

## RESEARCH ARTICLE

# Neurogenomic signatures of spatiotemporal memories in time-trained forager honey bees

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## SUMMARY

Honey bees can form distinct spatiotemporal memories that allow them to return repeatedly to different food sources at different times of day. Although it is becoming increasingly clear that different behavioral states are associated with different profiles of brain gene expression, it is not known whether this relationship extends to states that are as dynamic and specific as those associated with foraging-related spatiotemporal memories. We tested this hypothesis by training different groups of foragers from the same colony to collect sucrose solution from one of two artificial feeders; each feeder was in a different location and had sucrose available at a different time, either in the morning or afternoon. Bees from both training groups were collected at both the morning and afternoon training times to result in one set of bees that was undergoing stereotypical food anticipatory behavior and another that was inactive for each time of day. Between the two groups with the different spatiotemporal memories, microarray analysis revealed that 1329 genes were differentially expressed in the brains of honey bees. Many of these genes also varied with time of day, time of training or state of food anticipation. Some of these genes are known to be involved in a variety of biological processes, including metabolism and behavior. These results indicate that distinct spatiotemporal foraging memories in honey bees are associated with distinct neurogenomic signatures, and the decomposition of these signatures into sets of genes that are also influenced by time or activity state hints at the modular composition of this complex neurogenomic phenotype.

Supplementary material available online at <http://jeb.biologists.org/cgi/content/full/214/6/979/DC1>

Key words: circadian, appetitive behavior, honey bee, memory, microarray.

## INTRODUCTION

Honey bees (*Apis mellifera*) have the remarkable ability to form and use spatiotemporal memories in order to direct their foraging behavior. This is adaptive because different species of flowering plants bloom at different times of day (Linnaeus, 1751), and nectar and pollen are available for pollinators only during discrete, but consistent, windows of time (Gimenes et al., 1993; Doorn and Meeteren, 2003; Matile, 2006). Honey bees can learn not only where to fly to collect food, but also when to do so (Beling, 1929; Wahl, 1932). This ability is based, in part, on an endogenous time-keeping mechanism (Beling, 1929; Renner, 1957).

Although much is known about the molecular basis of basic clock function in animals, including honey bees (Ruben et al., 2006), less is known about the molecular mechanisms underlying food-related activity rhythms. Like honey bees, mice and other animals can be trained to forage at specific times of day, resulting in rhythmic anticipatory appetitive behavior (reviewed by Mistlberger, 2009). It now appears that clocks in various parts of the brain (Angeles-Castellanos et al., 2007) and the liver (Vollmers et al., 2009) are necessary for circadian food

anticipatory activity in mammals. The molecular basis of food anticipatory states has yet to be elucidated in insects, perhaps because it has not been possible to train *Drosophila* to anticipate food availability in a circadian manner (Oishi et al., 2004). Honey bees are thus an attractive organism for such studies.

Although it is becoming increasingly clear that different behavioral states are associated with different profiles of brain gene expression (Robinson et al., 2008), it is not known whether this relationship extends to states that are as dynamic and specific as those associated with foraging-related spatiotemporal memories. Transcriptional profiles in honey bee brains have been shown to be strongly correlated with certain behaviors such as nursing (brood care) and foraging (Whitfield et al., 2003; Alaux et al., 2009a; Alaux et al., 2009b). The duration of the nursing and foraging states in honey bees is 1 week or longer, and comparisons between the brains of nurses and foragers revealed large gene expression differences [~20 to 40% of transcripts tested in Alaux et al. (Alaux et al., 2009b) and Whitfield et al. (Whitfield et al., 2003), respectively]. Other behaviors that are more transient in duration, such as guarding the hive, comb building and undertaking (the removal of dead bees from the colony), each last for only a day or two and are associated with

fewer differences in gene expression (Cash et al., 2005). These findings, plus the known influences of gene expression on rhythmic behavior (Allada and Chung, 2010) and various forms of learned behavior (Barrett and Wood, 2008) in other organisms, led us to hypothesize that transcriptional differences exist between bees trained to have distinct foraging-related spatiotemporal memories.

MATERIALS AND METHODS

We trained different bees from the same hive to forage at different times of day, at different locations and with different scents. Our experimental design enabled us to search separately for gene expression patterns associated with differences in activity state, time of day or specific spatiotemporal memories.

Bees, training and collection

Two unrelated typical colonies of European honey bees (*Apis mellifera* Linnaeus 1758, a mixture of European subspecies, largely *ligustica*) were each placed into a clear plastic-walled observation hive (97×96 cm) with eight frames of honeycombs. Each colony was headed by a naturally mated queen and was composed of ~12,000 workers at the start of the study. The hives were kept at the former Marine Corps Armory site, warrant of East Tennessee State University, Johnson City, TN, USA. Each hive was kept in a small wooden shed to allow for observations without exposing the colony to direct sunlight, and the bees were allowed to adjust to the new environment for at least 1 week before training commenced. The sides of the hive were constructed of 16 clear plastic windows that could be opened to allow for the collection of bees with minimal disturbance to the colony. Two replicates of the experiment were performed, one for each colony, from 14 to 19 and 21 to 28 August 2007. Over the duration of the experiment, several different species of wildflowers were in bloom at the study site.

The field component of this study consisted of three phases: orientation, training and collection. The orientation phase lasted 1 or 2 days, during which the bees learned the location of the artificial feeder. Filter paper soaked with sucrose solution was used to lure bees out of the hive and to transfer them from the hive entrance to a feeder located 1 m away. The feeder consisted of a 96-well tissue culture plate that was filled with 2 mol l<sup>-1</sup> sucrose solution and placed on a piece of scented filter paper disk situated on top of a short (40 cm) table. Once a steady number of bees (~5 min<sup>-1</sup>) were observed flying to the feeder on their own, the filter paper transfer was stopped and the feeder was moved several meters away from the hive. When the foragers again found the feeder at a steady rate, the feeder was moved again several more meters away. This procedure was repeated until the feeder reached the desired training location, ~120 m away from the hive. Training was performed at two different times of day, once in the morning from 09:00 to 10:15 h and again in the afternoon from 17:00 to 18:15 h. At the end of each training period, the feeder was emptied and washed, and the filter paper was replaced with a new unscented sheet. Different locations and different scents were used for each of the two training times, morning and afternoon. Essential oil extracts of lilac and lavender flowers were used for scents in the morning and afternoon, respectively.

The training phase consisted of adding scent and sucrose solution to the feeder during the training time and marking – with an individual identifying paint code (see von Frisch, 1967) – foragers that were recruited to the training location by the trained bees from the orientation phase. Their time of arrival was recorded for each foraging trip. Although most bees displayed fidelity to a single training time, some did not; any bee bearing the paint code

indicating that she had obtained a food reward at the other training time was removed to prevent cross-recruitment based on residual smells. Training continued for 4–7 days until there were a sufficient number (*N*≈40) of trained bees at both training times.

On collection day, the feeder was left empty and unscented to ensure that any gene expression differences were a result of memory and not recent experience with the food source. All bees used for molecular analysis were collected from the hive, 15–45 min before the onset of the training time. The bees were collected directly off the combs to help prevent confounding effects associated with the energy expenditure from flying to the feeder. Once focal bees were located and identified by their paint code, the windows of the observation hive were flipped up and soft forceps were used to collect the bees directly into liquid nitrogen to flash-freeze the transcriptional profile. Both ‘anticipating bees’ (bees whose training time was soon approaching) and ‘inactive bees’ (bees trained to the other time) were collected simultaneously out of the hive at both times, resulting in four experimental groups (Fig. 1).

Anticipating bees were collected near the entrance of the hive, as described by Körner (Körner, 1939) and Moore et al. (Moore et al., 1989). These bees monitor returning foragers for scents or dances that indicate that the food source to which they are trained has already become available. If left undisturbed, these bees would have eventually flown to the feeding location even without exposure to other cues (von Frisch, 1967). Inactive bees were collected farther away from the hive entrance in areas associated with honey storage. These bees were not observed performing other behaviors and appeared to be in a sleep-like state, as described by previous investigators (von Frisch, 1967; Kaiser and Steiner-Kaiser, 1983). The four behavioral groups analyzed in this study were: morning-trained, morning-collected (tAM-cAM); morning-trained, afternoon-collected (tAM-cPM); afternoon-trained, afternoon-collected (tPM-cPM); and afternoon-trained, morning-collected (tPM-cAM).

Bees representing these four groups were collected for microarray analysis if they received at least 3 days of training. Furthermore, each bee included in the microarray analysis (*N*=76) flew to the feeder and received a food reward at least two, six and six times on the last 3 days of training, respectively. This criterion identified the most highly trained and motivated bees. Experiments performed

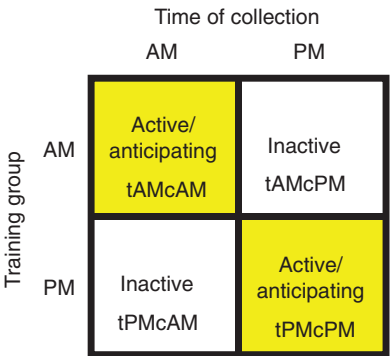


Fig. 1. Experimental design and the four behavioral groups analyzed in this study. Two groups of honey bees (*Apis mellifera*) from the same colony were trained to forage at an artificial feeder either in the morning (tAM) or in the afternoon (tPM). Bees from each training group were collected just prior to the onset of each training time, in the morning (cAM) or afternoon (cPM). Bees collected just before their training time demonstrated stereotypical anticipatory behavior that precedes flying to a food source whereas bees collected outside of their training time were inactive.

Table 1. Number of rewarded visits by honey bees *Apis mellifera* to the feeders by day

Bee ID	Training group	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
BWP	AM				6	6	9	13
PWY	AM			1	5	10	8	8
PYW	AM			13	17	13	8	12
YBB	AM					9	12	14
YPB	AM					7	8	12
YYW	AM				6	11	10	11
BYY	AM					3	12	13
WcPR	AM					12	13	8
WcWP	AM					13	11	14
WcWY	AM			12		14	13	13
BBW	AM				5	10	10	9
BPW	AM				6	13	14	15
YWW	AM				10	12	11	12
YYG	AM				5	4	10	10
BBY	AM					2	11	8
RWP	AM					5	7	10
WRR	AM					2	6	6
WcPW	AM					14	13	10
WcYA	AM			10		13	15	16
RBW	PM				7	5	13	20
RWP	PM		1	3	4	7	9	12
WPP	PM			5	18	15	14	18
WRP	PM		1	6	6	6	7	12
YcGG	PM			6	4	12	13	16
YcGW	PM			7	6	10	16	20
YcWB	PM				10	12	17	23
YcWY	PM			15	15	8	14	18
GGG	PM	1	2	2	14	15	18	20
RGG	PM	4	7	8	12	9	12	19
RWR	PM	4	8	22	21	10	17	17
WPRC	PM			10	10	10	10	18
WWW	PM	3	2	2	10	13	14	11
YcBY	PM			10	14	12	15	16
YcWP	PM			4	13	9	18	22
YcYR	PM			10	12	14	8	17
YcYW	PM			10	6	12	7	16
YcYY	PM			19	17	16	15	19

Bees visited the artificial feeder multiple times over the duration of the training time. The performance of the 37 bees used for the microarray analysis is shown here.

at the same study site in previous years (Moore and Doherty, 2009) (B.N.V.N., A. E. Wagner and D.M., unpublished) demonstrated that >90% of bees with this level of training will fly to the empty, unscented feeder at the appropriate time on the following day. Of the 76 bees identified according to this criterion, we selected the 12 bees from each of the four experimental groups (six from each replicate, two replicates) with the most visits to the feeder during the training phase ( $N=48$ ) for microarray analysis. Table 1 shows the distribution of visits by bees during the training phase, for all bees that were selected for microarray analysis.

#### Microarray analysis

The heads of the bees were freeze-dried ( $-80^{\circ}\text{C}$  and 40 Pa) and the brains dissected out while submerged in dry ice/ethanol (see Schulz and Robinson, 1999). The brains were homogenized and nucleic acids were extracted using phenol/chloroform and RNeasy kits (Qiagen, Valencia, CA, USA). Then samples were treated with DNase (Qiagen). From the resulting RNA, 500 ng was amplified using MessageAmpII kits (Ambion/Applied Biosystems, Austin, TX, USA). Amplified RNA was labeled with Cy3 or Cy5 dyes using a Kreatech ULS labeling system (Amsterdam, The Netherlands). The 48 individuals were hybridized in pairs with a loop design that used 113 microarrays (printed by W. M. Keck Center for

Comparative and Functional Genomics, University of Illinois Urbana-Champaign, Urbana, IL, USA). The microarrays contained 13,440 distinct 70-mer oligonucleotides based on information from the honey bee genome (Honeybee Genome Sequencing Consortium, 2006). The oligonucleotide sequences were chosen based on predicted gene models and expressed sequence tags, and each was spotted twice on the array. This second-generation honey bee microarray was characterized by Alaux et al. (Alaux et al., 2009b), and from this point on, each representative sequence will be referred to as a 'gene'. Hybridized arrays were scanned and analyzed using Axon GenePix 6.0 (Molecular Devices, Sunnyvale, CA, USA) software. As in Sen Sarma et al. (Sen Sarma et al., 2010), spots flagged with '-100' by Axon GenePix 6.0 were removed from the analysis and genes that are highly expressed in the hypopharyngeal glands were also excluded because of the risk of contamination during brain dissection (Alaux et al., 2009a). The remaining data were filtered using the median of control elements for microarray and dye as the threshold. LOWESS normalization was carried out on the  $\log_2$ -transformed intensities, and the measurements were then adjusted for global dye and microarray effects. A total of 8209 genes remained after these exclusions, representing the number of genes in the brain expressed above threshold for all individuals.

### Statistical analysis of microarray results

A linear mixed-effects model was used to analyze the normalized  $\log_2$ -transformed fluorescent intensities for each gene (Alaux et al., 2009a; Alaux et al., 2009b). The model accounted for the effects of dye, microarray, individual sample and source colony. Two-way ANOVA with *post hoc* contrasts was used to determine significant differences based on time of collection, training group and activity state.

Principal component and hierarchical clustering analyses of the gene expression values showed that 11 individuals, relatively evenly distributed across all four experimental groups, were clustered together. Investigation revealed that the RNA amplification of all 11 of these individuals took place on two sequential days, and the affected 11 yielded the lowest final quantity of RNA on those days. For this reason, these 11 individuals and the corresponding 37 arrays they were measured with were dropped and the ANOVA was rerun. Only results of this reanalysis are reported here. Genes showing differential expression at a false discovery rate (FDR)-corrected *P*-value of  $<0.01$  were considered significant.

Genes identified as differentially expressed in this experiment were compared with those from a previously published study (Alaux et al., 2009b) to probe for similarities and differences across different behavioral states. Gene list overlap analysis was performed using an exact hypergeometric probability test (one-tailed) in Microsoft Excel (Redmond, WA, USA). As in Alaux et al. (Alaux et al., 2009a), the representation factor (rf) was calculated as the number of observed overlapping genes divided by the expected number; the expected number of overlapping genes is defined as the product of the number of genes differentially expressed in each list, divided by the total number of genes analyzed.

Functional insights into differentially expressed genes were obtained by conducting a gene ontology (GO) term enrichment analysis. This analysis was performed using the FlyBase identification number representing the best BLAST hit for each honey bee gene (Honeybee Genome Sequencing Consortium, 2006) and the DAVID Bioinformatics Resources Functional Annotation tool (Huang et al., 2009). GO terms returned by DAVID with a modified Fisher's exact *P*-value  $<0.05$  were considered significantly enriched.

### Quantitative reverse-transcriptase PCR analysis and array validation

Quantitative reverse-transcriptase PCR (qRT-PCR) was used to confirm some of the results obtained from microarray analysis. Twenty-four genes were selected for validation based on significance, fold change and functions of interest (supplementary material Fig.S1). Two hundred nanograms of the original unamplified RNA from all original 76 individuals in the study were used to synthesize cDNAs using ArrayScript (Ambion) reverse transcriptase. qRT-PCR was performed in triplicate 10  $\mu$ l reactions in 384-well plates using PerfeCTa SYBR Green Fastmix (Quanta Biosystems, Gaithersburg, MD, USA). Reactions not within 0.5  $C_t$  (cycle threshold) agreement with the others were discounted. The results were analyzed by standard weighted means two-way ANOVA with *post hoc* contrasts using SAS (SAS Institute Inc., Cary, NC, USA). Agreement of gene expression results between microarray and qRT-PCR analysis was tested using standardized data ('standardize' procedure in SAS, mean=0, s.d.=1), which were then analyzed using linear discriminant analysis ('lda' function) in the MASS package (Venables and Ripley, 2002) for R (R Development Core Team, 2006).

## RESULTS

### Behavioral evidence for spatiotemporal memories

Over the course of the two replicates, 184 trained foragers were marked individually and followed throughout the experiment. Of these bees, 28 were captured upon their arrival at the wrong feeding station, i.e. the one to which they were not initially trained; they were removed from the study. Thus, the majority (85%) of the time-trained bees specialized and foraged exclusively at one training time or the other, as expected (Kleber, 1935; Moore et al., 1989). Twenty-five bees (14%) that were marked and trained did not appear on the final training day and were not found during the collections from the hive. These disappearances represent natural mortality expected among forager honey bees (Winston, 1987).

Honey bees clearly exhibited food anticipatory behavior over the course of the experiment. Outside of the training time, trained bees were typically found scattered in areas of the hive away from the entrance; however, as the training time approached, the trained bees began to cluster near the entrance and dance floor. Furthermore, on training days, bees frequently flew to the feeder before food or scent had been applied for that day. The frequency of these reconnaissance flights increased as the training time approached (Fig. 2). As previously reported (Moore and Rankin, 1983), afternoon-trained bees exhibited anticipatory behavior for a longer period of time before the onset of the training time than morning-trained bees.

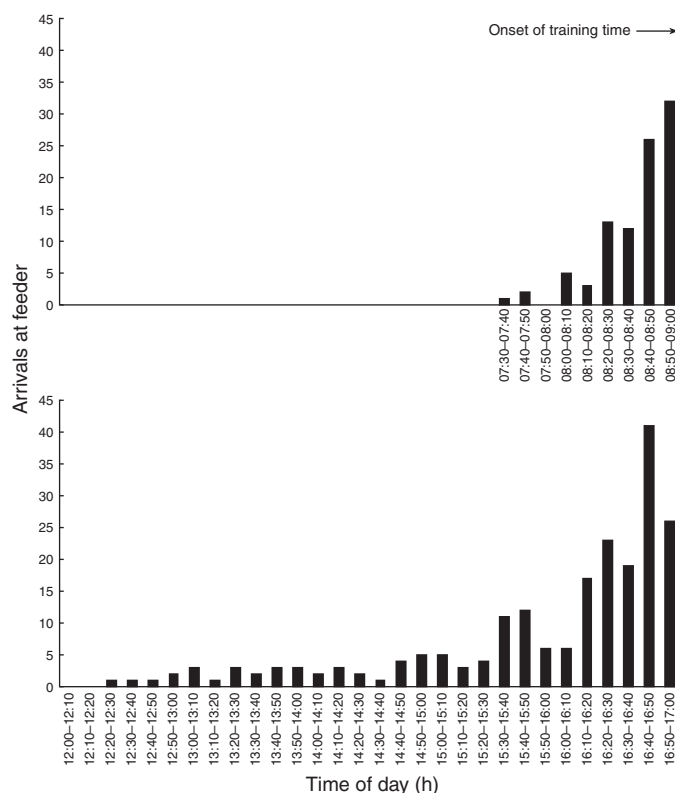


Fig. 2. Anticipatory behavior in honey bees. Trained bees frequently flew to the feeder in anticipation of food availability, and the frequency of these flights increased as the training time approached. Data are from 18 Aug 2007, the day prior to the day of collection for the first replicate of the experiment.



### Brain gene expression differences associated with time of collection

To determine whether there were genes showing brain expression differences associated with time of collection, independent of time of training, we used two-way ANOVA to contrast the gene expression values for tAM-cAM and tPM-cAM with tAM-cPM and tPM-cPM bees (Fig. 1). There were 798 genes showing differences in brain gene expression between morning- and afternoon-collected bees ( $FDR < 0.01$ ). This represents ~10% of the genes on the microarray analyzed in this study.

GO analysis revealed 80 categories significantly enriched (modified Fisher's exact test,  $P < 0.05$ ) for genes with differences in brain gene expression associated with time of day (supplementary material Table S1). Among enriched terms for genes with higher expression in the morning-collected bees were 'organelle ATP synthesis coupled electron transport' and numerous mitochondria-associated terms (supplementary material Table S2). There were also multiple terms associated with ribosomes and translation as well as proteasomes and catabolic processes. Enriched terms for genes more highly expressed in afternoon-collected bees included 'cell adhesion', 'lipid metabolic process', 'lipid transport' and 'protein localization' (supplementary material Table S3). Terms associated with circadian behavior – such as 'circadian rhythm', 'rhythmic behavior', 'locomotory behavior' and 'sleep' – all appear exclusively for the list of genes with higher expression in the afternoon.

### Brain gene expression differences associated with time of training

To determine whether there were genes showing brain expression differences associated with time of training, independent of time of collection, we contrasted tAM-cAM and tAM-cPM with tPM-cAM and tPM-cPM bees. Relative to other categories, fewer genes (229) were found to be differentially expressed between the two groups of bees trained to forage either in the morning or afternoon, regardless of whether they were collected in an anticipatory or inactive state ( $FDR < 0.01$ ). GO analysis showed an enrichment of upregulated genes involved with carbohydrate catabolic processes and kinase regulator activity in the morning-trained groups (supplementary material Tables S4, S5). Afternoon-trained groups showed enrichment for genes associated with 'response to heat' and 'oxidoreductase activity, acting on the CH-CH group of donors' (supplementary material Table S6). Among the genes showing time of training differences were *foraging* and *clock*, both of which had higher expression in morning-trained bees.

### Brain gene expression differences associated with state of food anticipation

To determine whether there were genes showing brain expression differences associated with food anticipatory state, independent of time of training or collection, we contrasted tAM-cAM and tPM-cPM with tAM-cPM and tPM-cAM bees. Bees collected 15–45 min prior to their time of training were actively anticipating food availability and preparing for flight whereas bees trained to the other time were inactive. This difference in activity state was associated with a very large number of genes: 2028 differentially expressed brain transcripts or ~25% of all genes on the microarray analyzed in this study.

