	0.02
		Nucleotide excision repair	KEGG	0.01
		cytoplasmic mRNA processing body	GO	0
		adrenocorticotropin receptor activity	GO	0.01
CG9932 / CG9932	265			
		4-d-old vs. 14-d-old	DEG	0.03
		Glycine, serine and threonine metabolism	KEGG	0.03
		Neuroactive ligand-receptor interaction	KEGG	0
		receptor signaling protein tyrosine phosphatase activity	GO	0
CrebB-17A / Cyclic-AMP response element binding protein B at 17A	185			
		10-d-old vs. 15-d-old	DEG	0
		Neuroactive ligand-receptor interaction	KEGG	0.03
		nucleotide receptor activity, G-protein coupled	GO	0.01
		growth hormone secretagogue receptor activity	GO	0.01
		platelet ADP receptor activity	GO	0.01
		super conserved receptor expressed in brain receptor activity	GO	0.01
		Mas proto-oncogene receptor activity	GO	0.01
		RDC1 receptor activity	GO	0.01
		Epstein-Barr Virus-induced receptor activity	GO	0.01
		nociceptin/orphanin-FQ receptor activity	GO	0.01
		gastropyloric receptor activity	GO	0.01
		pituitary adenylate cyclase-activating polypeptide receptor activity	GO	0.01

		calcitonin gene-related polypeptide receptor activity	GO	0.01
		adenylate cyclase inhibiting metabotropic glutamate receptor activity	GO	0.01
		G-protein coupled cytokinin receptor activity	GO	0.01
		actin binding	GO	0
		G-protein coupled receptor activity	GO	0.01
		calcitonin receptor activity	GO	0
		cannabinoid receptor activity	GO	0.01
		icosanoid receptor activity	GO	0.01
		glucagon receptor activity	GO	0.01
		leukotriene receptor activity	GO	0.01
		parathyroid hormone receptor activity	GO	0.01
		platelet activating factor receptor activity	GO	0.01
		thyrotropin-releasing hormone receptor activity	GO	0.01
		vasoactive intestinal polypeptide receptor activity	GO	0.01
		G-protein coupled photoreceptor activity	GO	0.01
		melatonin receptor activity	GO	0.01
		peptide receptor activity, G-protein coupled	GO	0
		secretin receptor activity	GO	0.01
		corticotrophin-releasing factor receptor activity	GO	0.01
		protein-hormone receptor activity	GO	0.01
		pheromone receptor activity	GO	0.01
		gastric inhibitory peptide receptor activity	GO	0.01
		growth hormone-releasing hormone receptor activity	GO	0.01
		brain-specific angiogenesis inhibitor activity	GO	0.01
		ecdysis-triggering hormone receptor activity	GO	0.01
		bioactive lipid receptor activity	GO	0.01
		nicotinic acid receptor activity	GO	0.01
CG17912 / CG17912	181			
		High vs. Low Pollen Hoarding	DEG	0
		Queen Mandibular Pheromone	DEG	0
		10-d-old vs. 15-d-old	DEG	0.01
		immune system process	GO	0.01
		channel regulator activity	GO	0.01
		metallochaperone activity	GO	0.01
		protein tag	GO	0.01
		locomotion	GO	0.01
		chemoattractant activity	GO	0.01
		translation regulator activity	GO	0
		chemorepellent activity	GO	0.01
		nutrient reservoir activity	GO	0.01
		response to stimulus	GO	0

br / broad	169			
		CAACAA	*cis*	0.07
		Alarm Pheromone	DEG	0.04
		lig vs. mel (10-d-old)	DEG	0.01
		lig vs. mel (15-d-old)	DEG	0.02
		Time Trained: Anticipating vs. Inactive	DEG	0
		prospore membrane	GO	0
		plasma membrane	GO	0.01
		membrane	GO	0.01
		outer membrane	GO	0
		organelle membrane	GO	0
		pre-autophagosomal structure membrane	GO	0
		photosynthetic membrane	GO	0
		nuclear membrane-endoplasmic reticulum network	GO	0
		photoreceptor outer segment membrane	GO	0
		presynaptic membrane	GO	0
		postsynaptic membrane	GO	0
		coated membrane	GO	0
		photoreceptor inner segment membrane	GO	0
		Broad Motif	Dmel	0
rn / rotund	162			
		AHB vs. EHB (4-d-old)	DEG	0.00E+00
		AHB vs. EHB (14-d-old)	DEG	0
		store-operated calcium entry	GO	0.01
		calcium ion transport	GO	0.01
		N-methyltransferase activity	GO	0.01
		folic acid and derivative biosynthetic process	GO	0.00E+00
		tetrahydrofolylpolyglutamate biosynthetic process	GO	0.00E+00
		cytosolic calcium ion transport	GO	0.01
		calcium ion transmembrane transport	GO	0.01
Myb / Myb oncogene-like	154			
		AHB vs. EHB (4-d-old)	DEG	0.03
		AHB vs. EHB (14-d-old)	DEG	0.04
		Cysteine and methionine metabolism	KEGG	0.03
		ATP-dependent DNA helicase activity	GO	0.01
		tropomyosin binding	GO	0.01
		Myb Network	Dmel	0.01
		Myb Motif	Dmel	0.09
MTA1-like / MTA1-like	146			
		Queen Mandibular Pheromone	DEG	0
		Time Trained: Anticipating vs. Inactive	DEG	0.00E+00

		Cysteine and methionine metabolism	KEGG	0.03
		Glycerolipid metabolism	KEGG	0.04
		actin monomer binding	GO	0.01
		signaling pathway	GO	0.01
		actin filament binding	GO	0.01
ftz-f1 / ftz transcription factor 1	146			
		V_CDP_02	*cis*	0.05
		AHB vs. EHB (4-d-old)	DEG	0.01
		AHB vs. EHB (14-d-old)	DEG	0.03
		Rich vs. Poor Diet	DEG	0.05
		Time Trained: Anticipating vs. Inactive	DEG	0.00E+00
		Vibration Signalers	DEG	0.01
		Arachidonic acid metabolism	KEGG	0.01
		Ether lipid metabolism	KEGG	0.03
		Glycerophospholipid metabolism	KEGG	0.04
		Linoleic acid metabolism	KEGG	0.02
		alpha-Linolenic acid metabolism	KEGG	0.01
		neuromuscular junction development	GO	0
		learning or memory	GO	0
		learning	GO	0
		memory	GO	0.01
		neuromuscular junction	GO	0.01
		synapse organization	GO	0
		cognition	GO	0
		synaptic growth at neuromuscular junction	GO	0
dl / dorsal	134			
		AHB vs. EHB Soldiers (Indiv.)	DEG	0.04
		Time Trained: Anticipating vs. Inactive	DEG	0.04
		Time Trained: Spatiotemporal Memories	DEG	0
		Time Trained: Spatiotemporal Memories	DEG	0.00E+00
		10-d-old vs. 15-d-old	DEG	0.04
bun / bunched	133			
		dl	*cis*	0.01
		Scout vs. Recruit	DEG	0.05
		Time Trained: Anticipating vs. Inactive	DEG	0.02
		Folate biosynthesis	KEGG	0.01
		Spliceosome	KEGG	0.04
		pole plasm oskar mRNA localization	GO	0.01
Trl / Trithorax-like	131			
		Neuroactive ligand-receptor interaction	KEGG	0.01
		diuretic hormone receptor activity	GO	0.01

		3-5 exonuclease activity	GO	0.01
		glial cell development	GO	0
		establishment of glial blood-brain barrier	GO	0
		Trl Network	Dmel	0.04
cg / combgap	131			
		4-d-old vs. 14-d-old	DEG	0.01
		amino acid transmembrane transport	GO	0
		G-protein coupled receptor activity	GO	0.01
		integral to plasma membrane	GO	0.01
		sodium ion transport	GO	0
		drug transmembrane transport	GO	0
		taste receptor activity	GO	0.01
		monovalent inorganic cation transport	GO	0
		biotin transport	GO	0
		integral to membrane	GO	0.01
		metal ion transport	GO	0
		carbohydrate transmembrane transport	GO	0
		ion transmembrane transport	GO	0
		vacuolar transmembrane transport	GO	0
		glutathione transmembrane transport	GO	0
		acetyl-CoA transmembrane transport	GO	0
		coenzyme A transmembrane transport	GO	0
		FAD transmembrane transport	GO	0
		heme transmembrane transport	GO	0
		NAD transmembrane transport	GO	0
		nicotinamide mononucleotide transmembrane transport	GO	0
		carbon dioxide transmembrane transport	GO	0
		sterol transmembrane transport	GO	0
		purine nucleoside transmembrane transport	GO	0
		dipeptide transmembrane transport	GO	0
		tripeptide transmembrane transport	GO	0
		boron transmembrane transport	GO	0
		vitamin transmembrane transport	GO	0
		transmembrane transport	GO	0
		intracellular protein transmembrane transport	GO	0.01
CG9776 / CG9776	108			
		Queen Mandibular Pheromone	DEG	0
		Time Trained: Spatiotemporal Memories	DEG	0.04
Vps45 / Vps45	106			
		V_DEC_Q1	*cis*	0.01
		Vitellogenin RNAi	DEG	0

		AHB vs. EHB Forager (Indiv.)	DEG	0.05
		Aminoacyl-tRNA biosynthesis	KEGG	0.04
		Fatty acid metabolism	KEGG	0.02
		tRNA 5-leader removal	GO	0
		tRNA splicing, via endonucleolytic cleavage and ligation	GO	0
		tRNA metabolic process	GO	0
		tRNA modification	GO	0.00E+00
		tRNA processing	GO	0.00E+00
		cation-transporting ATPase activity	GO	0
		ncRNA processing	GO	0
		tRNA 3-end processing	GO	0
CG7786 / CG7786	98			
		10-d-old vs. 15-d-old	DEG	0.03
		4-d-old vs. 14-d-old	DEG	0.01
		Neuroactive ligand-receptor interaction	KEGG	0
		taste receptor activity	GO	0
CG6769 / CG6769	97			
		Cysteine and methionine metabolism	KEGG	0.02
		RNA polymerase	KEGG	0.03
		Spliceosome	KEGG	0.03
		DNA helicase activity	GO	0.01
		DNA metabolic process	GO	0.01
		DNA amplification	GO	0.01
		S-methyltransferase activity	GO	0
		S-adenosylmethionine-dependent methyltransferase activity	GO	0
		selenocysteine methyltransferase activity	GO	0
		transferase activity, transferring one-carbon groups	GO	0.01
		ATP-dependent 5-3 RNA helicase activity	GO	0.01
		ATP-dependent 3-5 RNA helicase activity	GO	0.01
fd85E / fd85E	94			
		AHB vs. EHB (4-d-old)	DEG	0
		AHB vs. EHB (14-d-old)	DEG	0
		Butanoate metabolism	KEGG	0.03
		Fatty acid elongation in mitochondria	KEGG	0
		Fatty acid metabolism	KEGG	0.04
		Valine, leucine and isoleucine degradation	KEGG	0.05
Su(var)2-10 / Suppressor of variegation 2-10	94			
		V_E2F1_Q4_01	*cis*	0.00E+00
		AHB vs. EHB Soldiers (Colony)	DEG	0.00E+00
		Alarm Pheromone	DEG	0
		Foraging Experience	DEG	0

		Glycerolipid metabolism	KEGG	0.04
		DNA helicase activity	GO	0
		RNA helicase activity	GO	0.01
		peptidyl-prolyl cis-trans isomerase activity	GO	0
		endosome	GO	0
		protein folding	GO	0
		de novo protein folding	GO	0
		protein thiol-disulfide exchange	GO	0
		post-chaperonin tubulin folding pathway	GO	0
		cis-trans isomerase activity	GO	0
		protein maturation by protein folding	GO	0
		ATP-dependent 5-3 RNA helicase activity	GO	0
		ATP-dependent 3-5 RNA helicase activity	GO	0
		protein folding in endoplasmic reticulum	GO	0
		protein refolding	GO	0
		chaperone-mediated protein folding	GO	0
		Su(var)2-10 Network	Dmel	0
YL-1	84			
		YL-1 Network	Dmel	0
su(Hw) / suppressor of Hairy wing	80			
		Valine, leucine and isoleucine degradation	KEGG	0.03
		cellular protein complex assembly	GO	0
ewg / erect wing	79			
		Fructose and mannose metabolism	KEGG	0.01
CG15011 / CG15011	78			
		Time Trained: Anticipating vs. Inactive	DEG	0.05
		Time Trained: Spatiotemporal Memories	DEG	0
HLH106 / Helix loop helix protein 106	76			
		V_STAT1_02	*cis*	0
		Vitellogenin RNAi	DEG	0
		Citrate cycle (TCA cycle)	KEGG	0.03
		Glycine, serine and threonine metabolism	KEGG	0.01
		Pyruvate metabolism	KEGG	0.02
		structural constituent of cytoskeleton	GO	0
		serine-type peptidase activity	GO	0.01
		mitochondrial part	GO	0.01
		oxidation reduction	GO	0
		cellular metabolic process	GO	0.01
woc / without children	76			
		Endocytosis	KEGG	0.04
		Peroxisome	KEGG	0.02

		serine-type peptidase activity	GO	0.01
		protein import	GO	0
sd / scalloped	73			
		Time Trained: Anticipating vs. Inactive	DEG	0.00E+00
		Time Trained: Spatiotemporal Memories	DEG	0
		5,10-methylenetetrahydrofolate-dependent methyltransferase activity	GO	0
tgo / tango	73			
		VNRYTAATGRBM	*cis*	0
		lig vs. mel (10-d-old)	DEG	0.02
		Glutathione metabolism	KEGG	0.01
CG15715 / CG15715	71			
		Queen Mandibular Pheromone	DEG	0
		structural molecule activity	GO	0
		translation regulator activity	GO	0.01
adp / adipose	71			
		Vitellogenin RNAi	DEG	0
		Methane metabolism	KEGG	0
		synaptic transmission	GO	0.01
CG17829 / CG17829	70			
		V_MAF_Q6_01	*cis*	0.04
E2f / E2F transcription factor	68			
		4-d-old vs. 14-d-old	DEG	0
		integral to plasma membrane	GO	0
		integral to membrane	GO	0
		intrinsic to membrane	GO	0
		integral to organelle membrane	GO	0
		integral to thylakoid membrane	GO	0
		integral to cell outer membrane	GO	0
Ets97D / Ets at 97D	68			
		kni	*cis*	0
		AHB vs. EHB Soldiers (Colony)	DEG	0.01
		Alarm Pheromone	DEG	0.02
		Foraging Experience	DEG	0
		flight behavior	GO	0
		adult locomotory behavior	GO	0.01
CG9139 / CG9139	65			
		V_TEF1_Q6	*cis*	0
		Foraging Experience	DEG	0.04
		4-d-old vs. 14-d-old	DEG	0.01
		Aminoacyl-tRNA biosynthesis	KEGG	0
		Endocytosis	KEGG	0.01

