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Blood Meal Induced Regulation of Gene Expression in the Maxillary Palps, a Chemosensory Organ of the Mosquito *Aedes aegypti*

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Background: *Aedes aegypti* is a prominent and highly competent vector of several arboviral diseases, including dengue, yellow fever, and Zika. Behaviors associated with reproductive feeding, both pre- and post-blood meal, directly influence disease transmission capacity. Odors mediate host seeking pre-blood meal, while post-blood meal females are refractory to host odors for at least 24 h. During this time, flight activity is substantially reduced. Two key host odors, carbon dioxide and (*R*)-1-octen-3-ol, are detected by the maxillary palps in mosquitoes. In the search for future vector control tools, the identification of genes that are regulated in the maxillary palps between host seeking and 24 h post-blood meal may provide an informative pool of targets.

Results: The blood meal-induced regulation of chemosensory, neuromodulatory, and other signal transduction genes was investigated in the maxillary palps of 24 h post-blood fed *Ae. aegypti* females, 6 days after emergence, and compared to host-seeking females of the same age using a transcriptomic approach. Genes-of-interest implicated in the behavioral switch from host seeking to post-blood meal quiescence were identified from multiple gene families investigated: odorant receptors, ionotropic receptors, pickpocket receptors, transient receptor potential receptors, odorant binding proteins, chemosensory proteins, neuromodulators, and their receptors, as well as constituents of second messenger signaling pathways. Reflecting the change in transcript abundance of families involved in CO_2 signaling, the neural sensitivity to this key kairomone compound was found to decrease in blood fed mosquitoes compare with their on-blood fed counter parts.

Conclusions: Sensory-associated gene expression is regulated in the maxillary palps of *Ae. aegypti* females in response to blood feeding. The concerted regulation of multiple genes within the sensory pathways of the maxillary palps likely play a key role in modulating the behavioral changes observed post-blood meal. Future functional characterization of the proteins generated by the genes-of-interest identified in this study may provide both a better understanding of the regulation of gonotrophic feeding and a pool of potential targets for vector control strategies.

Keywords: chemosensory, mosquito, transcriptome, host seeking, blood feeding, resting

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INTRODUCTION

The host-seeking behavior of mosquitoes depends heavily on the olfactory system, and is intimately linked to disease transmission (Mescher and De Moraes, 2017). Mosquito activation and medium-to-long range attraction to, and recognition of, hosts is partly mediated by two odors, carbon dioxide (CO_2) (Dekker and Cardé, 2011; Majeed et al., 2017) and (*R*)-1-octen-3-ol (Majeed et al., 2016). These volatiles are detected by a series of chemosensory proteins expressed in an olfactory organ of relatively low complexity, the maxillary palps (Lu et al., 2007; Bohbot and Dickens, 2009; Grant and Dickens, 2011; Erdelyan et al., 2012; Manoharan et al., 2013; McMeniman et al., 2014; Hill et al., 2015). Short range attraction and the ultimate acceptance of a host also rely on other sensory modalities, including thermal and mechanosensory detection, and gustation (Montell and Zwiebel, 2016). In this study, we identify potential candidate genes important for generating the behavioral switch between host seeking and resting through their differential abundance in the maxillary palps of host-seeking and 24 h post-blood fed female *Ae. aegypti* mosquitoes.

Capitate peg sensilla, the only chemosensory sensillum type found on the maxillary palps, house three olfactory sensory neurons (OSNs) (Grant et al., 1995), and cover the fourth of the five segments comprising the maxillary palp in female *Ae. aegypti* (McIver and Charlton, 1970). Fourteen chemosensory gene families are expressed by the OSNs and accessory cells of these sensilla (Bohbot et al., 2014; Matthews et al., 2016). Of the chemoreceptors, the odorant receptors (Ors), the ionotropic receptors (Irs), and the gustatory receptors (Grs) are well-characterized (Hallem and Carlson, 2004; Hallem et al., 2004; Kent et al., 2008; Benton et al., 2009; Carey et al., 2010; Croset et al., 2010; Wang et al., 2010; Pitts et al., 2017). The Ors constitute a family of seven transmembrane domain proteins that function as heteromeric ionotropic and/or metabotropic receptors (for review see Stengl and Funk, 2013) composed of the highly conserved Or co-receptor (Orco) and unique Or-subunits, tuned to a defined set of odorants (Hallem and Carlson, 2004; Hallem et al., 2004; Ditzen et al., 2008; Carey et al., 2010; Wang et al., 2010; Bohbot et al., 2011a,b; Xu et al., 2013; McBride et al., 2014). Within this rapidly evolving gene family (Benton, 2015), only three are expressed in the maxillary palps of *Ae. aegypti*; Orco, the (*R*)-1-octen-3-ol-sensitive Or8 (Cook et al., 2011; Grant and Dickens, 2011) and the as yet orphan Or49 (Bohbot et al., 2014). While originally described as "antennal Irs" (Croset et al., 2010), a subset of Irs, including two (Ir25a, Ir76b) of the three Ir co-receptors are expressed in the maxillary palps (Matthews et al., 2016; Pitts et al., 2017). Although the primary role described for Grs is the detection of tastants, two of the three Grs expressed in the maxillary palp are involved in transducing CO_2 reception, Gr1 and Gr3, but not Gr2 (Erdelyan et al., 2012). Carbon dioxide elicits activation of host-seeking behavior and long-range attraction in mosquitoes (Grant et al., 1995; Dekker and Cardé, 2011; Majeed et al., 2014, 2017).

Besides Ors, Irs, and Grs, two additional receptor protein families, the pickpocket and the transient receptor potential receptors (Pkps and Trps, respectively), have recently received attention for their involvement in chemosensation. The Ppks robustly expressed in the maxillary palps of *Ae. aegypti* (AAEL008053, AAEL000926) (Bohbot et al., 2014) are from subfamily V, and are likely to be involved in mechanosensation (Walker et al., 2000; Tracey et al., 2003; Johnson and Carder, 2012; Kim et al., 2012; Zelle et al., 2013; Guo et al., 2014). The Trps are involved in sensing auditory, proprioreceptive, geotactic, thermal and chemical cues (Fowler and Montell, 2013), and references therein). The TrpA, Trpc, and TrpL subfamilies are involved in chemosensation, and appear to be sensitive to ligands that can be modulated by Or and/or Gr activated G-protein G_q activating phospholipase C (PLC) signaling cascades (Kang et al., 2010; Kim et al., 2010; Kwon et al., 2010; Badsha et al., 2012; Fowler and Montell, 2013). In addition, *painless* is required for the avoidance of isothiocyanate (Al-Anzi et al., 2006). Of these, *Trp*, *painless,* and *TrpA1* are expressed in the maxillary palps of *Ae. aegypti* (Bohbot et al., 2014).

While not recognized as receptors, sensory neuron membrane proteins (SNMPs), are expressed in the OSN membranes (Vogt, 2003; Nichols and Vogt, 2008). Sensory neuron membrane proteins are involved in modulating the interaction between odorants, particularly pheromones, and Ors (Benton et al., 2007; Jin et al., 2008). Orthologs of both Snmp1 and Snmp2 have been identified in mosquitoes (Nichols and Vogt, 2008), and shown to be expressed in the maxillary palps of host-seeking female *Ae. aegypti* (Bohbot et al., 2014; Matthews et al., 2016).

Signaling cascades amplify and modulate chemical signals, producing the final electrical output of the OSN. All current models indicate that the Or/Orco complex comprises a ligand binding receptor, a cation permeable ion channel, and connection to metabotropic pathways (Sato et al., 2008; Nakagawa and Vosshall, 2009; Sargsyan et al., 2011; Getahun et al., 2013). Of the two main metabotropic cascades, the first involves the G-protein G_q activating phospholipase c-ß (Plc-ß) to increase intracellular calcium. This follows the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to inositol trisphosphate (IP_3) and diacylglycerol (DAG), leading to IP_3-dependant calcium channel activation, increasing intracellular Ca^{2+}, and activating protein kinase C (Pkc) phosphorylating cation channels, including Orco. The second cascade involves the G-protein G_s, which activates adenylate cyclase and triggers the cAMP signaling cascade, activating ion channels, including Orco, through Plc and Pkc pathways (Getahun et al., 2013). The activation of protein kinase A (Pka) by these pathways can lead to long-term effects through the activation of transcription factors that regulate gene expression.

Members of three chemosensory protein families have been shown to be secreted by the accessory cells into the sensillum lymph surrounding the OSNs: the odorant binding proteins (Obps), the chemosensory proteins (Csps) and the odorant degrading enzymes (Odes) (Pelosi et al., 2005; Leal, 2013). The Obps and Csps are small soluble proteins that play a role in the selective recognition, gain control and transport of odorants from the cuticular pores to the membrane receptors on the OSNs (Vogt et al., 1999; Vogt, 2003; Pelosi et al., 2005; Larter et al., 2016). There are three subfamilies of Obps, the classical, the Plus-C and the two-domain Obps (Zhou et al., 2008; Manoharan

et al., 2013). The most robustly expressed Obps in the *Ae. aegypti* maxillary palps are from the classical and Plus-C subfamilies (Bohbot et al., 2014). Half of the identified Csps are expressed in the maxillary palps of host-seeking *Ae. aegypti* females (Matthews et al., 2016). Following the dissociation of the odorants from the receptors, the Odes act to rapidly clear the lymph of odorants (Younus et al., 2014). The Odes are not well-defined and are generally described by those members of the cytochrome P450 and carboxylic esterases expressed in olfactory tissues.

The sensory tuning of the peripheral olfactory system in mosquitoes is likely generated by the sensitivity and specificity of these proteins, acting in concert (Carey et al., 2010; Wang et al., 2010; Rinker et al., 2013; Bohbot et al., 2014; McBride et al., 2014; Omondi et al., 2015). In this study, the chemosensory genes that are modulated in the maxillary palps of host seeking (nbf) and 24 h post-blood meal (bf) female *Ae. aegypti* of the same age, 6 days-post-emergence (dpe), are identified as genes-of-interest for further functional investigation. The function and role in signal transduction are discussed for genes subject to modulation, and put in the context of the neuronal activity of the maxillary palp capitate peg neurons. In particular, the differential expression of the chemosensory-related genes is discussed in the context of the ligand sensitivity of their concordant OSNs, in both host-seeking and 24 h pbm states.