Genes upregulated in anticipating bees showed significant GO enrichment for many categories associated with nervous system function, including 'cation transport', 'transmission of nerve impulse', 'neurotransmitter secretion' and 'synaptic transmission' (supplementary material Tables S7, S8). Behavioral categories were

also present, including 'rhythmic process' and 'circadian behavior', as well as 'response to chemical stimulus', 'response to light stimulus' and 'memory', even though the bees had not recently encountered the environment outside the hive. *ankyrin*, *GB16541*, the heat shock proteins *hsp8* and *hsp90a*, and the insulin receptor *inR* were all confirmed as correlated with the state of food anticipation via qRT-PCR (see Validation of microarray results with qRT-PCR).

Inactive bees showed a remarkably strong signature of ribosome-related genes in their transcriptional profile (supplementary material Table S9). In all, 99 genes annotated as being associated with the ribonucleoprotein complex were significantly upregulated in inactive bees, with the GO term 'structural constituent of the ribosome' having the strongest enrichment found in the entire experiment (modified Fisher's exact test,  $P = 1.07E-46$ ). Other significantly enriched terms include 'mitochondrial part', 'mitochondrial ribosome', 'proteasome complex', 'DNA-directed RNA polymerase activity' and 'spliceosome'. Some of the genes upregulated in inactive bees and confirmed by qRT-PCR include the histone H1 and the canonical clock genes *period* and *cryptochrome* (Ruben et al., 2006).

The list of genes differentially expressed between anticipating and inactive groups was compared with a list of genes generated by Alaux et al. (Alaux et al., 2009b) for differences between (non-foraging) nurse and forager honey bees. There was significant overlap for these lists ( $rf = 1.2$ ,  $P < 0.0001$ ), with 66% of genes either up- or down-regulated in a concordant way for both anticipating bees in this study and foragers in Alaux et al. (Alaux et al., 2009b).

### Brain gene expression differences associated with distinct spatiotemporal memories

To determine whether there were genes showing brain expression differences associated with distinct spatiotemporal memories, we contrasted tAM-cAM bees with tPM-cPM bees. These two groups

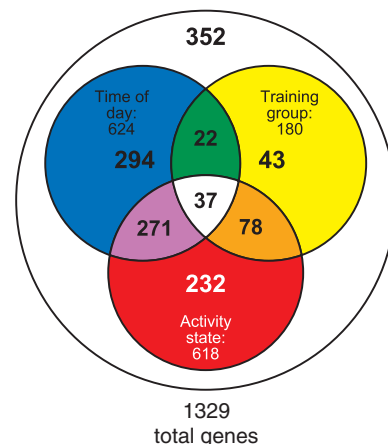


Fig. 3. Decomposition of brain gene expression differences associated with distinct spatiotemporal memories in honey bees: effects of time of collection (Time of day), time of training (Training group) and activity state. A total of 1329 genes were differentially expressed between the two active groups with distinct spatiotemporal memories. Expression of a majority of these genes was also influenced by time of day, time of training and/or activity state. Three-hundred fifty-two of the 1329 genes did not have significant differences in expression in these other analyses and represent unique transcriptional signatures associated with a unique spatiotemporal memory.

Table 2. Selected results of gene ontology (GO) enrichment analysis to identify molecular functions and biological processes that are associated with the lists of genes showing expression differences in association with time of collection, time of training, activity state or differences in spatiotemporal memories

GO term	Time of day	Time of training	Activity state	Different spatiotemporal memories
Carbohydrate catabolic process		tAM		tPMcPM
Cation transport			Active	
Cell adhesion	cPM			tAMcAM
Cellular macromolecule catabolic process	cAM			tPMcPM
Chemosensory behavior	cPM		Active	Overall
Lipid metabolic process	cPM			tAMcAM
Mitochondrial part	cAM	tPM	Inactive	tPMcPM
Neurotransmitter secretion			Active	
Organelle ATP synthesis coupled electron transport	cAM		Inactive	
Response to heat		tPM		
Rhythmic behavior	cPM		Active	
Structural constituent of ribosome	cAM		Inactive	tPMcPM
Synaptic transmission			Active	

Full data with all enriched categories are available in the supplementary material Tables S1–S15.

were both anticipating food availability, but at a different time of day and for a different location. A total of 1329 genes were differentially expressed in the brains between these two groups (*t*-test,  $P<0.01$ ). This large number of genes included many genes that were differentially expressed in the analyses described above. Of the 1329 differentially expressed genes, 624 also varied significantly with time of day, 180 also varied significantly with time of training and 618 also varied significantly with state of food anticipation (Fig. 3). The same pattern of decomposition is reflected in the GO enrichment analysis. For example, terms such as ‘cell adhesion’ and ‘cellular macromolecule catabolic process’ appeared in the analysis of tAM-cAM vs tPM-cPM bees as well as in the time of day analysis (Table 2). GO enrichment also revealed other differences between the tAM-cAM and tPM-cPM bees, including ‘lipid metabolic process’, ‘carbohydrate metabolic process’ and ‘chemosensory behavior’ (supplementary material Tables S10–S12).

Removing all the genes that varied with time of day, time of training and/or anticipatory state still left 352 genes that showed significant differences in brain expression between tAM-cAM and tPM-cPM bees. These expression differences are associated with other unknown aspects that either contribute to the formation of the two unique spatiotemporal memories or reflect their existence. GO enriched terms for these putative spatiotemporal memory-related genes include ‘extracellular structure organization and biogenesis’ and ‘synaptogenesis’ (supplementary material Tables S13–S15).

**Effects of time of day, time of training and anticipatory state on brain expression of clock genes**

The molecular basis of circadian function involves a small set of interacting transcription factors, some with negative and others with positive feedback effects on each other’s expression (Allada and Chung, 2010). *cycle* (*cyc*), a gene comprising part of the positive feedback mechanism in the bee’s circadian clock, was robustly differentially expressed in terms of time of day of collection (ANOVA,  $P<0.0001$ ) but was not affected by time of training or activity state (Fig. 5). Another canonical clock gene in the positive feedback mechanism, *clock* (*clk*), showed a significant difference based on time of training ( $P=0.0035$ ) but no effect based on anticipatory state or time of collection; there was higher expression among morning-trained bees, both active and inactive. By contrast, *period* (*per*), a gene with negative feedback effects, was differentially expressed as a function of both time of day of collection ( $P=0.03$ ) and activity state ( $P<0.0001$ ), but not time of

training. *cryptochrome* (*cry*), which is also involved in negative feedback regulation of the clock, was differentially expressed based on both time of day of collection ( $P<0.0001$ ) and activity state ( $P<0.0001$ ) but not time of training.

**Validation of microarray results with qRT-PCR**

qRT-PCR validation of the microarray results was challenging in this study because each gene could show significant expression differences for each of the time of collection, time of training, activity state and/or unique memory analyses. We therefore used linear discriminant analysis (LDA) to examine the gestalt of similarities

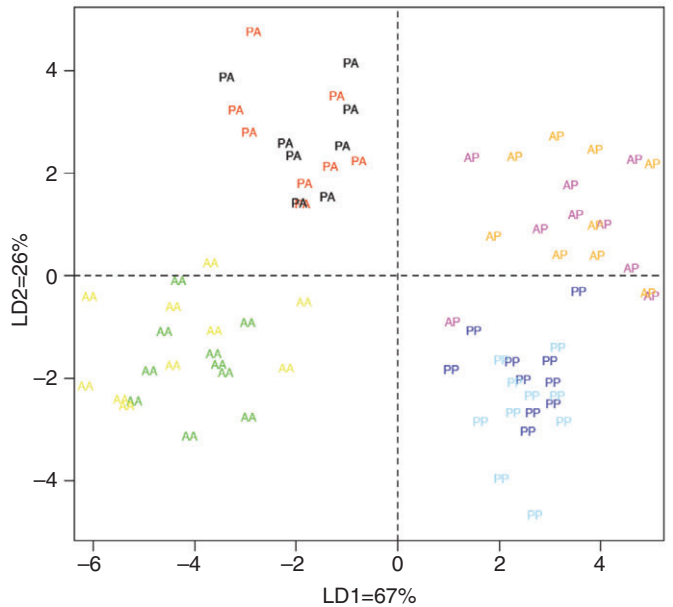


Fig. 4. Linear discriminant analysis indicates strong similarities between results obtained with microarrays and qRT-PCR and supports the hypothesis that distinct neurogenomic states exist for each of the four honey bee groups in the experiment. Microarray and qRT-PCR data for the same 37 individuals are shown here. For clarity of presentation, abbreviations were condensed from what appears in Fig. 1: AA, AM (morning) trained, AM collected; AP, AM trained, PM (afternoon) collected; PA, PM trained, AM collected; PP, PM trained, PM collected. Black, orange, yellow and light blue represent the microarray data whereas red, pink, green and dark blue represent the qRT-PCR data.

and differences between the four experimental groups across the two different expression measurement platforms. Expression values for each of the genes, generated by both microarray and qRT-PCR, are found in supplementary material Fig. S1. LDA revealed that overall expression patterns were similar between microarray and qRT-PCR analysis (Fig. 4). The qRT-PCR results showed strong clustering for individuals within the four experimental groups and nice separation of the four groups, with only the data from the 24 genes chosen for validation. This result further reflects the robustness of the differences between the four groups and supports the hypothesis that spatiotemporal foraging rhythms are associated with unique neurogenomic states.

### DISCUSSION

The principal significance of these results is that they demonstrate that the ability of honey bees to form distinct spatiotemporal foraging memories is associated with the formation of distinct neurogenomic signatures of gene expression in the brain. Furthermore, these signatures can be partially decomposed into sets of genes that are influenced by time or activity state, and still others that are not influenced by these variables. This finding hints at the modular composition of complex neurogenomic phenotypes. It is not known which, if any, of these extensive gene expression changes are causal. However, differences in gene expression are known to have causal effects on various forms of both circadian (Allada and Chung, 2010) and learned behavior (Barrett and Wood, 2008) in the laboratory, suggesting that at least some of the changes we detected function

to enable bees to form and utilize different spatiotemporal memories during natural foraging behavior.

This study identified a relatively large number of genes that showed differences in expression as a function of time of collection, i.e. genes whose expression varied in the morning *vs* the afternoon. Ueda et al. (Ueda et al., 2002) reported that a similar number of genes varied in expression as a function of light–dark conditions in *Drosophila melanogaster* heads. In our study, genes upregulated in the morning included those associated with ATP synthesis, mitochondria and NADH oxoreductase activity, as well as the canonical clock gene *cycle* (*bmal1* in mammals). These findings are consistent with previous reports of a link between the positive feedback element of the molecular clock and the regulation of basic metabolic processes (Eckel-Mahan and Sassone-Corsi, 2009; Ramsey et al., 2009). Genes upregulated in the afternoon included cell adhesion molecules, which have been implicated in the synchronization of circadian oscillators (Shen et al., 1997; Miche and Colwell, 2001) and the regulation of learning and memory (Welzl and Stork, 2003). Perhaps this finding reflects the occurrence of memory consolidation in foragers during the night.

Some genes showed differences associated with the time at which the bee was trained, regardless of whether it was then collected in the morning or afternoon, or was anticipating or inactive. This might reflect either differences in the environment encountered as a result of the different training times or a natural proclivity to respond to training at one time *versus* another. Regarding the latter, Kraus et al. (Kraus et al., in press) have reported genotypic differences in

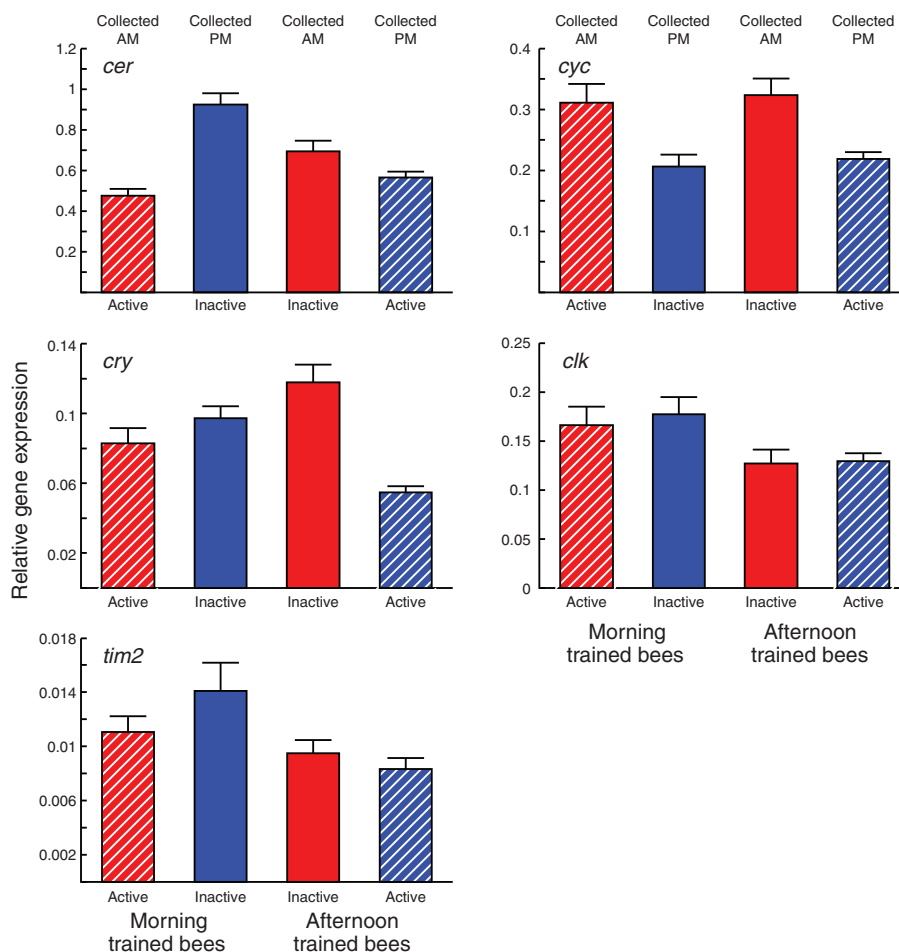


Fig. 5. Expression of canonical clock genes as a function of time training in honey bees. Genes representing the set of transcription factors comprising the negative feedback element of the molecular clock (*per*, *cry* and *tim2*) have expression patterns that are more closely associated with the activity state of the bee rather than time of day. Genes representing the positive feedback element of the molecular clock (*cyc* and *clk*) do not show any significant differences based on activity state, although *cyc* shows clear differences based on the time of day. See supplementary material Table S16 for significance values.

the preferences of bees to forage either in the morning or afternoon. Among the genes differentially expressed based on the time of training were genes associated with metabolism as well as *foraging* and *clock*. Upregulation of *foraging* has been implicated in increased phototactic behavior in honey bees (Ben-Shahar et al., 2003), and increased phototaxis could make the foragers more likely to respond to the morning feeder (which coincides more closely with sunrise) than the afternoon feeder. Other genetic differences for similar phenomena include polymorphisms in human *clock* associated with differences in the times at which individuals prefer to either be active or sleeping (Katzenberg et al., 1998).

The largest differences in brain gene expression detected in this study were related to differences in the activity state of the bees, either anticipating flight to a specific food reward or inactive. Bees collected just before their time of training were observed displaying the stereotypical anticipatory behavior that precedes exiting the hive to fly to the feeder. Renner demonstrated that anticipatory behavior in bees arises as a function of an internal circadian clock that allows the bees to time the availability of food (Renner, 1957). Results of GO analyses suggest that mechanisms involved in stimulus perception and response are reactivated in a circadian manner during the anticipatory state, perhaps 'priming' the brain for subsequent activity, after over 22 h of inactivity. In addition, GO categories such as 'cation transport,' 'transmission of nerve impulse' and 'neurotransmitter secretion' point toward possible mechanisms that the brain would use to initiate behavior. Mammalian pacemaker neurons found in the suprachiasmatic nucleus generate autonomous circadian activity patterns when isolated (Green and Gillette, 1982), and cation channels play a role in this autoactivation (Meredith et al., 2006; Wang and Huang, 2006; Kononenko and Berezetskaya, 2010).

There was also a significant enrichment of genes encoding the heat shock proteins that are upregulated in anticipating bees. These genes could play a role in preparing foragers for the stresses of foraging (Williams et al., 2008). It is also possible that heat shock proteins play more of a direct role in behavior; knock-down of heat shock proteins affects behavioral rhythmicity in flies, even though the core molecular oscillators are unaffected (Hung et al., 2009).

A large number of the genes upregulated in inactive bees were associated with transcription, translation and proteasome function. Inactive foragers have long been thought to enter into sleep-like states (von Frisch, 1967; Kaiser and Steiner-Kaiser, 1983; Kaiser, 1988; Eban-Rothschild and Bloch, 2008; Klein et al., 2008); this speculation is strengthened by work in *Drosophila* rigorously demonstrating that insects sleep (Hendricks et al., 2000; Shaw et al., 2000) (reviewed by Harbison et al., 2009). Perhaps inactive bees are showing evidence for the kind of purging of old proteins and building of new proteins that has been associated with sleep in other organisms (reviewed by Mackiewicz et al., 2008).

Further insights into the possible significance of the molecular differences between active and inactive bees were gained by comparing their differences in brain gene expression with the differences between nurse and forager bees (Alaux et al., 2009b). Anticipating bees have a brain expression profile that resembles the forager profile whereas the profile of inactive bees more closely resembles that of the nurse. Among the genes differentially expressed in both contexts is the insulin receptor (*inR*), which shows higher expression in both foragers compared with nurses and in anticipating foragers compared with inactive foragers. Insulin and other nutritional signaling pathways play a part in the division of labor among honey bee workers (Ament et al., 2008), and RNAi knock-down of the insulin

receptor alters honey bee foraging behavior (Wang et al., 2010). These results imply that some of the same genes involved in the regulation of foraging over the lifetime of the bee are also involved in the regulation of daily foraging rhythms.

Genes involved in synaptogenesis are significantly overrepresented in the group of genes that vary with spatiotemporal memory but not with time of training, time of collection or activity state. This is consistent with the idea that different spatiotemporal memories have unique representations in the brain. In addition, the overrepresentation of genes previously shown to be involved in chemosensory behavior perhaps reflects the possibility that some aspects of the olfactory system are differentially utilized in the learning of the unique scents used in the spatiotemporal training.

Some of the genes well known to be involved in the regulation of circadian rhythms in other organisms showed interesting patterns of expression in this study. Expression of *per* and *cry* in bees trained in the morning was consistent with patterns reported by Ruben et al. (Ruben et al., 2006) under *ad libitum* feeding conditions; however, an altered pattern was seen in the afternoon-trained bees. This is perhaps because the time-training procedures used in this experiment uncoupled activity state from time of day, and food acted as a zeitgeber to set elements of the internal clock, which then dictated foraging rhythms on subsequent days. Intriguingly, another clock gene, *cyc*, did not show this altered expression pattern. The differences observed in the time-trained bees suggest that, in the honey bee, the two sets of molecular regulatory feedback loops have become uncoupled, with the positive element synchronizing gene expression oscillations with day–night cycles whereas the negative element synchronizes expression oscillations with the time of food availability. This is supported by the recent finding that genes involved in insulin signaling are involved in setting the pace of the negative element of the clock in *Drosophila* (Zheng and Sehgal, 2010).

It should be noted that this study was performed using whole brains. The altered pattern of gene expression due to spatiotemporal training that we report here could have occurred only in a subset of neurons, and different neural circuits could have different expression profiles. Previous studies in mice have found that restricted feeding schedules shifted expression of *per*, but only in specific brain regions (Angeles-Costellanos et al., 2007) (reviewed by Webb et al., 2009). Furthermore, peripheral oscillators in other tissues such as the insect fat body (a metabolic tissue analogous to vertebrate livers) could play a role in behavioral rhythmicity as it relates to feeding (Xu et al., 2008; Vollmers et al., 2009) (reviewed by Escobar et al., 2009). Additional studies that probe the specific neuroanatomical location of the differences in gene expression reported here might help clarify the neurobiological basis of these findings and will contribute to our understanding of learning and memory under natural conditions.

This study is the first to separate genes involved with activity rhythms from genes influenced by time of day in an insect without the confounding effects of sleep deprivation. We identified genes associated with motivation in the form of food anticipation, and we have demonstrated that spatiotemporal training creates neurogenomic profiles that are distinct for each training regime. These findings highlight the surprisingly close relationship between changes in brain gene expression and naturally occurring behavioral plasticity (Robinson et al., 2005; Robinson et al., 2008). A challenge for the future is to learn how time training exerts these effects on the genome and how, in turn, spatiotemporally encoded neurogenomic profiles impact foraging behavior.