		ATP-dependent DNA helicase activity	GO	0.01
		aminoacyl-tRNA ligase activity	GO	0
		tRNA aminoacylation for protein translation	GO	0
Dp / DP transcription factor	65			
		Eip74EF	*cis*	0.03
		AHB vs. EHB (4-d-old)	DEG	0
		AHB vs. EHB (14-d-old)	DEG	0
		Time Trained: Anticipating vs. Inactive	DEG	0.01
		Endocytosis	KEGG	0.03
		epidermal cell differentiation	GO	0
		cell differentiation	GO	0.01
		actin nucleation	GO	0.01
		Dp Network	Dmel	0.01
CG32121 / CG32121	63			
		Queen Mandibular Pheromone	DEG	0.01
		Time Trained: Anticipating vs. Inactive	DEG	0
		Endocytosis	KEGG	0.04
		activation of MAPKK activity	GO	0.01
		activation of MAPK activity	GO	0.01
		inhibitory G-protein coupled receptor phosphorylation	GO	0.01
		protein kinase activity	GO	0
		protein histidine kinase activity	GO	0
		protein serine/threonine kinase activity	GO	0
		protein serine/threonine/tyrosine kinase activity	GO	0
		protein tyrosine kinase activity	GO	0.01
		pyruvate dehydrogenase (acetyl-transferring) kinase activity	GO	0
		DNA damage induced protein phosphorylation	GO	0
		common-partner SMAD protein phosphorylation	GO	0.01
		I-kappaB phosphorylation	GO	0.01
		JUN phosphorylation	GO	0.01
		activation of caspase activity by protein amino acid phosphorylation	GO	0.01
		[isocitrate dehydrogenase (NADP+)] kinase activity	GO	0
		calcium-dependent protein kinase activity	GO	0
		regulation of translational initiation by eIF2 alpha phosphorylation	GO	0.01
		histone phosphorylation	GO	0.01
		phosphotransferase activity, alcohol group as acceptor	GO	0
		peptidyl-serine phosphorylation	GO	0.01
		peptidyl-histidine phosphorylation	GO	0.01
		peptidyl-threonine phosphorylation	GO	0.01
		peptidyl-tyrosine phosphorylation	GO	0.01
		peptidyl-arginine phosphorylation	GO	0.01

		peptidyl-aspartic acid phosphorylation	GO	0.01
		peptidyl-cysteine phosphorylation	GO	0.01
		transmembrane receptor protein kinase activity	GO	0.01
		cyclin-dependent protein kinase activating kinase activity	GO	0
		actin phosphorylation	GO	0.01
		histone kinase activity	GO	0
		post-translational protein modification	GO	0.01
		regulation of imaginal disc growth	GO	0
		regulation of organ growth	GO	0
		protein amino acid autophosphorylation	GO	0.01
		[hydroxymethylglutaryl-CoA reductase (NADPH)] kinase activity	GO	0
		[3-methyl-2-oxobutanoate dehydrogenase (acetyl-transferring)] kinase activity	GO	0
		beta-adrenergic receptor kinase activity	GO	0
		caldesmon kinase activity	GO	0
		dephospho-[reductase kinase] kinase activity	GO	0
		low-density-lipoprotein-receptor kinase activity	GO	0
		rhodopsin kinase activity	GO	0
		tau-protein kinase activity	GO	0
		tropomyosin kinase activity	GO	0
		[tyrosine 3-monooxygenase] kinase activity	GO	0
		[acetyl-CoA carboxylase] kinase activity	GO	0
		pathway-restricted SMAD protein phosphorylation	GO	0.01
NC2&bgr; / NC2&bgr;	63			
		Vitellogenin RNAi	DEG	0.02
		Time Trained: Anticipating vs. Inactive	DEG	0.01
		Time Trained: Spatiotemporal Memories	DEG	0.00E+00
		Endocytosis	KEGG	0.04
		RNA degradation	KEGG	0.03
		actin filament-based process	GO	0
		plasma membrane part	GO	0
fru / fruitless	61			
		YYWWTTA	*cis*	0.00E+00
		AHB vs. EHB (4-d-old)	DEG	0.00E+00
		AHB vs. EHB (14-d-old)	DEG	0.00E+00
		Rich vs. Poor Diet	DEG	0.01
		Vitellogenin RNAi	DEG	0.01
		Time Trained: Anticipating vs. Inactive	DEG	0.00E+00
		Vibration Signalers	DEG	0.01
		Time Trained: Morning vs. Afternoon	DEG	0.03
		Wnt signaling pathway	KEGG	0.02
		plasma membrane part	GO	0

Pbp95 / Pbp95	59			
		V_CREL_01	*cis*	0
		AHB vs. EHB Soldiers (Colony)	DEG	0.01
		metallopeptidase activity	GO	0.01
		protein methyltransferase activity	GO	0
bigmax / bigmax	59			
		Time Trained: Anticipating vs. Inactive	DEG	0.03
CG14711 / CG14711	58			
		MTTWGRCTT	*cis*	0.04
		AHB vs. EHB Soldiers (Colony)	DEG	0.01
		Forager vs. Nurse (old, SCC)	DEG	0.01
		anatomical structure formation involved in morphogenesis	GO	0
		CG14711 Network	Dmel	0
CG11294 / CG11294	55			
		4-d-old vs. 14-d-old	DEG	0.01
		Homologous recombination	KEGG	0.01
nej / nejire	53			
		RAKWYWAV	*cis*	0.01
		Vitellogenin RNAi	DEG	0.01
		Time Trained: Anticipating vs. Inactive	DEG	0
		AHB vs. EHB (4-d-old)	DEG	0.01
		AHB vs. EHB (14-d-old)	DEG	0.01
		Vitellogenin RNAi	DEG	0
		Time Trained: Anticipating vs. Inactive	DEG	0
		Wnt signaling pathway	KEGG	0.02
		behavior	GO	0
		adult behavior	GO	0
		sarcolemma	GO	0
		plasma membrane part	GO	0
		unfolded protein binding	GO	0.01
		chaperone binding	GO	0.01
Deaf1 / Deformed epidermal autoregulatory factor-1	52			
		Alarm Pheromone	DEG	0.04
		Foraging Experience	DEG	0
		nitrogen compound metabolic process	GO	0
		catabolic process	GO	0
		biosynthetic process	GO	0
		oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	GO	0
		organophosphate metabolic process	GO	0
		secondary metabolic process	GO	0

		pigment metabolic process	GO	0
		hormone metabolic process	GO	0
		macromolecule metabolic process	GO	0
		multi-organism metabolic process	GO	0
		multicellular organismal metabolic process	GO	0
		primary metabolic process	GO	0
		small molecule metabolic process	GO	0
		respiratory burst	GO	0
		organic substance metabolic process	GO	0
		Deaf1 Network	Dmel	0
lilli / lilliputian	52			
		TAATTAA	*cis*	0.02
		AHB vs. EHB Soldiers (Indiv.)	DEG	0.03
		AHB vs. EHB (4-d-old)	DEG	0.01
		AHB vs. EHB (14-d-old)	DEG	0.01
		Time Trained: Anticipating vs. Inactive	DEG	0
pnt / pointed	49			
		MMRCAWGT	*cis*	0.03
		AHB vs. EHB (4-d-old)	DEG	0
		AHB vs. EHB (14-d-old)	DEG	0.01
		Queen Mandibular Pheromone	DEG	0.01
		Time Trained: Anticipating vs. Inactive	DEG	0.00E+00
		Vibration Signalers	DEG	0.02
		behavior	GO	0.01
		learning or memory	GO	0
		learning	GO	0
		memory	GO	0
		associative learning	GO	0
		olfactory learning	GO	0
		olfactory behavior	GO	0
		neuron projection	GO	0
		cognition	GO	0
Bgb / Big brother	47			
		hunchback	*cis*	0
		Queen Mandibular Pheromone	DEG	0
		Time Trained: Anticipating vs. Inactive	DEG	0.00E+00
		Wnt signaling pathway	KEGG	0
CTCF / CTCF	47			
		V_STAT1_02	*cis*	0.02
		AHB vs. EHB Soldiers (Colony)	DEG	0.00E+00
		protein import into nucleus, translocation	GO	0.01

		receptor signaling protein activity	GO	0.01
		phagocytosis, engulfment	GO	0.01
		protein complex	GO	0.01
		engulfment of apoptotic cell	GO	0.01
		CTCF Network	Dmel	0
Ssb-c31a / Single stranded-binding protein c31A	46			
		sna	*cis*	0.06
		lig vs. mel (10-d-old)	DEG	0.03
		lig vs. mel (15-d-old)	DEG	0
		AHB vs. EHB (4-d-old)	DEG	0.00E+00
		AHB vs. EHB (14-d-old)	DEG	0.00E+00
		Time Trained: Anticipating vs. Inactive	DEG	0.01
		Basal transcription factors	KEGG	0.01
		N-Glycan biosynthesis	KEGG	0.03
		Nucleotide excision repair	KEGG	0.04
		structural constituent of ribosome	GO	0.01
		transcription initiation	GO	0
		transcription initiation from RNA polymerase II promoter	GO	0
		RNA polymerase II transcription mediator activity	GO	0
		dephosphorylation of RNA polymerase II C-terminal domain	GO	0.01
Tkr / Tyrosine kinase-related protein	46			
		G-protein coupled receptor protein signaling pathway	GO	0.01
		signaling pathway	GO	0
		intracellular signal transduction	GO	0.01
pan / pangolin	46			
		WRNATKTNT	*cis*	0.00E+00
		Time Trained: Anticipating vs. Inactive	DEG	0.00E+00
		Alarm Pheromone	DEG	0.02
		intrinsic to plasma membrane	GO	0
		pan motif	Dmel	0.08
l(2)k10201 / lethal (2) k10201	45			
		pan	*cis*	0
		Queen Mandibular Pheromone	DEG	0
		Time Trained: Anticipating vs. Inactive	DEG	0.04
CG9876 / CG9876	44			
		V_EGR3_01	*cis*	0.03
		actin binding	GO	0.01
		actin monomer binding	GO	0
		cytoskeleton organization	GO	0
		actin cytoskeleton organization	GO	0.01
		actin filament binding	GO	0

Dsp1 / Dorsal switch protein 1	44			
		V_CDP_02	*cis*	0.02
		Time Trained: Anticipating vs. Inactive	DEG	0.00E+00
		calcium ion binding	GO	0.01
		locomotory behavior	GO	0
		Dsp1 Network	Dmel	0.04
Dref / DNA replication-related element factor	42			
		CAYNTGT	*cis*	0.06
		Queen Mandibular Pheromone	DEG	0.05
		Dref Network	Dmel	0.04
slp2 / sloppy paired 2	42			
		cad	*cis*	0.03
		AHB vs. EHB (4-d-old)	DEG	0.02
		N-Glycan biosynthesis	KEGG	0.02
		Proteasome	KEGG	0
		protein secretion	GO	0
		carbohydrate biosynthetic process	GO	0
		protein import	GO	0.01
		lactoferrin transport	GO	0
		transferrin transport	GO	0
		glycoprotein transport	GO	0
		lipoprotein transport	GO	0
		bacteriocin transport	GO	0
		protein transport by the Sec complex	GO	0
		protein transport by the Tat complex	GO	0
		establishment of protein localization	GO	0
		translocation of peptides or proteins into other organism during symbiotic interaction	GO	0
		protein transport in within extracellular region	GO	0
aop / anterior open	41			
		br.Z4	*cis*	0
		AHB vs. EHB (4-d-old)	DEG	0.03
		Rich vs. Poor Diet	DEG	0
		Time Trained: Anticipating vs. Inactive	DEG	0.00E+00
		DNA replication checkpoint	GO	0.01
		signal transduction during filamentous growth	GO	0.01
		prospore membrane	GO	0
		plasma membrane	GO	0
		cytoskeleton organization	GO	0
		dorsal closure	GO	0
		membrane	GO	0

		morphogenesis of embryonic epithelium	GO	0.01
		outer membrane	GO	0
		neuronal signal transduction	GO	0.01
		signal transmission	GO	0.01
		organelle membrane	GO	0
		regulation of conjugation with cellular fusion by signal transduction	GO	0.01
		pre-autophagosomal structure membrane	GO	0
		photosynthetic membrane	GO	0
		nuclear membrane-endoplasmic reticulum network	GO	0
		photoreceptor outer segment membrane	GO	0
		presynaptic membrane	GO	0
		postsynaptic membrane	GO	0
		coated membrane	GO	0
		regulation of cellular process	GO	0
		photoreceptor inner segment membrane	GO	0
		SMAD protein signal transduction	GO	0.01
		regulation of floral organ abscission by signal transduction	GO	0.01
		peptidase activity, acting on L-amino acid peptides	GO	0
		aop Network	Dmel	0.01
oc / ocelliless	41			
		V_LEF1_Q2	*cis*	0.04
		4-d-old vs. 14-d-old	DEG	0.00E+00
		Glycerolipid metabolism	KEGG	0
		Glycerophospholipid metabolism	KEGG	0
		Phosphatidylinositol signaling system	KEGG	0.00E+00
CG30420 / CG30420	40			
		YGYGGTY	*cis*	0
kay / kayak	40			
		Time Trained: Anticipating vs. Inactive	DEG	0.00E+00
		Time Trained: Spatiotemporal Memories	DEG	0.00E+00
		Forager vs. Nurse (young, SCC)	DEG	0.04
vri / vrille	40			
		I_EN_Q6	*cis*	0.05
		Aminoacyl-tRNA biosynthesis	KEGG	0.03
		Glycerophospholipid metabolism	KEGG	0.05
		cellular homeostasis	GO	0.01
Mtp / Mtp	39			
		ap	*cis*	0
		AHB vs. EHB (4-d-old)	DEG	0.03
		Queen Mandibular Pheromone	DEG	0.02
		Time Trained: Anticipating vs. Inactive	DEG	0.00E+00