MATERIALS AND METHODS

Animal Rearing and Tissue Collection

Aedes aegypti (Rockefeller strain) were reared at 27 ± 1°C, 65 ± 5% relative humidity under a 12 h:12 h light:dark period, as previously described (Majeed et al., 2017). To control for potential age- and circadian-related differences in all experiments, 6-day post-emergence (dpe) adult female mosquitoes with *ad libitum* access to sucrose (10%) were used between ZT 5 and 8. The mosquitoes were anesthetized on ice for at least 1.5 min prior to dissection. Maxillary palps were dissected from 6-dpe females under two conditions: those blood fed 24 h prior to dissection (bf) and those which were not (nbf). All mosquitoes had *ad libitum* access to 10% sucrose throughout. Tissues (500 pairs per condition) were stored at −20°C in RNAlater (Life Technologies, Sweden). Blood meals were provided to the mosquitoes using a Hemotek membrane feeder. The blood used was from donor sheep and was commercially purchased from a fully certified facility (Håtunalab, Bro, SE). No additional ethical approval from the university was required.

RNA Extraction and Illumina Sequencing

Tissues were sonicated (Vibra-Cell sonicator, Sonics and Materials, VCX-130; 10 cycles; 70% amplitude; 1 s pulses), incubated (30 s) on ice, and then the whole cycle was repeated twice. Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Sweden), including the RNase-free DNase I on-column treatment, according to the manufacturer's protocol, and then stored at −80°C. Total RNA was quantified using fluorometric analysis (Qubit, Invitrogen, Sweden), and the quality assessed by NanoDrop and standard gel electrophoresis, prior to sending to Eurofins (Germany) for 3′ fragment cDNA library generation and sequencing on an Illumina HiSeq 2500 (single read, 1 × 100 nt). Bar coded fragment libraries were generated following the standard Illumina protocol. Four libraries were constructed, two replicates per condition, which were run in separate channels. Each channel contained a single replicate of the maxillary palp from female host-seeking (nbf), and 24 h post-blood meal (24 h pbm) bar coded libraries (channel 003: nbf 15505650 and bf 11263612 reads; channel 004: nbf 15927640 and bf 11518245 reads).

Read Mapping

Prior to mapping, the raw reads were analyzed and quality filtered. The adapter sequences and low-quality bases (Phred score < 20) were removed from both ends of each single read. Reads that did not fulfill an average quality threshold were clipped (sliding window, window size 20 nt, minimum quality 4) using Trimmomatic 0.20 (http://www.usadellab.org, u.d.), and reads shorter than 40 nt were removed. Reads were mapped using CLC Genomics Workbench 10 (http://www.clcbio.com, Qiagen), and the new *Ae. aegypti* Liverpool AGWG annotation, genome assembly AaegL5 and gene set AaegL5.1 (https://www.vectorbase.org/organisms/aedes-aegypti/lvp_agwg/aaegl5). While this genome database represents the current overall annotation, there has been a considerable upheaval in the chemosensory gene annotations, which has removed several long-standing, and empirically supported genes. Of note for the current study is the lack of annotation of the conserved culicine odorant receptor, Or49 (AAEL001303-RA in AaegL3.5), previously demonstrated to be expressed in the adult maxillary palps (Bohbot et al., 2007, 2014) and labrum (Jung et al., 2015), as well as in the larval antennae (Mysore et al., 2014). Indeed, RNAi knockdown of Or49 results in delayed blood feeding behavior (Jung et al., 2015). To account for this lack of annotation, reads mapping to the AaegL5 genome region, where the three-prime sequence of Or49 is located (2:448700040-448700246;448699296-448699784), were counted manually. Equally, the 3′ untranslated regions (UTRs) of multiple genes are truncated. Of note for this study, the 3′ UTR of *Ir76b* (AAEL006360), *Ir93a* (AAEL021659), *Ir41a* (AAEL000041), *Csp05687* (AAEL005687), *Scrb9* (AAEL000256), *Scrb3* (AAEL005979), and *Obp60* (AAEL008012) are truncated in the L5 annotation, and as we did quantitative sequencing of 3′ transcript ends, an incorrect 3′ annotation can result in false negatives (i.e., *Irs*), and underrepresented read abundances (i.e., *Csp, Scrbs,* and *Obps*). The 3′ UTRs are extended by 4850 bp (3: 396663291-396668151), 1356 bp (3:325201503-325202859), 1101 bp (3:103203960-103205061), 1159 bp (3:115197984-115199143), 3210 bp (2:227337205-227340415), 233 bp (2:8159347-8159580), and 1367 bp (1:164107819-164109186) from the L5 annotation, respectively. Additionally, the truncation of the 3′ UTR annotation of AAEL002924-RC, may have resulted in false positives attributed to *Ir8a*, as the 3′ UTRs of *Ir8a* and AAEL002924-RC overlap (1:85989081-85989566), albeit on complementary strands. In this study, all several hundred reads in this region are attributed to

AAEL002924-RC, according to the strand onto which the reads mapped.

Abundance and Differential Analyses

Single-end RNA-Seq libraries produced reads from the 3′ end of each transcript present. To quantify transcript abundance in each library, the mean read counts were normalized using an upper-quantile-based normalization (Bolstad et al., 2003) prior to between-library comparisons (see below). Transcripts with a mean abundance of more than 20 normalized read counts were considered detectible and thus included in further analysis. This threshold is similar to the <1 RPKM filter that is commonly employed to reduce noise (Rinker et al., 2013). The upper-quantile-based normalization was performed on each library to increase the detection sensitivity of differential expression for low abundance transcripts (Bolstad et al., 2003). Kal's Z-type test, which assumes a *ß*-binomial distribution on the normalized read counts, generated fold changes (FC) and probability estimates that were used to detect differential expression (Kal et al., 1999). Comparisons were made between nbf *vs.* bf maxillary palp libraries. Strict criteria were used to determine significant differential abundance. Genes that exhibited a FC>2 and a *P*-value corrected for false discovery rate (FDR) <0.05 were considered to be significantly differentially abundant. Genes with $1.5 > FC < 2$ and an *FDR P*-value <0.05, were considered potential genes-of-interest. The means of normalized read counts were transformed with the addition of a constant (1.1) to prevent values of infinitive fold change when one of the two libraries being compared exhibited no detectible reads.

Quantitative Real Time PCR

The MIQE guidelines were adhered to in this study (Bustin et al., 2009). Selected genes that demonstrated differential abundance according to the transcriptome analyses were assessed and verified using quantitative real time polymerase chain reaction (qPCR). Primer pairs for qPCR were designed using Primer 3 (http://bioinfo.ut.ee/primer3-0.4.0/primer3/) (20-22 nt, T_m ∼60°C, GC 50% ± 10%, GC-clamp 2 nt, amplicon 80–150 nt spanning exon-exon regions) and supplied by Eurofins Genomics (Munich, Germany) (**Supplementary File 1**). Six new cDNA libraries (nbf: 3; bf: 3) were constructed by Oligo(dT) priming from the total RNA extracted from 6 × 300 pairs of maxillary palps (extraction protocol described above in section RNA Extraction and Illumina Sequencing), using SuperScript III First-Strand Synthesis System for RT-PCR (Thermo Fischer Scientific, CA, USA), according to the manufacturer's protocol, and stored at−20°C. This resulted in three biological replicates for each of the nbf and bf conditions. Quantitative PCR was performed with minor modifications, according to manufacturers' protocol (Platinum SYBR Green qPCR SuperMix-UDG w/ROX, Bio-Rad Laboratories, CA, USA), in a total volume of 25 µl (12.5 µl Supermix, 0.5 µl each primer, 1 µl of cDNA, 11 µl DNAse-RNAse free water). Amplification was performed on a BioRad CFX 96 (Bio-Rad Laboratories, CA, United States), using the following parameters: 2 min at 50°C, then 2 min at 95°C, followed by 40 cycles (30 s at 95, 58, and 72°C, respectively). During elongation at 72°C, fluorescence was measured for each cycle. After the final cycle, specificity of the product amplification was assessed using melting curve analyses (65–94°C in 0.5°C increments). Controls (water alone and samples containing all reactants except cDNA) were included for each plate. For each of the three biological replicates for both nbf and bf, every primer pair was assessed three times (technical replicates), resulting in a total of nine replicates for each cohort. Levels of gene expression were determined using the ΔΔCq method (Livak and Schmittgen, 2001), and normalized to a reference factor. The geometric mean of three verified stable reference genes, *Elfa1* (AAEL017096), *RpL8* (AAEL000987), and *RpS7* (AAEL009496) (Christ et al., 2017) comprised the reference factor for each replicate. The level of differential gene abundance was presented relative to that of the nbf female maxillary palps, verified for normality and homogeneity of residuals using D'Agostino-Pearson test, and then compared using a two-tailed paired Student's *t*-test with significance that was adjusted for multiple comparisons.

Electrophysiology

Each capitate peg sensillum contains three OSNs (McIver and Charlton, 1970). The highest and intermediate spiking amplitude neurons, by convention referred to as the A and B cells, respond to CO_2 (Grant et al., 1995) and (*R*)-1-octen-3-ol (Lu et al., 2007; Cook et al., 2011), respectively. Electrophysiological recordings from these neurons were made using single sensillum recordings, as described previously (Majeed et al., 2014, 2017). The OSNs were stimulated by introducing various concentrations of either CO_2, from gas cylinders containing metered amounts of CO_2 and oxygen (20%) balanced by nitrogen (Strandmöllen, AB, Ljungby, Sweden) (Majeed et al., 2017), or racemic 1-octen-3-ol (Sigma Aldrich, Sweden) in a background of 150 ppm CO_2. This background of 150 ppm was used to clarify the visualization of the B cell response by reducing the response in the A cell. The stimuli were introduced into the humid airstream passing over the preparation 11 cm upstream of the maxillary palps. The resulting dose response curves were generated by non-linear regression, with a variable slope for nbf and bf mosquitoes using maximum likelihood, and compared using the extra sum-of-squares F-test (GraphPad Prism v. 5.0a, GraphPad Software, San Diego, US).