## LIST OF ABBREVIATIONS

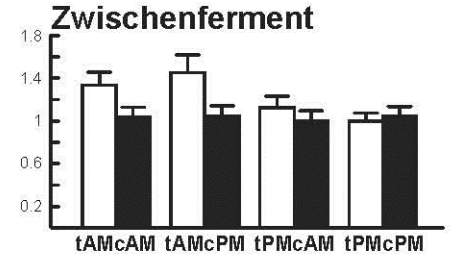
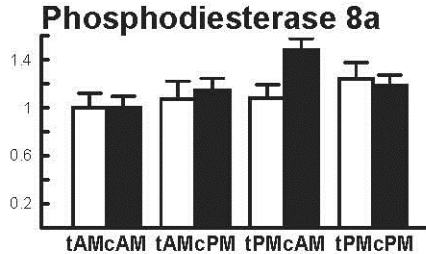
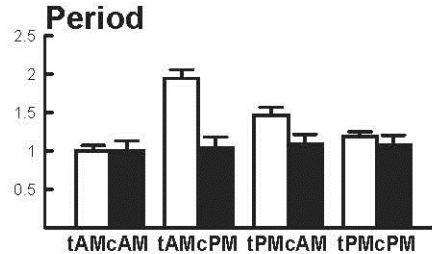
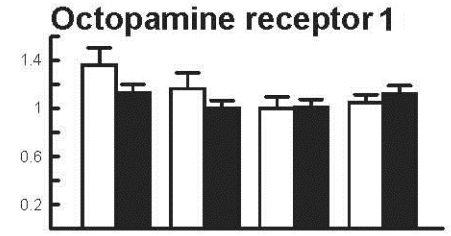
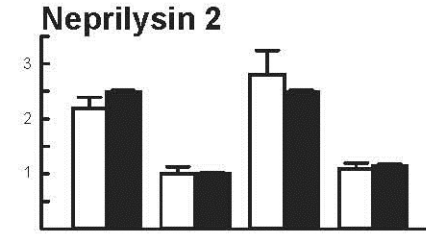
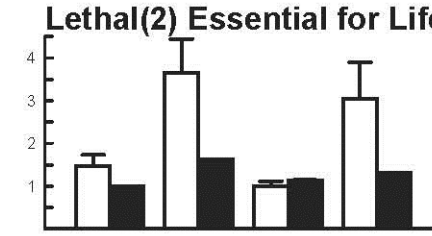
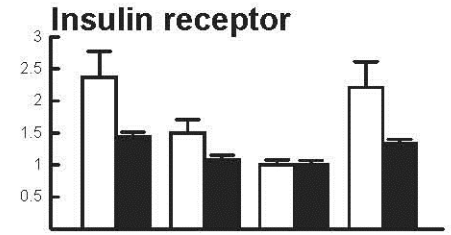
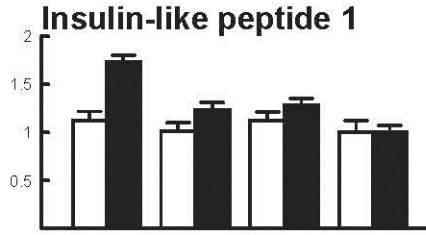
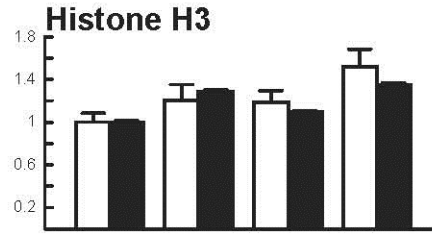
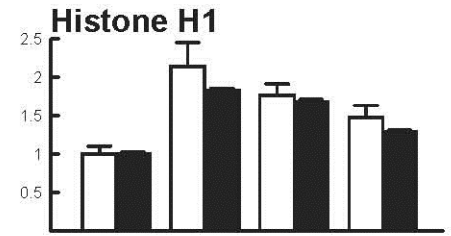
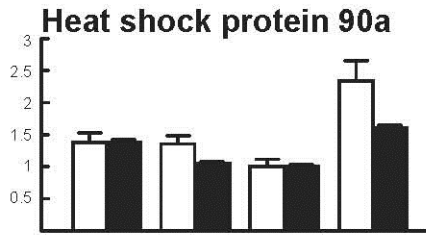
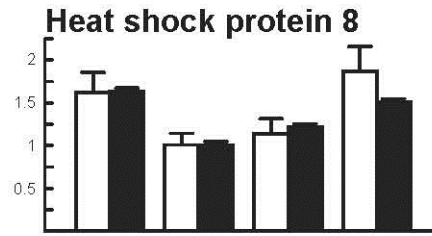
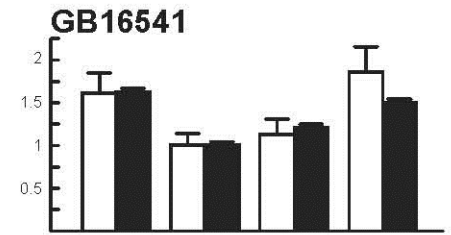
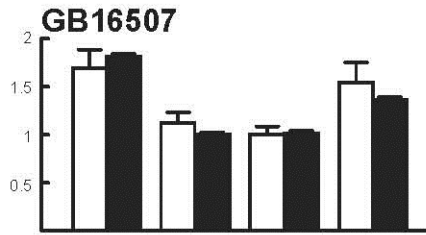
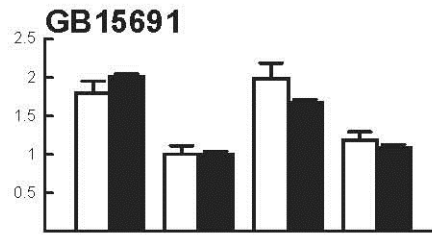
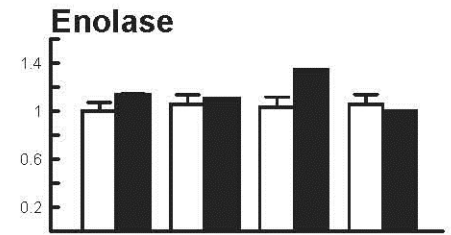
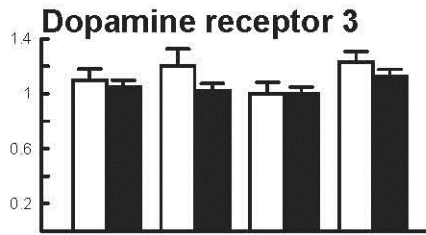
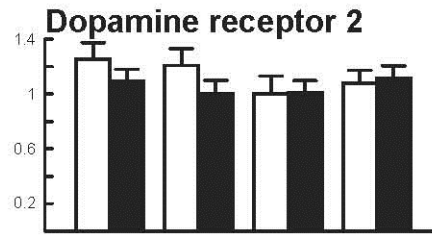
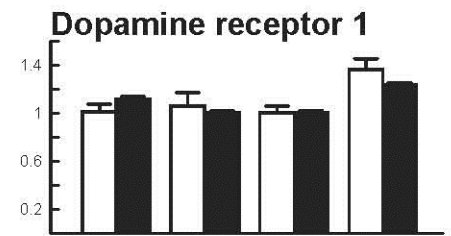
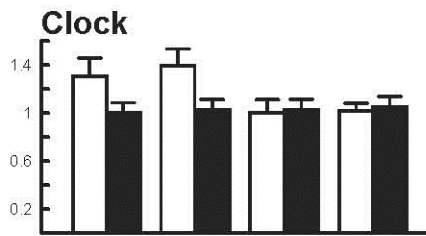
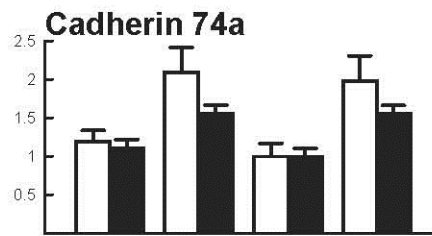
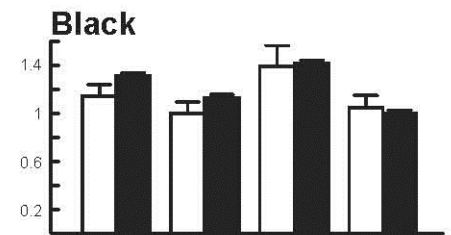
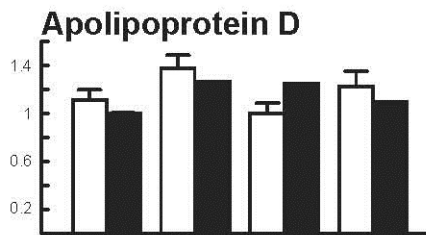
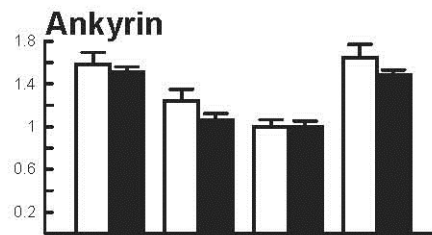
tAM-cAM	a bee trained to the morning feeder and collected in the morning
tAM-cPM	a bee trained to the morning feeder and collected in the afternoon
tPM-cAM	a bee trained to the afternoon feeder and collected in the morning
tPM-cPM	a bee trained to the afternoon feeder and collected in the afternoon

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**Table S1:** Significantly enriched GO terms for genes differentially expressed between morning- and afternoon-collected foragers.

GO category	GO Term	Count	%	P-value
Cell Component level 1	extracellular region	20	4.55%	6.23E-04
Mol Function level 2	structural constituent of ribosome	25	5.68%	0.00151909
Mol Function level 2	isomerase activity	13	2.95%	0.00184953
Cell Component level 3	cytoplasm	132	30.00%	0.00193726
Biol Process level 3	cellular catabolic process	29	6.59%	0.00232346
Biol Process level 2	catabolic process	31	7.05%	0.00274664
Cell Component level 5	cytoplasm	132	30.00%	0.0038595
Biol Process level 3	cellular biosynthetic process	62	14.09%	0.00409179
Cell Component level 4	cytoplasm	132	30.00%	0.00419286
Cell Component level 4	cytosolic ribosome (sensu Eukaryota)	15	3.41%	0.0042346
Cell Component level 5	cytosolic ribosome (sensu Eukaryota)	15	3.41%	0.00424633
Cell Component level 3	proteasome core complex (sensu Eukaryota)	6	1.36%	0.00531343
Cell Component level 4	proteasome core complex (sensu Eukaryota)	6	1.36%	0.00589536
Cell Component level 5	proteasome core complex (sensu Eukaryota)	6	1.36%	0.00590931
Cell Component level 4	cytosol	27	6.14%	0.00736113
Cell Component level 5	cytosol	27	6.14%	0.00737195
Cell Component level 3	ribosome	25	5.68%	0.00787205
Biol Process level 2	cell adhesion	19	4.32%	0.00838361
Biol Process level 1	biological adhesion	19	4.32%	0.0098868
Cell Component level 4	ribosome	25	5.68%	0.01037015
Cell Component level 5	ribosome	25	5.68%	0.01039154
Cell Component level 4	cytosolic part	17	3.86%	0.01073856
Cell Component level 3	ribosomal subunit	22	5.00%	0.01074598
Cell Component level 3	cytoplasmic part	108	24.55%	0.01075052
Cell Component level 5	cytosolic part	17	3.86%	0.01076926
Biol Process level 2	biosynthetic process	68	15.45%	0.01243578
Cell Component level 4	ribosomal subunit	22	5.00%	0.01377623
Cell Component level 5	ribosomal subunit	22	5.00%	0.01381185
Cell Component level 3	proteasome complex (sensu Eukaryota)	10	2.27%	0.01456207
Biol Process level 2	maintenance of protein localization	4	0.91%	0.01641689
Biol Process level 3	maintenance of protein localization	4	0.91%	0.01669994
Cell Component level 4	proteasome complex (sensu Eukaryota)	10	2.27%	0.01683375
Cell Component level 5	proteasome complex (sensu Eukaryota)	10	2.27%	0.01688178
Biol Process level 4	maintenance of protein localization	4	0.91%	0.01728508
Mol Function level 4	magnesium ion binding	7	1.59%	0.01925159
Biol Process level 2	maintenance of localization	5	1.14%	0.01930233
Cell Component level 5	cytoplasmic part	108	24.55%	0.01975791
Cell Component level 4	cytoplasmic part	108	24.55%	0.02029637
Biol Process level 1	maintenance of localization	5	1.14%	0.020394
Mol Function level 1	antioxidant activity	5	1.14%	0.02080644
Cell Component level 3	small ribosomal subunit	11	2.50%	0.02155214
Biol Process level 4	cellular macromolecule catabolic process	17	3.86%	0.0220579
Mol Function level 5	nucleoside diphosphate kinase activity	3	0.68%	0.02347454
Biol Process level 3	cell redox homeostasis	7	1.59%	0.02436663
Biol Process level 4	oocyte microtubule cytoskeleton organization	6	1.36%	0.02490946

Biol Process level 5	establishment and/or maintenance of cytoskeleton polarity	6	1.36%	0.02499097
Cell Component level 4	small ribosomal subunit	11	2.50%	0.0250229
Cell Component level 5	small ribosomal subunit	11	2.50%	0.02509591
Biol Process level 3	macromolecule catabolic process	19	4.32%	0.02547623
Biol Process level 5	cell redox homeostasis	7	1.59%	0.02584167
Mol Function level 2	oxidoreductase activity	38	8.64%	0.02643695
Biol Process level 2	translation	34	7.73%	0.02658954
Cell Component level 3	germline ring canal	4	0.91%	0.02936536
Cell Component level 3	intercellular bridge	4	0.91%	0.02936536
Cell Component level 2	intercellular bridge	4	0.91%	0.02965062
Biol Process level 2	cell fate commitment	17	3.86%	0.03003595
Cell Component level 4	cytosolic small ribosomal subunit (sensu Eukaryota)	7	1.59%	0.03096888
Cell Component level 5	cytosolic small ribosomal subunit (sensu Eukaryota)	7	1.59%	0.03104421
Cell Component level 4	germline ring canal	4	0.91%	0.0313204
Mol Function level 2	peroxidase activity	4	0.91%	0.03267941
Mol Function level 2	transmembrane transporter activity	41	9.32%	0.0327761
Biol Process level 4	translation	34	7.73%	0.03347212
Biol Process level 5	translation	34	7.73%	0.03363527
Cell Component level 4	lipid particle	17	3.86%	0.03392016
Cell Component level 5	lipid particle	17	3.86%	0.03402637
Biol Process level 3	cell-cell adhesion	9	2.05%	0.03456282
Biol Process level 4	cell fate commitment	17	3.86%	0.03485578
Mol Function level 4	peroxidase activity	4	0.91%	0.03498355
Mol Function level 3	oxidoreductase activity, acting on peroxide as acceptor	4	0.91%	0.03663431
Mol Function level 2	substrate-specific transporter activity	40	9.09%	0.03744975
Biol Process level 3	macromolecule biosynthetic process	42	9.55%	0.03773692
Biol Process level 3	lipid metabolic process	25	5.68%	0.03904056
Cell Component level 3	proton-transporting two-sector ATPase complex	5	1.14%	0.04003851
Mol Function level 3	substrate-specific transmembrane transporter activity	37	8.41%	0.04320409
Cell Component level 4	proton-transporting two-sector ATPase complex	5	1.14%	0.04325495
Cell Component level 5	proton-transporting two-sector ATPase complex	5	1.14%	0.0433397
Mol Function level 4	endopeptidase activity	19	4.32%	0.04377121
Mol Function level 5	glutathione peroxidase activity	3	0.68%	0.04413503
Biol Process level 2	behavior	21	4.77%	0.04449898
Mol Function level 4	rRNA binding	4	0.91%	0.04879272



**Table S2.** Significantly enriched GO terms for genes with significantly higher gene expression in morning-collected foragers as compared to afternoon-collected foragers.

GO Category	GO Term	Count	%	PValue
Cell Component level 4	cytosol	49	11.29%	2.80E-14
Cell Component level 5	cytosol	49	11.29%	4.56E-14
Mol Function level 2	structural constituent of ribosome	44	10.14%	5.34E-14
Cell Component level 4	cytosolic ribosome (sensu Eukaryota)	30	6.91%	7.15E-14
Cell Component level 5	cytosolic ribosome (sensu Eukaryota)	30	6.91%	1.00E-13
Cell Component level 3	ribosomal subunit	41	9.45%	2.61E-13
Cell Component level 3	ribosome	44	10.14%	5.96E-13
Cell Component level 4	ribosomal subunit	41	9.45%	1.22E-12
Cell Component level 5	ribosomal subunit	41	9.45%	1.86E-12
Cell Component level 4	cytosolic part	33	7.60%	1.93E-12
Cell Component level 5	cytosolic part	33	7.60%	2.75E-12
Cell Component level 4	ribosome	44	10.14%	2.99E-12
Cell Component level 5	ribosome	44	10.14%	4.62E-12
Biol Process level 2	translation	54	12.44%	9.42E-11
Biol Process level 4	translation	54	12.44%	2.54E-10
Biol Process level 5	translation	54	12.44%	2.86E-10
Cell Component level 3	cytoplasm	149	34.33%	3.14E-10
Biol Process level 3	cellular biosynthetic process	80	18.43%	3.40E-10
Biol Process level 3	macromolecule biosynthetic process	62	14.29%	1.55E-09
Biol Process level 2	biosynthetic process	86	19.82%	4.92E-09
Cell Component level 4	cytoplasm	149	34.33%	8.49E-09
Cell Component level 5	cytoplasm	149	34.33%	2.14E-08
Cell Component level 2	ribonucleoprotein complex	49	11.29%	2.51E-08
Cell Component level 3	ribonucleoprotein complex	49	11.29%	2.55E-08
Cell Component level 3	cytoplasmic part	125	28.80%	2.57E-08
Cell Component level 5	cytosolic large ribosomal subunit (sensu Eukaryota)	18	4.15%	5.69E-08
Cell Component level 4	ribonucleoprotein complex	49	11.29%	1.16E-07
Cell Component level 3	small ribosomal subunit	19	4.38%	1.57E-07
Cell Component level 5	ribonucleoprotein complex	49	11.29%	1.73E-07
Mol Function level 1	structural molecule activity	48	11.06%	1.75E-07
Biol Process level 2	cellular metabolic process	217	50.00%	1.78E-07
Biol Process level 1	metabolic process	235	54.15%	2.30E-07
Cell Component level 4	small ribosomal subunit	19	4.38%	3.33E-07
Cell Component level 5	small ribosomal subunit	19	4.38%	4.07E-07
Cell Component level 4	cytoplasmic part	125	28.80%	4.24E-07
Cell Component level 4	large ribosomal subunit	24	5.53%	7.97E-07
Cell Component level 5	cytoplasmic part	125	28.80%	9.10E-07
Cell Component level 5	large ribosomal subunit	24	5.53%	1.01E-06
Cell Component level 1	macromolecular complex	120	27.65%	1.87E-06
Cell Component level 4	cytosolic small ribosomal subunit (sensu Eukaryota)	12	2.76%	5.63E-06
Cell Component level 5	cytosolic small ribosomal subunit (sensu Eukaryota)	12	2.76%	6.43E-06
Cell Component level 3	mitochondrial membrane part	24	5.53%	7.30E-06
Cell Component level 4	mitochondrial membrane part	24	5.53%	1.66E-05
Cell Component level 3	organelle inner membrane	29	6.68%	1.81E-05
Cell Component level 5	mitochondrial membrane part	24	5.53%	2.07E-05

Biol Process level 3	oxidative phosphorylation	22	5.07%	2.50E-05
Cell Component level 3	mitochondrial part	46	10.60%	3.21E-05
Cell Component level 3	mitochondrial envelope	32	7.37%	3.50E-05
Mol Function level 4	rRNA binding	7	1.61%	3.69E-05
Cell Component level 4	mitochondrial inner membrane	29	6.68%	4.50E-05
Cell Component level 4	organelle inner membrane	29	6.68%	4.50E-05
Cell Component level 5	mitochondrial inner membrane	29	6.68%	5.74E-05
Cell Component level 5	organelle inner membrane	29	6.68%	5.74E-05
Cell Component level 2	non-membrane-bound organelle	60	13.82%	8.70E-05
Cell Component level 3	intracellular non-membrane-bound organelle	60	13.82%	8.82E-05
Cell Component level 4	mitochondrial envelope	32	7.37%	9.04E-05
Cell Component level 3	mitochondrial membrane	29	6.68%	9.32E-05
Cell Component level 4	mitochondrial part	46	10.60%	1.07E-04
Cell Component level 2	intracellular part	193	44.47%	1.14E-04
Cell Component level 5	mitochondrial envelope	32	7.37%	1.16E-04
Cell Component level 3	intracellular part	193	44.47%	1.19E-04
Cell Component level 5	mitochondrial part	46	10.60%	1.47E-04
Cell Component level 3	proteasome complex (sensu Eukaryota)	13	3.00%	1.82E-04
Biol Process level 3	electron transport	25	5.76%	2.11E-04
Cell Component level 4	mitochondrial membrane	29	6.68%	2.20E-04
Cell Component level 4	mitochondrial respiratory chain	16	3.69%	2.56E-04
Cell Component level 5	mitochondrial membrane	29	6.68%	2.76E-04
Biol Process level 2	generation of precursor metabolites and energy	31	7.14%	2.80E-04
Cell Component level 4	proteasome complex (sensu Eukaryota)	13	3.00%	2.93E-04
Cell Component level 5	mitochondrial respiratory chain	16	3.69%	2.98E-04
Cell Component level 5	proteasome complex (sensu Eukaryota)	13	3.00%	3.33E-04
Cell Component level 4	intracellular non-membrane-bound organelle	60	13.82%	3.49E-04
Biol Process level 4	ATP synthesis coupled electron transport	14	3.23%	4.66E-04
Biol Process level 5	organelle ATP synthesis coupled electron transport	14	3.23%	4.89E-04
Cell Component level 5	intracellular non-membrane-bound organelle	60	13.82%	5.00E-04
Cell Component level 3	proteasome core complex (sensu Eukaryota)	7	1.61%	5.01E-04
Cell Component level 4	mitochondrion	51	11.75%	5.91E-04
Biol Process level 3	cellular catabolic process	29	6.68%	5.95E-04
Biol Process level 2	catabolic process	31	7.14%	6.39E-04
Cell Component level 4	proteasome core complex (sensu Eukaryota)	7	1.61%	6.61E-04
Biol Process level 4	cellular protein metabolic process	113	26.04%	6.93E-04
Cell Component level 5	proteasome core complex (sensu Eukaryota)	7	1.61%	7.12E-04
Cell Component level 2	intracellular organelle part	106	24.42%	7.80E-04
Cell Component level 2	organelle envelope	33	7.60%	7.88E-04
Cell Component level 3	intracellular organelle part	106	24.42%	7.93E-04
Cell Component level 3	organelle envelope	33	7.60%	7.94E-04
Cell Component level 5	mitochondrion	51	11.75%	8.09E-04
Biol Process level 3	cellular macromolecule metabolic process	113	26.04%	8.58E-04
Cell Component level 2	organelle part	106	24.42%	8.62E-04
Biol Process level 3	macromolecule catabolic process	22	5.07%	8.90E-04
Cell Component level 4	lipid particle	21	4.84%	8.96E-04
Cell Component level 1	envelope	33	7.60%	9.25E-04
Cell Component level 5	lipid particle	21	4.84%	0.00106364
Biol Process level 3	protein metabolic process	114	26.27%	0.00106524
Cell Component level 2	protein complex	90	20.74%	0.00119323