		Scout vs. Recruit	DEG	0.01
		Wnt signaling pathway	KEGG	0.01
		cell adhesion involved in retrograde extension	GO	0.01
		dorsal closure	GO	0
		enzyme activator activity	GO	0
		morphogenesis of embryonic epithelium	GO	0
		biological adhesion	GO	0.01
		cell-substrate adhesion	GO	0.01
		cell adhesion mediated by integrin	GO	0.01
		cell adhesion involved in biofilm formation	GO	0.01
		plasma membrane part	GO	0.01
		embryonic morphogenesis	GO	0.01
		cell adhesion involved in prostatic bud elongation	GO	0.01
maf-S /	39			
		sna	*cis*	0
		lig vs. mel (10-d-old)	DEG	0
		lig vs. mel (15-d-old)	DEG	0
		RNA polymerase	KEGG	0.01
rib / ribbon	37			
CG9890 / CG9890	36			
		Bapx1	*cis*	0.04
		AHB vs. EHB (4-d-old)	DEG	0
		AHB vs. EHB (14-d-old)	DEG	0
		intrinsic to plasma membrane	GO	0.01
		integral to external side of plasma membrane	GO	0.01
Eip93F /	36			
		electron transport chain	GO	0.01
		oxidative demethylation	GO	0.01
		oxidative deethylation	GO	0.01
crc / cryptocephal	36			
		I_OVO_01	*cis*	0.01
		High vs. Low Pollen Hoarding	DEG	0.04
		Vitellogenin RNAi	DEG	0
		AHB vs. EHB Forager (Colony)	DEG	0.04
		Time Trained: Anticipating vs. Inactive	DEG	0
		Peroxisome	KEGG	0.01
ap / apterous	34			
		I_DRI_01	*cis*	0
		Time Trained: Anticipating vs. Inactive	DEG	0.04
		endopeptidase activity	GO	0.01
		metalloendopeptidase activity	GO	0

		metal ion binding	GO	0
		ap Motif	Dmel	0
CG12029 / CG12029	29			
		V_CDPCR1_01	*cis*	0.04
		Neuroactive ligand-receptor interaction	KEGG	0
CG16779 / CG16779	27			
HLHm&bgr; / E(spl) region transcript m&bgr;	25			
		MTTWGRCTT	*cis*	0.02
		Rich vs. Poor Diet	DEG	0
		Queen Mandibular Pheromone	DEG	0.01
		Time Trained: Anticipating vs. Inactive	DEG	0.00E+00
gem / gemini	25			
		grh	*cis*	0.02
		Queen Mandibular Pheromone	DEG	0.03
		Time Trained: Anticipating vs. Inactive	DEG	0.02
		Vibration Signalers	DEG	0.01
		Time Trained: Anticipating vs. Inactive	DEG	0.01
CG34422 / CG34422	24			
		br.Z3	*cis*	0.04
		AHB vs. EHB Guards (Colony)	DEG	0
		AHB vs. EHB Soldiers (Colony)	DEG	0.00E+00
		Vitellogenin RNAi	DEG	0.01
		AHB vs. EHB Forager (Colony)	DEG	0
		oocyte fate determination	GO	0.00E+00
AP-2 /	23			
		VSNKTDATKRCNV	*cis*	0
fd59A / forkhead domain 59A	23			
		TAATTAA	*cis*	0.01
Alh / Alhambra	22			
		br.Z1	*cis*	0
		Time Trained: Anticipating vs. Inactive	DEG	0.00E+00
CG32532 / CG32532	22			
		I_GAGAFACTOR_Q6	*cis*	0.02
		High vs. Low Pollen Hoarding	DEG	0.01
		Vitellogenin RNAi	DEG	0.02
CG33695 / CG33695	22			
		V_TCF11_01	*cis*	0.02
		AHB vs. EHB (4-d-old)	DEG	0.01
		AHB vs. EHB (14-d-old)	DEG	0.01
		Forager vs. Nurse (typical)	DEG	0.04
		Time Trained: Anticipating vs. Inactive	DEG	0.02

		Proteasome	KEGG	0.01
CG6854 / CG6854	22			
		V_HMEF2_Q6	*cis*	0.01
		Vitellogenin RNAi	DEG	0.02
		CG6854 Motif	Dmel	0.01
ecd / ecdysoneless	21			
psq / pipsqueak	21			
		br.Z1	*cis*	0.01
AM04324	20			
		Adf1	*cis*	0.08
Max /	20			
		V_AML_Q6	*cis*	0
		Max Network	Dmel	0
abo / abnormal oocyte	20			
		V_GR_01	*cis*	0.06
blos1 / blos1	19			
		V_CREL_01	*cis*	0
		lig vs. mel (10-d-old)	DEG	0
		lig vs. mel (15-d-old)	DEG	0.01
		AHB vs. EHB (4-d-old)	DEG	0.03
		AHB vs. EHB (14-d-old)	DEG	0
		Queen Mandibular Pheromone	DEG	0.02
		Scout vs. Recruit	DEG	0.02
		RNA degradation	KEGG	0
		Spliceosome	KEGG	0
Oli / Olig family	19			
		VTKASTCAK	*cis*	0.02
		Queen Mandibular Pheromone	DEG	0.02
Fer2 / 48 related 2	18			
		V_TCF11MAFG_01	*cis*	0.01
jing /	18			
		br.Z4	*cis*	0.04
mbf1 / multiprotein bridging factor 1	18			
		CTYAAGTGG	*cis*	0.06
		Time Trained: Anticipating vs. Inactive	DEG	0.03
		Vibration Signalers	DEG	0.01
		Oxidative phosphorylation	KEGG	0.02
		mitochondrial membrane part	GO	0
CG11414 / CG11414	17			
CG11641 / CG11641	17			
		RAKWYWAV	*cis*	0.01

E2f2 / E2F transcription factor 2	17			
		I_BRCZ1_01	*cis*	0.03
		Alarm Pheromone	DEG	0.01
Stat92E / Signal-transducer and activator of transcription protein at 92E	17			
		byn	*cis*	0
		AHB vs. EHB (4-d-old)	DEG	0.01
		AHB vs. EHB (14-d-old)	DEG	0.05
		structural constituent of ribosome	GO	0
		translation	GO	0.01
		mitochondrial translation	GO	0.01
		plastid translation	GO	0.01
Xbp1 / X box binding protein-1	17			
		V_PIT1_Q6	*cis*	0.02
		Forager vs. Nurse (typical)	DEG	0.03
		AHB vs. EHB Forager (Colony)	DEG	0.02
		Time Trained: Anticipating vs. Inactive	DEG	0
		Vibration Signalers	DEG	0.01
A3-3 / A3-3	16			
		V_AP1_Q6_01	*cis*	0.09
		Time Trained: Anticipating vs. Inactive	DEG	0.01
		reproductive developmental process	GO	0
BtbVII / BTB-protein-VII	16			
		Scout vs. Recruit	DEG	0
CG12071 / CG12071	16			
		I_ELF1_01	*cis*	0.04
		Vitellogenin RNAi	DEG	0.03
		neurotransmitter transport	GO	0
		protein transport	GO	0
MEP-1 / MEP-1	16			
		CACGCG	*cis*	0
		Vitellogenin RNAi	DEG	0.01
		4-d-old vs. 14-d-old	DEG	0.01
		chromosomal part	GO	0
D / Dichaete	16			
		RTATATRTA	*cis*	0.07
		4-d-old vs. 14-d-old	DEG	0
Ets65A / Ets at 65A	16			
		V_FOXD3_01	*cis*	0.00E+00
		Rich vs. Poor Diet	DEG	0.01
		Vitellogenin RNAi	DEG	0.01
		Queen Mandibular Pheromone	DEG	0.01

		Time Trained: Anticipating vs. Inactive	DEG	0.00E+00
		Scout vs. Recruit	DEG	0.02
eg / eagle	16			
		Adf1	*cis*	0.05
		Endocytosis	KEGG	0
Mio / Mlx interactor	15			
		SPI1	*cis*	0.02
		Alarm Pheromone	DEG	0
		Queen Mandibular Pheromone	DEG	0
crp / cropped	15			
		exd	*cis*	0
		AHB vs. EHB (4-d-old)	DEG	0.01
		AHB vs. EHB (14-d-old)	DEG	0
		microtubule associated complex	GO	0
emc / extra macrochaetae	15			
		I_EVE_Q6	*cis*	0.02
unpg / unplugged	15			
		Dref	*cis*	0
		4-d-old vs. 14-d-old	DEG	0
Eip74EF / Ecdysone-induced protein 74EF	14			
		V_STAT5B_01	*cis*	0.01
		Time Trained: Anticipating vs. Inactive	DEG	0
		Eip74EF Motif	Dmel	0.09
CG42748 / CG42748	13			
		VVVBTAATCC	*cis*	0.01
		lig vs. mel (10-d-old)	DEG	0.01
		lig vs. mel (15-d-old)	DEG	0
		Time Trained: Anticipating vs. Inactive	DEG	0.01
Nf-YA / Nf-YA	13			
		KNMTTATSVNH	*cis*	0
		Vitellogenin RNAi	DEG	0.03
Med / Medea	12			
		V_TFIIA_Q6	*cis*	0.01
Optix / Optix	12			
		pan	*cis*	0.01
		4-d-old vs. 14-d-old	DEG	0
		establishment of localization	GO	0.01
al / aristaless	12			
		GGRNWTTCC	*cis*	0
		4-d-old vs. 14-d-old	DEG	0
chn / charlatan	12			

		RXR.VDR	*cis*	0.02
vnd / ventral nervous system defective	12			
		Pbx	*cis*	0
		AHB vs. EHB (4-d-old)	DEG	0.01
		AHB vs. EHB (14-d-old)	DEG	0.05
		Scout vs. Recruit	DEG	0.02
		Vibration Signalers	DEG	0.04
CG7368 / CG7368	11			
		RKAAASA	*cis*	0.01
MBD-like / MBD-like	11			
		RTGRGAR	*cis*	0.03
		Scout vs. Recruit	DEG	0
stc / shuttle craft	11			
		RTAAMA	*cis*	0
Ada2b / Ada2b	10			
		byn	*cis*	0.07
		Vitellogenin RNAi	DEG	0.03
		AHB vs. EHB Forager (Colony)	DEG	0.04
CG12769 / CG12769	10			
		E2F1	*cis*	0.06
CG3136 / CG3136	10			
		DDNBKGTDTHDHV	*cis*	0
sima / similar	10			
		AHB vs. EHB (4-d-old)	DEG	0
		AHB vs. EHB (14-d-old)	DEG	0
CG13296 / CG13296	9			
		I_OVO_01	*cis*	0.03
Camta / Calmodulin-binding transcription activator	9			
		AHB vs. EHB (4-d-old)	DEG	0.01
		AHB vs. EHB (14-d-old)	DEG	0.01
		Endocytosis	KEGG	0.01
SoxN / SoxNeuro	9			
		Antp	*cis*	0.01
		AHB vs. EHB (14-d-old)	DEG	0.05
		AHB vs. EHB Forager (Colony)	DEG	0.04
		Time Trained: Anticipating vs. Inactive	DEG	0
p53 /	9			
		AAHKMTHBCA	*cis*	0.03
		Scout vs. Recruit	DEG	0.02
trh / trachealess	9			
		4-d-old vs. 14-d-old	DEG	0.02

tup / tailup	9			
		pan	*cis*	0
Fer3 / 48 related 3	8			
		AAHKMTHBCA	*cis*	0.06
		Time Trained: Anticipating vs. Inactive	DEG	0.04
ash1 / absent, small, or homeotic discs 1	8			
		ANHDDBHGATAASSDNNB	*cis*	0
bs / blistered	8			
		En1	*cis*	0
		AHB vs. EHB (4-d-old)	DEG	0.03
		AHB vs. EHB (14-d-old)	DEG	0.03
cnc / cap-n-collar	8			
		I_CF1_01	*cis*	0.01
		Rich vs. Poor Diet	DEG	0.03
exd / extradenticle	8			
		Cf2.II	*cis*	0.01
		Time Trained: Anticipating vs. Inactive	DEG	0.01
		Time Trained: Spatiotemporal Memories	DEG	0.02
		Exd Motif	Dmel	0.06
CG7015 / CG7015	7			
		AMHGGGTTAH	*cis*	0.01
CG8506 / CG8506	7			
		V_HNF1_Q6_01	*cis*	0.03
HLH4C / Helix loop helix protein 4C	7			
Jra / Jun-related antigen	7			
		p120	*cis*	0
		Queen Mandibular Pheromone	DEG	0.02
Mi-2 /	7			
		HSRGAAAAHYV	*cis*	0.03
Su(H) / Suppressor of Hairless	7			
		br.Z4	*cis*	0.02
		Vibration Signalers	DEG	0.02
onecut / onecut	7			
		AHB vs. EHB (4-d-old)	DEG	0.03
		Scout vs. Recruit	DEG	0.05
CG3815 / CG3815	6			
		br.Z4	*cis*	0.05
		Forager vs. Nurse (old, SCC)	DEG	0.03
Su(var)205 / Suppressor of variegation 205	6			
Usf / Usf	6			
		Arnt.Ahr	*cis*	0.01

bab1 / bric a brac 1	6			
		BEAF.32	*cis*	0.05
		4-d-old vs. 14-d-old	DEG	0.02
		bab1 network	Dmel	0
dac / dachshund	6			
		CTYAAGTGG	*cis*	0
		lig vs. mel (10-d-old)	DEG	0.02
		lig vs. mel (15-d-old)	DEG	0.03
		Time Trained: Anticipating vs. Inactive	DEG	0.04
pros / prospero	6			
		Scout vs. Recruit	DEG	0.03
sim / single-minded	6			
CG32105 / CG32105	5			
CG9418 / CG9418	5			
Clk / Clock	5			
		intracellular membrane-bounded organelle	GO	0.01
MBD-R2 / MBD-R2	5			
		V_GATA6_01	*cis*	0
kin17 / kin17	5			
		Espl	*cis*	0.02
		Alarm Pheromone	DEG	0.02
CG12701 / CG12701	4			
CG3407 / CG3407	4			
		ESR1	*cis*	0.01
		10-d-old vs. 15-d-old	DEG	0.03
CG7099 / CG7099	4			
		CKCAKCWCT	*cis*	0.07
		AHB vs. EHB Soldiers (Colony)	DEG	0
		AHB vs. EHB (14-d-old)	DEG	0.04
Tusp / Tusp	4			
crol / crooked legs	4			
		BHTAAKCYSBV	*cis*	0.01
dmrt93B / doublesex-Mab related 93B	4			
		abd.A	*cis*	0.01
ey / eyeless	4			
		AAATTAA	*cis*	0.06
		Scout vs. Recruit	DEG	0.01
mip120 / Myb-interacting protein 120	4			
		Scout vs. Recruit	DEG	0.04
repo / reversed polarity	4			
CG11456 / CG11456	3			

		ANHDDBHGATAASSDNNB	*cis*	0.02
CG9215 / CG9215	3			
		AHB vs. EHB Soldiers (Colony)	DEG	0.01
Eip78C / Ecdysone-induced protein 78C	3			
Sox100B / Sox100B	3			
		byn	*cis*	0.03
bon / bonus	3			
nau / nautilus	3			
ttk / tramtrack	3			
CG11085 / CG11085	2			
		Rich vs. Poor Diet	DEG	0
CG1832 / CG1832	2			
CG31670 / CG31670	2			
GATAd / GATAd	2			
Hsf / Heat shock factor	2			
Mnf / Mnf	2			
Smox / Smad on X	2			
cbt / cbt	2			
cic / capicua	2			
d4 / d4	2			
pita /	2			
CG1620 / CG1620	1			
CG1845 / CG1845	1			
CG18619 / CG18619	1			
CG30443 / CG30443	1			
CG31224 / CG31224	1			
Gug / Grunge	1			
Hr39 / Hormone receptor-like in 39	1			
Hr96 / Hormone receptor-like in 96	1			
Lmpt / Limpet	1			
Pdp1 / PAR-domain protein 1	1			
cyc / cycle	1			
h / hairy	1			
usp / ultraspiracle	1			
zfh1 / Zn finger homeodomain 1	1			

Table S6. Network hubs influence many different behavioral states.