RESULTS AND DISCUSSION

Transcript Abundance in Varies With Blood Meal Status

Transcriptome Quality

The CEGMA gene set is a set of core eukaryotic genes (CEGs) identified as expressed in all eukaryotic animals and likely involved in essential cell processes (Parra et al., 2007). These genes were assessed to determine whether the depth of sequencing was sufficient to reliably identify expressed transcripts and to assess the stability of transcript abundance across libraries (Taparia et al., 2017). Ninety-nine percent of the CEGs were found at detectable levels in the maxillary palps, indicating that the sequencing depth was sufficient to reliably identify gene expression (**Figure 1**; **Supplementary File 2**). The fold change in abundance for 99%

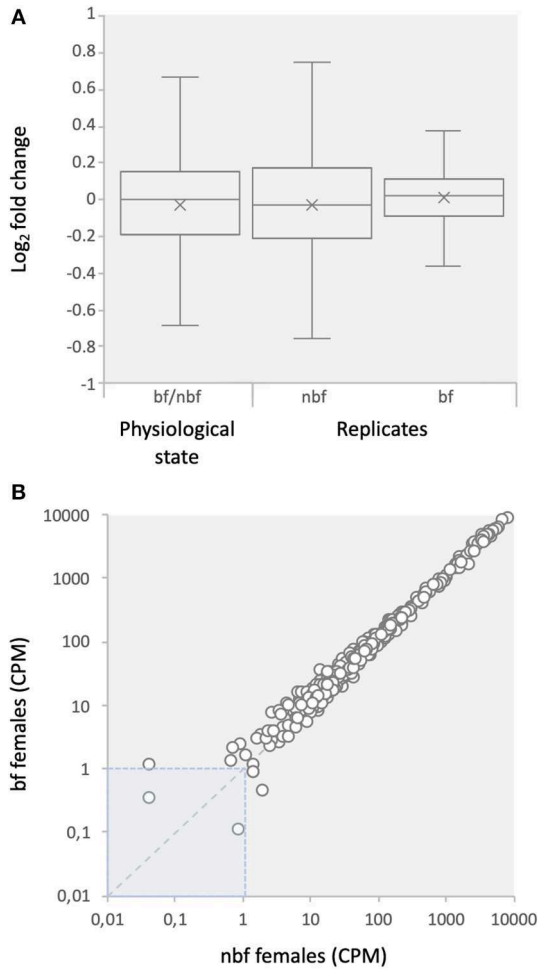

FIGURE 1 | Expression of core eukaryotic genes (CEGs). **(A)** Comparison of fold changes between blood fed (bf) and non-blood fed (nbf) female mosquitoes, and between replicates of the maxillary palp transcript libraries of *Aedes aegypti*. **(B)** Comparison of counts per million (CPM) between bf and nbf females.

and >92% of the CEGs transcripts, between physiological states and between replicates, was <2.0 and <1.5, respectively, as would be expected for housekeeping genes (**Figure 1**) (Dzaki et al., 2017; Shakeel et al., 2017).

Transcript Abundance

A total of 17,330 genes are annotated in the *Ae. aegypti* genome database (AaegL5.1), of which 9,252 were reliably detected in the maxillary palps of the 6-dpe females. Previous studies report between 10,796 (Bohbot et al., 2014) and 11,052 (Matthews et al., 2016) transcripts, from earlier annotations, to be expressed in the maxillary palps of host-seeking females. The observed discrepancy in expression found between our study and those previous is likely due to methodological differences, including the wider age range assayed, earlier reference gene sets, and the paired-end libraries generated in previous studies. A mapping to the AaegRU database used in Matthews et al. (2016) resulted in the reliable detection of 363 more genes. While paired-end libraries are likely to identify more transcripts, since the sequencing covers the entire length of the transcript, the accuracy of the abundance estimates is reduced compared to the 3′ end quantitative sequencing used in this study (Moll et al., 2014).

The number of genes with detectable transcript abundance in the maxillary palps of bf females (8,899) was similar to that found in nbf females (8,834). An analysis of the transcripts with annotated gene ontologies (GOs) (nbf: 8,879, bf: 8,803) revealed that the most represented molecular functions in these olfactory organs, both by number and by read abundance were genes encoding for proteins with ligand binding capabilities (small molecule-, protein-, DNA-, and cofactor binding), enzyme (hydrolases, transferases, and oxidoreductases) and enzyme inhibitor activities, as well as transmembrane transporter activity (**Figures 2A,B**). The only GO class demonstrating differential overall read abundance was odorant binding (GO:0005549; **Figure 2B**). As maxillary palps are involved in olfaction, it is not surprising that this organ has a relatively high number of odorant binding genes expressed (2–3%). The proportion of odorant binding genes demonstrated significantly higher overall normalized read abundance [$t\text{-ratio}_{(2)} = 151.6$; $q = 6.590 \times 10^{-4}$; **Figure 2B**], and significantly more genes with >2-fold higher abundance, in the maxillary palps of bf than in nbf females [$\chi^2 = 3.89$; $P < 0.0486$; **Figure 2C**]. The only other GO class showing differential numbers of genes with >2-fold difference in normalized read abundance was the structural constituent of cuticle (GO:0042302), which was more abundant in the maxillary palps of nbf than bf females [$\chi^2 = 9.50$; $P < 0.0021$; **Figure 2C**]. This is likely a reflection of an overall down-regulation of cuticular proteins in post-blood fed mosquitoes (Dana et al., 2005).

The 20 genes with the highest transcript abundance in the maxillary palps include the large ribosomal RNA subunit (*lsu rRNA*; AAEL018689), the most highly represented gene in both libraries, and 11 other ribosomal proteins present in both physiological states. As none of these ribosomal transcripts differed in abundance between nbf and bf mosquitoes, this suggests that protein translation is a major and ongoing process in both physiological states, indicating that the observed differences expression of other genes may be a result of targeted regulation and not an overall regulation of gene expression. The other top abundance transcripts included an Obp (*Obp63*), a *haemolymph juvenile hormone binding protein* (AAEL001306) and an *ADP/ATP carrier protein* (AAEL004855), similar to that previously described (Bohbot et al., 2014; Matthews et al., 2016).

Chemosensation

Host preference, driven by the necessity to detect and locate hosts to obtain blood meals, has exerted a strong selection pressure on the olfactory system of vector mosquitoes (Takken and Knols, 1999). The olfactory system of female mosquitoes is highly tuned to salient host volatiles (Takken and Knols, 1999; McBride et al., 2014), together with generic cues, such as CO_2 and (*R*)-1-octen-3-ol, which are detected by two of the three maxillary palp OSNs, providing sufficient information to enable identification of, and

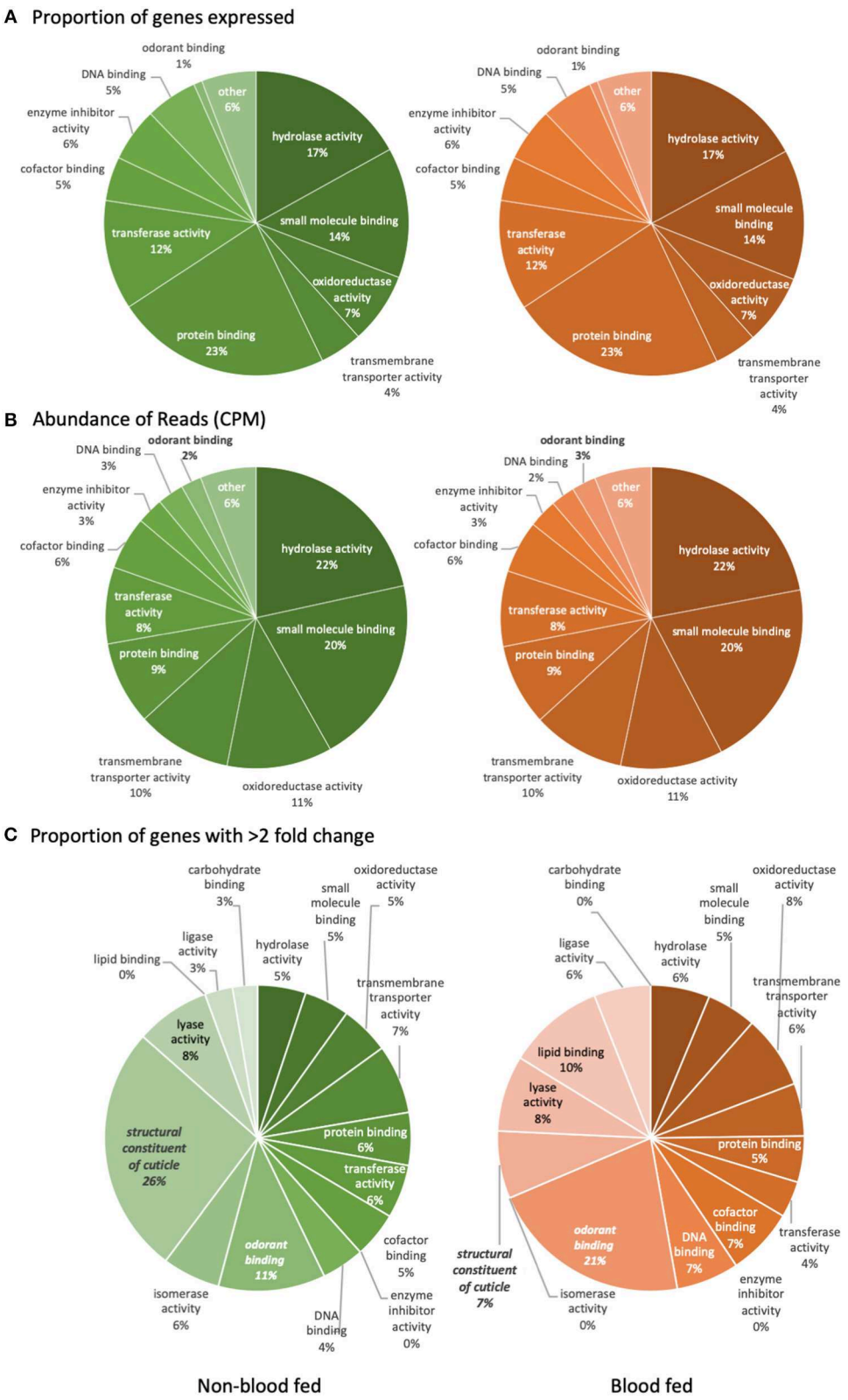

FIGURE 2 | Level three gene ontology analysis of molecular functions. The analysis represents **(A)** the proportion of reliably expressed genes, **(B)** the abundance of reads (CPM), and **(C)** the proportion of genes with >2-fold change in the maxillary palps transcriptomes of non-blood fed (left) and blood fed (right) female *Aedes aegypti*. Significant difference ($q < 0.05$; multiple *t*-tests) is indicated in bold/italics. Gene ontology classes that were represented by <1% of the total were not named.

discrimination among, potential host species (Majeed et al., 2016, 2017). The third OSN is not yet functionally characterized.