Mol Function level 4	endopeptidase activity	23	5.30%	0.00120338
Cell Component level 1	organelle part	106	24.42%	0.00131232
Cell Component level 4	mitochondrial proton-transporting ATP synthase complex	7	1.61%	0.00179345
Cell Component level 2	intracellular	201	46.31%	0.001805
Cell Component level 4	organelle envelope	33	7.60%	0.00183999
Cell Component level 3	intracellular	201	46.31%	0.00187765
Cell Component level 5	mitochondrial proton-transporting ATP synthase complex	7	1.61%	0.00192713
Cell Component level 3	proton-transporting ATP synthase complex	7	1.61%	0.00209902
Cell Component level 5	organelle envelope	33	7.60%	0.00229539
Cell Component level 4	proton-transporting ATP synthase complex	7	1.61%	0.00273108
Cell Component level 5	proton-transporting ATP synthase complex	7	1.61%	0.00293093
Cell Component level 4	intracellular part	193	44.47%	0.00389141
Biol Process level 2	macromolecule metabolic process	168	38.71%	0.00393127
Biol Process level 2	primary metabolic process	195	44.93%	0.00406562
Biol Process level 4	cellular macromolecule catabolic process	18	4.15%	0.00469333
Cell Component level 4	intracellular organelle part	106	24.42%	0.00487684
Biol Process level 5	cellular protein catabolic process	11	2.53%	0.00517759
Biol Process level 5	modification-dependent macromolecule catabolic process	11	2.53%	0.00517759
Mol Function level 4	NADH dehydrogenase activity	9	2.07%	0.00539759
Mol Function level 3	oxidoreductase activity, acting on NADH or NADPH	10	2.30%	0.00560987
Biol Process level 4	protein catabolic process	12	2.76%	0.00625428
Biol Process level 5	protein catabolic process	12	2.76%	0.00649991
Cell Component level 5	intracellular organelle part	106	24.42%	0.00775429
Cell Component level 3	proteasome regulatory particle, base subcomplex	5	1.15%	0.0080292
Biol Process level 4	mitochondrial electron transport, NADH to ubiquinone	9	2.07%	0.00861519
Cell Component level 3	respiratory chain complex I	9	2.07%	0.00881711
Cell Component level 3	NADH dehydrogenase complex (quinone)	9	2.07%	0.00881711
Cell Component level 4	proteasome regulatory particle, base subcomplex	5	1.15%	0.00963485
Cell Component level 5	proteasome regulatory particle, base subcomplex	5	1.15%	0.0101182
Biol Process level 1	gene expression	86	19.82%	0.01025009
Biol Process level 4	biopolymer catabolic process	15	3.46%	0.01126867
Cell Component level 4	NADH dehydrogenase complex (quinone)	9	2.07%	0.01186613
Cell Component level 4	respiratory chain complex I	9	2.07%	0.01186613
Cell Component level 4	mitochondrial respiratory chain complex I	9	2.07%	0.01186613
Mol Function level 3	peptidase activity	27	6.22%	0.01220242
Cell Component level 5	respiratory chain complex I	9	2.07%	0.0128434
Cell Component level 5	NADH dehydrogenase complex (quinone)	9	2.07%	0.0128434
Cell Component level 5	mitochondrial respiratory chain complex I	9	2.07%	0.0128434
Mol Function level 2	oxidoreductase activity	39	8.99%	0.01325982
Mol Function level 4	magnesium ion binding	7	1.61%	0.0147554
Mol Function level 1	antioxidant activity	5	1.15%	0.01940609
Mol Function level 5	nucleoside diphosphate kinase activity	3	0.69%	0.02160293
Biol Process level 3	translational initiation	8	1.84%	0.02162925
Cell Component level 3	proton-transporting two-sector ATPase complex	9	2.07%	0.02188416
Biol Process level 4	alcohol biosynthetic process	4	0.92%	0.02260778
Mol Function level 3	translation factor activity, nucleic acid binding	10	2.30%	0.02279624
Biol Process level 5	monosaccharide biosynthetic process	4	0.92%	0.0229404
Mol Function level 5	ATPase activity, coupled to transmembrane movement	12	2.76%	0.02308302
Biol Process level 5	translational initiation	8	1.84%	0.02450978
Mol Function level 5	threonine endopeptidase activity	4	0.92%	0.02665398

Mol Function level 2	translation factor activity, nucleic acid binding	10	2.30%	0.02676061
Cell Component level 4	mitochondrial proton-transporting ATP synthase complex	4	0.92%	0.02879394
Cell Component level 4	proton-transporting two-sector ATPase complex	9	2.07%	0.02879806
Cell Component level 5	mitochondrial proton-transporting ATP synthase complex	4	0.92%	0.02987292
Biol Process level 3	cofactor metabolic process	19	4.38%	0.03046757
Cell Component level 5	proton-transporting two-sector ATPase complex	9	2.07%	0.03097504
Mol Function level 3	electron carrier activity	14	3.23%	0.03102381
Biol Process level 4	cellular carbohydrate metabolic process	18	4.15%	0.03208603
Cell Component level 4	organellar small ribosomal subunit	7	1.61%	0.03288201
Cell Component level 4	mitochondrial small ribosomal subunit	7	1.61%	0.03288201
Cell Component level 3	proton-transporting two-sector ATPase complex	5	1.15%	0.03312621
Cell Component level 2	organelle membrane	35	8.06%	0.03342026
Cell Component level 3	organelle membrane	35	8.06%	0.03360501
Mol Function level 4	hydrolase activity catalyzing transmembrane movement	12	2.76%	0.0347517
Cell Component level 5	organellar small ribosomal subunit	7	1.61%	0.03489716
Cell Component level 5	mitochondrial small ribosomal subunit	7	1.61%	0.03489716
Mol Function level 1	translation regulator activity	10	2.30%	0.03668182
Cell Component level 3	proton-transporting ATP synthase complex, coupling factor F(o)	4	0.92%	0.0372693
Cell Component level 4	proton-transporting two-sector ATPase complex	5	1.15%	0.03908941
Mol Function level 5	glutathione peroxidase activity	3	0.69%	0.04072489
Cell Component level 5	proton-transporting two-sector ATPase complex	5	1.15%	0.04085865
Cell Component level 4	proton-transporting ATP synthase complex, coupling factor F(o)	4	0.92%	0.04257474
Mol Function level 3	glutathione peroxidase activity	3	0.69%	0.04349528
Cell Component level 5	proton-transporting ATP synthase complex, coupling factor F(o)	4	0.92%	0.04412134
Biol Process level 3	carbohydrate metabolic process	24	5.53%	0.04495144
Biol Process level 5	nucleoside triphosphate biosynthetic process	8	1.84%	0.04508237



**Table S3.** Significantly enriched GO terms for genes with significantly higher gene expression in afternoon-collected foragers as compared to morning-collected foragers.

GO Category	GO Term	Count	%	PValue
Biol Process level 3	lipid metabolic process	31	7.51%	4.03E-05
Biol Process level 2	behavior	26	6.30%	1.21E-04
Biol Process level 1	localization	101	24.46%	1.29E-04
Mol Function level 5	peptide-methionine-(S)-S-oxide reductase activity	5	1.21%	2.09E-04
Mol Function level 4	oxidoreductase activity, sulfur donors and disulfide acceptors	5	1.21%	2.42E-04
Biol Process level 1	rhythmic process	10	2.42%	2.74E-04
Cell Component level 4	endoplasmic reticulum	22	5.33%	5.30E-04
Cell Component level 5	endoplasmic reticulum	22	5.33%	6.17E-04
Mol Function level 2	isomerase activity	13	3.15%	6.27E-04
Biol Process level 2	circadian rhythm	9	2.18%	0.0011106
Biol Process level 1	maintenance of localization	6	1.45%	0.00139154
Biol Process level 2	maintenance of localization	6	1.45%	0.00139674
Biol Process level 4	cellular lipid metabolic process	23	5.57%	0.00146618
Biol Process level 3	cellular lipid metabolic process	23	5.57%	0.00181973
Biol Process level 2	rhythmic behavior	8	1.94%	0.00183813
Biol Process level 3	rhythmic behavior	8	1.94%	0.00188592
Biol Process level 1	response to stimulus	48	11.62%	0.00449752
Mol Function level 3	oxidoreductase activity, acting on sulfur group of donors	5	1.21%	0.00479187
Mol Function level 3	protein kinase regulator activity	7	1.69%	0.00531099
Mol Function level 2	kinase regulator activity	8	1.94%	0.00548635
Biol Process level 4	circadian behavior	7	1.69%	0.00668732
Biol Process level 3	circadian behavior	7	1.69%	0.00721931
Biol Process level 1	biological adhesion	17	4.12%	0.00878023
Biol Process level 2	cell adhesion	17	4.12%	0.00883815
Biol Process level 4	maintenance of protein localization	4	0.97%	0.0102032
Biol Process level 5	prosthetic group metabolic process	5	1.21%	0.01024123
Biol Process level 3	chemosensory behavior	9	2.18%	0.01031096
Biol Process level 2	maintenance of protein localization	4	0.97%	0.01052335
Biol Process level 3	maintenance of protein localization	4	0.97%	0.01065562
Biol Process level 4	learning	7	1.69%	0.01075861
Biol Process level 5	establishment and/or maintenance of cytoskeleton polarity	6	1.45%	0.01102951
Biol Process level 3	prosthetic group metabolic process	5	1.21%	0.01127528
Biol Process level 4	oocyte microtubule cytoskeleton organization	6	1.45%	0.01158577
Cell Component level 4	endoplasmic reticulum part	10	2.42%	0.0117409
Cell Component level 3	endoplasmic reticulum part	10	2.42%	0.01181833
Cell Component level 5	endoplasmic reticulum part	10	2.42%	0.01262726
Biol Process level 3	learning and/or memory	9	2.18%	0.0143174
Mol Function level 3	lipid transporter activity	5	1.21%	0.01530148
Biol Process level 2	response to abiotic stimulus	14	3.39%	0.01646947
Biol Process level 5	oocyte axis determination	11	2.66%	0.0170473
Biol Process level 5	olfactory learning	6	1.45%	0.01802728
Biol Process level 4	oocyte axis determination	11	2.66%	0.01842518
Mol Function level 5	protein-methionine-R-oxide reductase activity	3	0.73%	0.01914839
Cell Component level 4	germline ring canal	4	0.97%	0.02017843
Cell Component level 3	germline ring canal	4	0.97%	0.0201915

Cell Component level 3	intercellular bridge	4	0.97%	0.0201915
Cell Component level 2	intercellular bridge	4	0.97%	0.02042452
Biol Process level 4	sequestering of lipid	3	0.73%	0.02066872
Biol Process level 5	oocyte construction	11	2.66%	0.02151818
Biol Process level 2	response to chemical stimulus	17	4.12%	0.02155979
Biol Process level 3	locomotory behavior	11	2.66%	0.02289151
Biol Process level 1	establishment of localization	79	19.13%	0.02345202
Biol Process level 2	transport	78	18.89%	0.02353548
Biol Process level 2	establishment of localization	79	19.13%	0.02366241
Biol Process level 5	oocyte development	11	2.66%	0.02404916
Biol Process level 2	cell motility	17	4.12%	0.02526339
Biol Process level 3	oocyte construction	11	2.66%	0.02561518
Biol Process level 3	transport	78	18.89%	0.02584794
Biol Process level 2	sleep	4	0.97%	0.02585659
Biol Process level 4	oocyte development	11	2.66%	0.025918
Biol Process level 3	cell motility	17	4.12%	0.02619635
Biol Process level 4	lipid transport	6	1.45%	0.02874319
Biol Process level 3	lipid transport	6	1.45%	0.03051321
Biol Process level 1	multicellular organismal process	90	21.79%	0.03051333
Biol Process level 5	germ cell development	13	3.15%	0.03137042
Mol Function level 2	oxidoreductase activity	34	8.23%	0.03230924
Cell Component level 1	extracellular region	14	3.39%	0.03286903
Biol Process level 4	germ cell development	13	3.15%	0.03404026
Biol Process level 2	localization of cell	17	4.12%	0.03408011
Biol Process level 1	developmental process	85	20.58%	0.03416661
Cell Component level 4	integral to membrane	53	12.83%	0.0349069
Biol Process level 3	response to wounding	4	0.97%	0.03677415
Cell Component level 4	intrinsic to membrane	53	12.83%	0.03768409
Biol Process level 3	germ cell development	13	3.15%	0.03783865
Biol Process level 2	response to external stimulus	12	2.91%	0.03926042
Biol Process level 5	cell redox homeostasis	6	1.45%	0.03954847
Cell Component level 3	intrinsic to membrane	53	12.83%	0.03983332
Biol Process level 4	axis specification	13	3.15%	0.04037106
Cell Component level 5	integral to membrane	53	12.83%	0.04234256
Biol Process level 4	oocyte differentiation	11	2.66%	0.0428938
Biol Process level 3	cell redox homeostasis	6	1.45%	0.04377332
Biol Process level 3	axis specification	13	3.15%	0.04476133
Biol Process level 5	membrane lipid metabolic process	9	2.18%	0.04540093
Cell Component level 5	intrinsic to membrane	53	12.83%	0.04561559
Biol Process level 5	nerve-nerve synaptic transmission	4	0.97%	0.04594905
Biol Process level 4	membrane lipid metabolic process	9	2.18%	0.04818339

**Table S4.** Significantly enriched GO terms for genes differentially expressed between morning- and afternoon-trained foragers.

GO Category	GO Term	Count	%	PValue
Biol Process level 5	mesoderm development	6	8.70%	0.00125436
Biol Process level 4	mesoderm development	6	8.70%	0.00155513
Biol Process level 2	catabolic process	10	14.49%	0.00370188
Mol Function level 5	phosphoric diester hydrolase activity	4	5.80%	0.0040843
Mol Function level 2	kinase regulator activity	4	5.80%	0.00883697
Biol Process level 3	macromolecule catabolic process	7	10.14%	0.01226181
Biol Process level 3	cellular catabolic process	8	11.59%	0.02176575
Biol Process level 5	tissue development	6	8.70%	0.02217571
Biol Process level 4	tissue development	6	8.70%	0.02661243
Mol Function level 3	oxidoreductase activity, acting on CH-CH group of donors	3	4.35%	0.02812104
Biol Process level 3	tissue development	6	8.70%	0.02948445
Biol Process level 4	carbohydrate catabolic process	4	5.80%	0.0315342
Mol Function level 2	two-component response regulator activity	2	2.90%	0.03198469
Mol Function level 5	fructose-bisphosphate aldolase activity	2	2.90%	0.03513494
Mol Function level 3	two-component response regulator activity	2	2.90%	0.03527912
Biol Process level 4	response to heat	3	4.35%	0.04237111
Cell Component level 3	organelle inner membrane	6	8.70%	0.04409891
Biol Process level 3	response to heat	3	4.35%	0.0444005
Mol Function level 3	protein kinase regulator activity	3	4.35%	0.04809975

**Table S5.** Significantly enriched GO terms for genes with significantly higher gene expression in morning-trained foragers as compared to afternoon-trained foragers.

GO Category	GO Term	Count	%	PValue
Mol Function level 2	kinase regulator activity	3	12.00%	0.01142202
Mol Function level 5	fructose-bisphosphate aldolase activity	2	8.00%	0.0152706
Biol Process level 2	catabolic process	5	20.00%	0.02684746
Biol Process level 4	carbohydrate catabolic process	3	12.00%	0.02913609
Mol Function level 4	aldehyde-lyase activity	2	8.00%	0.03447943
Biol Process level 3	macromolecule catabolic process	4	16.00%	0.03520985
Mol Function level 3	kinase inhibitor activity	2	8.00%	0.04118181
Biol Process level 4	mesoderm development	3	12.00%	0.04396019
Biol Process level 5	mesoderm development	3	12.00%	0.04722053



**Table S6.** Significantly enriched GO terms for genes with significantly higher gene expression in afternoon-trained foragers as compared to morning-trained foragers.

GO Category	GO Term	Count	%	PValue
Biol Process level 5	nucleotide metabolic process	5	11.11%	0.00811953
Mol Function level 3	oxidoreductase activity, acting on the CH-CH group of donors	3	6.67%	0.0108935
Biol Process level 2	response to abiotic stimulus	5	11.11%	0.01128647
Biol Process level 4	response to heat	3	6.67%	0.01691561
Biol Process level 3	response to heat	3	6.67%	0.01835818
Biol Process level 4	nucleobase, nucleoside and nucleotide metabolic process	5	11.11%	0.01915743
Mol Function level 2	two-component response regulator activity	2	4.44%	0.01991376
Mol Function level 3	two-component response regulator activity	2	4.44%	0.02151968
Biol Process level 3	response to temperature stimulus	3	6.67%	0.02428874
Biol Process level 5	establishment and/or maintenance of chromatin architecture	4	8.89%	0.02540583
Biol Process level 5	DNA packaging	4	8.89%	0.02540583
Cell Component level 3	organelle inner membrane	5	11.11%	0.04087235
Biol Process level 4	two-component signal transduction system (phosphorelay)	2	4.44%	0.04215106
Cell Component level 4	organelle inner membrane	5	11.11%	0.04441127
Cell Component level 4	mitochondrial inner membrane	5	11.11%	0.04441127
Cell Component level 2	organelle membrane	7	15.56%	0.04446571
Cell Component level 3	organelle membrane	7	15.56%	0.0445454
Cell Component level 3	mitochondrial part	7	15.56%	0.04529472
Cell Component level 5	mitochondrial inner membrane	5	11.11%	0.04837859
Cell Component level 5	organelle inner membrane	5	11.11%	0.04837859
Cell Component level 4	organelle membrane	7	15.56%	0.04944547

**Table S7.** Significantly enriched GO terms for genes differentially expressed between active (anticipating a food reward) and inactive foragers.

GO Category	GO Term	Count	%	PValue
Mol Function level 2	structural constituent of ribosome	91	7.97%	5.70E-28
Cell Component level 3	ribosome	90	7.88%	3.53E-26
Cell Component level 4	ribosome	90	7.88%	6.54E-25
Cell Component level 3	ribosomal subunit	80	7.01%	1.82E-24
Cell Component level 5	ribosome	90	7.88%	2.45E-24
Cell Component level 4	ribosomal subunit	80	7.01%	2.57E-23
Cell Component level 5	ribosomal subunit	80	7.01%	8.46E-23
Cell Component level 4	cytosolic ribosome (sensu Eukaryota)	51	4.47%	1.36E-21
Cell Component level 5	cytosolic ribosome (sensu Eukaryota)	51	4.47%	3.11E-21
Cell Component level 2	ribonucleoprotein complex	109	9.54%	2.08E-18
Cell Component level 3	ribonucleoprotein complex	109	9.54%	2.15E-18
Cell Component level 4	cytosolic part	57	4.99%	6.49E-18
Cell Component level 5	cytosolic part	57	4.99%	1.53E-17
Cell Component level 4	ribonucleoprotein complex	109	9.54%	4.26E-17
Biol Process level 2	translation	111	9.72%	1.08E-16
Cell Component level 5	ribonucleoprotein complex	109	9.54%	2.75E-16
Cell Component level 1	macromolecular complex	285	24.96%	2.80E-16
Biol Process level 5	translation	111	9.72%	3.17E-16
Biol Process level 4	translation	111	9.72%	4.11E-16
Biol Process level 3	cellular biosynthetic process	174	15.24%	5.28E-16
Mol Function level 1	structural molecule activity	109	9.54%	5.67E-15
Cell Component level 3	cytoplasmic part	276	24.17%	1.34E-13
Cell Component level 3	cytoplasm	319	27.93%	4.17E-13
Cell Component level 3	small ribosomal subunit	35	3.06%	1.23E-12
Biol Process level 3	macromolecule biosynthetic process	126	11.03%	1.99E-12
Cell Component level 5	cytosolic large ribosomal subunit (sensu Eukaryota)	31	2.71%	2.88E-12
Cell Component level 4	small ribosomal subunit	35	3.06%	4.24E-12
Cell Component level 5	small ribosomal subunit	35	3.06%	7.25E-12
Biol Process level 2	biosynthetic process	184	16.11%	8.12E-12
Cell Component level 4	cytoplasmic part	276	24.17%	1.78E-11
Cell Component level 4	large ribosomal subunit	46	4.03%	3.27E-11
Cell Component level 5	large ribosomal subunit	46	4.03%	6.20E-11
Cell Component level 4	cytoplasm	319	27.93%	8.49E-11
Cell Component level 5	cytoplasmic part	276	24.17%	1.86E-10
Cell Component level 3	mitochondrial part	102	8.93%	6.03E-10
Cell Component level 5	cytoplasm	319	27.93%	1.18E-09
Cell Component level 2	non-membrane-bound organelle	137	12.00%	1.43E-09
Cell Component level 3	intracellular non-membrane-bound organelle	137	12.00%	1.47E-09
Cell Component level 2	organelle part	256	22.42%	1.81E-09
Cell Component level 2	intracellular organelle part	255	22.33%	2.42E-09
Cell Component level 3	intracellular organelle part	255	22.33%	2.57E-09
Cell Component level 1	organelle part	256	22.42%	3.86E-09
Cell Component level 4	cytosol	69	6.04%	4.98E-09
Cell Component level 4	mitochondrial part	102	8.93%	6.20E-09
Cell Component level 4	cytosolic small ribosomal subunit (sensu Eukaryota)	20	1.75%	6.65E-09