We list the number of targets for each of the top 20 network hubs that were differentially expressed in each of the 27 behavioral comparisons. For each of the three behavioral categories (aggression, maturation, and foraging) we also describe the average percentage of differentially expressed genes that were members of each of these modules.

		Total DEGs	DEGs in TRN	*Lag1*	*CG9932*	*CrebB*	*CG17912*	*br*	*rn*	*Myb*	*MTA1-like*	*ftz-f1*	*dl*	*bun*	*Trl*	*cg*	*CG9776*	*CG8228*	*CG7786*	*CG6769*	*CG16999*	*Su(var)2-10*	*YL-1*
Aggression	AHB vs. EHB Guards (Ind. Genotype)	217	29	8	4	5	1	0	0	3	4	7	0	3	2	4	0	1	0	1	1	0	2
	AHB vs. EHB Soldiers (Ind. Genotype)	510	72	13	6	6	1	4	3	5	5	2	9	7	3	6	4	2	2	0	1	8	4
	AHB vs. EHB Guards (Col. Genotype)	450	62	7	7	8	1	5	2	8	5	8	2	2	2	4	2	4	1	1	2	7	6
	AHB vs. EHB Soldiers (Col. Genotype)	757	137	17	9	10	3	8	5	17	8	10	5	7	8	6	3	10	1	7	3	23	21
	Alarm Pheromone	430	80	13	3	7	2	7	2	7	12	2	2	7	3	3	2	0	1	2	3	13	5
Maturation	AHB vs. EHB (4-d-old)	3485	804	134	71	58	41	54	83	71	65	72	35	59	58	46	43	46	20	32	48	39	30
	AHB vs. EHB (14-d-old)	3660	804	143	74	61	44	51	83	73	67	71	35	59	55	47	43	48	18	32	47	41	31
	lig. vs. mel. (10-d-old)	2893	804	81	57	37	32	40	50	43	45	49	23	46	34	28	30	42	12	28	23	28	29
	lig. vs. mel. (15-d-old)	2980	804	84	57	37	32	40	55	44	50	52	22	46	33	24	34	44	12	27	23	31	32
	High vs. Low Pollen Hoarding	444	103	16	11	14	19	7	9	12	12	7	3	8	3	5	9	9	1	6	4	8	5
	Young Nurse vs. Old Forager	1278	231	47	24	16	13	16	20	21	26	22	14	25	16	11	11	16	2	9	7	20	13
	Nurse vs. Forager (young)	166	21	2	3	1	0	3	2	3	3	5	0	2	2	2	1	1	0	1	1	1	0
	Nurse vs. Forager (old)	402	73	14	5	6	7	5	3	7	6	6	2	4	4	7	1	2	2	4	6	3	1
	Queen Mandibular Pheromone	740	209	26	18	10	48	7	10	11	24	14	8	16	8	13	17	6	3	6	2	13	2
	Rich vs. Poor Diet	331	804	7	4	1	3	2	3	5	5	10	1	3	4	3	2	0	0	3	2	5	1
	Vitellogenin RNAi	2901	690	119	71	48	42	36	37	38	46	54	36	37	39	33	37	38	26	26	35	37	28
	10- vs. 15-d-old	576	130	24	16	24	19	9	11	5	14	10	12	13	7	10	5	7	10	4	6	1	1
	4- vs. 14-d-old	2131	492	90	70	47	30	35	32	31	30	33	23	29	34	42	25	8	28	29	13	30	18
Foraging	AHB vs. EHB Foragers (Ind. Genotype)	60	5	1	1	0	0	0	1	1	1	0	1	0	0	0	1	2	0	0	0	0	0
	AHB vs. EHB Foragers (Col. Genotype)	316	48	4	2	2	2	4	1	3	8	2	1	3	0	5	1	3	2	1	0	6	4
	Scout vs. Recruit	1041	226	28	11	18	17	15	9	9	15	13	10	22	6	13	10	9	5	9	12	16	12
	Vibration Signalers	711	155	34	9	5	3	6	9	11	12	20	8	8	13	8	7	5	2	0	9	5	3
	Anticipation vs. Inactive	3222	804	132	57	50	43	56	39	47	61	69	47	59	37	44	34	20	15	20	30	30	19
	Morning vs. Afternoon	784	804	35	14	10	6	5	11	13	12	19	11	12	11	7	13	8	1	3	10	4	2
	Spatiotemporal Memories	224	804	16	10	7	1	1	2	0	3	2	16	2	0	5	6	3	3	0	1	1	1
	Foraging Experience	190	804	9	4	3	3	4	1	6	2	1	2	1	2	2	1	0	0	1	2	7	2
	Distance Perception	52	8	1	1	0	1	0	0	0	1	1	0	1	0	1	0	0	1	0	0	1	0
Summary	Targets			394	265	185	181	169	162	154	146	146	134	133	131	131	108	106	98	97	94	94	84
	% of Full TRN			18	12	9	8	8	7	7	7	7	6	6	6	6	5	5	5	4	4	4	4
	Mean % DEGs in Aggression Comp.			15	7	9	2	7	3	10	9	6	5	7	4	6	3	4	2	2	3	14	9
	Mean % DEGs in Maturation Comp.			16	10	8	9	6	7	8	9	9	4	7	6	6	5	5	3	5	4	6	3
	Mean % DEGs in Foraging Comp.			19	8	7	4	6	4	6	8	7	8	6	4	6	5	4	2	2	4	7	4

Table S7. Associations between TFs and behavioral comparisons

This table shows the enrichment P-values summarized Fig. 3C. The rows correspond to each TF and the columns correspond to various conditions. The rows are sorted in descending order based on the number of enrichments for each TF. The subdivisions in the rows correspond to different class of TFs: the top 4 are the global regulators with targets enriched in all 3 behavior classes, followed by those TFs involved in more than 1 class and the last 3 are specific to each behavior.

	MATURATION													AGGRESSION				FORAGING							
dl	1	1	1	1	1	1	1	1	0.04	1	1	1	1	0.04	1	1	1	1	1	0	1	1	1	0.04	1
CG7274	1	1	1	1	1	1	1	1	1	1	0.01	1	1	1	1	0	0	1	1	1	1	0	1	1	1
br	1	1	0.01	0.02	1	1	1	1	1	1	1	1	1	1	0.04	1	1	1	1	1	1	1	1	0	1
lilli	0.01	0.01	1	1	1	1	1	1	1	1	1	1	1	0.03	1	1	1	1	1	1	1	1	1	0	1
fru	0	0	1	1	1	1	0.01	1	1	1	0.01	1	1	1	1	1	1	1	0.03	1	1	1	1	0	0.01
CG30077	0.03	0	0	0.01	1	1	1	1	1	0.02	1	1	1	1	1	1	1	1	1	1	1	1	0.02	1	1
nej	0.01	0.01	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Ets65A	1	1	1	1	1	1	0.01	1	1	0.01	0.01	1	1	1	1	1	1	1	1	1	1	1	0.02	0	1
Ssb-c31a	0	0	0.03	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.01	1
ftz-f1	0.01	0.03	1	1	1	1	0.05	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0.01
pnt	0	0.01	1	1	1	1	1	1	1	0.01	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0.02
CG33695	0.01	0.01	1	1	1	1	1	0.04	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.02	1
CG9342	0.03	1	1	1	1	1	1	1	1	0.02	1	1	1	1	1	1	1	1	1	1	1	1	0.01	0	1
Xbp1	1	1	1	1	1	1	1	0.03	1	1	1	1	1	1	1	1	1	1	1	1	1	0.02	1	0	0.01
crc	1	1	1	1	0.04	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0.04	1	0	1
gem	1	1	1	1	1	1	1	1	1	0.03	1	1	1	1	1	1	1	1	1	1	1	1	1	0.01	0.01
vnd	0.01	0.05	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.02	1	0.04
CG15216	1	1	0.01	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.01	1
Dp	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.01	1
HLHm&bgr;	1	1	1	1	1	1	0	1	1	0.01	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
NC2&bgr;	1	1	1	1	1	1	1	1	1	1	0.02	1	1	1	1	1	1	1	1	0	1	1	1	0.01	1
SoxN	1	0.05	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.04	1	0	1
aop	0.03	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
dac	1	1	0.02	0.03	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.04	1
kay	1	1	1	1	1	1	1	1	1	1	1	1	0.04	1	1	1	1	1	1	0	1	1	1	0	1
Ada2b	1	1	1	1	1	1	1	1	1	1	0.03	1	1	1	1	1	1	1	1	1	1	0.04	1	1	1
Bgb	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
CG32121	1	1	1	1	1	1	1	1	1	0.01	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
CG8228	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0.05	1	1	1	1	1	1	1
CG9139	1	1	1	1	1	0.01	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.04	1	1	1	1
CG9776	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0.04	1	1	1	1	1
MTA1-like	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
l(2)k10201	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0.04	1
onecut	0.03	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.05	1	1
CG14711	1	1	1	1	1	1	1	1	1	1	1	0.01	1	1	1	1	0.01	1	1	1	1	1	1	1	1
CG7099	1	0.04	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
Mio	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
Ets97D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.02	1	0.01	1	1	1	0	1	1	1	1
Su(var)2-10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	0	1	1	1	1
Deaf1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.04	1	1	1	1	1	0	1	1	1	1
pan	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.02	1	1	1	1	1	1	1	1	0	1
CG17912	1	1	1	1	0	1	1	1	0.01	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CG1244	1	1	1	1	1	0.01	1	1	1	1	0.01	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CG16899	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CG32532	1	1	1	1	0.01	1	1	1	1	1	0.02	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CG7786	1	1	1	1	1	0.01	1	1	0.03	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CG9890	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Camta	0.01	0.01	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Myb	0.03	0.04	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Stat92E	0.01	0.05	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
bs	0.03	0.03	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
crp	0.01	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
maf-S	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
rn	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
sima	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
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CG12071	1	1	1	1	1	1	1	1	1	1	0.03	1	1	1	1	1	1	1	1	1	1	1	1	1	1

CG15715	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
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CG3891	1	1	1	1	1	1	1	1	1	1	0.03	1	1	1	1	1	1	1	1	1	1	1	1	1	1
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sd	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1
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Su(H)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.02
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pros	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.03	1	1
AM09256	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
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CG9215	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.01	1	1	1	1	1	1	1	1
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E2f2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.01	1	1	1	1	1	1	1	1	1	1
kin17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.02	1	1	1	1	1	1	1	1	1	1

Table S8. Measures of network dynamics and regulation for the 27 behavioral comparisons

To analyze the possibility of differences in transcriptional regulation acting over different timescales, we divided the 27 comparisons into those that influence behavior over long (hereditary differences, which influence behavior over evolutionary time), medium (maturation and other environmentally-induced differences occurring over weeks), or short (e.g., changes in foraging activity occurring over hours or a few days) timescales (1st column). For each comparison, we list the number of differentially expressed target genes in the TRN (DEGs); the number of modules for which there was significant between-state 'shuffling' in the relative expression of target genes; the average number of TFs regulating each differentially expressed target gene (in-degree); and the number of differentially expressed target genes for each TF (out-degree). These data are summarized in Fig. S9.

	Comparison	DEGs	Shuffled Modules	k_{in} of Targets	k_{out} of TFs
long (hereditary)	**AHB vs. EHB (4-d-old)**	804	0	3.19	14.02
	AHB vs. EHB (14-d-old)	834	0	3.19	14.46
	AHB vs. EHB Soldiers (Individual Genotype)	72	0	3.11	2.43
	AHB vs. EHB Foragers (Individual Genotype)	5	0	3.60	1.06
	***lig.* vs. *mel.* (10-d-old)**	582	5	3.14	10.56
	***lig.* vs. *mel.* (15-d-old)**	593	12	3.15	10.92
	High vs. Low Pollen Hoarding	103	0	3.22	3.22
medium (weeks)	**AHB vs. EHB Guards (Colony Genotype)**	62	0	3.16	2.45
	AHB vs. EHB Soldiers (Colony Genotype)	137	6	3.01	3.90
	AHB vs. EHB Foragers (Colony Genotype)	48	1	3.04	1.83
	10- vs. 15-d-old	130	62	3.15	3.47
	4- vs. 14-d-old	492	87	3.07	8.98
	Young Nurse vs. Old Forager	231	42	3.15	5.64
	Nurse vs. Forager (old)	73	0	2.89	2.24
	Nurse vs. Forager (young)	21	0	3.19	1.43
	Foraging Experience	45	14	3.04	1.90
short (hours/days)	**Alarm Pheromone**	80	0	3.15	2.68
	Queen Mandibular Pheromone	209	0	2.89	4.79
	Rich vs. Poor Diet	66	0	2.83	2.31
	Vitellogenin RNAi	690	0	3.00	11.65
	Vibration Signalers	155	0	2.82	3.77
	Scout vs. Recruit	226	0	3.01	4.93
	Morning vs. Afternoon	146	0	3.10	3.91
	Spatiotemporal Memories	60	0	2.95	2.33
	Anticipation vs. Inactive	804	0	3.05	13.35

Table S9. The hormonally-related TF *Ultraspiracle* binds genomic regions near foraging- and maturation-related TFs

16 of the TFs predicted to regulate maturation and/or foraging are themselves direct targets of the hormonally-related TF *ultraspiracle (usp)*. Genome-wide targets of *usp* protein in the bee were characterized using chromatin immunoprecipitation—genomic tiling microarrays (Ament et al., submitted).

	Foraging-related TFs	**Maturation-related TFs**
1	Eip74EF / Ecdysone-induced protein 74EF	D / Dichaete
2	Ets65A / Ets at 65A	Ets65A / Ets at 65A
3	SoxN / SoxNeuro	SoxN / SoxNeuro
4	ey / eyeless	Stat92E / Signal-transducer and activator of transcription protein at 92E
5	fru / fruitless	bs / blistered
6	lilli / lilliputian	fru / fruitless
7	pan / pangolin	lilli / lilliputian
8	pnt / pointed	oc / ocelliless
9	vnd / ventral nervous system defective	pnt / pointed
10		sima / similar
11		slp2 / sloppy paired 2
12		trh / trachealess
13		vnd / ventral nervous system defective

Table S10. Mushroom body or optic-lobe specific gene expression does not predict TRN membership or accurate modeling of mushroom body gene expression.