Modulation of Carbon Dioxide Signaling

The *Grs* involved in transducing CO_2 reception in female *Ae. aegypti* mosquitoes are *Gr1* (AAEL002380) and *Gr3* (AAEL010058) (Erdelyan et al., 2012; McMeniman et al., 2014). Both of these CO_2-sensitive receptors are up-regulated during adult maturation, indicating a role for the peripheral regulation of host seeking in both *Ae. aegypti* (Bohbot et al., 2013) and *An. gambiae* (Omondi et al., 2015). Interestingly, the transcript abundance of both of these receptor genes was significantly reduced in the maxillary palps of bf compared with that of nbf females of the same age (1.2 and 1.7-fold; *FDR P* < 0.0001, respectively; **Figure 3A**). This change in transcript abundance was confirmed by qPCR (**Supplementary Data Sheet 1abc**); in fact, there was an overall congruence between the qPCR and RNA-seq datasets amongst all genes tested. To assess the biological relevance of this <2-fold reduction in *Gr* expression, the response of the CO_2-sensitive OSN, the A-cell, was analyzed in both nbf and bf females using single sensillum recordings. This showed that the sensitivity of the A-cell was reduced

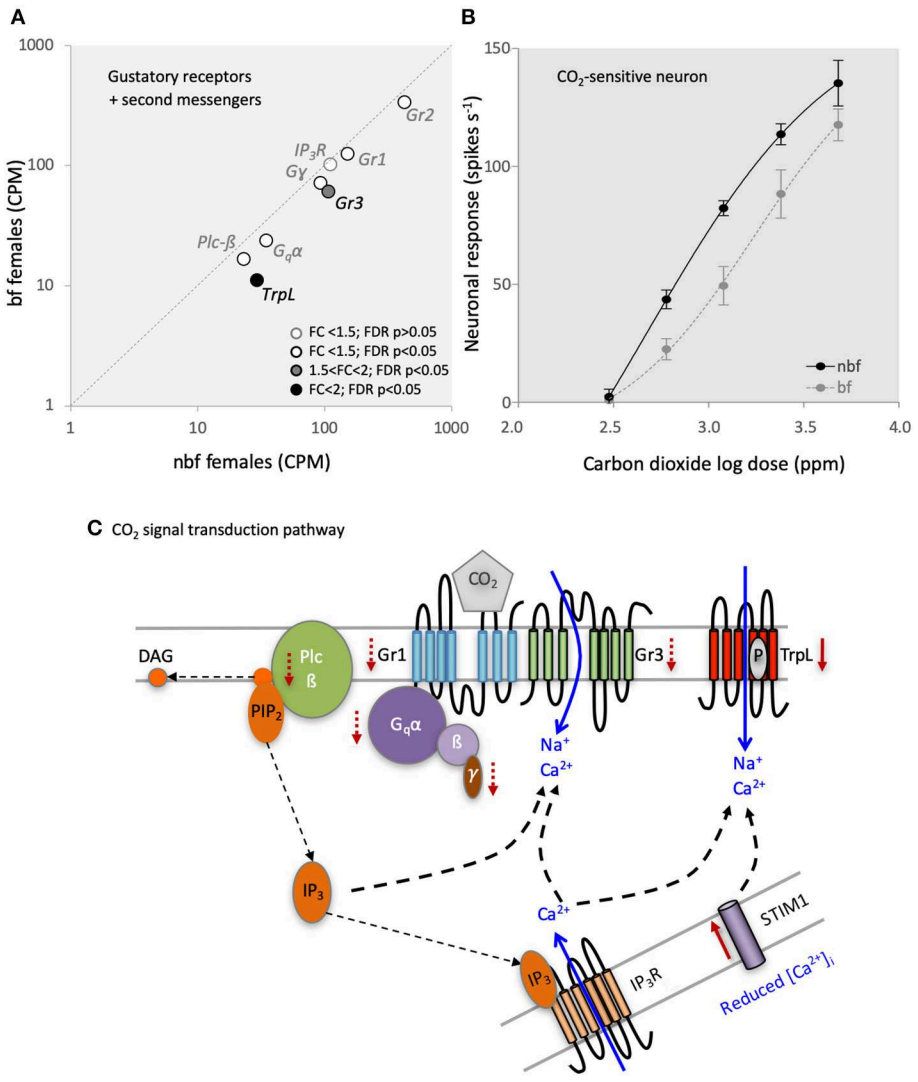

FIGURE 3 | Carbon dioxide (CO_2) signaling in the maxillary palps. **(A)** Differential transcript regulation of CO_2-sensitive *gustatory receptors* (*Gr1* and *Gr3*), *Gr2* and the second messenger genes, *phospholipase c beta* (*Plc-ß*), *G_q-protein alpha subunit* ($G_q\alpha$), *G-protein gamma subunit* ($G\gamma$), and *inositol trisphosphate receptor* (IP_3R) in the maxillary palps of blood fed (bf) and non-blood fed (nbf) *Aedes aegypti* females is shown. Transcripts that exhibit significant differences in abundance (Kal's test; *FDR P* < 0.05), are denoted according to their fold change (FC); FC > 2 in black-filled circles; 1.5 < FC < 2 in gray-filled circles; and FC < 1.5 in unfilled circles. **(B)** Differential response of the CO_2-sensitive neuron to various concentrations of CO_2 is shown. **(C)** Model of the putative CO_2 signaling pathway. Solid red arrows indicate significant (FC > 2; *FDR P* > 0.05) and dashed red arrows indicate potential (FC < 2; *FDR P* > 0.05) changes in regulation. Diacylglycerol (DAG); phosphatidylinositol 4,5-bisphosphate (PIP2); G-protein beta subunit (ß); G-protein gamma subunit (γ); Stromal interaction molecule 1 (STIM1); transient receptor potential receptor L (TrpL).

in bf compared to nbf females [$F_{(4,77)} = 5.958$, *FDR P* = 0.0003; **Figure 3B**], reflecting the reduction in *Gr1* (150.80 to 125.66 CPM) and *Gr3* (106.79 to 60.64 CPM) abundance (**Supplementary File 2**). Alternatively, the observed changes in the neuronal sensitivity may have been generated by post-transcriptional regulation, such as regulation of translation and other cellular regulation processes such as phosphorylation.

The third *Gr* to be detected in the maxillary palps of both nbf and bf females was *Gr2* (**Figure 3A**), a paralogue of *Gr1*, which also was less abundant in the maxillary palps of bf females (1.3-fold; *FDR P* = 0.0257; confirmed by qPCR, **Supplementary Data Sheet 1b**). The *Gr2* appears not to be required for CO_2 reception in *Ae. aegypti* (Erdelyan et al., 2012), and the function of this receptor has yet to be determined.

Since the abundance of the CO_2-sensitive *Gr* transcripts was significantly lower in bf females, yet the fold change was <2-fold, the observed differential response in the CO_2-sensitive OSNs 24 h post-blood meal may be a result of regulatory changes in both the translation and translocation of the receptors, and/or changes in the internal amplification of the signal via the second messenger cascades. The signal transduction pathway for CO_2 detection has been partially described in *D. melanogaster*, implicating G-protein signaling via a $G\alpha_q$ (*DmGα49B*) (Yao and Carlson, 2010), which activates the phospholipase c-ß (Plc-ß) pathway via DmPlc21c to modulate the downstream Trps, DmTRP, and DmTRPL. The knockdown/knockout of any of the genes in this pathway results in a reduction/loss of the CO_2 behavioral phenotype in *D. melanogaster*. Recently, Bohbot et al. (2014) proposed that a similar transduction pathway underlies CO_2 detection in female *Ae. aegypti* mosquitoes, citing similar transcript abundances of the homologous genes: *Gr1, Gr3, Gα49B* (AAEL010506), *Plc-ß* (AAEL009380), and *TrpL* (AAEL005575). The comparison here between 6 dpe nbf and bf female maxillary palp transcriptomes lends support to this hypothesis (**Figure 3A**; **Supplementary Data Sheet 1**). The transcript abundance of the G-proteins *Gα49B* (AAEL010506; 1.4-fold; *FDR P* = 0.0002) and G_γ (AAEL006685; 1.3-fold; *FDR P* = 0.0024) was significantly lower in bf females (confirmed by qPCR; **Supplementary Data Sheet 1de**), while $G_ß$ (AAEL019779) maintained a consistent abundance (*FDR P* = 0.1906). In the phospholipase c pathway, *Plc-ß-A* (AAEL009380) transcript abundance was significantly lower in bf females (1.3-fold change; *FDR P* = 0.0060), confirmed in subsequent qPCR analyses (**Supplementary Data Sheet 1f**), while the *inositol 1,4,5-triphosphate receptor* (AAEL02744) was not regulated in either the transcriptomic (1.1-fold, *FDR P* = 0.6572; **Figure 3A**) or qPCR analysis (**Supplementary Data Sheet 1g**). Of the downstream Trps, *TrpL* (AAEL005575) displayed a reduced abundance in the maxillary palps of bf females (2.6-fold; *FDR P* < 0.0001; confirmed by qPCR, **Supplementary Data Sheet 1f**), while *trp* (AAEL005437) was not expressed in the maxillary palps of 6-dpe females. In 6-dpe Orlando strain females, *trp* was reported to be robustly expressed, however it is not known whether the abundance is regulated post-blood meal (Bohbot et al., 2014). The other homologous gene implicated in CO_2 signal transduction in *D. melanogaster* is *Stim1* (AAEL018261), which displayed a higher abundance in bf compared to nbf (4-fold; *FDR P* = 0.0226). Combined, our results demonstrate that most of the transcripts in the putative CO_2 signal transduction pathway are less abundant 24 h post-blood meal (**Figures 3A,C**), corresponding with the physiological reduction in sensitivity to CO_2 (**Figure 3B**), when females no longer seek for hosts (Klowden and Blackmer, 1987; Christ et al., 2017). As genetic manipulation of non-model organisms becomes more accessible, it is becoming possible to directly test the link between the CO_2 physiology/behavior and the CO_2-related signal transduction pathway gene expression (McMeniman et al., 2014).