Cell Component level 5	cytosolic small ribosomal subunit (sensu Eukaryota)	20	1.75%	9.13E-09
Cell Component level 5	cytosol	69	6.04%	1.09E-08
Cell Component level 5	mitochondrial part	102	8.93%	1.71E-08
Biol Process level 3	oxidative phosphorylation	45	3.94%	2.04E-08
Cell Component level 4	intracellular non-membrane-bound organelle	137	12.00%	2.32E-08
Cell Component level 3	mitochondrial membrane part	45	3.94%	3.56E-08
Cell Component level 2	protein complex	214	18.74%	4.29E-08
Cell Component level 5	intracellular non-membrane-bound organelle	137	12.00%	7.73E-08
Cell Component level 4	mitochondrial membrane part	45	3.94%	1.28E-07
Cell Component level 4	intracellular organelle part	255	22.33%	1.52E-07
Cell Component level 4	mitochondrion	115	10.07%	1.77E-07
Cell Component level 5	mitochondrial membrane part	45	3.94%	2.22E-07
Cell Component level 5	mitochondrion	115	10.07%	4.89E-07
Cell Component level 5	intracellular organelle part	255	22.33%	9.48E-07
Mol Function level 3	substrate-specific transmembrane transporter activity	94	8.23%	1.83E-06
Biol Process level 2	generation of precursor metabolites and energy	67	5.87%	2.82E-06
Cell Component level 4	mitochondrial respiratory chain	29	2.54%	1.15E-05
Cell Component level 4	lipid particle	42	3.68%	1.50E-05
Cell Component level 5	mitochondrial respiratory chain	29	2.54%	1.65E-05
Cell Component level 3	mitochondrial lumen	45	3.94%	2.17E-05
Cell Component level 4	mitochondrial ribosome	31	2.71%	2.18E-05
Cell Component level 4	organellar ribosome	31	2.71%	2.18E-05
Cell Component level 5	lipid particle	42	3.68%	2.38E-05
Mol Function level 5	cation transmembrane transporter activity	64	5.60%	2.89E-05
Cell Component level 3	mitochondrial envelope	58	5.08%	3.15E-05
Cell Component level 5	mitochondrial ribosome	31	2.71%	3.15E-05
Cell Component level 5	organellar ribosome	31	2.71%	3.15E-05
Cell Component level 3	mitochondrial membrane	53	4.64%	5.91E-05
Cell Component level 4	mitochondrial matrix	45	3.94%	6.35E-05
Cell Component level 4	mitochondrial lumen	45	3.94%	6.35E-05
Cell Component level 5	mitochondrial matrix	45	3.94%	9.99E-05
Cell Component level 5	mitochondrial lumen	45	3.94%	9.99E-05
Cell Component level 3	organelle inner membrane	49	4.29%	1.02E-04
Biol Process level 5	organelle ATP synthesis coupled electron transport	25	2.19%	1.02E-04
Biol Process level 4	ATP synthesis coupled electron transport	25	2.19%	1.08E-04
Cell Component level 4	mitochondrial envelope	58	5.08%	1.09E-04
Mol Function level 2	substrate-specific transporter activity	101	8.84%	1.20E-04
Cell Component level 2	intracellular organelle	367	32.14%	1.41E-04
Cell Component level 3	intracellular organelle	367	32.14%	1.49E-04
Cell Component level 2	intracellular part	434	38.00%	1.57E-04
Mol Function level 4	ion transmembrane transporter activity	72	6.30%	1.60E-04
Cell Component level 3	intracellular part	434	38.00%	1.68E-04
Cell Component level 5	mitochondrial envelope	58	5.08%	1.83E-04
Cell Component level 4	mitochondrial membrane	53	4.64%	1.85E-04
Biol Process level 3	electron transport	47	4.12%	1.91E-04
Cell Component level 1	organelle	368	32.22%	2.48E-04
Cell Component level 4	mitochondrial inner membrane	49	4.29%	2.94E-04
Cell Component level 4	organelle inner membrane	49	4.29%	2.94E-04
Cell Component level 5	mitochondrial membrane	53	4.64%	2.98E-04
Mol Function level 2	oxidoreductase activity	92	8.06%	3.30E-04

Cell Component level 4	mitochondrial small ribosomal subunit	15	1.31%	4.07E-04
Cell Component level 4	organellar small ribosomal subunit	15	1.31%	4.07E-04
Cell Component level 5	mitochondrial inner membrane	49	4.29%	4.59E-04
Cell Component level 5	organelle inner membrane	49	4.29%	4.59E-04
Mol Function level 4	primary active transmembrane transporter activity	33	2.89%	4.92E-04
Mol Function level 5	P-P-bond-hydrolysis-driven transmembrane transport	33	2.89%	4.92E-04
Cell Component level 5	mitochondrial small ribosomal subunit	15	1.31%	4.96E-04
Cell Component level 5	organellar small ribosomal subunit	15	1.31%	4.96E-04
Mol Function level 2	transmembrane transporter activity	99	8.67%	6.91E-04
Biol Process level 3	protein metabolic process	261	22.85%	0.00126707
Biol Process level 5	phosphorylation	78	6.83%	0.00178591
Mol Function level 3	active transmembrane transporter activity	51	4.47%	0.00191717
Mol Function level 1	transporter activity	115	10.07%	0.00207422
Mol Function level 5	ATPase activity, coupled to transmembrane movement	22	1.93%	0.00211804
Biol Process level 2	behavior	47	4.12%	0.00223009
Cell Component level 2	organelle envelope	61	5.34%	0.00234895
Cell Component level 3	organelle envelope	61	5.34%	0.0023744
Biol Process level 1	metabolic process	526	46.06%	0.00260611
Cell Component level 1	envelope	61	5.34%	0.00265565
Cell Component level 2	organelle membrane	79	6.92%	0.00281059
Cell Component level 3	organelle membrane	79	6.92%	0.00284594
Biol Process level 5	protein import into mitochondrion	7	0.61%	0.003261
Biol Process level 5	inner mitochondrial membrane organization and biogenesis	6	0.53%	0.00371197
Biol Process level 2	cellular metabolic process	483	42.29%	0.00374652
Cell Component level 3	proton-transporting two-sector ATPase complex	17	1.49%	0.00445366
Biol Process level 4	cellular protein metabolic process	249	21.80%	0.0050558
Mol Function level 3	heme-copper terminal oxidase activity	7	0.61%	0.00509952
Mol Function level 3	oxidoreductase activity, acting on heme group of donors	7	0.61%	0.00509952
Biol Process level 5	ATP biosynthetic process	15	1.31%	0.00512462
Mol Function level 4	cytochrome-c oxidase activity	7	0.61%	0.00579376
Mol Function level 4	oxidoreductase activity, acting on diphenols	7	0.61%	0.00579376
Mol Function level 4	oxidoreductase activity, acting on heme group of donors	7	0.61%	0.00579376
Mol Function level 5	cytochrome-c oxidase activity	7	0.61%	0.00585609
Mol Function level 5	ubiquinol-cytochrome-c reductase activity	7	0.61%	0.00585609
Mol Function level 4	P-P-bond-hydrolysis-driven protein transmembrane	10	0.88%	0.00600908
Mol Function level 4	macromolecule transmembrane transporter activity	10	0.88%	0.00600908
Mol Function level 5	P-P-bond-hydrolysis-driven protein transmembrane	10	0.88%	0.00608475
Cell Component level 4	organelle envelope	61	5.34%	0.00643863
Cell Component level 4	proton-transporting two-sector ATPase complex	17	1.49%	0.00688363
Biol Process level 4	ATP synthesis coupled proton transport	14	1.23%	0.00751167
Biol Process level 4	ATP metabolic process	15	1.31%	0.00755139
Cell Component level 5	proton-transporting two-sector ATPase complex	17	1.49%	0.00826083
Cell Component level 4	intracellular organelle	367	32.14%	0.00833753
Cell Component level 4	organelle membrane	79	6.92%	0.00891679
Biol Process level 3	cellular macromolecule metabolic process	250	21.89%	0.00897113
Cell Component level 2	intracellular	455	39.84%	0.00931126
Cell Component level 5	organelle envelope	61	5.34%	0.00966937
Cell Component level 3	receptor complex	6	0.53%	0.00983784
Cell Component level 3	intracellular	455	39.84%	0.00985202
Mol Function level 3	oxidoreductase activity, acting on diphenols	7	0.61%	0.01039086

Biol Process level 5	mitochondrial membrane organization and biogenesis	6	0.53%	0.0104365
Biol Process level 4	mitochondrial membrane organization and biogenesis	6	0.53%	0.01057777
Mol Function level 4	oxidoreductase activity, acting on sulfur group	5	0.44%	0.01060067
Mol Function level 5	glycerol kinase activity	5	0.44%	0.01069085
Mol Function level 5	peptide-methionine-(S)-S-oxide reductase activity	5	0.44%	0.01069085
Cell Component level 3	proton-transporting two-sector ATPase complex	9	0.79%	0.01190875
Mol Function level 3	protein transmembrane transporter activity	11	0.96%	0.01216651
Mol Function level 4	NADH dehydrogenase activity	14	1.23%	0.01222356
Cell Component level 3	mitochondrial intermembrane space protein transporter	5	0.44%	0.01249826
Biol Process level 3	chemosensory behavior	16	1.40%	0.01266062
Biol Process level 1	rhythmic process	13	1.14%	0.01362369
Biol Process level 3	phosphorus metabolic process	88	7.71%	0.01441563
Mol Function level 4	protein transmembrane transporter activity	11	0.96%	0.01441993
Biol Process level 5	second-messenger-mediated signaling	14	1.23%	0.01446285
Cell Component level 3	respiratory chain complex III	7	0.61%	0.0146282
Cell Component level 4	mitochondrial intermembrane space protein transporter	5	0.44%	0.01463859
Mol Function level 4	hydrolase activity, acting on acid anhydrides	24	2.10%	0.01464271
Biol Process level 5	purine nucleotide biosynthetic process	19	1.66%	0.01473813
Cell Component level 4	proton-transporting two-sector ATPase complex	9	0.79%	0.01542169
Cell Component level 5	mitochondrial intermembrane space protein transport	5	0.44%	0.01565371
Cell Component level 5	proton-transporting two-sector ATPase complex	9	0.79%	0.01719572
Cell Component level 3	respiratory chain complex I	14	1.23%	0.01757455
Cell Component level 3	NADH dehydrogenase complex (quinone)	14	1.23%	0.01757455
Cell Component level 4	respiratory chain complex III	7	0.61%	0.01803561
Cell Component level 4	mitochondrial respiratory chain complex III	7	0.61%	0.01803561
Biol Process level 3	response to organic substance	8	0.70%	0.01816016
Biol Process level 4	phosphate metabolic process	88	7.71%	0.01817947
Biol Process level 3	cofactor metabolic process	40	3.50%	0.01884778
Biol Process level 2	rhythmic behavior	11	0.96%	0.01963692
Cell Component level 5	respiratory chain complex III	7	0.61%	0.01970325
Cell Component level 5	mitochondrial respiratory chain complex III	7	0.61%	0.01970325
Cell Component level 2	membrane part	177	15.50%	0.02037799
Biol Process level 3	rhythmic behavior	11	0.96%	0.02048539
Cell Component level 3	membrane part	177	15.50%	0.02059484
Cell Component level 3	respiratory chain complex IV	6	0.53%	0.02117925
Biol Process level 3	hydrogen transport	16	1.40%	0.02177763
Biol Process level 4	mitochondrial electron transport, ubiquinol to cyt	6	0.53%	0.02268189
Biol Process level 5	proton transport	16	1.40%	0.02300102
Cell Component level 4	intracellular part	434	38.00%	0.02312098
Cell Component level 3	nuclear envelope-endoplasmic reticulum network	14	1.23%	0.02340239
Cell Component level 3	endoplasmic reticulum membrane	14	1.23%	0.02340239
Biol Process level 4	hydrogen transport	16	1.40%	0.023625
Biol Process level 4	proton transport	16	1.40%	0.023625
Mol Function level 5	ATPase activity, coupled to transmembrane movement	23	2.01%	0.02412427
Biol Process level 4	cofactor biosynthetic process	24	2.10%	0.02426565
Biol Process level 5	nucleoside triphosphate biosynthetic process	15	1.31%	0.02450949
Cell Component level 4	respiratory chain complex I	14	1.23%	0.02466606
Cell Component level 4	mitochondrial respiratory chain complex I	14	1.23%	0.02466606
Cell Component level 4	NADH dehydrogenase complex (quinone)	14	1.23%	0.02466606
Cell Component level 4	respiratory chain complex IV	6	0.53%	0.02533392

Cell Component level 4	mitochondrial respiratory chain complex IV	6	0.53%	0.02533392
Biol Process level 2	maintenance of localization	7	0.61%	0.02592449
Biol Process level 1	maintenance of localization	7	0.61%	0.02617627
Biol Process level 2	circadian rhythm	12	1.05%	0.02640219
Mol Function level 3	oxidoreductase activity, acting on NADH or NADPH	15	1.31%	0.02646726
Biol Process level 4	mitochondrial electron transport, NADH to ubiquinone	14	1.23%	0.02664953
Cell Component level 5	respiratory chain complex IV	6	0.53%	0.02732597
Cell Component level 5	mitochondrial respiratory chain complex IV	6	0.53%	0.02732597
Biol Process level 1	gene expression	192	16.81%	0.02737367
Biol Process level 5	male mating behavior	7	0.61%	0.0275266
Biol Process level 5	male courtship behavior	7	0.61%	0.0275266
Biol Process level 4	male mating behavior	7	0.61%	0.02791984
Cell Component level 5	respiratory chain complex I	14	1.23%	0.02841158
Cell Component level 5	mitochondrial respiratory chain complex I	14	1.23%	0.02841158
Cell Component level 5	NADH dehydrogenase complex (quinone)	14	1.23%	0.02841158
Mol Function level 3	oxidoreductase activity, acting on sulfur group of donors	6	0.53%	0.02921014
Biol Process level 2	maintenance of protein localization	5	0.44%	0.03055749
Biol Process level 5	protein targeting to mitochondrion	10	0.88%	0.0307241
Biol Process level 5	fatty acid biosynthetic process	9	0.79%	0.03086923
Biol Process level 3	maintenance of protein localization	5	0.44%	0.03123295
Biol Process level 4	fatty acid biosynthetic process	9	0.79%	0.03140034
Mol Function level 4	rRNA binding	6	0.53%	0.03225806
Biol Process level 4	maintenance of protein localization	5	0.44%	0.03229064
Cell Component level 4	endoplasmic reticulum membrane	14	1.23%	0.03249691
Cell Component level 4	nuclear envelope-endoplasmic reticulum network	14	1.23%	0.03249691
Biol Process level 4	coenzyme metabolic process	36	3.15%	0.03479011
Biol Process level 3	ion transport	49	4.29%	0.03709245
Cell Component level 5	endoplasmic reticulum membrane	14	1.23%	0.03725468
Cell Component level 5	nuclear envelope-endoplasmic reticulum network	14	1.23%	0.03725468
Mol Function level 4	oxidoreductase activity, acting on NADH or NADPH	11	0.96%	0.03780262
Mol Function level 4	hydrolase activity, acting on carbon-nitrogen	4	0.35%	0.03819229
Mol Function level 5	NADH dehydrogenase (quinone) activity	11	0.96%	0.0382706
Cell Component level 5	intracellular organelle	367	32.14%	0.03933067
Mol Function level 3	transferase activity, transferring alkyl or aryl	9	0.79%	0.03960956
Biol Process level 2	behavioral interaction between organisms	10	0.88%	0.03965328
Biol Process level 3	behavioral interaction between organisms	10	0.88%	0.04112891
Biol Process level 3	circadian behavior	10	0.88%	0.04112891
Biol Process level 3	mating behavior	10	0.88%	0.04112891
Cell Component level 3	mitochondrial inner membrane presequence translocation	7	0.61%	0.04162373
Biol Process level 5	cation transport	42	3.68%	0.04181734
Biol Process level 4	ion transport	49	4.29%	0.04297774
Biol Process level 4	mating behavior	10	0.88%	0.04346654
Biol Process level 4	circadian behavior	10	0.88%	0.04346654
Biol Process level 5	coenzyme biosynthetic process	21	1.84%	0.04355447
Biol Process level 4	cation transport	42	3.68%	0.04379238
Biol Process level 5	carboxylic acid biosynthetic process	9	0.79%	0.04425238
Biol Process level 5	polyamine metabolic process	4	0.35%	0.0447209
Biol Process level 4	organic acid biosynthetic process	9	0.79%	0.04497894
Mol Function level 3	electron carrier activity	26	2.28%	0.04503005
Biol Process level 5	ribonucleotide biosynthetic process	18	1.58%	0.04722269



Cell Component level 4	mitochondrial large ribosomal subunit	15	1.31%	0.04984605
Cell Component level 4	mitochondrial inner membrane presequence translocation	7	0.61%	0.05029691
Mol Function level 2	cofactor binding	20	1.75%	0.05228223
Cell Component level 5	mitochondrial inner membrane presequence translocation	7	0.61%	0.05446045
Biol Process level 2	response to chemical stimulus	34	2.98%	0.05570218
Mol Function level 2	vitamin binding	8	0.70%	0.0559328
Cell Component level 2	organelle lumen	78	6.83%	0.05593952
Cell Component level 3	organelle lumen	78	6.83%	0.05642301
Cell Component level 5	mitochondrial large ribosomal subunit	15	1.31%	0.05701616
Cell Component level 5	organellar large ribosomal subunit	15	1.31%	0.05701616
Cell Component level 3	proton-transporting ATP synthase complex	5	0.44%	0.05809564
Biol Process level 5	courtship behavior	9	0.79%	0.06094721
Biol Process level 5	olfactory learning	9	0.79%	0.06094721
Biol Process level 4	mitochondrial electron transport, cytochrome c	5	0.44%	0.06124114
Cell Component level 1	membrane-enclosed lumen	78	6.83%	0.06154189
Biol Process level 4	courtship behavior	9	0.79%	0.06190089
Biol Process level 5	monocarboxylic acid metabolic process	20	1.75%	0.06658385
Cell Component level 4	proton-transporting ATP synthase complex	5	0.44%	0.06675536
Cell Component level 4	rough endoplasmic reticulum membrane	5	0.44%	0.06675536
Cell Component level 5	proton-transporting ATP synthase complex	5	0.44%	0.07078346
Cell Component level 5	rough endoplasmic reticulum	5	0.44%	0.07078346
Cell Component level 5	rough endoplasmic reticulum membrane	5	0.44%	0.07078346
Biol Process level 4	olfactory behavior	13	1.14%	0.07475797
Cell Component level 2	cell part	553	48.42%	0.07946075
Mol Function level 4	ARF guanyl-nucleotide exchange factor activity	4	0.35%	0.07957972
Biol Process level 5	mitochondrial transport	12	1.05%	0.0801425
Biol Process level 1	cellular process	646	56.57%	0.08049274
Biol Process level 4	mitochondrial transport	12	1.05%	0.08160447
Biol Process level 2	reproductive behavior	10	0.88%	0.08972687
Cell Component level 4	membrane part	177	15.50%	0.0905211
Biol Process level 3	response to drug	4	0.35%	0.0905822
Biol Process level 3	regulation of tube length, open tracheal system	4	0.35%	0.0905822
Mol Function level 3	oxidoreductase activity, acting on the CH-CH group	7	0.61%	0.09076292
Biol Process level 1	response to stimulus	101	8.84%	0.09128955
Biol Process level 5	pole plasm protein localization	4	0.35%	0.09211279
Biol Process level 5	regulation of tube length, open tracheal system	4	0.35%	0.09211279
Biol Process level 5	regulation of tube diameter, open tracheal system	4	0.35%	0.09211279
Biol Process level 5	nucleotide metabolic process	27	2.36%	0.09260104
Biol Process level 3	reproductive behavior	10	0.88%	0.09266211
Biol Process level 4	regulation of tube diameter, open tracheal system	4	0.35%	0.09280972
Biol Process level 5	regulation of tube size, open tracheal system	7	0.61%	0.09280991
Biol Process level 4	regulation of tube size, open tracheal system	7	0.61%	0.09393068
Biol Process level 3	learning and/or memory	14	1.23%	0.09439472
Biol Process level 3	translational elongation	6	0.53%	0.0951375
Biol Process level 5	monovalent inorganic cation transport	28	2.45%	0.09548737
Cell Component level 3	endoplasmic reticulum part	15	1.31%	0.09717176
Biol Process level 5	translational elongation	6	0.53%	0.09737539
Biol Process level 5	glycerol metabolic process	5	0.44%	0.09889698
Biol Process level 4	polyol metabolic process	5	0.44%	0.09980038

**Table S8.** Significantly enriched GO terms for genes with significantly higher gene expression in active bees, as compared to inactive bees.