Gene co-expression modules in the mammalian brain have been linked to expression in specific sub-regions and cell types the brain. To understand the relationship between whole-brain TRN performance and expression in sub-regions of the honey bee brain, we looked for enrichment of the TRN with genes that were strongly differentially expressed (FDR < 0.05; > 5-fold difference in expression) between two of the largest sub-regions of the brain: the mushroom bodies (MB) and optic lobes (OL). We looked for overlap between these region-specific gene lists among the 2176 genes in the TRN to determine whether region-specific expression improved or reduced our ability to model genes accurately. We also looked for overlap between region-specific gene expression and a list of 550 genes for which the whole brain TRN model could accurately predict ($r > 0.5$) MB gene expression (see Fig. S10). For each pair of gene lists, we report the hypergeometric P-value for enrichment. We found no relationship between region-specific gene expression and our ability to accurately model gene expression. Among the TRN modules, there were a few that were enriched for genes differentially expressed between the two regions, but the TRN as a whole was not dominated by brain region-specific patterns of expression[29]. It was neither enriched nor depleted for genes known to show MB- or OL-specific patterns of gene expression (Table S10, Fig. S10). Moreover, the model performed more poorly when predicting MB-specific gene expression compared to whole brain gene expression (Fig. S11).

Gene lists compared (# of genes)	*P-value*
MB> OL (70) vs. all TRN genes (2176)	0.13
OL > MB (30) vs. all TRN genes (2176)	0.32
MB > OL (70) vs. accurately modeled in MB (550)	0.41
OL > MB (30) vs. accurately modeled in MB (550)	0.26

Table S12. ArrayExpress Accession IDs and microarray count for each of the experiments

The experiments that are in the process of submission are marked as **'pending'**.

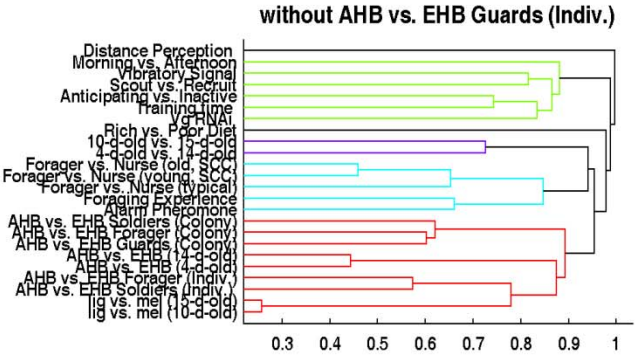
Experiment Name	ArrayExpress Accession	Array Count
AHB vs EHB foragers	E-TABM-605	60
AHB vs EHB guards	E-TABM-606	60
AHB vs EHB soldiers	E-TABM-607	58
alarm pheromone	E-TABM-604	64
Behavioral maturation (AHB-EHB)	E-TABM-953	112
Behavioral maturation (*ligustica/mellifera*)	E-TABM-952	224
distance perception	E-TABM-910	40
Drone-Worker	Pending	100
foraging experience	E-MTAB-482	152
high v. low pollen hoarding	E-MEXP-3079	19
nurse-forager	E-TABM-658	90
queen pheromone	Pending	32
Rich vs. poor diet brains	E-MTAB-507	48
scout behavior	E-MTAB-491	58
time training	E-MTAB-489	76
Vibration signal	E-TABM-608	28
vitellogenin RNAi brains	E-MTAB-490	84
Total		**1305**

Supplementary Figures

Fig. S1. Global analysis of bee brain gene expression and social behavior – Controls and Statistical Significance

We performed several statistics to verify the significance of the hierarchical clustering (Methods, Table S3 and Fig S1) which showed that the obtained behavior-specific clusters were robust. As mentioned in the S. Methods section, we used the used the cophenetic correlation metric to measure how faithfully the tree represents the dissimilarities among observations and found it to be at a very high value of 0.89, suggesting an accurate fit to the data.

We performed clustering with each of the 25 phenotype comparisons removed to check the stability of the clusters. We found that in all the clustering runs, there were clusters specific for aggression and foraging phenotypes, and the maturation phenotypes spread across other remaining clusters. The aggression subcluster for environmental influences sometimes grouped into a separate cluster of their own; nevertheless, all its members were retained in the same cluster. Additional validation and discussion in Table S3 and Methods section on '*Global analysis of bee brain gene expression and social behavior*'.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Distance Perception
Morning vs. Afternoon
Vibratory Signal
Scout vs. Recruit
Anticipating vs. Inactive
Training Time
Vg RNAi
Rich vs. Poor Diet
10-d-old vs. 15-d-old
4-d-old vs. 14-d-old
Forager vs. Nurse (old, SCC)
Forager vs. Nurse (young, SCC)
Forager vs. Nurse (typical)
Foraging Experience
Alarm Pheromone
AHB vs. EHB Soldiers (Colony)
AHB vs. EHB Forager (Colony)
AHB vs. EHB Guards (Colony)
AHB vs. EHB (14-d-old)
AHB vs. EHB (4-d-old)
AHB vs. EHB Forager (Indiv.)
AHB vs. EHB Soldiers (Indiv.)
lig vs. mel (15-d-old)
lig vs. mel (10-d-old)
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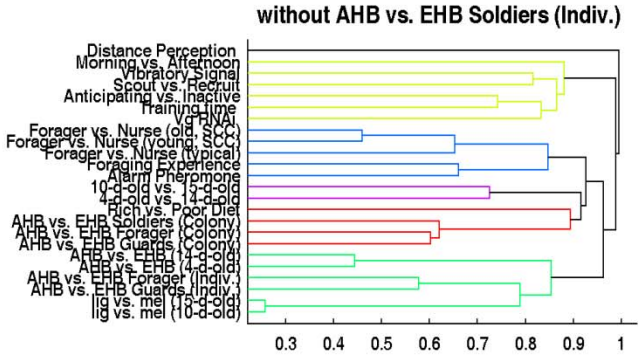
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Vibratory Signal
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Training Time
Vg RNAi
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AHB vs. EHB Guards (Colony)
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AHB vs. EHB (4-d-old)
AHB vs. EHB Forager (Indiv.)
AHB vs. EHB Guards (Indiv.)
lig vs. mel (15-d-old)
lig vs. mel (10-d-old)
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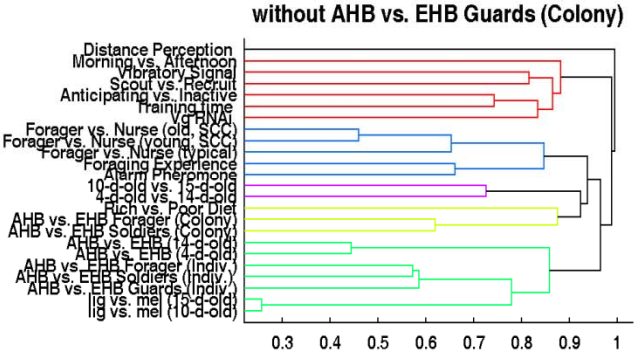
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lig vs. mel (15-d-old)
lig vs. mel (10-d-old)
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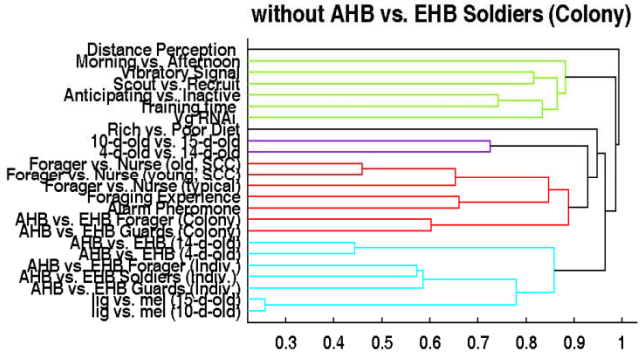
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Training Time
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AHB vs. EHB (4-d-old)
AHB vs. EHB Forager (Indiv.)
AHB vs. EHB Soldiers (Indiv.)
AHB vs. EHB Guards (Indiv.)
lig vs. mel (15-d-old)
lig vs. mel (10-d-old)
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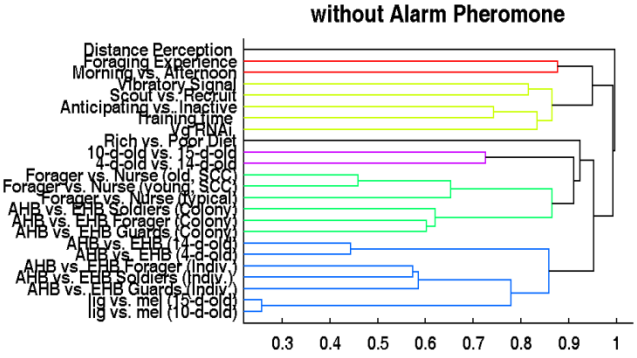
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Training Time
Vg RNAi
Rich vs. Poor Diet
10-d-old vs. 15-d-old
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Forager vs. Nurse (young, SCC)
Forager vs. Nurse (typical)
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AHB vs. EHB (4-d-old)
AHB vs. EHB Forager (Indiv.)
AHB vs. EHB Soldiers (Indiv.)
AHB vs. EHB Guards (Indiv.)
lig vs. mel (15-d-old)
lig vs. mel (10-d-old)
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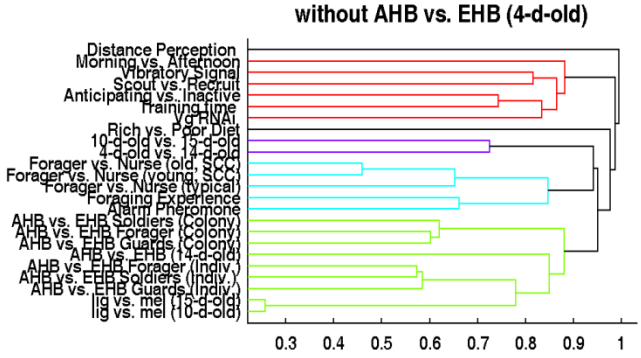
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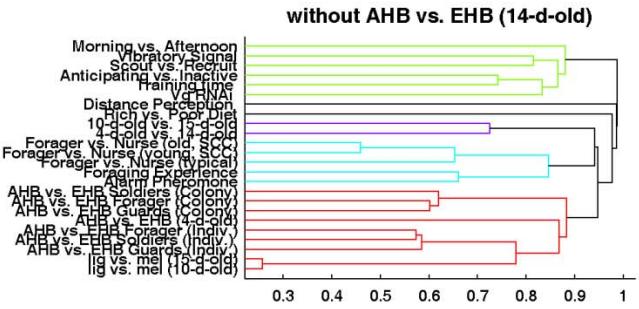
Morning vs. Afternoon
Vibratory Signal
Scout vs. Recruit
Anticipating vs. Inactive
Training Time
Vg RNAi
Rich vs. Poor Diet
10-d-old vs. 15-d-old
4-d-old vs. 14-d-old
Forager vs. Nurse (old, SCC)
Forager vs. Nurse (young, SCC)
Forager vs. Nurse (typical)
Foraging Experience
Alarm Pheromone
AHB vs. EHB Soldiers (Colony)
AHB vs. EHB Forager (Colony)
AHB vs. EHB Guards (Colony)
AHB vs. EHB (14-d-old)
AHB vs. EHB Forager (Indiv.)
AHB vs. EHB Soldiers (Indiv.)
AHB vs. EHB Guards (Indiv.)
lig vs. mel (15-d-old)
lig vs. mel (10-d-old)
0.3 0.4 0.5 0.6 0.7 0.8 0.9 1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Scout vs. Recruit
Anticipating vs. Inactive
Training Time
Vg RNAi
Distance Perception
Rich vs. Poor Diet
10-d-old vs. 15-d-old
4-d-old vs. 14-d-old
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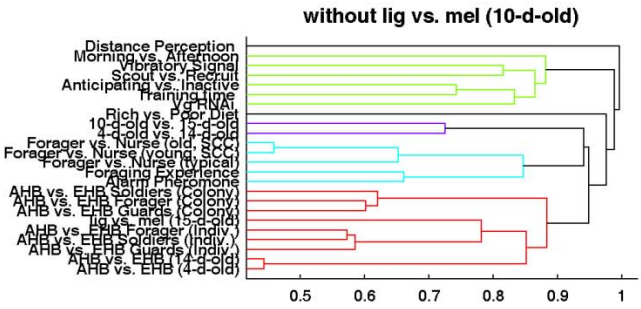
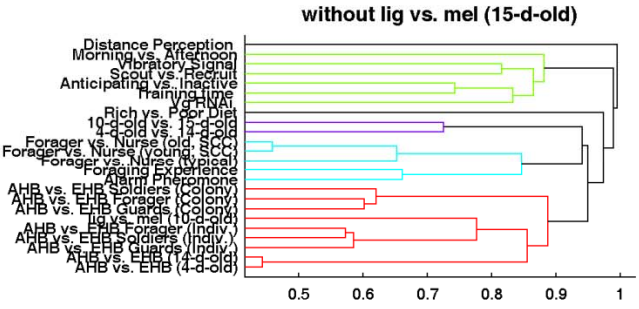
AHB vs. EHB Soldiers (Indiv.)
AHB vs. EHB Guards (Indiv.)
lig vs. mel (15-d-old)
lig vs. mel (10-d-old)
0.3 0.4 0.5 0.6 0.7 0.8 0.9 1