Differential Modulation of Or-Mediated Signaling

Ligand transduction in the B- and C-cells of *Ae. aegypti* is mediated by Or8, and tentatively by Or49, together with Orco, respectively (Bohbot and Dickens, 2009; Bohbot et al., 2013). Similar to the CO_2-sensitive *Grs*, the gene expression of these *Ors* is up-regulated during adult maturation (Bohbot et al., 2013), emphasizing the importance of Or8 in host recognition (Majeed et al., 2016) and reflecting a likely role for Or49 in the acquisition of host seeking. The modulation in expression of these *Ors* in bf maxillary palps, however, differs between *Or8* (1.2-fold change; *FDR P* = 0.1511; **Figure 4A**) and *Or49* (1.2-fold change; *FDR P* = 0.0007; **Figure 4A**), as also confirmed by qPCR (**Supplementary Data Sheet 1ik**). This demonstrates that these *Ors* are differentially regulated in bf maxillary palps. Both Or8 and its cognate OSN, the B-cell, selectively detect (*R*)-1-octen-3-ol (Lu et al., 2007; Bohbot and Dickens, 2009; Hill et al., 2015), one of the most extensively studied mosquito host cues (e.g., Bohbot and Dickens, 2009; Cook et al., 2011; Bohbot et al., 2013; Majeed et al., 2016). Reflecting the consistent levels of transcript expression for *Or8*, as well as *Orco* (1.1-fold change; *FDR P* = 0.6517; **Figure 4A**; confirmed by qPCR, **Supplementary Data Sheet 1j**), the (*R*)-1-octen-3-ol-sensitive OSN, the B-cell, was not differentially sensitive to racemic 1-octen-3-ol in nbf and bf females (**Figure 4B**). Despite efforts to identify the ecologically relevant ligand for Or49, the function of Or49 and the C-cell is not yet known.

Whilst the second messenger signaling pathway has not been determined for either Or8 or Or49, several second messengers have been implicated in Or signaling cascades, particularly in *D. melanogaster* and the hawkmoth, *Manduca sexta* (Sato et al., 2008; Nakagawa and Vosshall, 2009; Sargsyan et al., 2011; Getahun et al., 2013; Stengl and Funk, 2013). In addition to the pathway described for CO_2 signal transduction above, the G-protein coupled cyclic AMP-dependent pathway (Wicher et al., 2008; Getahun et al., 2013), as well as feedback onto the Or complex from phosphokinase c (Pkc) (Sargsyan et al., 2011; Getahun et al., 2013), have also been proposed for the regulation of Ors (for review see Stengl, 2010; Stengl and Funk, 2013). In the adenylate cyclase cascade, the only regulated member in the maxillary palps was *rho-GTPase-activating protein* (AAEL011253), which demonstrated a lower abundance in bf than nbf (1.4-fold; *FDR P* = 0.0030; **Figure 4C**). This suggests that this pathway may not be a major pathway in regulating Ors in the maxillary palps. On the other hand, two (AAEL001549, AAEL002892) of the six *Pkcs* expressed in the maxillary palps exhibited changes in abundance between nbf and bf, albeit not above the 1.5-fold cutoff (1.2-fold higher in bf, *FDR P* = 0.0206; 1.4-fold high in nbf, *FDR P* = 0.0425; respectively; **Figure 4C**).

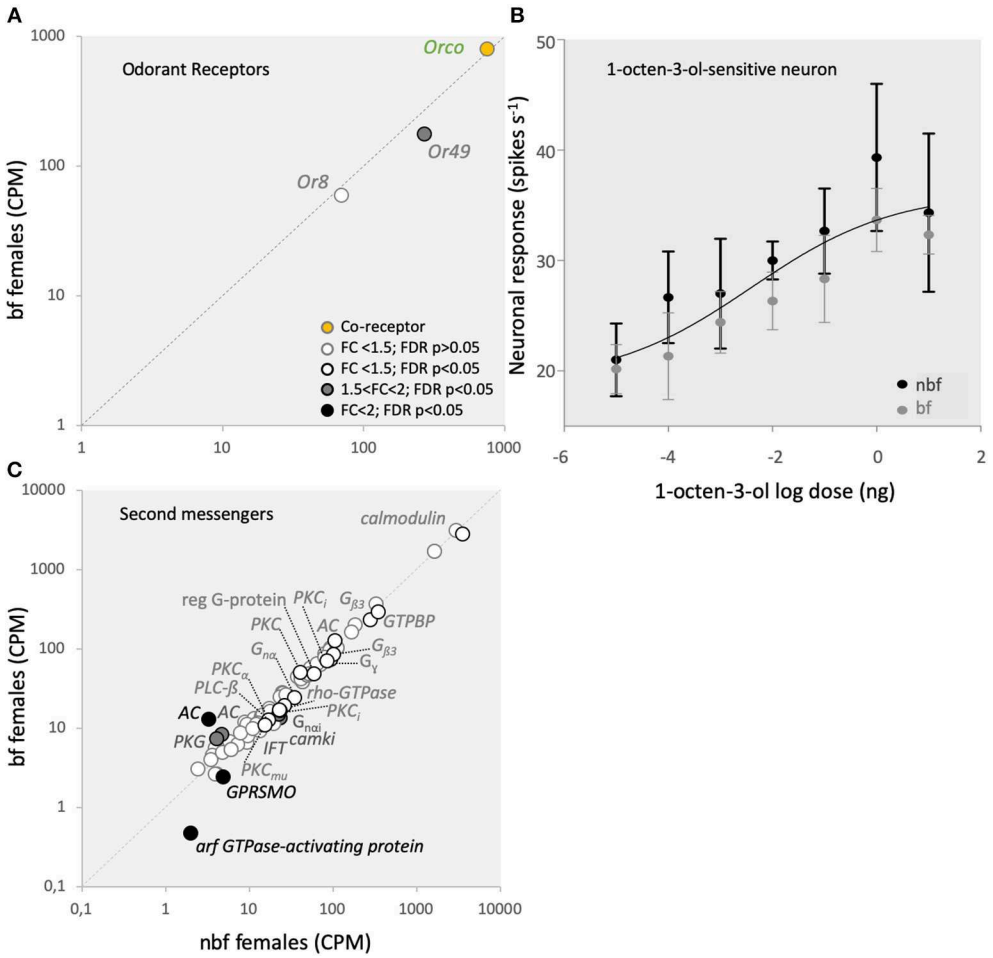

FIGURE 4 | Odorant receptor (Or) mediated signaling. **(A)** Transcript regulation in the maxillary palps of blood fed (bf) and non-blood fed (nbf) *Aedes aegypti* females of the co-receptor, Orco (orange-filled circle), the (*R*)-1-octen-3-ol-sensitive Or8, and the orphan receptor Or49. **(B)** Response of the (*R*)-1-octen-3-ol-sensitive neuron to various concentrations of (*R*)-1-octen-3-ol. The trendline represents the non-linear regression among all responses and concentrations. **(C)** Differential regulation of second messenger gene expression in the maxillary palps. Transcripts that exhibit significant differences in abundance (Kal's test; *FDR P* < 0.05) are denoted according to their fold change (FC); FC > 2 in black-filled circles; 1.5 < FC < 2 in gray-filled circles; and FC < 1.5 in unfilled circles. The gray dotted line indicates a hypothetical equal transcript abundance between the states. Chimerin (rho-GTPase-activating protein; *rho-GTPase*); guanylate cyclase (*GC*); nucleolar GTP-binding protein (*GTPBP*); cGMP-dependent protein kinase (*PKG*); cAMP-dependent protein kinase type ii regulatory subunit (*PKA*); smoothened G-protein coupled receptor (*GPRSMO*); intraflagellar transport protein (*IFT*); G-protein, gamma-subunit ($G\gamma$); GTP-binding protein alpha subunit ($Gn\alpha$); GTP-binding protein (i) alpha subunit ($Gn\alpha i$); protein kinase C inhibitor (*PKCi*); phospholipase c beta (*PLC-ß*); G-protein *β3* ($G_{\beta 3}$); calcium/calmodulin-dependent protein kinase type 1 (*camki*); regulator of g protein signaling (*reg G-protein*); protein kinase C (*PKC*; PKC_{mu}; PKC_{α}); adenylate cyclase (*AC*); calmodulin.