GO Category	GO Term	Count	%	PValue
Biol Process level 2	behavior	39	7.20%	1.04E-07
Biol Process level 3	neurological system process	55	10.15%	2.86E-07
Mol Function level 2	substrate-specific transporter activity	65	11.99%	9.26E-07
Biol Process level 2	system process	55	10.15%	9.78E-07
Cell Component level 4	plasma membrane part	40	7.38%	1.22E-06
Cell Component level 3	plasma membrane part	40	7.38%	1.54E-06
Biol Process level 5	synaptic transmission	36	6.64%	1.57E-06
Cell Component level 5	plasma membrane part	40	7.38%	1.58E-06
Biol Process level 4	synaptic transmission	36	6.64%	2.89E-06
Biol Process level 1	multicellular organismal process	143	26.38%	3.08E-06
Mol Function level 2	transmembrane transporter activity	64	11.81%	3.99E-06
Mol Function level 3	substrate-specific transmembrane transporter activity	61	11.25%	5.18E-06
Biol Process level 1	response to stimulus	74	13.65%	5.71E-06
Mol Function level 1	transporter activity	74	13.65%	6.20E-06
Biol Process level 4	transmission of nerve impulse	41	7.56%	8.74E-06
Cell Component level 2	membrane	140	25.83%	1.21E-05
Cell Component level 3	membrane	140	25.83%	1.24E-05
Biol Process level 2	cell communication	113	20.85%	1.33E-05
Cell Component level 4	membrane part	107	19.74%	1.38E-05
Biol Process level 3	ion transport	39	7.20%	1.51E-05
Biol Process level 4	ion transport	39	7.20%	1.85E-05
Cell Component level 4	intrinsic to plasma membrane	24	4.43%	1.89E-05
Cell Component level 5	intrinsic to plasma membrane	24	4.43%	2.24E-05
Cell Component level 2	membrane part	107	19.74%	2.75E-05
Cell Component level 3	membrane part	107	19.74%	2.80E-05
Biol Process level 3	chemosensory behavior	15	2.77%	3.18E-05
Mol Function level 4	ion transmembrane transporter activity	50	9.23%	3.48E-05
Biol Process level 3	cell-cell signaling	42	7.75%	3.53E-05
Biol Process level 4	intracellular signaling cascade	52	9.59%	6.91E-05
Biol Process level 2	response to chemical stimulus	28	5.17%	1.11E-04
Cell Component level 4	plasma membrane	51	9.41%	1.13E-04
Biol Process level 5	cation transport	32	5.90%	1.20E-04
Biol Process level 1	establishment of localization	119	21.96%	1.23E-04
Cell Component level 3	plasma membrane	51	9.41%	1.44E-04
Mol Function level 5	cation transmembrane transporter activity	42	7.75%	1.48E-04
Cell Component level 5	integral to plasma membrane	22	4.06%	1.50E-04
Biol Process level 2	establishment of localization	119	21.96%	1.51E-04
Biol Process level 3	transport	117	21.59%	1.83E-04
Biol Process level 4	cation transport	32	5.90%	1.92E-04
Biol Process level 2	transport	117	21.59%	1.96E-04
Biol Process level 4	olfactory behavior	13	2.40%	2.75E-04
Biol Process level 3	learning and/or memory	14	2.58%	2.81E-04
Mol Function level 3	active transmembrane transporter activity	36	6.64%	3.08E-04
Cell Component level 4	intrinsic to membrane	75	13.84%	3.27E-04
Biol Process level 5	second-messenger-mediated signaling	12	2.21%	3.68E-04

Cell Component level 5	intrinsic to membrane	75	13.84%	4.51E-04
Cell Component level 3	intrinsic to membrane	75	13.84%	4.58E-04
Cell Component level 4	integral to membrane	74	13.65%	4.95E-04
Biol Process level 2	anatomical structure development	98	18.08%	6.02E-04
Cell Component level 5	integral to membrane	74	13.65%	6.77E-04
Biol Process level 1	rhythmic process	11	2.03%	6.79E-04
Biol Process level 1	localization	129	23.80%	7.38E-04
Biol Process level 5	olfactory learning	9	1.66%	8.24E-04
Biol Process level 3	system development	82	15.13%	8.33E-04
Biol Process level 2	multicellular organismal development	108	19.93%	0.0013859
Biol Process level 1	developmental process	123	22.69%	0.00175605
Biol Process level 3	signal transduction	87	16.05%	0.00184147
Biol Process level 2	response to abiotic stimulus	20	3.69%	0.00191741
Mol Function level 5	ATPase activity, coupled to transmembrane movement of ions	16	2.95%	0.00191758
Biol Process level 2	circadian rhythm	10	1.85%	0.00227139
Biol Process level 3	response to organic substance	7	1.29%	0.00235819
Biol Process level 3	cytoplasm organization and biogenesis	15	2.77%	0.00242516
Biol Process level 3	rhythmic behavior	9	1.66%	0.00278366
Biol Process level 2	rhythmic behavior	9	1.66%	0.00279722
Biol Process level 5	monovalent inorganic cation transport	21	3.87%	0.00287792
Biol Process level 5	regulation of tube size, open tracheal system	7	1.29%	0.00344583
Biol Process level 4	regulation of tube size, open tracheal system	7	1.29%	0.00390487
Mol Function level 4	hydrolase activity,catalyzing transmembrane movement	18	3.32%	0.00391829
Biol Process level 4	learning	9	1.66%	0.00407085
Biol Process level 5	tissue development	22	4.06%	0.00418252
Biol Process level 4	ATP synthesis coupled proton transport	10	1.85%	0.00428516
Biol Process level 3	organ development	63	11.62%	0.00489054
Biol Process level 3	tissue development	22	4.06%	0.00502332
Biol Process level 2	cell development	63	11.62%	0.00555809
Biol Process level 4	tissue development	22	4.06%	0.00560725
Cell Component level 4	proton-transporting two-sector ATPase complex	11	2.03%	0.00570918
Cell Component level 3	proton-transporting two-sector ATPase complex	11	2.03%	0.00592453
Biol Process level 5	ATP biosynthetic process	10	1.85%	0.00605735
Biol Process level 4	organ development	63	11.62%	0.00607755
Cell Component level 5	proton-transporting two-sector ATPase complex	11	2.03%	0.00616257
Mol Function level 2	vitamin binding	7	1.29%	0.00645055
Biol Process level 4	cell development	63	11.62%	0.00651843
Cell Component level 4	lipid particle	20	3.69%	0.00684821
Biol Process level 3	cell differentiation	71	13.10%	0.00744787
Cell Component level 5	lipid particle	20	3.69%	0.00767196
Biol Process level 5	phosphorylation	42	7.75%	0.00810737
Biol Process level 5	intercellular junction assembly	8	1.48%	0.00812269
Mol Function level 4	hydrolase activity, acting on carbon-nitrogen bonds in cyclic amides	4	0.74%	0.00816037
Biol Process level 3	circadian behavior	8	1.48%	0.00879981
Biol Process level 4	ATP metabolic process	10	1.85%	0.00899093
Biol Process level 4	circadian behavior	8	1.48%	0.00927774
Mol Function level 5	ATPase activity coupled to transmembrane movement of substances	17	3.14%	0.0095283
Biol Process level 3	adult behavior	11	2.03%	0.00963478
Biol Process level 5	protein complex assembly	16	2.95%	0.00974533
Biol Process level 2	cellular developmental process	71	13.10%	0.01001314

Biol Process level 5	regulation of tube size	7	1.29%	0.01042758
Biol Process level 3	carbohydrate metabolic process	32	5.90%	0.01049239
Mol Function level 1	catalytic activity	220	40.59%	0.01050471
Biol Process level 5	regulation of tube architecture, open tracheal system	8	1.48%	0.01073225
Biol Process level 3	regulation of tube size	7	1.29%	0.01118593
Biol Process level 5	synaptic vesicle transport	16	2.95%	0.01119718
Biol Process level 3	regulation of tube architecture, open tracheal system	8	1.48%	0.01160735
Biol Process level 4	regulation of tube size	7	1.29%	0.01172217
Biol Process level 4	protein complex assembly	16	2.95%	0.0121286
Biol Process level 5	regulation of neurotransmitter levels	19	3.51%	0.01271652
Biol Process level 5	regulation of tube length, open tracheal system	4	0.74%	0.01370086
Biol Process level 5	regulation of tube diameter, open tracheal system	4	0.74%	0.01370086
Biol Process level 4	synaptic vesicle transport	16	2.95%	0.01389492
Biol Process level 3	response to drug	4	0.74%	0.0142645
Biol Process level 3	regulation of tube length, open tracheal system	4	0.74%	0.0142645
Biol Process level 4	regulation of tube diameter, open tracheal system	4	0.74%	0.01466531
Biol Process level 3	regulation of neurotransmitter levels	19	3.51%	0.0147412
Biol Process level 5	endothelial cell development	5	0.92%	0.01496099
Biol Process level 4	response to light stimulus	11	2.03%	0.01506812
Biol Process level 3	locomotory behavior	14	2.58%	0.01510004
Mol Function level 2	transferase activity	75	13.84%	0.01538312
Biol Process level 3	endothelial cell development	5	0.92%	0.01573451
Biol Process level 4	intercellular junction assembly and maintenance	8	1.48%	0.01579282
Biol Process level 1	biological regulation	119	21.96%	0.01615847
Biol Process level 4	endothelial cell differentiation	5	0.92%	0.01628353
Biol Process level 3	secretion	27	4.98%	0.01628809
Biol Process level 2	secretion	27	4.98%	0.016455
Biol Process level 3	phosphorus metabolic process	49	9.04%	0.01716676
Mol Function level 4	primary active transmembrane transporter activity	19	3.51%	0.01737838
Biol Process level 5	secretory pathway	26	4.80%	0.01739449
Biol Process level 5	nucleoside triphosphate biosynthetic process	10	1.85%	0.01753073
Biol Process level 5	lipid biosynthetic process	14	2.58%	0.01782878
Cell Component level 5	microtubule cytoskeleton	16	2.95%	0.01794401
Mol Function level 4	ARF guanyl-nucleotide exchange factor activity	4	0.74%	0.01841673
Biol Process level 5	courtship behavior	7	1.29%	0.01863233
Biol Process level 5	secretion by cell	26	4.80%	0.01895758
Biol Process level 3	lipid biosynthetic process	14	2.58%	0.01999065
Biol Process level 5	regulated secretory pathway	17	3.14%	0.02004646
Biol Process level 5	neurotransmitter secretion	17	3.14%	0.02004646
Biol Process level 4	phosphate metabolic process	49	9.04%	0.02014568
Mol Function level 5	P-P-bond-hydrolysis-driven transmembrane transporter activity	19	3.51%	0.0203706
Biol Process level 3	secretory pathway	26	4.80%	0.02075888
Biol Process level 4	courtship behavior	7	1.29%	0.02083572
Biol Process level 4	memory	7	1.29%	0.02083572
Biol Process level 4	cofactor biosynthetic process	15	2.77%	0.02103134
Biol Process level 4	lipid biosynthetic process	14	2.58%	0.02150861
Biol Process level 3	instar larval or pupal development	38	7.01%	0.021839
Biol Process level 5	small GTPase mediated signal transduction	19	3.51%	0.02198384
Cell Component level 4	proton-transporting two-sector ATPase complex, catalytic domain	6	1.11%	0.02210687
Biol Process level 5	metal ion transport	17	3.14%	0.02244241

Cell Component level 3	proton-transporting two-sector ATPase complex, catalytic domain	6	1.11%	0.02251312
Biol Process level 1	reproduction	44	8.12%	0.02251738
Biol Process level 3	secretion by cell	26	4.80%	0.02257874
Biol Process level 5	nerve-nerve synaptic transmission	5	0.92%	0.02264817
Biol Process level 3	neurotransmitter secretion	17	3.14%	0.02281744
Mol Function level 4	kinase activity	40	7.38%	0.02297067
Biol Process level 4	cellular carbohydrate metabolic process	22	4.06%	0.02300154
Cell Component level 5	proton-transporting two-sector ATPase complex, catalytic domain	6	1.11%	0.02307959
Biol Process level 4	secretory pathway	26	4.80%	0.02309985
Biol Process level 3	tube morphogenesis	12	2.21%	0.02367407
Mol Function level 4	secondary active transmembrane transporter activity	17	3.14%	0.02455564
Mol Function level 3	transferase activity, on alkyl or aryl (other than methyl) groups	7	1.29%	0.02475034
Biol Process level 4	regulated secretory pathway	17	3.14%	0.02475881
Biol Process level 4	neurotransmitter secretion	17	3.14%	0.02475881
Biol Process level 4	instar larval or pupal development	38	7.01%	0.02493535
Biol Process level 5	behavioral response to ethanol	4	0.74%	0.02501843
Biol Process level 4	secretion by cell	26	4.80%	0.0250993
Biol Process level 4	tube morphogenesis	12	2.21%	0.0252631
Biol Process level 2	generation of a signal involved in cell-cell signaling	17	3.14%	0.02566659
Mol Function level 3	hydrolase activity, acting on acid anhydrides	45	8.30%	0.02595822
Biol Process level 3	maintenance of protein localization	4	0.74%	0.0260109
Biol Process level 2	maintenance of protein localization	4	0.74%	0.02605926
Biol Process level 4	response to ethanol	4	0.74%	0.02671695
Biol Process level 4	maintenance of protein localization	4	0.74%	0.02671695
Biol Process level 4	behavioral response to ethanol	4	0.74%	0.02671695
Biol Process level 4	generation of a signal involved in cell-cell signaling	17	3.14%	0.02763837
Biol Process level 3	cofactor metabolic process	23	4.24%	0.02800836
Biol Process level 3	post-embryonic development	38	7.01%	0.02820744
Biol Process level 2	post-embryonic development	38	7.01%	0.02855266
Biol Process level 5	autophagic cell death	9	1.66%	0.02893802
Biol Process level 5	salivary gland histolysis	9	1.66%	0.02893802
Biol Process level 5	proton transport	10	1.85%	0.02944513
Mol Function level 2	lipid binding	12	2.21%	0.03078937
Biol Process level 3	hydrogen transport	10	1.85%	0.03203699
Biol Process level 5	synaptic vesicle priming	5	0.92%	0.03234102
Biol Process level 5	male mating behavior	5	0.92%	0.03234102
Biol Process level 5	male courtship behavior	5	0.92%	0.03234102
Biol Process level 3	behavioral interaction between organisms	7	1.29%	0.03241047
Biol Process level 3	mating behavior	7	1.29%	0.03241047
Biol Process level 2	behavioral interaction between organisms	7	1.29%	0.03251182
Mol Function level 5	manganese ion binding	7	1.29%	0.03256421
Biol Process level 1	maintenance of localization	5	0.92%	0.03342065
Biol Process level 4	mating behavior	7	1.29%	0.03382869
Biol Process level 4	hydrogen transport	10	1.85%	0.03386635
Biol Process level 4	proton transport	10	1.85%	0.03386635
Biol Process level 2	maintenance of localization	5	0.92%	0.03398946
Biol Process level 5	histolysis	9	1.66%	0.0344067
Biol Process level 3	regulation of catalytic activity	13	2.40%	0.03467086
Biol Process level 4	male mating behavior	5	0.92%	0.03502784
Cell Component level 4	sodium:potassium-exchanging ATPase complex	3	0.55%	0.03664332

Mol Function level 2	molecular adaptor activity	4	0.74%	0.03690546
Cell Component level 4	cytoskeleton	23	4.24%	0.03690547
Biol Process level 3	response to radiation	11	2.03%	0.03692167
Cell Component level 3	sodium:potassium-exchanging ATPase complex	3	0.55%	0.03693877
Biol Process level 4	nervous system development	40	7.38%	0.03720745
Cell Component level 5	sodium:potassium-exchanging ATPase complex	3	0.55%	0.03741441
Cell Component level 3	receptor complex	4	0.74%	0.03757044
Biol Process level 5	tricarboxylic acid cycle	7	1.29%	0.03778659
Biol Process level 5	aerobic respiration	7	1.29%	0.03778659
Biol Process level 4	open tracheal system development	15	2.77%	0.03802547
Biol Process level 5	maintenance of imaginal disc-derived wing hair orientation	3	0.55%	0.03865436
Biol Process level 5	purine nucleotide biosynthetic process	11	2.03%	0.0388072
Biol Process level 4	histolysis	9	1.66%	0.03907348
Biol Process level 2	response to external stimulus	15	2.77%	0.03968662
Mol Function level 3	carbon-carbon lyase activity	7	1.29%	0.03985494
Biol Process level 4	sensory perception	14	2.58%	0.03994635
Biol Process level 5	ectoderm development	13	2.40%	0.03996766
Biol Process level 5	regulation of tube diameter	4	0.74%	0.03999906
Cell Component level 5	cytoskeleton	23	4.24%	0.04093648
Biol Process level 3	regulation of tube diameter	4	0.74%	0.0415281
Biol Process level 5	fatty acid biosynthetic process	6	1.11%	0.04189856
Biol Process level 4	cellular respiration	7	1.29%	0.04192997
Biol Process level 4	regulation of tube diameter	4	0.74%	0.04261634
Biol Process level 1	reproductive process	13	2.40%	0.04282815
Mol Function level 3	transferase activity, transferring phosphorus-containing groups	46	8.49%	0.04307663
Mol Function level 5	gated channel activity	13	2.40%	0.04316098
Biol Process level 2	reproductive process	13	2.40%	0.04428687
Mol Function level 4	prenyltransferase activity	5	0.92%	0.04563853
Biol Process level 4	fatty acid biosynthetic process	6	1.11%	0.04588489
Biol Process level 5	acetyl-CoA metabolic process	7	1.29%	0.04621666
Biol Process level 5	coenzyme catabolic process	7	1.29%	0.04621666
Biol Process level 3	tube development	12	2.21%	0.0462743
Biol Process level 2	regulation of a molecular function	14	2.58%	0.04683963
Biol Process level 4	ectoderm development	13	2.40%	0.04695952
Biol Process level 5	monosaccharide catabolic process	8	1.48%	0.04761862
Biol Process level 4	female gamete generation	31	5.72%	0.0476202

**Table S9.** Significantly enriched GO terms for genes with significantly higher gene expression in inactive bees, as compared to active bees.

GO Category	GO Term	Count	%	PValue
Mol Function level 2	structural constituent of ribosome	85	14.14%	1.07E-46
Cell Component level 3	ribosome	84	13.98%	3.34E-45
Cell Component level 3	ribosomal subunit	76	12.65%	1.12E-42
Cell Component level 4	ribosome	84	13.98%	1.86E-42
Cell Component level 5	ribosome	84	13.98%	1.17E-41
Cell Component level 4	ribosomal subunit	76	12.65%	3.61E-40
Cell Component level 5	ribosomal subunit	76	12.65%	1.92E-39
Cell Component level 2	ribonucleoprotein complex	99	16.47%	1.99E-38
Cell Component level 3	ribonucleoprotein complex	99	16.47%	2.07E-38
Cell Component level 4	ribonucleoprotein complex	99	16.47%	2.23E-35
Cell Component level 5	ribonucleoprotein complex	99	16.47%	1.70E-34
Biol Process level 2	translation	93	15.47%	3.68E-31
Cell Component level 4	cytosolic ribosome (sensu Eukaryota)	48	7.99%	4.24E-31
Cell Component level 5	cytosolic ribosome (sensu Eukaryota)	48	7.99%	1.25E-30
Biol Process level 4	translation	93	15.47%	1.36E-30
Biol Process level 5	translation	93	15.47%	4.69E-30
Cell Component level 1	macromolecular complex	192	31.95%	1.26E-29
Cell Component level 2	intracellular organelle part	188	31.28%	1.67E-29
Cell Component level 3	intracellular organelle part	188	31.28%	1.78E-29
Cell Component level 2	organelle part	188	31.28%	2.44E-29
Cell Component level 1	organelle part	188	31.28%	6.37E-29
Cell Component level 3	cytoplasmic part	188	31.28%	1.83E-27
Cell Component level 4	cytosolic part	52	8.65%	6.27E-27
Mol Function level 1	structural molecule activity	90	14.98%	7.06E-27
Cell Component level 5	cytosolic part	52	8.65%	1.94E-26
Cell Component level 3	cytoplasm	206	34.28%	7.56E-25
Cell Component level 4	intracellular organelle part	188	31.28%	9.90E-25
Biol Process level 3	cellular biosynthetic process	121	20.13%	1.28E-23
Biol Process level 3	macromolecule biosynthetic process	98	16.31%	1.46E-23
Cell Component level 5	intracellular organelle part	188	31.28%	2.56E-23
Cell Component level 4	cytoplasmic part	188	31.28%	8.61E-23
Cell Component level 5	cytoplasmic part	188	31.28%	2.08E-21
Cell Component level 4	large ribosomal subunit	44	7.32%	1.99E-20
Cell Component level 5	large ribosomal subunit	44	7.32%	5.05E-20
Cell Component level 4	cytoplasm	206	34.28%	6.94E-20
Cell Component level 3	small ribosomal subunit	33	5.49%	1.12E-19
Cell Component level 3	mitochondrial part	82	13.64%	1.17E-19
Biol Process level 2	biosynthetic process	124	20.63%	6.51E-19
Cell Component level 4	small ribosomal subunit	33	5.49%	1.39E-18
Cell Component level 5	cytoplasm	206	34.28%	2.09E-18
Cell Component level 5	small ribosomal subunit	33	5.49%	2.86E-18
Cell Component level 2	intracellular part	261	43.43%	3.81E-18
Cell Component level 3	intracellular part	261	43.43%	4.18E-18
Cell Component level 5	cytosolic large ribosomal subunit (sensu Eukaryota)	29	4.83%	1.53E-17
Cell Component level 4	mitochondrial part	82	13.64%	1.90E-17



Cell Component level 2	non-membrane-bound organelle	100	16.64%	2.50E-17
Cell Component level 3	intracellular non-membrane-bound organelle	100	16.64%	2.57E-17
Cell Component level 4	mitochondrion	92	15.31%	7.88E-17
Cell Component level 5	mitochondrial part	82	13.64%	8.08E-17
Cell Component level 2	intracellular organelle	230	38.27%	3.09E-16
Cell Component level 3	intracellular organelle	230	38.27%	3.23E-16
Cell Component level 5	mitochondrion	92	15.31%	4.86E-16
Cell Component level 4	cytosol	58	9.65%	5.39E-16
Cell Component level 1	organelle	230	38.27%	1.28E-15
Cell Component level 5	cytosol	58	9.65%	1.60E-15
Cell Component level 4	intracellular non-membrane-bound organelle	100	16.64%	1.08E-14
Cell Component level 2	intracellular	267	44.43%	3.07E-14
Cell Component level 3	intracellular	267	44.43%	3.37E-14
Cell Component level 5	intracellular non-membrane-bound organelle	100	16.64%	5.40E-14
Cell Component level 4	cytosolic small ribosomal subunit (sensu Eukaryota)	19	3.16%	1.51E-12
Cell Component level 3	mitochondrial membrane part	37	6.16%	2.21E-12
Cell Component level 5	cytosolic small ribosomal subunit (sensu Eukaryota)	19	3.16%	2.29E-12
Biol Process level 2	cellular metabolic process	279	46.42%	3.06E-12
Cell Component level 4	intracellular part	261	43.43%	6.11E-12
Cell Component level 4	mitochondrial membrane part	37	6.16%	2.63E-11
Cell Component level 4	organellar ribosome	30	4.99%	3.11E-11
Cell Component level 4	mitochondrial ribosome	30	4.99%	3.11E-11
Cell Component level 4	intracellular organelle	230	38.27%	3.83E-11
Cell Component level 5	mitochondrial membrane part	37	6.16%	5.29E-11
Cell Component level 5	organellar ribosome	30	4.99%	5.62E-11
Cell Component level 5	mitochondrial ribosome	30	4.99%	5.62E-11
Biol Process level 1	gene expression	137	22.80%	6.15E-11
Biol Process level 1	metabolic process	296	49.25%	1.02E-10
Biol Process level 3	electron transport	41	6.82%	1.37E-10
Cell Component level 3	mitochondrial lumen	38	6.32%	2.83E-10
Cell Component level 5	intracellular organelle	230	38.27%	1.17E-09
Cell Component level 2	protein complex	128	21.30%	1.71E-09
Cell Component level 4	mitochondrial lumen	38	6.32%	3.06E-09
Cell Component level 4	mitochondrial matrix	38	6.32%	3.06E-09
Cell Component level 5	mitochondrial lumen	38	6.32%	5.96E-09
Cell Component level 5	mitochondrial matrix	38	6.32%	5.96E-09
Cell Component level 3	mitochondrial envelope	44	7.32%	1.05E-08
Mol Function level 2	oxidoreductase activity	67	11.15%	1.41E-08
Cell Component level 3	mitochondrial membrane	41	6.82%	1.65E-08
Biol Process level 3	oxidative phosphorylation	30	4.99%	4.22E-08
Cell Component level 3	organelle inner membrane	38	6.32%	5.04E-08
Cell Component level 4	mitochondrial respiratory chain	24	3.99%	5.41E-08
Cell Component level 5	mitochondrial respiratory chain	24	3.99%	8.49E-08
Cell Component level 2	organelle lumen	64	10.65%	8.98E-08
Cell Component level 3	organelle lumen	64	10.65%	9.12E-08
Cell Component level 1	membrane-enclosed lumen	64	10.65%	9.50E-08
Cell Component level 4	mitochondrial envelope	44	7.32%	1.27E-07
Cell Component level 4	mitochondrial membrane	41	6.82%	1.73E-07
Biol Process level 4	ATP synthesis coupled electron transport	21	3.49%	1.88E-07
Biol Process level 5	organelle ATP synthesis coupled electron transport	21	3.49%	2.53E-07