without lig vs. mel (10-d-old)
Distance Perception
Morning vs. Afternoon
Vibratory Signal
Scout vs. Recruit
Anticipating vs. Inactive
Training Time
Vg RNAi
Rich vs. Poor Diet
10-d-old vs. 15-d-old
4-d-old vs. 14-d-old
Forager vs. Nurse (old, SCC)
Forager vs. Nurse (young, SCC)
Forager vs. Nurse (typical)
Foraging Experience
Alarm Pheromone
AHB vs. EHB Soldiers (Colony)
AHB vs. EHB Forager (Colony)
AHB vs. EHB Guards (Colony)
lig vs. mel (15-d-old)
AHB vs. EHB Forager (Indiv.)
AHB vs. EHB Soldiers (Indiv.)
AHB vs. EHB Guards (Indiv.)
AHB vs. EHB (14-d-old)
AHB vs. EHB (4-d-old)
0.5 0.6 0.7 0.8 0.9 1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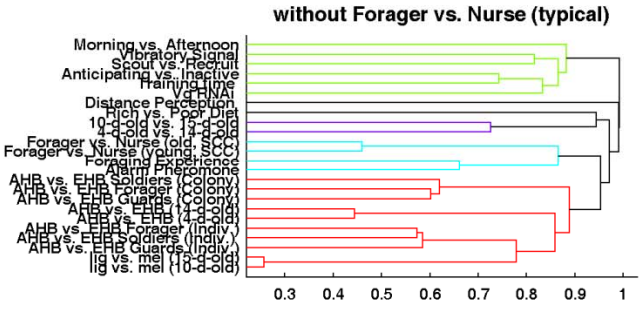
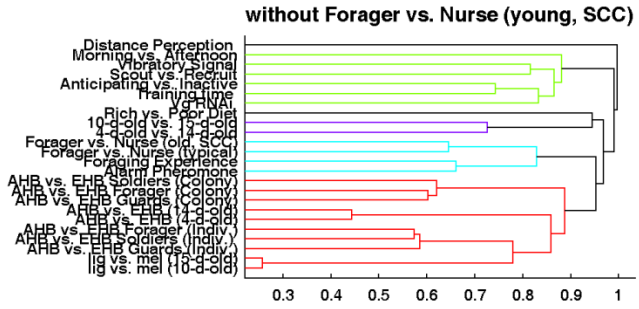
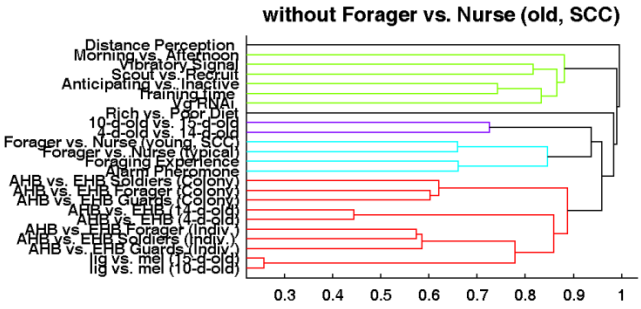
Distance Perception
Morning vs. Afternoon
Vibratory Signal
Scout vs. Recruit
Anticipating vs. Inactive
Training Time
Vg RNAi
Rich vs. Poor Diet
10-d-old vs. 15-d-old
4-d-old vs. 14-d-old
Forager vs. Nurse (old, SCC)
Forager vs. Nurse (young, SCC)
Forager vs. Nurse (typical)
Foraging Experience
Alarm Pheromone
AHB vs. EHB Soldiers (Colony)
AHB vs. EHB Forager (Colony)
AHB vs. EHB Guards (Colony)
lig vs. mel (10-d-old)
AHB vs. EHB Forager (Indiv.)
AHB vs. EHB Soldiers (Indiv.)
AHB vs. EHB Guards (Indiv.)
AHB vs. EHB (14-d-old)
AHB vs. EHB (4-d-old)
0.5 0.6 0.7 0.8 0.9 1


without Forager vs. Nurse (typical)
Morning vs. Afternoon
Vibratory Signal
Scout vs. Recruit
Anticipating vs. Inactive
Training Time
Vg RNAi
Distance Perception
Rich vs. Poor Diet
10-d-old vs. 15-d-old
4-d-old vs. 14-d-old
Forager vs. Nurse (old, SCC)
Forager vs. Nurse (young, SCC)
Foraging Experience
Alarm Pheromone
AHB vs. EHB Soldiers (Colony)
AHB vs. EHB Forager (Colony)
AHB vs. EHB Guards (Colony)
AHB vs. EHB (14-d-old)
AHB vs. EHB (4-d-old)
AHB vs. EHB Forager (Indiv.)
AHB vs. EHB Soldiers (Indiv.)
AHB vs. EHB Guards (Indiv.)
lig vs. mel (15-d-old)
lig vs. mel (10-d-old)
0.3 0.4 0.5 0.6 0.7 0.8 0.9 1


without Forager vs. Nurse (young, SCC)
Distance Perception
Morning vs. Afternoon
Vibratory Signal
Scout vs. Recruit
Anticipating vs. Inactive
Training Time
Vg RNAi
Rich vs. Poor Diet
10-d-old vs. 15-d-old
4-d-old vs. 14-d-old
Forager vs. Nurse (old, SCC)
Forager vs. Nurse (typical)
Foraging Experience
Alarm Pheromone
AHB vs. EHB Soldiers (Colony)
AHB vs. EHB Forager (Colony)
AHB vs. EHB Guards (Colony)
AHB vs. EHB (14-d-old)
AHB vs. EHB (4-d-old)
AHB vs. EHB Forager (Indiv.)
AHB vs. EHB Soldiers (Indiv.)
AHB vs. EHB Guards (Indiv.)
lig vs. mel (15-d-old)
lig vs. mel (10-d-old)
0.3 0.4 0.5 0.6 0.7 0.8 0.9 1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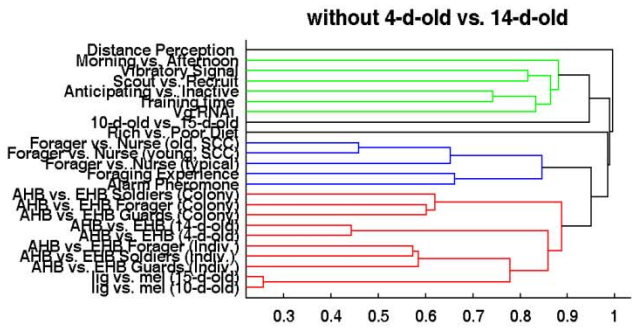
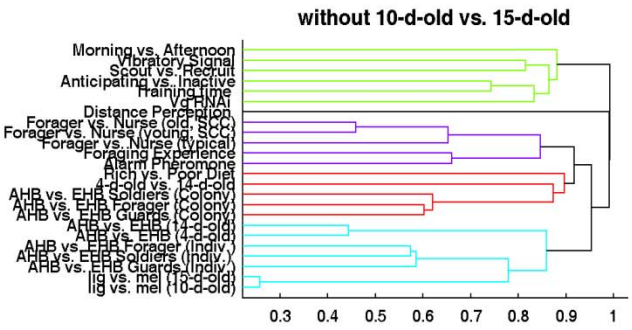
Distance Perception
Morning vs. Afternoon
Vibratory Signal
Scout vs. Recruit
Anticipating vs. Inactive
Training Time
Vg RNAi
Rich vs. Poor Diet
10-d-old vs. 15-d-old
4-d-old vs. 14-d-old
Forager vs. Nurse (young, SCC)
Forager vs. Nurse (typical)
Foraging Experience
Alarm Pheromone
AHB vs. EHB Soldiers (Colony)
AHB vs. EHB Forager (Colony)
AHB vs. EHB Guards (Colony)
AHB vs. EHB (14-d-old)
AHB vs. EHB (4-d-old)
AHB vs. EHB Forager (Indiv.)
AHB vs. EHB Soldiers (Indiv.)
AHB vs. EHB Guards (Indiv.)
lig vs. mel (15-d-old)
lig vs. mel (10-d-old)
0.3 0.4 0.5 0.6 0.7 0.8 0.9 1


without 4-d-old vs. 14-d-old
Distance Perception
Morning vs. Afternoon
Vibratory Signal
Scout vs. Recruit
Anticipating vs. Inactive
Training Time
Vg RNAi
10-d-old vs. 15-d-old
Rich vs. Poor Diet
Forager vs. Nurse (old, SCC)
Forager vs. Nurse (young, SCC)
Forager vs. Nurse (typical)
Foraging Experience
Alarm Pheromone
AHB vs. EHB Soldiers (Colony)
AHB vs. EHB Forager (Colony)
AHB vs. EHB Guards (Colony)
AHB vs. EHB (14-d-old)
AHB vs. EHB (4-d-old)
AHB vs. EHB Forager (Indiv.)
AHB vs. EHB Soldiers (Indiv.)
AHB vs. EHB Guards (Indiv.)
lig vs. mel (15-d-old)
lig vs. mel (10-d-old)
0.3 0.4 0.5 0.6 0.7 0.8 0.9 1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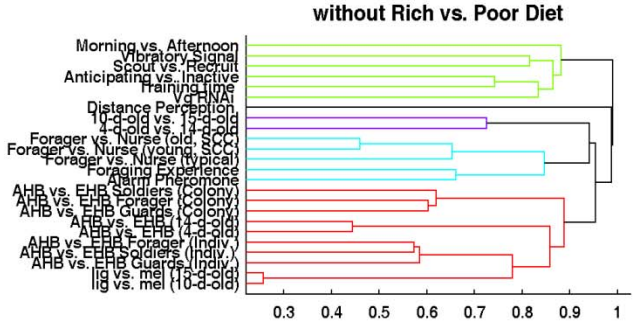
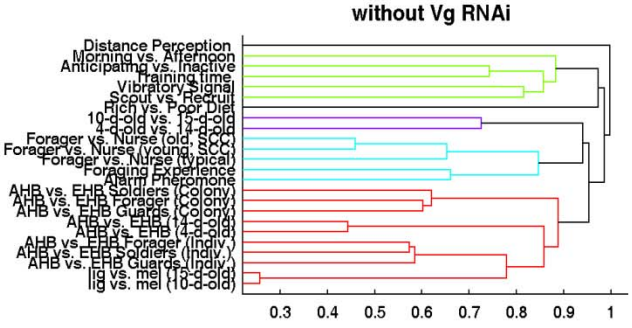
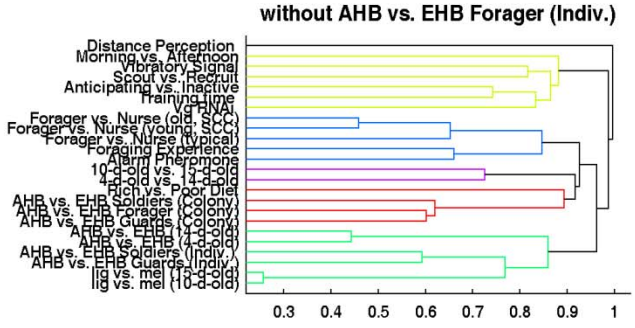
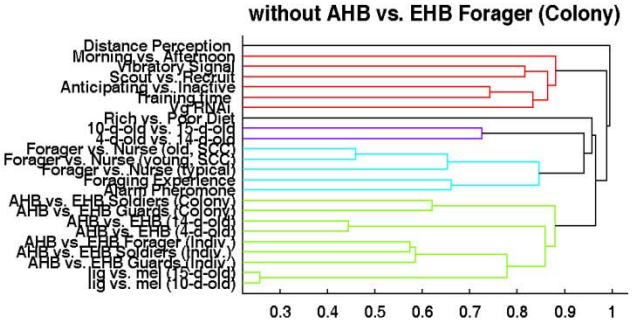
Morning vs. Afternoon
Vibratory Signal
Scout vs. Recruit
Anticipating vs. Inactive
Training Time
Vg RNAi
Distance Perception
Forager vs. Nurse (old, SCC)
Forager vs. Nurse (young, SCC)
Forager vs. Nurse (typical)
Foraging Experience
Alarm Pheromone
Rich vs. Poor Diet
4-d-old vs. 14-d-old
AHB vs. EHB Soldiers (Colony)
AHB vs. EHB Forager (Colony)
AHB vs. EHB Guards (Colony)
AHB vs. EHB (14-d-old)
AHB vs. EHB (4-d-old)
AHB vs. EHB Forager (Indiv.)
AHB vs. EHB Soldiers (Indiv.)
AHB vs. EHB Guards (Indiv.)
lig vs. mel (15-d-old)
lig vs. mel (10-d-old)
0.3 0.4 0.5 0.6 0.7 0.8 0.9 1


without Rich vs. Poor Diet
Morning vs. Afternoon
Vibratory Signal
Scout vs. Recruit
Anticipating vs. Inactive
Training Time
Vg RNAi
Distance Perception
10-d-old vs. 15-d-old
4-d-old vs. 14-d-old
Forager vs. Nurse (old, SCC)
Forager vs. Nurse (young, SCC)
Forager vs. Nurse (typical)
Foraging Experience
Alarm Pheromone
AHB vs. EHB Soldiers (Colony)
AHB vs. EHB Forager (Colony)
AHB vs. EHB Guards (Colony)
AHB vs. EHB (14-d-old)
AHB vs. EHB (4-d-old)
AHB vs. EHB Forager (Indiv.)
AHB vs. EHB Soldiers (Indiv.)
AHB vs. EHB Guards (Indiv.)
lig vs. mel (15-d-old)
lig vs. mel (10-d-old)
0.3 0.4 0.5 0.6 0.7 0.8 0.9 1


without Vg RNAi
Distance Perception
Morning vs. Afternoon
Anticipating vs. Inactive
Training Time
Vibratory Signal
Scout vs. Recruit
Rich vs. Poor Diet
10-d-old vs. 15-d-old
4-d-old vs. 14-d-old
Forager vs. Nurse (old, SCC)
Forager vs. Nurse (young, SCC)
Forager vs. Nurse (typical)
Foraging Experience
Alarm Pheromone
AHB vs. EHB Soldiers (Colony)
AHB vs. EHB Forager (Colony)
AHB vs. EHB Guards (Colony)
AHB vs. EHB (14-d-old)
AHB vs. EHB (4-d-old)
AHB vs. EHB Forager (Indiv.)
AHB vs. EHB Soldiers (Indiv.)
AHB vs. EHB Guards (Indiv.)
lig vs. mel (15-d-old)
lig vs. mel (10-d-old)
0.3 0.4 0.5 0.6 0.7 0.8 0.9 1


without AHB vs. EHB Forager (Indiv.)
Distance Perception
Morning vs. Afternoon
Vibratory Signal
Scout vs. Recruit
Anticipating vs. Inactive
Training Time
Vg RNAi
Forager vs. Nurse (old, SCC)
Forager vs. Nurse (young, SCC)
Forager vs. Nurse (typical)
Foraging Experience
Alarm Pheromone
10-d-old vs. 15-d-old
4-d-old vs. 14-d-old
Rich vs. Poor Diet
AHB vs. EHB Soldiers (Colony)
AHB vs. EHB Forager (Colony)
AHB vs. EHB Guards (Colony)
AHB vs. EHB (14-d-old)
AHB vs. EHB (4-d-old)
AHB vs. EHB Soldiers (Indiv.)
AHB vs. EHB Guards (Indiv.)
lig vs. mel (15-d-old)
lig vs. mel (10-d-old)
0.3 0.4 0.5 0.6 0.7 0.8 0.9 1


without AHB vs. EHB Forager (Colony)
Distance Perception
Morning vs. Afternoon
Vibratory Signal
Scout vs. Recruit
Anticipating vs. Inactive
Training Time
Vg RNAi
Rich vs. Poor Diet
10-d-old vs. 15-d-old
4-d-old vs. 14-d-old
Forager vs. Nurse (old, SCC)
Forager vs. Nurse (young, SCC)
Forager vs. Nurse (typical)
Foraging Experience
Alarm Pheromone
AHB vs. EHB Soldiers (Colony)
AHB vs. EHB Guards (Colony)
AHB vs. EHB (14-d-old)
AHB vs. EHB (4-d-old)
AHB vs. EHB Forager (Indiv.)
AHB vs. EHB Soldiers (Indiv.)
AHB vs. EHB Guards (Indiv.)
lig vs. mel (15-d-old)
lig vs. mel (10-d-old)
0.3 0.4 0.5 0.6 0.7 0.8 0.9 1

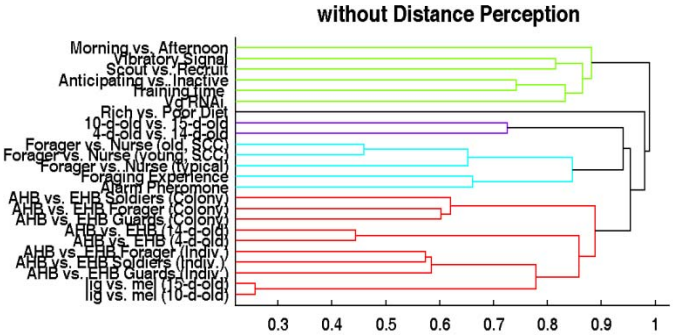

without Distance Perception
Morning vs. Afternoon
Vibratory Signal
Scout vs. Recruit
Anticipating vs. Inactive
Training Time
Vg RNAi
Rich vs. Poor Diet
10-d-old vs. 15-d-old
4-d-old vs. 14-d-old
Forager vs. Nurse (old, SCC)
Forager vs. Nurse (young, SCC)
Forager vs. Nurse (typical)
Foraging Experience
Alarm Pheromone
AHB vs. EHB Soldiers (Colony)
AHB vs. EHB Forager (Colony)
AHB vs. EHB Guards (Colony)
AHB vs. EHB (14-d-old)
AHB vs. EHB (4-d-old)
AHB vs. EHB Forager (Indiv.)
AHB vs. EHB Soldiers (Indiv.)
AHB vs. EHB Guards (Indiv.)
lig vs. mel (15-d-old)
lig vs. mel (10-d-old)
0.3 0.4 0.5 0.6 0.7 0.8 0.9 1

Fig. S2. Performance of the bee brain TRN in 10-fold cross validation

Histogram shows the distribution of Pearson correlations in test set for the expression values predicted by the TRN compared to actual gene expression, for the 2176 genes that were accurately predicted in the training set (training set, $r > 0.8$).