Other Receptor Families

Five *Ir* transcripts were detected in the maxillary palps of females (**Figure 5A**). The co-receptor *Ir25a* in *Ae. aegypti* was found to be the most abundant *Ir* in the maxillary palps (**Figure 5A**) (Bohbot et al., 2014; Matthews et al., 2016), suggesting the predominance of this co-receptor in Ir complexes in this olfactory organ. While *Ir8a* was not expressed above threshold levels in the maxillary palps, the third *Irco*, *Ir76b*, was the fourth highest *Ir* in abundance, one tenth of that of *Ir25a*, similar to what was shown previously (Matthews et al., 2016) (**Figure 5A**). Neither co-receptor was shown to be differentially abundant (**Figure 5A**). Five other Irs were consistently expressed in the maxillary palps of nbf and bf females, of which *Ir100e.1* demonstrated a >2-fold change in abundance in bf compared with nbf females (32.9-fold; *FDR P* < 0.0001), and *Ir75k.2* was shown to be abundant only in nbf females (3.85 ± 0.76 CPM; *FDR P* = 0.0472; **Figure 5A**).

In *Ae. aegypti*, members of the expanded *Ir75* subfamily are expressed in the maxillary palp (**Figure 5A**) (Bohbot et al., 2014; Matthews et al., 2016), antenna (Matthews et al., 2016), as well as in the labella and tarsi (Sparks et al., 2014), however the function of these receptors in *Ae. aegypti* is not known. In *An. gambiae* and *D. melanogaster*, the Ir75 subfamily responds to short chain carboxylic acids (Yao et al., 2005; Abuin et al., 2011; Pitts et al., 2017). The subfamily expansion in mosquitoes emphasizes the ecological significance of carboxylic acids in driving host attraction (Davis, 1984; Bosch et al., 2000; Smallegange et al.,

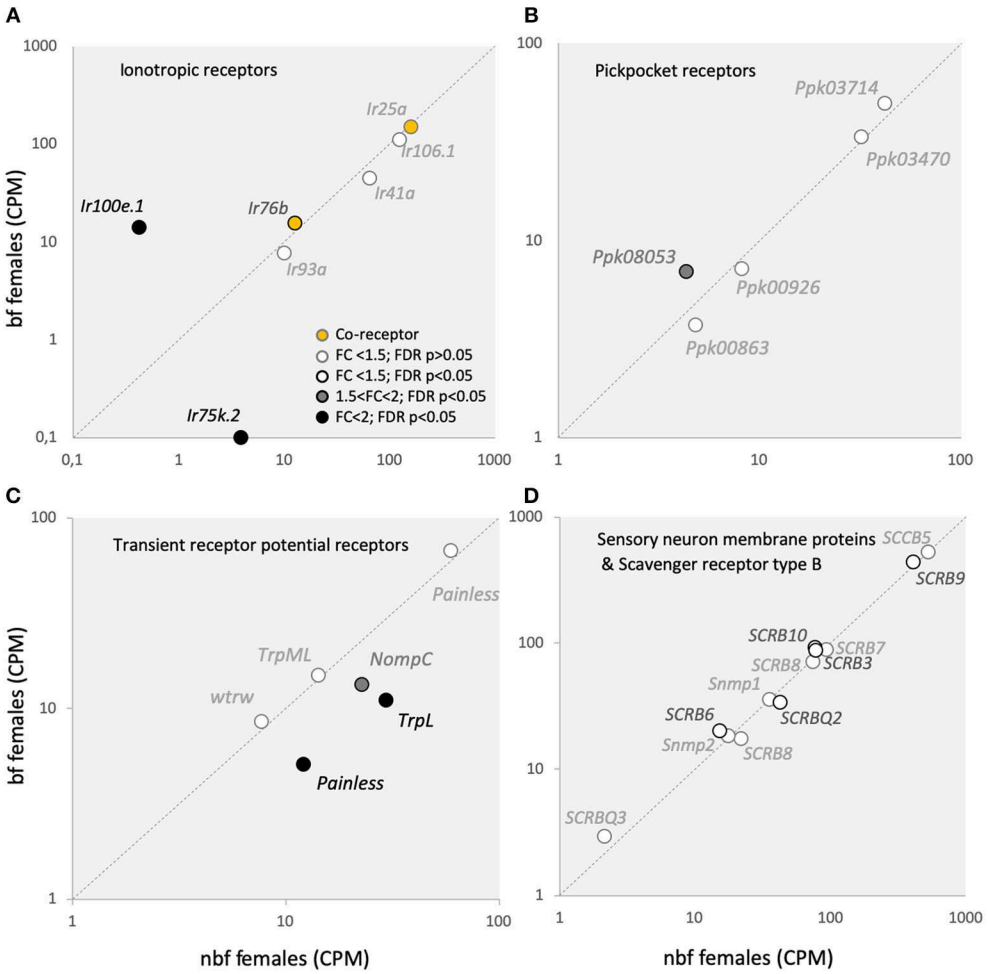

FIGURE 5 | Sensory receptor transcript abundance. Transcript regulation of **(A)** ionotropic receptor (Ir) co-receptors, *Ir25a* and *Ir76b* (orange-filled circles), as well as other Irs; **(B)** pickpocket receptors (*Ppks*); **(C)** transient receptor potential receptors (*Trps*); and **(D)** sensory neuron membrane proteins (*Snmps*) and scavenger receptors type B (*SCRBs*) is presented as the normalized number of reads in the maxillary palps of blood fed (bf) and non-blood fed (nbf) *Aedes aegypti* females. Transcripts that exhibit significant differences in abundance (Kal's test; *FDR P* < 0.05) are denoted according to their fold change (FC); FC > 2 in black-filled circles; 1.5 < FC < 2 in gray-filled circles; and FC < 1.5 in unfilled circles. The gray dotted line indicates a hypothetical equal transcript abundance between the states.

2005). The lack of expression of *Ir75k.2* in the maxillary palps of bf females, and its presence in nbf females, suggests a role for this receptor in host detection. The *Ir100*s subfamily is expressed in the larval and adult internal gustatory organs of *D. melanogaster* (Croset et al., 2010), and may therefore play some role in the detection of taste compounds. In *Ae. aegypti*, *Ir100e* has been found to be expressed in the maxillary palps (**Figure 5A**) (Bohbot et al., 2014; Matthews et al., 2016) and in the antennae (Matthews et al., 2016), but not in the labella or tarsi (Sparks et al., 2014), suggesting that while the role in taste detection may be conserved in the pharyngeal organ, it is likely that the role for Ir100e has expanded. Since *Ir100e.1* was more abundant in the maxillary palps of bf females (**Figure 5A**), it will be of future interest to determine the ligand sensitivity of this Ir, together with its co-receptor *Ir76b*, and its role in the maxillary palps of *Ae. aegypti* following a blood meal. While the results reported here are from the Rockefeller strain, those from the Liverpool strain (LVPIB12) (Matthews et al., 2016), and from those reported from the Orlando strain (Bohbot et al., 2014) each differ from one another. The sole unique *Ir* detected in the maxillary palps of all three strains was *Ir100e*. Of the other five *Irs* reported expressed in the Orlando strain (*Ir92a, Ir41a.1, Ir75l, Ir7y.2,* and *Ir7h.2*), but not in the Rockefeller strain, only *Ir92a* and *Ir41a.1* were found to be consistently detected above threshold levels in the Liverpool strain. Whether these differences are strain-related, or whether they are a result of the ongoing re-annotation of the *Ae. aegypti* genome, will need to be further addressed as time and study concretise the annotations. For example, *Ir106.1* represents the collapsing of 7 putative genes (AAEL005231; AAEL008226; AAEL008231; AAEL017163; AAEL018066; AAEL018068; AAEL018069) from previous annotations (Matthews et al., 2016) into a single gene (AAEL019784) in the current L5 annotation. Two of these putative genes from the earlier annotation were shown

to have abundances above threshold in the maxillary palps of nbf females (Matthews et al., 2016). Additionally, the *Ir75* subfamily has been extensively re-annotated, particularly the *Ir75k* paralogues, so that specific, direct comparisons between the transcriptomes are not feasible. In addition, those genes previously identified as *Ir41a.1* (L3 AAEL000007) and *Ir41a.2* (L3 AAEL000031) are no longer supported in the L5 annotation, however, AAEL000041 (currently annotated as a forkhead protein) shares strong similarity with the other mosquito *Ir41a*s and with *Ir41a* in *Drosophila*, and is reported here as *Ir41a*.

The Ppks and Trps have recently been added to the list of chemosensory receptors (Al-Anzi et al., 2006; Kang et al., 2010; Badsha et al., 2012; Fowler and Montell, 2013). None of the five *Ppk* transcripts expressed in the maxillary palps (**Figure 5B**) are homologous to those shown to be chemosensitive in *D. melanogaster* (Joseph and Carlson, 2015). However, of the six *Trps* expressed in the maxillary palps of *Ae. aegypti* (**Figure 5C**), only *painless* (AAEL006835; AAEL004397), and *TRPL* (AAEL005575) have been implicated in chemosensation (Al-Anzi et al., 2006; Kang et al., 2010; Badsha et al., 2012; Fowler and Montell, 2013). In *Ae. aegypti* maxillary palps, of the two *painless* transcripts expressed, one had higher in abundance in nbf females (AAEL004397: 2.4-fold; *FDR P* < 0.0001). In *D. melanogaster*, *painless* is involved in noxious chemical sensing, particularly of isothiocyanate (Al-Anzi et al., 2006; Kang et al., 2010). The transcript abundance of *TrpL*, which likely plays a role as Gq/Plc activated ion channels downstream of Ors or Grs (Badsha et al., 2012; Fowler and Montell, 2013), was reduced in bf females (see above). The other *Trp* that exhibited a lower abundance in the maxillary palps of nbf females, *NompC* (1.7-fold; *FDR P* < 0.0001), likely plays a role in mechanosensation, together with *painless* and *Ppk0863* (Joseph and Carlson, 2015). Taken together, this expression pattern of the *Trps* and *Ppks* suggests a decrease in sensitivity to chemical and mechanical stimuli in the maxillary palp in bf females, which reflects the ecology of the mosquito during this period in the gonotrophic cycle.