Cell Component level 5	mitochondrial envelope	44	7.32%	2.54E-07
Cell Component level 5	mitochondrial membrane	41	6.82%	3.32E-07
Cell Component level 4	mitochondrial inner membrane	38	6.32%	4.46E-07
Cell Component level 4	organelle inner membrane	38	6.32%	4.46E-07
Biol Process level 2	generation of precursor metabolites and energy	43	7.15%	5.32E-07
Cell Component level 5	mitochondrial inner membrane	38	6.32%	8.17E-07
Cell Component level 5	organelle inner membrane	38	6.32%	8.17E-07
Biol Process level 4	cellular protein metabolic process	149	24.79%	1.10E-06
Cell Component level 4	organellar small ribosomal subunit	14	2.33%	1.73E-06
Cell Component level 4	mitochondrial small ribosomal subunit	14	2.33%	1.73E-06
Biol Process level 3	protein metabolic process	152	25.29%	1.97E-06
Cell Component level 2	organelle envelope	45	7.49%	2.25E-06
Cell Component level 3	organelle envelope	45	7.49%	2.28E-06
Cell Component level 5	organellar small ribosomal subunit	14	2.33%	2.28E-06
Cell Component level 5	mitochondrial small ribosomal subunit	14	2.33%	2.28E-06
Cell Component level 1	envelope	45	7.49%	2.33E-06
Mol Function level 3	electron carrier activity	23	3.83%	3.45E-06
Biol Process level 3	cellular macromolecule metabolic process	149	24.79%	3.67E-06
Cell Component level 2	organelle membrane	55	9.15%	5.45E-06
Cell Component level 3	organelle membrane	55	9.15%	5.52E-06
Cell Component level 4	organelle envelope	45	7.49%	2.10E-05
Mol Function level 5	cytochrome-c oxidase activity	7	1.16%	3.84E-05
Cell Component level 5	organelle envelope	45	7.49%	3.85E-05
Mol Function level 4	cytochrome-c oxidase activity	7	1.16%	4.37E-05
Mol Function level 4	oxidoreductase activity, heme donors and oxygen acceptor	7	1.16%	4.37E-05
Mol Function level 3	oxidoreductase activity, acting on heme group of donors	7	1.16%	4.46E-05
Mol Function level 3	heme-copper terminal oxidase activity	7	1.16%	4.46E-05
Mol Function level 4	NADH dehydrogenase activity	12	2.00%	6.45E-05
Cell Component level 4	organelle membrane	55	9.15%	6.48E-05
Biol Process level 5	protein import into mitochondrion	7	1.16%	6.69E-05
Mol Function level 3	oxidoreductase activity, acting on NADH or NADPH	13	2.16%	1.15E-04
Cell Component level 4	mitochondrial large ribosomal subunit	15	2.50%	1.20E-04
Biol Process level 5	inner mitochondrial membrane organization and biogenesis	6	1.00%	1.34E-04
Cell Component level 5	organellar large ribosomal subunit	15	2.50%	1.56E-04
Cell Component level 5	mitochondrial large ribosomal subunit	15	2.50%	1.56E-04
Biol Process level 4	mitochondrial membrane organization and biogenesis	6	1.00%	3.88E-04
Biol Process level 5	mitochondrial membrane organization and biogenesis	6	1.00%	4.24E-04
Mol Function level 5	P-P-bond-hydrolysis-driven transmembrane transport activity	8	1.33%	4.51E-04
Mol Function level 4	P-P-bond-hydrolysis-driven transmembrane transport activity	8	1.33%	5.19E-04
Mol Function level 4	macromolecule transmembrane transporter activity	8	1.33%	5.19E-04
Mol Function level 4	protein transmembrane transporter activity	9	1.50%	5.54E-04
Mol Function level 3	protein transmembrane transporter activity	9	1.50%	5.67E-04
Cell Component level 3	respiratory chain complex I	12	2.00%	6.00E-04
Cell Component level 3	NADH dehydrogenase complex (quinone)	12	2.00%	6.00E-04
Mol Function level 4	rRNA binding	6	1.00%	6.58E-04
Biol Process level 4	mitochondrial electron transport, NADH to ubiquinone	12	2.00%	7.01E-04
Cell Component level 3	mitochondrial intermembrane space protein transporter	5	0.83%	9.45E-04
Biol Process level 1	cellular process	332	55.24%	9.61E-04
Cell Component level 3	respiratory chain complex IV	6	1.00%	0.00102126
Cell Component level 3	intracellular membrane-bound organelle	171	28.45%	0.00109474

Cell Component level 2	membrane-bound organelle	171	28.45%	0.00118101
Cell Component level 4	NADH dehydrogenase complex (quinone)	12	2.00%	0.00123128
Cell Component level 4	respiratory chain complex I	12	2.00%	0.00123128
Cell Component level 4	mitochondrial respiratory chain complex I	12	2.00%	0.00123128
Mol Function level 5	NADH dehydrogenase (quinone) activity	9	1.50%	0.00125405
Biol Process level 2	primary metabolic process	243	40.43%	0.00129923
Cell Component level 4	mitochondrial intermembrane space protein transporter	5	0.83%	0.00131273
Biol Process level 5	protein targeting to mitochondrion	9	1.50%	0.00139812
Cell Component level 5	mitochondrial intermembrane space protein transporter	5	0.83%	0.00144083
Cell Component level 3	mitochondrial inner membrane presequence translocase	7	1.16%	0.0014445
Mol Function level 4	oxidoreductase activity, with NADH and others as acceptor	9	1.50%	0.00145735
Cell Component level 5	respiratory chain complex I	12	2.00%	0.00150501
Cell Component level 5	NADH dehydrogenase complex (quinone)	12	2.00%	0.00150501
Cell Component level 5	mitochondrial respiratory chain complex I	12	2.00%	0.00150501
Cell Component level 4	respiratory chain complex IV	6	1.00%	0.0015139
Cell Component level 4	mitochondrial respiratory chain complex IV	6	1.00%	0.0015139
Cell Component level 5	respiratory chain complex IV	6	1.00%	0.0016919
Cell Component level 5	mitochondrial respiratory chain complex IV	6	1.00%	0.0016919
Cell Component level 4	mitochondrial inner membrane presequence translocase	7	1.16%	0.00226036
Cell Component level 5	mitochondrial inner membrane presequence translocase	7	1.16%	0.0025639
Biol Process level 2	macromolecule metabolic process	207	34.44%	0.00292304
Mol Function level 3	protein transporter activity	11	1.83%	0.00461324
Biol Process level 4	mitochondrial electron transport, cytochrome c to oxygen	5	0.83%	0.00505213
Mol Function level 5	ubiquinol-cytochrome-c reductase activity	5	0.83%	0.00614869
Cell Component level 4	lipid particle	22	3.66%	0.00636429
Mol Function level 4	oxidoreductase, diphenol donors, cytochrome as acceptor	5	0.83%	0.0066464
Cell Component level 5	lipid particle	22	3.66%	0.00837595
Mol Function level 3	oxidoreductase, acting on diphenols and related substances	5	0.83%	0.01037018
Cell Component level 3	endoplasmic reticulum membrane	10	1.66%	0.01082283
Cell Component level 3	nuclear envelope-endoplasmic reticulum network	10	1.66%	0.01082283
Cell Component level 3	organelle envelope lumen	5	0.83%	0.01601095
Cell Component level 4	nuclear envelope-endoplasmic reticulum network	10	1.66%	0.01832549
Cell Component level 4	endoplasmic reticulum membrane	10	1.66%	0.01832549
Biol Process level 4	mitochondrial transport	9	1.50%	0.01991573
Cell Component level 5	nuclear envelope-endoplasmic reticulum network	10	1.66%	0.02119503
Cell Component level 5	endoplasmic reticulum membrane	10	1.66%	0.02119503
Cell Component level 4	organelle envelope lumen	5	0.83%	0.02145007
Cell Component level 4	mitochondrial intermembrane space	5	0.83%	0.02145007
Biol Process level 5	mitochondrial transport	9	1.50%	0.02203223
Cell Component level 5	organelle envelope lumen	5	0.83%	0.0232864
Cell Component level 5	mitochondrial intermembrane space	5	0.83%	0.0232864
Mol Function level 5	protein-methionine-R-oxide reductase activity	3	0.50%	0.02395373
Cell Component level 3	respiratory chain complex III	5	0.83%	0.02419272
Cell Component level 3	endoplasmic reticulum part	11	1.83%	0.0242213
Mol Function level 5	DNA-directed RNA polymerase activity	6	1.00%	0.02543036
Biol Process level 3	oxygen and reactive oxygen species metabolic process	5	0.83%	0.03145202
Cell Component level 4	respiratory chain complex III	5	0.83%	0.03212523
Cell Component level 4	mitochondrial respiratory chain complex III	5	0.83%	0.03212523
Cell Component level 3	proteasome complex (sensu Eukaryota)	10	1.66%	0.03285816
Cell Component level 4	snRNP U6	4	0.67%	0.03313642

Mol Function level 5	P-P-bond-hydrolysis-driven transmembrane transporter activity	14	2.33%	0.03463696
Cell Component level 5	respiratory chain complex III	5	0.83%	0.0347827
Cell Component level 5	mitochondrial respiratory chain complex III	5	0.83%	0.0347827
Cell Component level 5	snRNP U6	4	0.67%	0.03533276
Cell Component level 3	small nuclear ribonucleoprotein complex	10	1.66%	0.0383595
Mol Function level 4	primary active transmembrane transporter activity	14	2.33%	0.04063478
Cell Component level 4	endoplasmic reticulum part	11	1.83%	0.04071234
Mol Function level 3	oxidoreductase activity, acting on sulfur group of donors	4	0.67%	0.0456782
Mol Function level 3	oxidoreductase activity, acting on the CH-CH group of donors	5	0.83%	0.04622646
Cell Component level 5	endoplasmic reticulum part	11	1.83%	0.04691973
Cell Component level 3	spliceosome	12	2.00%	0.04753455

**Table S10.** Significantly enriched GO terms for genes differentially expressed between anticipating bees with different spatiotemporal memories (tAM-cAM contrasted with tPM-cPM).

GO Category	GO Term	Count	%	PValue
Biol Process level 3	lipid metabolic process	42	5.75%	8.05E-04
Mol Function level 2	structural constituent of ribosome	35	4.79%	0.00200062
Cell Component level 3	cytoplasm	192	26.27%	0.00208546
Biol Process level 5	monosaccharide catabolic process	12	1.64%	0.00268226
Biol Process level 5	hexose metabolic process	17	2.33%	0.00317228
Cell Component level 4	cytosolic part	24	3.28%	0.00351826
Mol Function level 4	calcium ion binding	26	3.56%	0.0035555
Mol Function level 3	active transmembrane transporter activity	38	5.20%	0.00356755
Biol Process level 4	alcohol catabolic process	12	1.64%	0.0038089
Cell Component level 5	cytosolic part	24	3.28%	0.00398486
Biol Process level 3	carbohydrate metabolic process	41	5.61%	0.00400294
Cell Component level 4	cytoplasm	192	26.27%	0.00410058
Cell Component level 3	ribosome	35	4.79%	0.00436265
Biol Process level 4	carbohydrate catabolic process	14	1.92%	0.00568365
Cell Component level 4	ribosome	35	4.79%	0.00569202
Cell Component level 4	cytosol	37	5.06%	0.00628659
Biol Process level 5	pyrimidine nucleotide biosynthetic process	6	0.82%	0.00644245
Cell Component level 5	ribosome	35	4.79%	0.00662572
Cell Component level 5	cytoplasm	192	26.27%	0.00670151
Mol Function level 5	anion transmembrane transporter activity	13	1.78%	0.00716578
Biol Process level 5	monosaccharide metabolic process	17	2.33%	0.0072322
Cell Component level 5	cytosol	37	5.06%	0.00734672
Biol Process level 4	monosaccharide metabolic process	17	2.33%	0.0076026
Biol Process level 5	cellular carbohydrate catabolic process	13	1.78%	0.00761115
Biol Process level 3	cellular biosynthetic process	87	11.90%	0.00761492
Biol Process level 3	alcohol metabolic process	23	3.15%	0.00916836
Biol Process level 4	cellular carbohydrate metabolic process	28	3.83%	0.00958695
Cell Component level 3	ribosomal subunit	30	4.10%	0.00971101
Biol Process level 4	cellular lipid metabolic process	32	4.38%	0.00974306
Biol Process level 3	cellular lipid metabolic process	32	4.38%	0.01018456
Biol Process level 2	biosynthetic process	99	13.54%	0.01175508
Mol Function level 5	glutathione peroxidase activity	4	0.55%	0.0118742
Cell Component level 4	ribosomal subunit	30	4.10%	0.01219923
Biol Process level 2	response to chemical stimulus	27	3.69%	0.0128572
Mol Function level 3	glutathione peroxidase activity	4	0.55%	0.01299465
Biol Process level 5	pyrimidine base metabolic process	7	0.96%	0.01334113
Mol Function level 4	secondary active transmembrane transporter activity	20	2.74%	0.01334127
Biol Process level 2	cell adhesion	25	3.42%	0.01338154
Cell Component level 5	ribosomal subunit	30	4.10%	0.01387391
Cell Component level 4	cytosolic ribosome (sensu Eukaryota)	18	2.46%	0.01402308
Biol Process level 1	biological adhesion	25	3.42%	0.01492475
Cell Component level 5	cytosolic ribosome (sensu Eukaryota)	18	2.46%	0.01537466
Cell Component level 4	cullin-RING ubiquitin ligase complex	5	0.68%	0.01595386
Cell Component level 5	cullin-RING ubiquitin ligase complex	5	0.68%	0.01651868
Cell Component level 5	SCF ubiquitin ligase complex	5	0.68%	0.01651868

Mol Function level 2	isomerase activity	15	2.05%	0.01666654
Biol Process level 3	chemosensory behavior	12	1.64%	0.01771286
Mol Function level 2	transmembrane transporter activity	64	8.76%	0.01828169
Biol Process level 4	extracellular transport	7	0.96%	0.01984096
Biol Process level 3	extracellular transport	7	0.96%	0.02008912
Cell Component level 3	cytoplasmic part	155	21.20%	0.02155105
Mol Function level 3	oxidoreductase activity, acting on peroxide	5	0.68%	0.02350999
Mol Function level 2	peroxidase activity	5	0.68%	0.02381842
Mol Function level 4	peroxidase activity	5	0.68%	0.02407181
Mol Function level 3	identical protein binding	16	2.19%	0.02650256
Biol Process level 4	reproductive behavior in a multicellular organism	7	0.96%	0.02767539
Mol Function level 1	antioxidant activity	6	0.82%	0.02785755
Biol Process level 3	reproductive behavior in a multicellular organism	7	0.96%	0.02800974
Mol Function level 4	oxidoreductase activity, acting on aldehyde or oxo group	4	0.55%	0.02932374
Biol Process level 3	macromolecule biosynthetic process	61	8.34%	0.0302684
Biol Process level 2	catabolic process	38	5.20%	0.03252222
Biol Process level 2	behavior	30	4.10%	0.03322274
Mol Function level 5	glucuronosyltransferase activity	5	0.68%	0.03353392
Biol Process level 4	cellular macromolecule catabolic process	22	3.01%	0.03652507
Cell Component level 4	cytoplasmic part	155	21.20%	0.03687947
Biol Process level 5	translation	47	6.43%	0.04127017
Biol Process level 4	translation	47	6.43%	0.04468114
Biol Process level 3	macromolecule catabolic process	25	3.42%	0.0455264
Biol Process level 2	translation	47	6.43%	0.04614265
Biol Process level 3	cellular catabolic process	34	4.65%	0.04654915
Mol Function level 5	symporter activity	11	1.50%	0.04768353

**Table S11.** Significantly enriched GO terms for genes more highly expressed in morning-anticipating bees (tAM-cAM) in contrast to afternoon-anticipating bees (tPM-cPM).

GO Category	GO Term	Count	%	PValue
Biol Process level 3	lipid metabolic process	28	7.98%	1.81E-05
Biol Process level 4	cellular lipid metabolic process	21	5.98%	6.38E-04
Biol Process level 3	cellular lipid metabolic process	21	5.98%	7.98E-04
Biol Process level 5	pyrimidine base metabolic process	6	1.71%	0.00161489
Biol Process level 5	prosthetic group metabolic process	5	1.42%	0.00456538
Biol Process level 3	prosthetic group metabolic process	5	1.42%	0.0054897
Biol Process level 2	behavior	18	5.13%	0.01193817
Biol Process level 5	lipid biosynthetic process	10	2.85%	0.01588302
Mol Function level 3	identical protein binding	10	2.85%	0.01641969
Biol Process level 2	response to chemical stimulus	15	4.27%	0.01936687
Biol Process level 4	lipid biosynthetic process	10	2.85%	0.01958843
Biol Process level 2	cell adhesion	14	3.99%	0.02000621
Biol Process level 5	nucleobase metabolic process	7	1.99%	0.0209387
Biol Process level 1	biological adhesion	14	3.99%	0.02110614
Mol Function level 2	isomerase activity	9	2.56%	0.02124222
Biol Process level 3	lipid biosynthetic process	10	2.85%	0.02158158
Biol Process level 2	response to abiotic stimulus	12	3.42%	0.02255012
Biol Process level 4	nucleobase metabolic process	7	1.99%	0.024441
Cell Component level 4	endoplasmic reticulum	15	4.27%	0.02527298
Cell Component level 5	endoplasmic reticulum	15	4.27%	0.02821051
Biol Process level 4	chromosome organization and biogenesis	13	3.70%	0.03307619
Biol Process level 5	isoprenoid metabolic process	4	1.14%	0.03342711
Biol Process level 4	isoprenoid metabolic process	4	1.14%	0.03656256
Mol Function level 5	iron ion binding	9	2.56%	0.04602303
Biol Process level 5	central nervous system development	10	2.85%	0.04693733



**Table S12.** Significantly enriched GO terms for genes more highly expressed in afternoon-anticipating bees (tPM-cPM) in contrast to morning-anticipating bees (tAM-cAM).