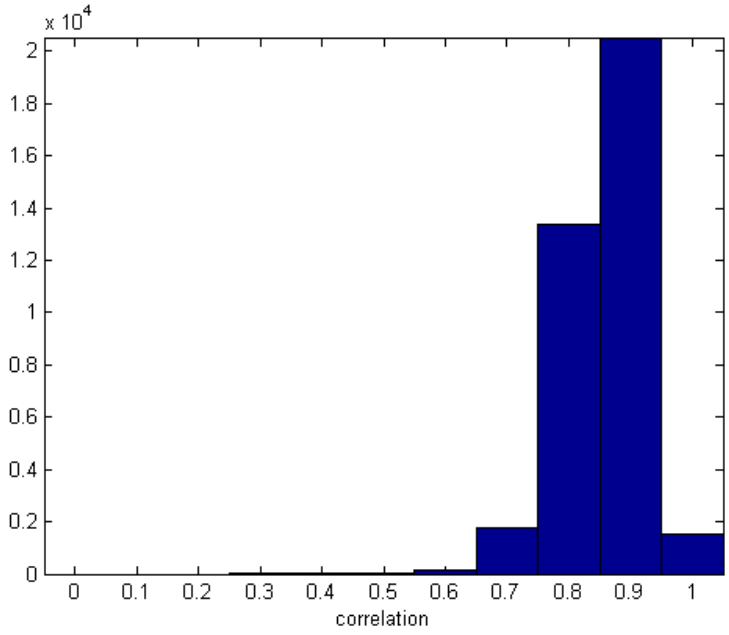


Fig. S3. Performance of the bee brain TRN in leave-one-state-out cross validation

Our algorithm (ARACNE+LARS) was trained using the samples from all but one state, and the samples from the remaining state were then used as a test set. Mean Root Mean Square Deviation (RMSD) in training sets (red line) and in test sets (black line) are shown, using each of the 48 behavioral states as the test condition. We included in each test condition those genes that were predicted with high accuracy in that training set (training RMSD < 0.5).

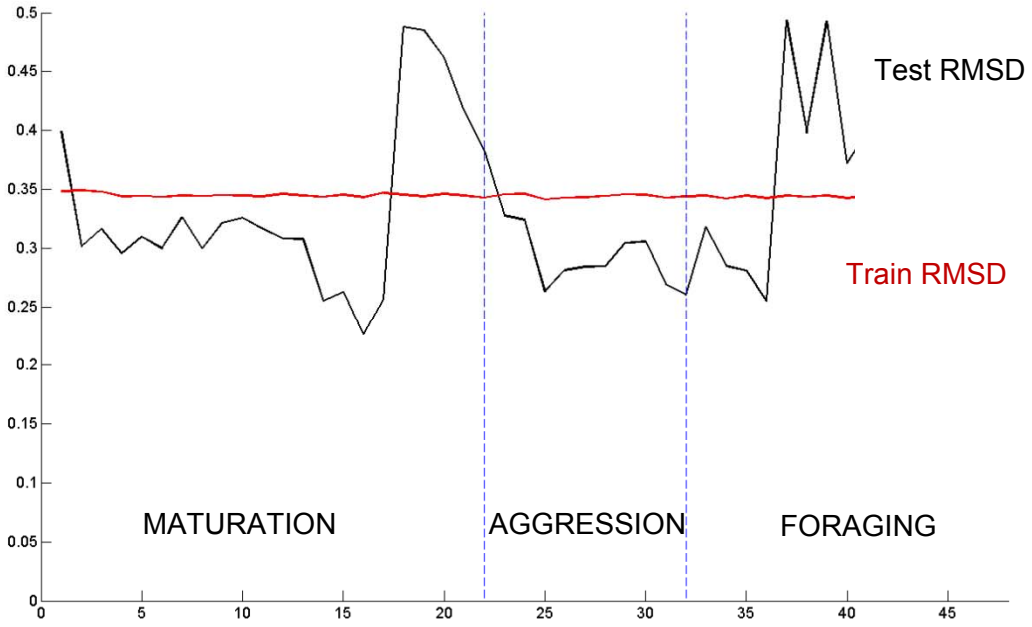


Fig S4. TF expression permutation across phenotypes for estimating background correlation

We performed permutation tests to estimate background correlation levels in the data. Left column shows performance of the TRN using 'normal' data; right column shows performance in 'shuffled' data. The first and second rows of histograms show the distribution of correlations in training and in test sets, respectively. In the shuffled data the correlation distribution appears to be randomly distributed. The plots at the bottom of the figure show the relationship between training set correlations and test set correlations for each gene. Training set and test set accuracy were more closely related in the normal data; correlation between training and test set accuracy was especially high (r = 0.92) for the accurately predicted genes (right of the sold line) that were retained in the final TRN model. See Methods section on *'Estimation of background accuracy and controls*' for additional details on permutation tests.

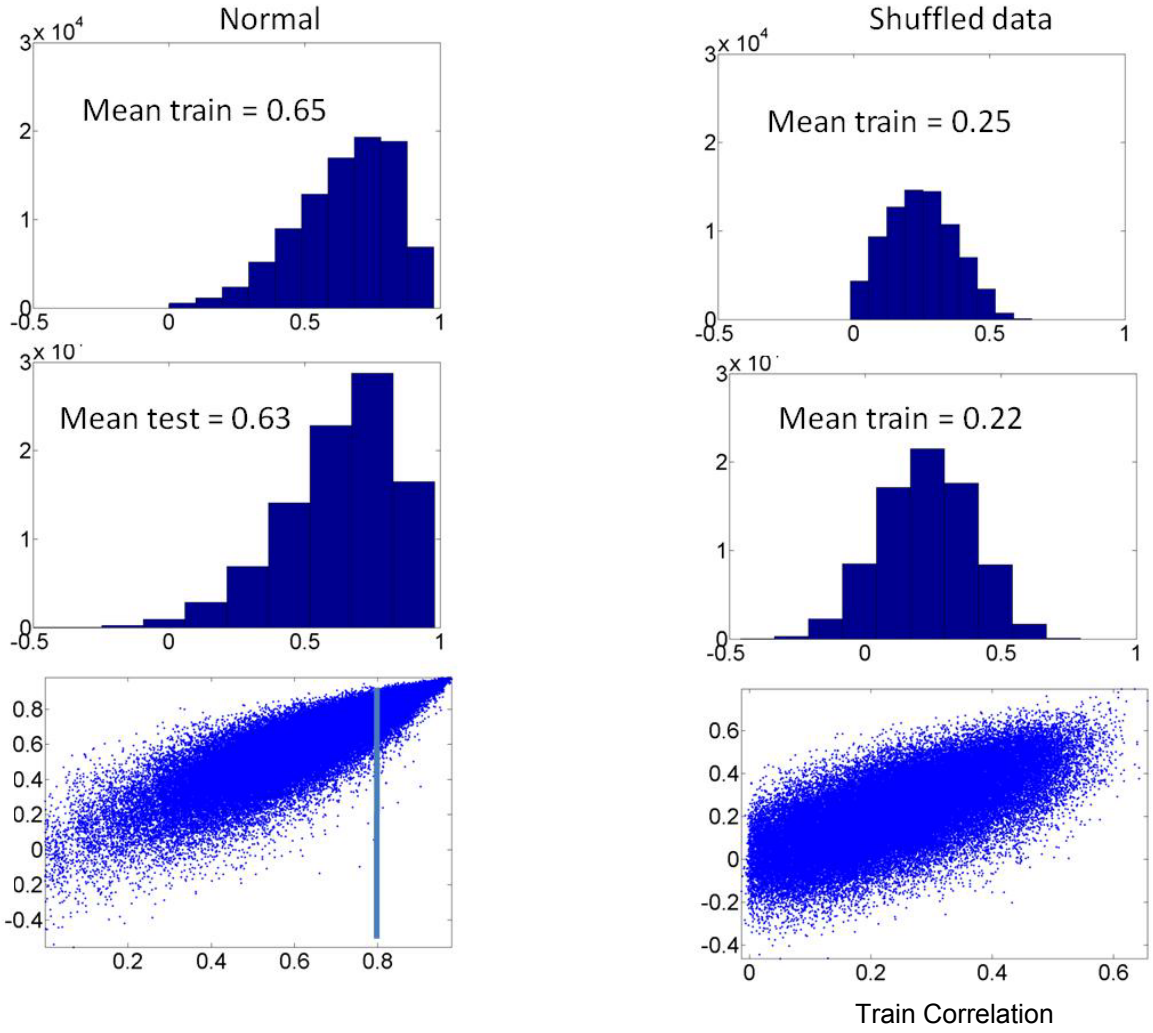


Fig. S5. Distribution of targets regulated by individual TFs

Most TFs were predicted to regulate only a few genes; a few network hubs (shown at the left side of the graph) regulate many targets.

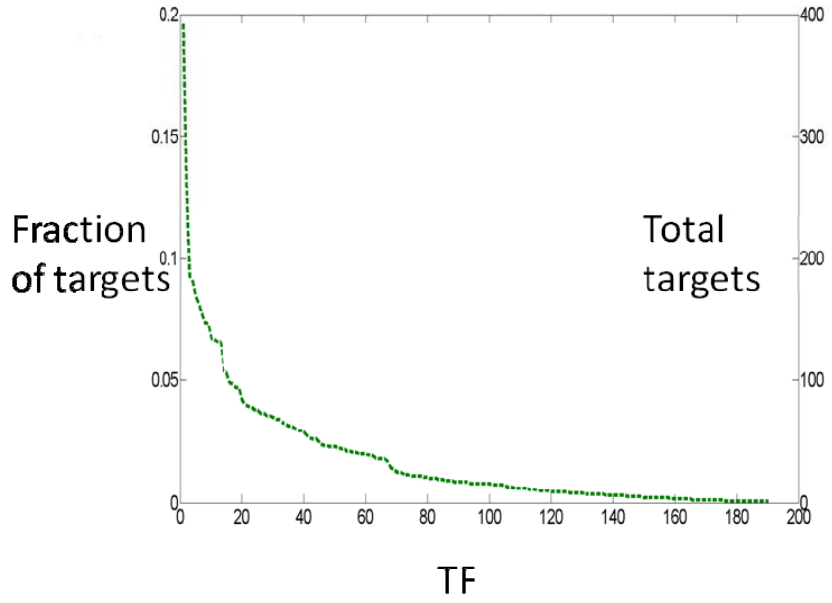


Fig. S6. A small number of network hubs regulate most genes in the network

The fraction of targets that are regulated with at least one interaction is shown as a function of the number of TFs included, added from most to least targets. The top seven TFs with the most targets together regulate 50% of the target genes; the top 40 together regulate 90% of all target genes.

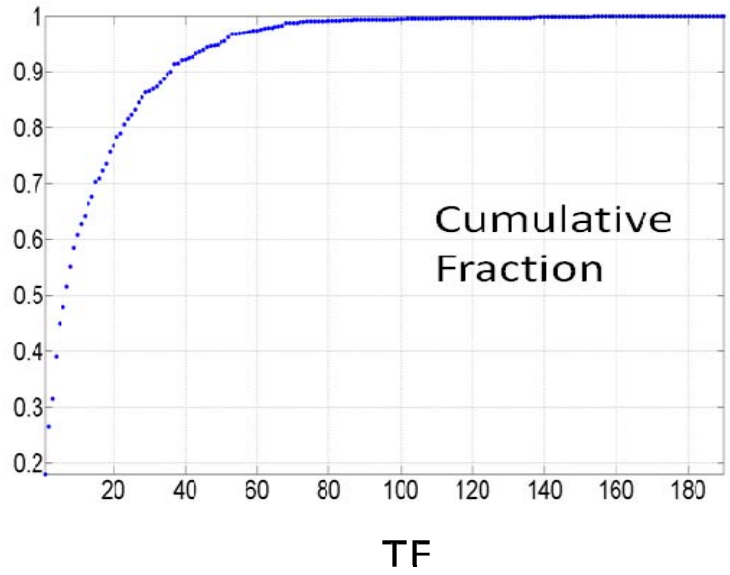


Fig. S7. Within-state consistency in the relative expression of target genes within each module

We used DIRAC to determine the extent to which the relative levels of expression for the target genes in each module were preserved across all the individuals (10-20 bees) in each of the 48 behavioral states. Relative expression of targets within most modules was highly conserved across most or all states. The rows correspond to each TF and the columns are the various pairwise comparisons.