None of the 13 ligand-binding scavenger receptor, type B, genes (*SCRB/CD36*) (Vogt and Riddiford, 1981; Vogt et al., 2009), including the two chemosensory-related members of the gene family, the *Snmps* (*Snmp1* and *Snmp2*) were regulated in the maxillary palps of nbf and bf females (**Figure 5D**). The SNMPs have been implicated in mediating pheromone detection, acting as molecular bridges (SNMP1) (Bohbot et al., 2010), and in the clearance of lipophilic components from the sensillum lymph (SNMP2) (Forstner et al., 2008; Jiang et al., 2016). The expression of the *Snmps* in the maxillary palps of the Rockefeller strain (**Figure 5D**) was found to be in line with that reported in the Liverpool strain (Matthews et al., 2016), however *Snmp1* transcripts were reported as absent from the maxillary palps in the host-seeking females of the Orlando strain (Bohbot et al., 2014). The presence of *Snmp* transcripts in the maxillary palps suggests that this chemosensory organ may play a role in the proposed aggregation and oviposition pheromone detection by *Ae. aegypti* females (Cabrera and Jaffe, 2007; Seenivasagan et al., 2009).

Soluble Odor Binding Proteins

Chemosensory proteins belong to a conserved arthropod protein family that is poorly understood, but may play a role in mediating the movement of lipophilic volatiles through the aqueous environment of the sensillum lymph (Vogt, 2003). Of the 18 identified *Csps* in *Ae. aegypti*, nine are expressed in the maxillary palps (**Figure 6A**) (Matthews et al., 2016). Three of the *Csps* were significantly more abundant in maxillary palps of bf females (AAEL002007: 8.2-fold; *FDR P* < 0.0001; AAEL002003: 6.2-fold; *FDR P* = 0.0288; AAEL001991: 20.9-fold; *FDR P* = 0.0105), and two more *Csps* were identified as genes-of-interest as being significantly more abundant 24 h pbm (AAEL005710: 1.6-fold; *FDR P* < 0.0001; AAEL012383: 1.5-fold; *FDR P* = 0.0008). The functional significance of the abundance of *Csps* is unknown.

Odorant binding proteins are the largest family of chaperone proteins, which may solubilise odorants, interact with chemoreceptors (Vogt, 2003), and play a role in gain control (Larter et al., 2016). In *Ae. aegypti*, there are 111 *Obp*s annotated among the three classes of *Obps*: the Classic, the Plus-C and the Two-Domain *Obps* (Zhou et al., 2008; Manoharan et al., 2013). Classic and Plus-C *Obp* transcripts were expressed in the maxillary palps of both nbf and bf female, almost half of which were differentially abundant (**Figure 6B**). No Two-Domain *Obp* transcripts were above threshold levels in the maxillary palps (**Figure 6B**) (Bohbot et al., 2014; Matthews et al., 2016). While actual functional data for these regulated Obps is not yet available in *Ae. aegypti*, homology modeling for the classic Obps has suggested that these regulated Obps are predominantly binding amine-containing compounds and permethrin (Manoharan et al., 2013).

Modulators and Their Receptors

Modulation of host-seeking behavior following blood feeding has been shown to be regulated by various neuromodulators, predominantly neuropeptides (Brown et al., 1994; Liesch et al., 2013; Christ et al., 2017). Of the 13 neuropeptide transcripts that were detected in the maxillary palps, four were significantly more abundant in the maxillary palps of bf females (*ITG-like*, AAEL010262, 1.6-fold, *FDR P* < 0.0001; *PK1*, AAEL012060, 4.7-fold, *FDR P* < 0.0001; *RYa*, AAEL011702, 8.2-fold, *FDR P* = 0.0055; *AKH*, AAEL011996, 47.5-fold, *FDR P* = 0.0004) and four in nbf females (*sulfakinin*, AAEL006451, 2.4-fold, *FDR P* = 0.0004; *ACP*, AAEL010950, 7.8-fold, *FDR P* < 0.0001; *PK2,* AAEL005444, 2.6-fold, *FDR P* = 0.004; *Agatoxin-like*, AAEL022190, 8.0-fold, *FDR P* = 0.0089), while two neuropetides were only found to be expressed in the maxillary palps of nbf females (*CCHa2*, AAEL026488; *PTTH*, AAEL026383). Four neuropeptides were found to be expressed together with their cognate receptors (*ASTC:GPRSMS, RYa:GPRNPY6, AKH:AKHR, PK2:GPRHP3*). The *ASTC* transcripts were not regulated in the maxillary palps post-blood meal (**Figure 7A**), which is in line with that described in the brain (Mayoral et al., 2010). However, one of the two mosquito *ASTC* cognate receptors, *GPRSMS*, was more abundant in bf females (2.3-fold, *FDR P* = 0.03; **Figure 7B**). While ASTC signaling is known to be involved in inhibiting juvenile hormone synthesis in the corpora allata, its role in the maxillary palps of *Ae. aegypti*

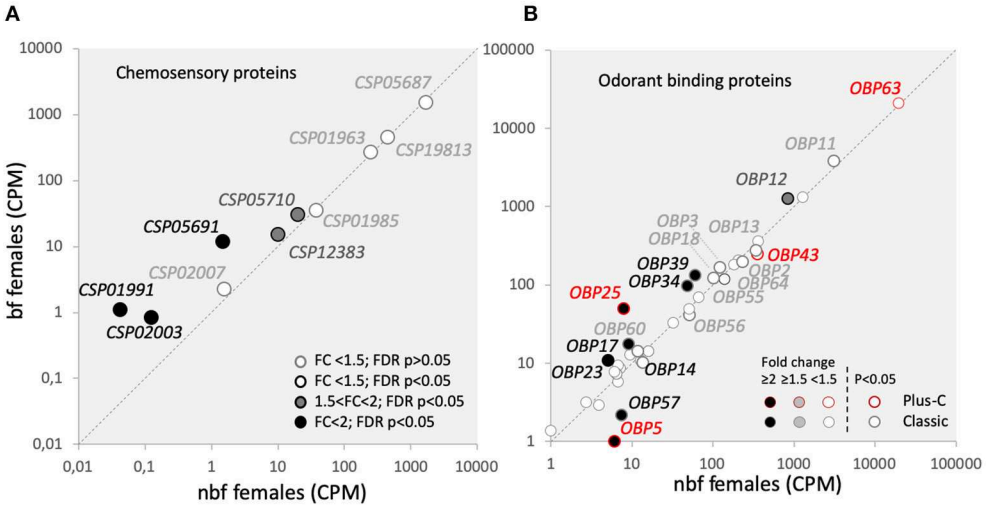

FIGURE 6 | Olfactory binding proteins transcript abundance. Transcript regulation in the maxillary palps of blood fed (bf) and non-blood fed (nbf) *Aedes aegypti* females is presented as the normalized number of reads of **(A)** chemosensory proteins (*Csps*); and **(B)** odorant binding proteins (*Obps*) of two classes, outlined in greyscale and red for classic and plus-C *Obps*, respectively. Transcripts that exhibit significant differences in abundance (Kal's test; *FDR P* < 0.05), are denoted according to their fold change (FC); FC > 2 in black-filled circles; 1.5 < FC < 2 in gray-filled circles; and FC < 1.5 in unfilled circles. The gray dotted line indicates a hypothetical equal transcript abundance between the states. So that **(B)** is legible, the cutoff for including Obps FC < 1.5 is 25 reads.

females is unknown. Recently identified, *RYamide* and its cognate receptor, *GPRGNPY6* (12.2-fold, *FDR P* = 0.0012), were more abundant in the maxillary palps of bf females. RYamide signaling has been suggested to be involved in regulating response to food (Ohno et al., 2017), which may indicate a role for its expression and regulation post-blood meal in the maxillary palps of *Ae. aegypti* females. Similarly, both of the regulated neuropeptides *Akh* and *Pyrokinin 2* were expressed along with their cognate receptors *AKHR* (AAEL011325) and *GPRGHP3* (AAEL012796), respectively, however, neither receptor exhibited differential abundance (**Figure 7B**). The abundance of transcripts of both peptide precursor and receptor in the maxillary palps suggests that the signaling of these peptide systems is located, at least in part, within the maxillary palps, and/or that individual cells in the maxillary palps express both the peptide and the receptor, creating the possibility for autoregulation, similar to that which is observed in the autoregulation of antennal OSNs in *D. melanogaster* (Ko et al., 2015). The cognate receptors of the remaining neuropeptide transcripts abundant in the maxillary palps (**Figure 7A**) were not detected (**Figure 7B**). The presence of the neuropeptide precursor transcripts, and the absence of the receptor transcripts, in the maxillary palps suggests that neuropeptide-expressing cells are afferent neurons which will release the neuropeptides within the CNS. The most likely cells that fit this category in the maxillary palps are the OSNs. Additionally, there are 18 neuropeptide/GPCR receptor transcripts present in the maxillary palps, without the detection of their cognate neuropeptide transcript (**Figure 7B**). Of these, four (*diuretic hormone CRF-like receptor, GPRDIH1,* 1.8-fold, *FDR P* = 0.0430; *vasopressin receptor*, *GPRVPR2*, 1.8-fold, *FDR P* = 0.0129; *Sex peptide receptor, Sex peptide R*, 2.7-fold, *FDR P* = 0.00104; *diuretic hormone CRF-like receptor*, *AAEL019757*, 15.9-fold, *FDR P* = 0.04711) were more abundant in nbf, and two (*diuretic hormone calcitonin-like receptor*, *GPRCAL1*, 1.7-fold, *FDR P* = 0.0331; *orphan GPCR class D*, *GPRNND3*, 2.6-fold, *FDR P* = 0.0070) were more abundant in bf females (**Figure 7B**). Detection of these receptors suggests that the maxillary palps are sensitive to modulation from neural and/or humoral neuropeptides.