GO Category	GO Term	Count	%	PValue
Cell Component level 4	cytosolic part	21	5.53%	1.09E-05
Cell Component level 5	cytosolic part	21	5.53%	1.18E-05
Mol Function level 2	structural constituent of ribosome	27	7.11%	4.31E-05
Cell Component level 4	ribosome	27	7.11%	9.13E-05
Cell Component level 3	ribosome	27	7.11%	9.74E-05
Cell Component level 5	ribosome	27	7.11%	9.89E-05
Cell Component level 4	cytosol	28	7.37%	1.33E-04
Cell Component level 5	cytosol	28	7.37%	1.45E-04
Cell Component level 4	cytosolic ribosome (sensu Eukaryota)	16	4.21%	1.55E-04
Cell Component level 4	ribosomal subunit	24	6.32%	1.61E-04
Cell Component level 5	cytosolic ribosome (sensu Eukaryota)	16	4.21%	1.65E-04
Cell Component level 3	ribosomal subunit	24	6.32%	1.70E-04
Cell Component level 5	ribosomal subunit	24	6.32%	1.74E-04
Biol Process level 5	hexose metabolic process	14	3.68%	2.86E-04
Biol Process level 4	cellular carbohydrate metabolic process	22	5.79%	3.31E-04
Biol Process level 3	carbohydrate metabolic process	29	7.63%	3.95E-04
Biol Process level 3	cellular biosynthetic process	57	15.00%	5.21E-04
Biol Process level 5	monosaccharide catabolic process	10	2.63%	5.31E-04
Biol Process level 4	monosaccharide metabolic process	14	3.68%	5.32E-04
Biol Process level 3	alcohol metabolic process	18	4.74%	5.84E-04
Biol Process level 4	alcohol catabolic process	10	2.63%	6.13E-04
Biol Process level 5	monosaccharide metabolic process	14	3.68%	6.33E-04
Cell Component level 4	ribonucleoprotein complex	34	8.95%	9.65E-04
Cell Component level 2	ribonucleoprotein complex	34	8.95%	0.00102731
Cell Component level 3	ribonucleoprotein complex	34	8.95%	0.00103501
Cell Component level 5	ribonucleoprotein complex	34	8.95%	0.00104963
Mol Function level 3	active transmembrane transporter activity	26	6.84%	0.00116642
Cell Component level 4	cytoplasm	112	29.47%	0.00129285
Cell Component level 5	cytoplasm	112	29.47%	0.00148189
Biol Process level 4	carbohydrate catabolic process	11	2.89%	0.00148397
Biol Process level 3	macromolecule biosynthetic process	42	11.05%	0.00156071
Biol Process level 2	translation	34	8.95%	0.00164901
Biol Process level 4	translation	34	8.95%	0.00196887
Cell Component level 3	cytoplasm	112	29.47%	0.00215171
Biol Process level 5	translation	34	8.95%	0.0026455
Mol Function level 1	structural molecule activity	34	8.95%	0.00271773
Cell Component level 4	cytoplasmic part	94	24.74%	0.00320299
Biol Process level 2	biosynthetic process	61	16.05%	0.00344974
Biol Process level 5	cellular carbohydrate catabolic process	10	2.63%	0.00354369
Cell Component level 5	cytoplasmic part	94	24.74%	0.00365441
Cell Component level 3	cytoplasmic part	94	24.74%	0.00431977
Cell Component level 4	mitochondrial envelope	24	6.32%	0.00460593
Cell Component level 4	large ribosomal subunit	15	3.95%	0.00461153
Cell Component level 5	cytosolic large ribosomal subunit (sensu Eukaryota)	10	2.63%	0.00461654
Cell Component level 3	mitochondrial envelope	24	6.32%	0.00475149

Cell Component level 4	intracellular non-membrane-bound organelle	48	12.63%	0.00481766
Cell Component level 5	large ribosomal subunit	15	3.95%	0.00483338
Cell Component level 5	mitochondrial envelope	24	6.32%	0.00490479
Cell Component level 2	non-membrane-bound organelle	48	12.63%	0.00521769
Cell Component level 3	intracellular non-membrane-bound organelle	48	12.63%	0.00526177
Cell Component level 5	intracellular non-membrane-bound organelle	48	12.63%	0.00528654
Mol Function level 4	oxidoreductase, aldehyde or oxo donors, disulfide acceptor	4	1.05%	0.00554199
Mol Function level 2	transmembrane transporter activity	41	10.79%	0.00562482
Mol Function level 5	ATPase, coupled to transmembrane movement of substances	13	3.42%	0.00581967
Cell Component level 4	small ribosomal subunit	11	2.89%	0.00626725
Cell Component level 3	small ribosomal subunit	11	2.89%	0.00631698
Cell Component level 5	small ribosomal subunit	11	2.89%	0.00650607
Mol Function level 4	ion transmembrane transporter activity	30	7.89%	0.00808737
Mol Function level 4	hydrolase, on acid anhydrides, transmembrane movement	13	3.42%	0.00923936
Cell Component level 4	mitochondrial part	34	8.95%	0.00996965
Cell Component level 3	mitochondrial part	34	8.95%	0.01041806
Mol Function level 5	anion transmembrane transporter activity	9	2.37%	0.01064342
Cell Component level 5	mitochondrial part	34	8.95%	0.01072279
Biol Process level 2	cellular metabolic process	180	47.37%	0.01077897
Biol Process level 1	metabolic process	196	51.58%	0.01267637
Mol Function level 5	NADH dehydrogenase (quinone) activity	7	1.84%	0.01387875
Cell Component level 4	mitochondrion	39	10.26%	0.01643238
Biol Process level 4	alcohol biosynthetic process	4	1.05%	0.01673675
Biol Process level 4	female germ-line cyst formation	4	1.05%	0.01673675
Mol Function level 4	oxidoreductase activity, acting on NADH or similar as receptor	7	1.84%	0.01678527
Biol Process level 5	monosaccharide biosynthetic process	4	1.05%	0.01760611
Biol Process level 5	female germ-line cyst formation	4	1.05%	0.01760611
Cell Component level 5	mitochondrion	39	10.26%	0.0177219
Mol Function level 5	nucleoside diphosphate kinase activity	3	0.79%	0.0193658
Biol Process level 3	macromolecule catabolic process	17	4.47%	0.01979296
Mol Function level 4	secondary active transmembrane transporter activity	13	3.42%	0.02012102
Mol Function level 5	ATPase activity, coupled to transmembrane movement of ions	10	2.63%	0.02160631
Mol Function level 3	substrate-specific transmembrane transporter activity	34	8.95%	0.02412947
Biol Process level 4	polyol metabolic process	4	1.05%	0.02510839
Biol Process level 5	glycerol metabolic process	4	1.05%	0.02638059
Cell Component level 1	envelope	25	6.58%	0.02725718
Cell Component level 4	organelle envelope	25	6.58%	0.02747082
Cell Component level 2	organelle envelope	25	6.58%	0.02790184
Cell Component level 3	organelle envelope	25	6.58%	0.02802884
Mol Function level 4	calcium ion binding	15	3.95%	0.0280402
Cell Component level 5	organelle envelope	25	6.58%	0.02903373
Mol Function level 5	glucuronosyltransferase activity	4	1.05%	0.03232795
Mol Function level 5	P-P-bond-hydrolysis-driven transmembrane transporter activity	13	3.42%	0.03447162
Mol Function level 4	rRNA binding	4	1.05%	0.03590491
Biol Process level 4	female gamete generation	24	6.32%	0.03591493
Mol Function level 5	glutathione peroxidase activity	3	0.79%	0.03662947
Mol Function level 3	oxidoreductase activity, acting on NADH or NADPH	8	2.11%	0.03814346
Biol Process level 5	oogenesis	24	6.32%	0.03852929
Mol Function level 3	glutathione peroxidase activity	3	0.79%	0.03859867
Biol Process level 2	catabolic process	23	6.05%	0.03956529

Mol Function level 2	vitamin binding	5	1.32%	0.04071686
Mol Function level 4	magnesium ion binding	6	1.58%	0.04227459
Cell Component level 4	mitochondrial inner membrane	18	4.74%	0.04233296
Cell Component level 4	organelle inner membrane	18	4.74%	0.04233296
Cell Component level 3	organelle inner membrane	18	4.74%	0.04275836
Biol Process level 4	cellular macromolecule catabolic process	14	3.68%	0.04355369
Cell Component level 5	mitochondrial inner membrane	18	4.74%	0.04419715
Cell Component level 5	organelle inner membrane	18	4.74%	0.04419715
Biol Process level 5	acetyl-CoA metabolic process	6	1.58%	0.04424803
Mol Function level 4	NADH dehydrogenase activity	7	1.84%	0.04526462
Cell Component level 4	cytosolic small ribosomal subunit (sensu Eukaryota)	6	1.58%	0.04540216
Biol Process level 1	reproduction	32	8.42%	0.04546934
Mol Function level 4	primary active transmembrane transporter activity	13	3.42%	0.0454952
Cell Component level 5	cytosolic small ribosomal subunit (sensu Eukaryota)	6	1.58%	0.0463302
Mol Function level 3	ligase activity, forming carbon-sulfur bonds	4	1.05%	0.04666897

**Table S13.** Significantly enriched GO terms for the 352 differentially expressed genes associated with unique spatiotemporal memories and not with time of day, training group, and/or activity state.

GO Category	GO Term	Count	%	PValue
Biol Process level 3	extracellular structure organization and biogenesis	7	3.12%	0.00441799
Biol Process level 4	regulation of synaptic growth at neuromuscular junction	4	1.79%	0.008722
Biol Process level 3	regulation of synaptic growth at neuromuscular junction	4	1.79%	0.00928487
Biol Process level 4	ovarian nurse cell to oocyte transport	5	2.23%	0.00936606
Biol Process level 4	synapse organization and biogenesis	6	2.68%	0.01007037
Biol Process level 3	ovarian nurse cell to oocyte transport	5	2.23%	0.01013575
Biol Process level 5	synaptogenesis	5	2.23%	0.01318377
Biol Process level 5	oogenesis	15	6.70%	0.0149086
Biol Process level 3	synaptic growth at neuromuscular junction	4	1.79%	0.01513539
Biol Process level 4	female gamete generation	15	6.70%	0.01588337
Mol Function level 4	calcium ion binding	10	4.46%	0.01917808
Mol Function level 4	thiol-disulfide exchange intermediate activity	3	1.34%	0.02505865
Mol Function level 5	pyrophosphatase activity	18	8.04%	0.02707767
Biol Process level 4	female germ-line cyst formation	3	1.34%	0.03061361
Biol Process level 5	female germ-line cyst formation	3	1.34%	0.03085084
Biol Process level 3	gamete generation	17	7.59%	0.0318054
Biol Process level 2	sexual reproduction	17	7.59%	0.03643064
Cell Component level 4	cullin-RING ubiquitin ligase complex	3	1.34%	0.03837186
Mol Function level 5	acetylglucosaminyltransferase activity	4	1.79%	0.03937506
Cell Component level 5	cullin-RING ubiquitin ligase complex	3	1.34%	0.04037742
Cell Component level 5	SCF ubiquitin ligase complex	3	1.34%	0.04037742
Biol Process level 5	neuromuscular junction development	4	1.79%	0.04077406
Mol Function level 3	hydrolase activity, acting on acid anhydrides	18	8.04%	0.04549037
Mol Function level 5	ATPase, coupled to transmembrane movement of substances	7	3.12%	0.04599123
Biol Process level 4	regulation of apoptosis	6	2.68%	0.04823195
Biol Process level 5	regulation of apoptosis	6	2.68%	0.04891282

**Table S14.** Significantly enriched GO terms for genes found to be more highly expressed in morning-anticipating bees (tAM-cAM), as compared to afternoon-anticipating bees (tPM-cPM), after removal of genes significant for the time of day, training group, and/or activity state.

GO Category	GO Term	Count	%	PValue
Biol Process level 5	oogenesis	13	10.83%	0.00133761
Biol Process level 4	female gamete generation	13	10.83%	0.00136778
Biol Process level 4	ovarian nurse cell to oocyte transport	5	4.17%	0.00146758
Mol Function level 3	active transmembrane transporter activity	12	10.00%	0.00164725
Biol Process level 3	ovarian nurse cell to oocyte transport	5	4.17%	0.00164923
Mol Function level 4	calcium ion binding	9	7.50%	0.00211815
Biol Process level 3	extracellular structure organization and biogenesis	6	5.00%	0.00239965
Mol Function level 5	ATPase, coupled to transmembrane movement of substances	7	5.83%	0.00476689
Biol Process level 3	gamete generation	14	11.67%	0.00495429
Biol Process level 2	sexual reproduction	14	11.67%	0.00523005
Biol Process level 1	reproduction	15	12.50%	0.00540292
Mol Function level 4	hydrolase on acid anhydrides, transmembrane movement	7	5.83%	0.0056696
Biol Process level 4	synapse organization and biogenesis	5	4.17%	0.00803415
Mol Function level 5	pyrophosphatase activity	14	11.67%	0.00844505
Biol Process level 4	female germ-line cyst formation	3	2.50%	0.01157987
Biol Process level 5	female germ-line cyst formation	3	2.50%	0.0118057
Mol Function level 4	hydrolase, acting on phosphorus-containing anhydrides	14	11.67%	0.01222169
Mol Function level 3	hydrolase activity, acting on acid anhydrides	14	11.67%	0.01414431
Mol Function level 5	P-P-bond-hydrolysis-driven transmembrane transporter activity	7	5.83%	0.0146194
Mol Function level 2	transmembrane transporter activity	16	13.33%	0.01507815
Mol Function level 4	primary active transmembrane transporter activity	7	5.83%	0.01595288
Biol Process level 5	synaptogenesis	4	3.33%	0.01824488
Biol Process level 4	anatomical structure formation	7	5.83%	0.02083398
Biol Process level 2	anatomical structure formation	7	5.83%	0.02368547
Biol Process level 3	anatomical structure formation	7	5.83%	0.02395452
Biol Process level 5	ovarian follicle cell development	6	5.00%	0.02546711
Biol Process level 3	ovarian follicle cell development	6	5.00%	0.02766751
Biol Process level 4	regulation of synaptic growth at neuromuscular junction	3	2.50%	0.02846087
Biol Process level 3	regulation of synaptic growth at neuromuscular junction	3	2.50%	0.03012666
Mol Function level 5	ATPase activity, coupled to transmembrane movement of ions	5	4.17%	0.0360755
Biol Process level 4	germ-line cyst formation	3	2.50%	0.03910729
Biol Process level 5	germ-line cyst formation	3	2.50%	0.03983418
Biol Process level 3	germ-line cyst formation	3	2.50%	0.04135366
Biol Process level 3	synaptic growth at neuromuscular junction	3	2.50%	0.04135366
Cell Component level 4	mitochondrial envelope	9	7.50%	0.04315457
Cell Component level 2	membrane	34	28.33%	0.04628274
Cell Component level 3	membrane	34	28.33%	0.04656064
Biol Process level 4	regulation of transforming growth factor beta receptor signaling	2	1.67%	0.04863556
Biol Process level 4	ring canal formation, actin assembly	2	1.67%	0.04863556
Biol Process level 5	ring canal formation, actin assembly	2	1.67%	0.04913065
Biol Process level 5	negative regulation of transforming growth factor beta signaling	2	1.67%	0.04913065
Biol Process level 5	regulation of transforming growth factor beta receptor signaling	2	1.67%	0.04913065
Cell Component level 5	mitochondrial envelope	9	7.50%	0.0495029

**Table S15.** Significantly enriched GO terms for genes found to be more highly expressed in afternoon-anticipating bees (tPM-cPM), as compared to morning-anticipating bees (tAM-cAM), after removal of genes significant for the time of day, training group, and/or activity state.

GO Category	GO Term	Count	%	PValue
Biol Process level 5	lipid biosynthetic process	6	5.77%	0.00215915
Biol Process level 4	lipid biosynthetic process	6	5.77%	0.00223107
Biol Process level 3	lipid biosynthetic process	6	5.77%	0.00236788
Biol Process level 3	lipid metabolic process	9	8.65%	0.00409178
Biol Process level 4	cellular lipid metabolic process	8	7.69%	0.00443555
Biol Process level 3	cellular lipid metabolic process	8	7.69%	0.00479968
Mol Function level 2	transferase activity	17	16.35%	0.01983536
Mol Function level 3	transferase activity, transferring glycosyl groups	5	4.81%	0.02578568
Biol Process level 5	positive regulation of retinal cell programmed cell death	2	1.92%	0.03167386
Cell Component level 4	Golgi stack	3	2.88%	0.0319922
Mol Function level 5	acetylglucosaminyltransferase activity	3	2.88%	0.03308461
Cell Component level 5	Golgi stack	3	2.88%	0.03366131
Mol Function level 1	catalytic activity	40	38.46%	0.036336
Cell Component level 3	ubiquitin ligase complex	3	2.88%	0.04618184
Cell Component level 4	ubiquitin ligase complex	3	2.88%	0.04966404

**Table S16.** Results of statistical tests for gene expression differences in canonical clock genes as well as each gene analyzed by both qPCR (N=76) and microarray (N=37). p-values are shown here. Graphical representation of means is found in Figure 5 and supplemental Figure S1. *Cryptochrome* did not have high enough gene expression in the brain to pass filtering thresholds on the microarray. *Tim2* did not have a representing oligo probe on the microarray. “Time of day” contrasts tAM-cAM and tPM-cAM with tAM-cPM and tPM-cPM. “Training group” contrasts tAM-cAM and tAM-cPM with tPM-cAM and tPM-cPM. “Activity state” contrasts tAM-cAM and tPM-cPM with tAM-cPM and tPM-cAM. All three of these contrasts were performed using 2-way ANOVA with post-hoc contrasts. Finally, “Different spatiotemporal memories” contrasts tAM-cAM with tPM-cPM, using t-tests.

Gene	Time of day		Training group		Activity State		Different spatiotemporal memories	
	microarray	qPCR	microarray	qPCR	microarray	qPCR	microarray	qPCR
<i>Period</i>	0.9127	0.0309	0.6018	0.4227	0.0452	<.0001	0.2056	0.0547
<i>Cryptochrome</i>	---	<.0001	---	0.8804	---	<.0001	---	0.0072
<i>Tim2</i>	---	0.9607	---	0.0037	---	0.0768	---	0.0937
<i>Cycle</i>	0.1114	<.0001	0.5467	0.5986	0.0192	0.9890	0.5299	0.0108
<i>Clock</i>	0.7303	0.9994	0.6802	0.0035	0.2688	0.7599	0.2078	0.0866
<i>Ankyrin</i>	0.7511	0.0264	0.3021	0.2977	<.0001	<.0001	0.5714	0.7228
<i>Apolipoprotein D</i>	0.0063	0.0303	0.0741	0.1827	0.0003	0.9943	0.0001	0.4792
<i>Black</i>	<.0001	0.0555	0.6059	0.2461	<.0001	0.4493	<.0001	0.4893
<i>Cadherin74a</i>	<.0001	0.0014	0.6988	0.5921	0.0099	0.8996	<.0001	0.0394
<i>Dopamine receptor 1</i>	0.1873	0.0028	0.1882	0.1323	0.0002	0.0798	0.0012	0.0029
<i>Dopamine receptor 2</i>	0.9202	0.9592	0.8689	0.1104	0.0015	0.6108	0.5975	0.2565
<i>Dopamine receptor 3</i>	0.4663	0.0494	0.7893	0.6632	<.0001	0.4835	0.0865	0.2529
<i>Enolase</i>	<.0001	0.6506	0.0741	0.8111	<.0001	0.8503	<.0001	0.5874
<i>GB15691</i>	<.0001	<.0001	0.4319	0.2549	0.0001	0.9978	<.0001	0.0028
<i>GB16507</i>	0.5407	0.2521	0.6204	0.7415	<.0001	0.0632	0.4589	0.7290
<i>GB16541</i>	0.0156	0.5817	0.0512	0.3385	<.0001	0.0037	<.0001	0.5865
<i>Heat shock protein 8</i>	0.0054	0.2124	0.1739	0.4629	<.0001	0.0080	0.0453	0.5141
<i>Heat shock protein 90a</i>	0.0535	0.0009	0.5682	0.3364	<.0001	0.0112	0.0072	0.0114
<i>Histone H1</i>	0.0435	0.1306	0.4530	0.5899	<.0001	0.0002	0.0007	0.0155
<i>Histone H3</i>	<.0001	0.0263	0.0036	0.0931	0.0162	0.6584	<.0001	0.0089
<i>Insulin-like peptide 1</i>	<.0001	0.2950	<.0001	0.9477	0.0162	0.9519	<.0001	0.4395
<i>Insulin receptor</i>	0.9168	0.2803	0.1844	0.2615	<.0001	0.0045	0.0726	0.7764
<i>Lethal(2) essential for life 21</i>	<.0001	0.0025	0.3814	0.4305	<.0001	0.9256	<.0001	0.0940



<i>Neprilysin2</i>	<.0001	<.0001	0.2276	0.2056	0.7513	0.3698	<.0001	0.0001
<i>Octopamine receptor 1</i>	0.9985	0.4715	0.9866	0.0220	<.0001	0.2352	0.0087	0.0568
<i>Phosphodiesterase8a</i>	0.7220	0.0851	<.0001	0.2008	0.0024	0.3895	0.0038	0.2039
<i>Zwischenferment</i>	0.8537	0.4891	0.8826	0.0058	0.0751	0.2942	0.8398	0.0269

**Table S17.** Primer sequences for qPCR.

Gene	Forward sequence	Reverse sequence
<i>Period</i>	TGA AAG GGC CAG CGA ATC	CCT CGC TAA TGT TGG TTA ATT GG
<i>Cryptochrome</i>	TGG TGA CAA ACC TCC GTT AAC TT	GTT CCG GAG GAT CCA TGC T
<i>Tim2</i>	TCC AGA GCA GAG CCA CAG CTA	CAA TGG TAT AGG ATG GTC ACT GAT TT
<i>Cycle</i>	TGT GCC CCA AGG TGT ATC G	CTT CGA CTT TTC GCT TCA TTC TG
<i>Clock</i>	CAA GCG GGC GCA TTT ATT A	TCG TAA ACG GTC GTA TTT TCC A
<i>Ankyrin</i>	GTG ATC GAA AGG TTC GCC AG	TCA GGT TCG CTA CCC CAC C
<i>Apolipoprotein D</i>	AGT GTT CGC CAC GGA TTA CAA	TGA CGA TGA GCA AAT GTC AGC
<i>Black</i>	TCG AAA TTG CAG ATA TTT GCG A	CAA ACC ACC TCC CCA AGC T
<i>Cadherin74a</i>	GCC GTA CAT AAT GCG GAT CA	GCG TCA GAA TCA CGT CCG A
<i>Dopamine receptor 1</i>	ACA GAA TTC CGA GAA GCG TTC A	ATT CGC TAG TCG ACG GTT GAT TT
<i>Dopamine receptor 2</i>	ACA CGG AAT TGG TTC TCC ATC T	TCC CGT AAC CGG CTG TCA
<i>Dopamine receptor 3</i>	GCG CGG AGG GAA ATC TTA T	GGA TTC TTA CTG TGC GCT GTG T
<i>Enolase</i>	CAG ATG CTA TGA AAA TGG GTA CTG A	CCA ACA GAA GTA GCA TCA AGA CCA
<i>GB15691</i>	ATT TGA TCG TGT TCG CCT TCA	CGC TTG CTC GCA CTT ATC G
<i>GB16507</i>	CAC GAC ATC ACG CAG CCT T	CCT TGA TCA CGA ACA CCA CG
<i>GB16541</i>	TTG TGC CAT TCC TCC TGA ATT	CGC ATT AAC GCT GAT CGT CA
<i>Heat shock protein 8</i>	ATC CGT TTG CAA TCC TGT TGT	CAC CTG GAT GGA AGC CTC C
<i>Heat shock protein 90a</i>	TCG AAA CAC AAT GAT GAT GAA CAA	CAG TGA AAG AAC CAC CAG CAG A
<i>Histone H1</i>	CGG ACT TCT GAA ATG GTA AAT GC	AAT TGC TTG GAA AGA CGA TCC TT
<i>Histone H3</i>	TTC ACG CGA AAC GTG TTA CAA	GTT TAA GCA CGC TCG CCA C
<i>Insulin-like peptide 1</i>	GCT CAG GCT GTG CTC GAA AAG T	CGT TGT ATC CAC GAC CCT TGC
<i>Insulin receptor</i>	TTC CGT GTA CCA CGG CAG A	GGT GCA AAG TAC GCG TGA GA
<i>Lethal(2) essential for life</i>	TGT TCT CCG TCC TCA TCG ACT	TTG CTC GGG ATA TAA TCC CAA
<i>Nepriylsin2</i>	AAC CGA CGA TCC GAT TCG A	CGA CAA TCA AGA TAC GCG GAA
<i>Octopamine receptor 1</i>	ACG AAG GCG GCG AAG AC	GCG GCA CCA AGT ACA TTG TG
<i>Phosphodiesterase8a</i>	TGC GCT CAC GAA AGA ATC TTA A	TGT GTC CTC GGT AAT ATT CCG C
<i>Zwischenferment</i>	GGA GAA GCC AGC GTC TTG TC	CCA GCG TCA AAG TCT TTA TGC A