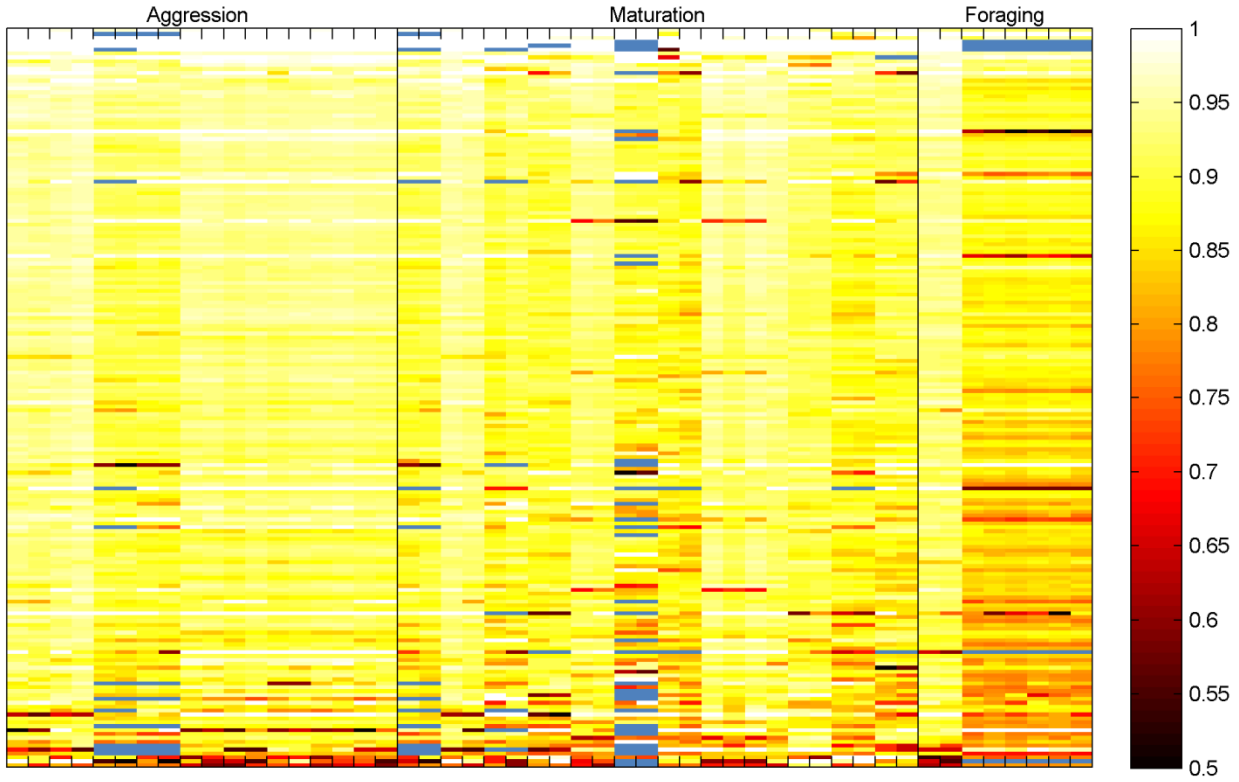


Fig. S8. Between-state reordering of relative gene expression increases with module size

For each TF-target module, we used DIRAC to identify between-state perturbations in the relative expression of target genes within a module, for each of the 27 behaviorally-related comparisons. DIRAC determines a probability for these 'shuffling' events based on the accuracy with which these changes in relative gene expression can be used to classify the two states in the comparison. There was a highly significant association between the number of target genes in a module and the degree of shuffling. There was no such association for random gene sets with the same size distribution (r^2 = 0.02), indicating that this is a specific feature to TF-target modules, rather than an artifact of the method.

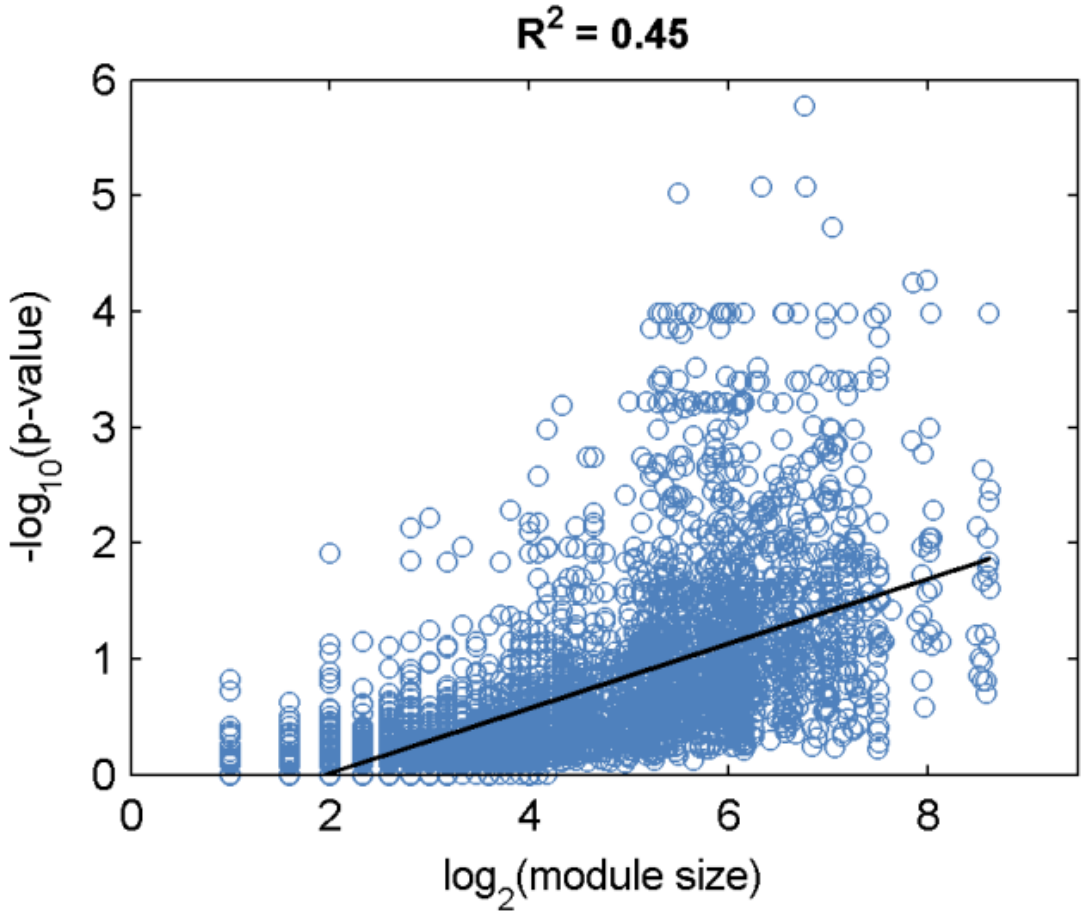


Fig. S9. Factors that influence behavior over long vs. short timescales influence network states differently

We subdivided the 27 comparisons into three categories based on the timescales over which they influence behavior (Table S8): *long-term differences* (hereditary differences between strains and sub-species that accumulate over evolutionary time); *medium-term differences* (changes occurring over a few weeks, primarily due to environmentally-induced changes); and *short-term differences* (factors that influence behavior for only hours or a few days; e.g., pheromones and the imprints of spatiotemporal memories on foraging). We compared the effects of these timescales on four aspects of network dynamics and regulation: the number of differentially expressed genes in the TRN (DEGs; A), the number of between-state perturbations in relative gene expression within modules ('shuffling', B), the number of TFs influencing each target gene (in-degree; C); and the number of target genes regulated by each TF (out-degree; D). Statistical significance for differences between timescales was determined using Kruskal-Wallis rank-sum tests.

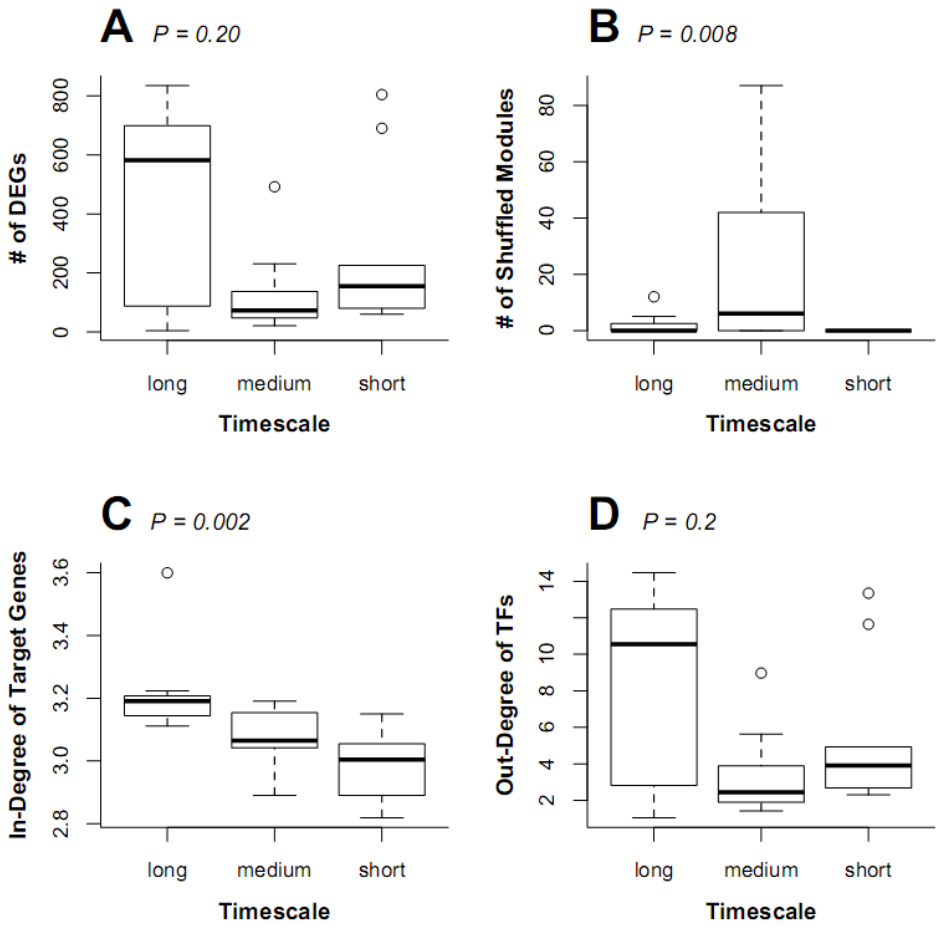


Fig. S10. Performance of the whole brain TRN in modeling mushroom body gene expression

Our model of the bee brain TRN accurately modeled the expression of ca. 25% of the genes expressed in the bee's brain, despite the fact that the brain is subdivided into ca. 20 sub-regions. To begin to characterize how whole brain gene expression relates to patterns of regulation within specific sub-regions, we used our whole-brain TRN to predict expression in the mushroom bodies (MB), the largest sub-region of the bee's brain, comprising ca. 40% by volume. We used the Foraging Experience dataset (Table S1), which includes expression profiles from the MB of 120 bees (primarily foragers). The whole brain TRN predicted MB expression for the 2176 genes in the model with a mean (Pearson) correlation accuracy, r = 0.36, including 550 genes with a correlation of 0.5 or higher (histogram in left panel). This performance was much better than if we used random sets of TFs as predictors of target gene expression (r = 0.02; histogram in right panel) or if we used the average expression of all TFs (r = -0.01). However, this performance was much worse than the TRN's performance on whole brain data. This suggests, as expected, that the regulation of some genes can deviate significantly within brain sub-regions from the 'average' expression predicted by the whole brain TRN.

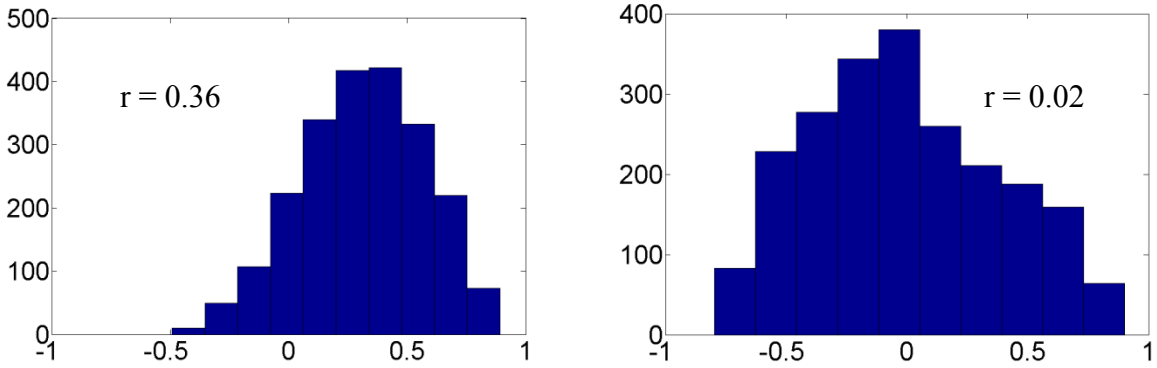
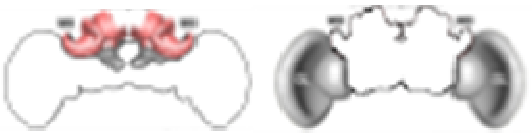


Fig. S11. Modules enriched for mushroom body- or optic lobe-specific expression

We looked for enrichment of the modules in the TRN with genes that were differentially expressed (FDR < 0.05) between two of the largest sub-regions of the brain: the mushroom bodies (MB, in pink) and optic lobes (OL, in grey). Several modules were enriched for genes with regional differences in expression, including genes with large fold differences (> 2- or > 5-fold difference between MB and OL). A few modules appear to be specifically associated with gene expression in one sub-region (e.g., the *kayak* module and MB-specific genes); this is consistent with anatomically defined regulation within the TRN. Surprisingly, several modules were separately enriched for genes with both MB- and OL-specific expression; these may represent region-specific responses to a broadly expressed TF. For instance, we speculate that *dl* (*NF-kB*) could respond to stress-related signals that simultaneously influence multiple brain regions but influence different targets depending on their baseline expression levels in each tissue. We report the number of region-specific genes and hypergeometric P-values for modules enriched (FDR < 0.1) in at least one test.



		MB > OL			OL > MB		
		All	2x	5x	All	2x	5x
		686	377	70	687	287	30
CG7786	98	18	14	4	15	7	
dl	105	19	11	4	20	9	
CG15011	78	15	7		14		
kay	40	10	5				
NC2&bgr;	63				13		
Lag1	394	46	27		39		
CG9932	265	31		5	33	18	3
cg	131	16	10		19		
bigmax	59	11	7				
sd	73	12					
CG9776	108	15			15		
CG17912	181	21					
CG16779	27		4				
D	16		3				
woc	76	10	7				
Eip93F	33		4				

P < 10-4
P < 10-3
P < 10-2
P < 0.05
P ≤ 1

Fig. S12. Comparison with *Drosophila* Transcriptional Regulatory Network

We compiled physical binding data for *Drosophila* from ModENCODE (21), REDfly (22), DROID (23) databases to compare TF targets with those predicted by our bee brain TRN. We found that 14 of the TF modules overlap with the corresponding TF module in *Drosophila*. To estimate the background overlap, we generated 100 random TRNs with the same module sizes as our TRN and overlapped with the *Drosophila* network. The average number of modules in the background set with significant overlap ($P < 0.05$) was 2.2 (shown as blue dots in the figure below). Hence our TRN's overlap (red star) is much more significant than could be observed by chance ($P < 10^{-15}$).