Another class of neuromodulators implicated in regulating mosquito behavior is the biogenic amines. Unlike neuropeptides, which are encoded in the genome, biogenic amines are synthesized from an array of enzymes. It is the abundance of these enzyme transcripts that are reported to indirectly indicate biogenic amine level in transcriptome analyses. Four of the seven detected biosynthetic enzymes are from the families responsible the generation of biogenic amines, *dopamine N-acetyltransferase* (*Dat*), *tyrosine hydroxylase* (*TH*), *phenylalanine-4-hydroxylase* (*FH*), and *dopa decarboxylase* (*Ddc*) (**Figure 7C**) (Matthews et al., 2016), none of which were differentially abundant (**Figure 7C**). Biogenic amine receptor transcripts were detected for serotonin (*5-HT7R*; *5-HT2BR*) and dopamine (*Dop1R1*), which also demonstrated consistent abundance pre- and post-blood meal (**Figure 7D**). These findings are supported by prior immunohistological investigations demonstrating that serotonergic neurons are present in the maxillary palps of female *Ae. aegypti* mosquitoes (Siju et al., 2008).

Neurotransmitters and their receptors are the primary signaling molecules at synapses, responsible for normal synaptic activity. Similar to biogenic amines, neurotransmitters are synthesized via enzymatic pathways, rather than being encoded in the genome. Three of these biosynthetic enzymes from the *glutamate decarboxylase* family (*Gad1, 2, and 4*), were detected in the maxillary palps (**Figure 7C**) (Matthews et al., 2016). Of these, only *Gad1* was found to be more abundant in the maxillary palps of nbf females (8.2-fold, *FDR P* <

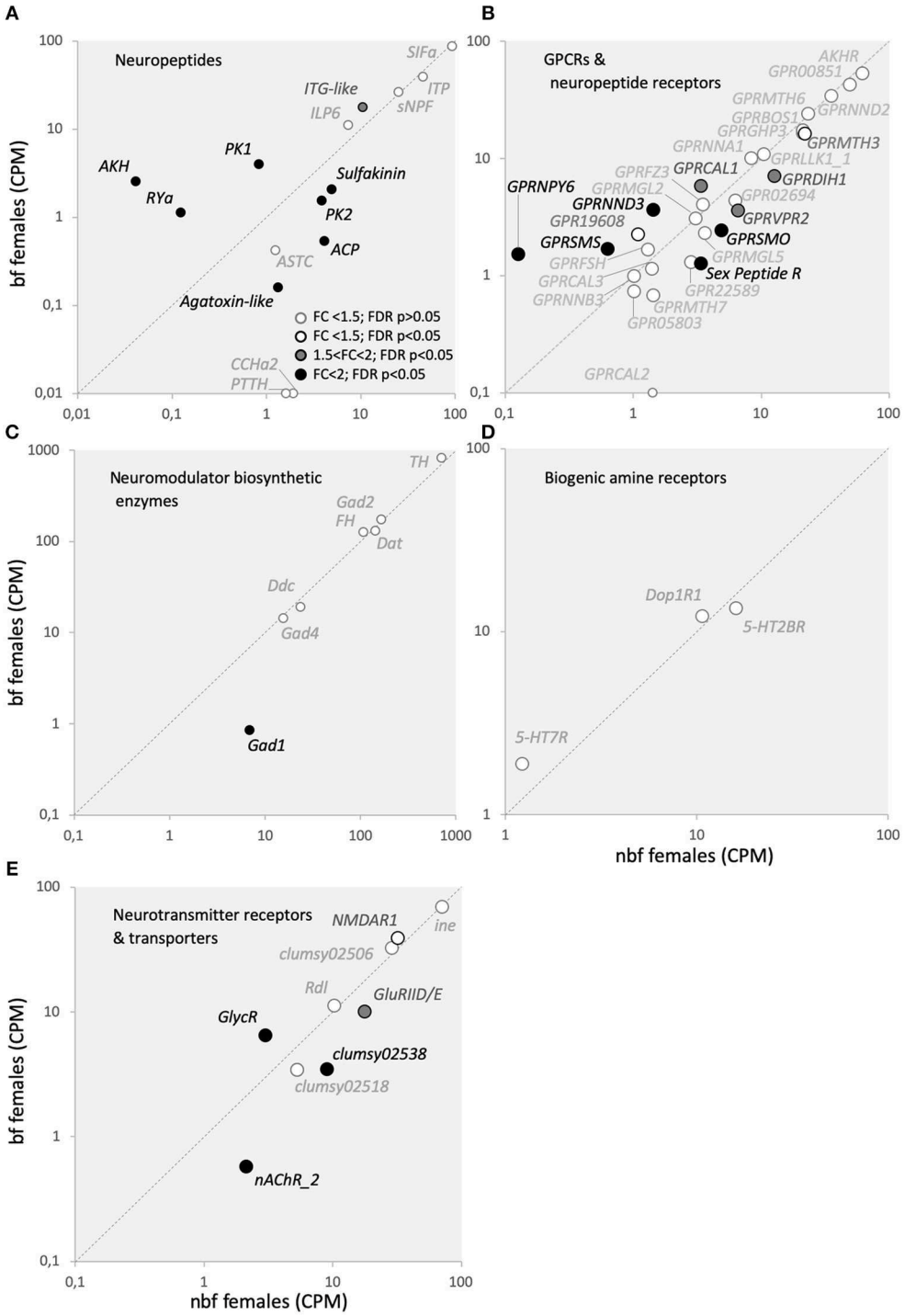

FIGURE 7 | Neurotransmitters and modulators with their cognate receptors, transporters, and biosynthetic enzymes transcript abundance. Transcript regulation in the maxillary palps of blood fed (bf) and non-blood fed (nbf) *Aedes aegypti* females is presented as the normalized number of reads of **(A)** neuropeptides, **(B)** GPCR and neuropeptide receptors, **(C)** neuromodulator biosynthetic enzymes, **(D)** biogenic amine receptors, **(E)** neurotransmitter receptors and transporters. Transcripts that exhibit significant differences in abundance (Kal's test; *FDR P* < 0.05), are denoted according to their fold change (FC); FC > 2 in black-filled circles; 1.5 < FC < 2 in gray-filled circles; and FC < 1.5 in unfilled circles. The gray dotted line indicates a hypothetical equal transcript abundance between the states. **(A)** *SIFamide* (*SIFa*; AAEL009858); *ion-transport peptide* (*IPT*; AAEL019725); *short neuropeptide F* (*sNPF*; AAEL012542); ITG-like peptide (ITG-like; AAEL010262); insulin-like peptide 6 (ILP6; AAEL004547); sulfakinin (sulfakinin; AAEL006451); adipokinetic/corazonin peptide (ACP; AAEL010950); *pyrokinin 2* (*PK2;* AAEL005444); CCHamide 2 (CCHa2; AAEL026488); prothoracicotropic hormone (PTTH; AAEL026383); Agatoxin-like neuropeptide (Agatoxin-like; AAEL022190); *allatostatin C* (*ASTC*; AAEL005747);

(Continued)

FIGURE 7 | pheromone/pyrokinin biosynthesis-activating neuropeptide (*PK1*; AAEL012060); RYamide (RYa; AAEL011702); adipokinetic hormone (AKH; AAEL011996); **(B)** gonadotropin-releasing hormone receptor (AKHR, AAEL011325); predicted G-protein coupled receptor (GPR00851, AAEL000851; GPR05803, AAEL005803; GPR22589, AAEL022589); GPCR Orphan/Putative Class A Family (GPRNNA1, AAEL003378; GPR19608, AAEL019608); GPCR Orphan/Putative Class B Family (GPRNNB3, AAEL001724); GPCR Orphan/Putative Class D Family (GPRNND2, AAEL001782; GPRNND3, AAEL06232); GPCR Growth Hormone Releasing Hormone Family (GPRGHP3, AAEL012796); GPCR Diuretic Insect Hormone (GPRDIH1, AAEL008292; GPRCAL1, AAEL010043; GPRCAL2, AAEL006490; GPRCAL3, AAEL009024); GPCR Glycoprotein Hormone Family (GPRFSH, AAEL004399); GPCR Somatostatin Family (GPRSMS, AAEL012356); GPCR Neuropeptide Y Family (GPRNPY6, AAEL017005); GPCR Leukokinin Family (GPRLLK1_1, AAEL006636); GPCR Vasopressin Family (GRPVPR2, AAEL008655); Sex Peptide Receptor (Sex Peptide R, AAEL019881); GPCR Metabotropic glutamate Family (GPRMGL2, AAEL004533; GPRMGL5, AAEL009822); GPCR Methuselah Family (GPRMTH3, AAEL017374; GPRMTH6, AAEL011521; GPRMTH7, AAEL000811); GPCR Bride of Sevenless Family (GPRBOS1, AAEL007004); GPCR Frizzled/Smoothened Family (GPRSMO, AAEL006669; GPRFZ4, AAEL008322); **(C)** tyrosine 3-monooxygenase (TH, AAEL017098); phenylalanine-4-hydroxylase (FH, AAEL017029); dopamine N-acetyltransferase (Dat, AAEL011088); aromatic L-amino acid decarboxylase (Ddc, AAEL014238); glutamic acid decarboxylase (Gad1, AAEL011981; Gad2, AAEL007542; Gad4, AAEL010951); **(D)** GPCR Serotonin Family (5-HT2BR, AAEL019805; 5-HT7R, AAEL027242); GPCR Dopamine Family (Dop1R1, AAEL019437); and **(E)** sodium- and chloride-dependent neurotransmitter transporter (ine, AAEL006412); glutamate receptors (NMDAR1, AAEL; clumsy02506, AAEL02506; clumsy02538; AAEL02538; clumsy02518, AAEL02518; GluRIID/E, AAEL022196); nicotinic acetylcholine receptor (nAChR_2, AAEL018352); glycine receptor beta precursor (GlyR, AAEL001568); GABA receptor (Rdl, AAEL008354).

0.0001; **Figure 7C**), which is indicative of the biogenesis of the neurotransmitter GABA. It is interesting to note that while the biosynthetic enzymes for GABA were present, none of those for acetylcholine (*choline O-acetyltransferase*, *ChAT,* AAEL010471) were detected, indicating that the primary neurotransmitter produced in the maxillary palps of 6 dpe female *Ae. aegypti* is GABA, which primarily is involved in inhibitory signaling pathways. While GABA receptors have been described in OSNs to be involved in gain control (Olsen and Wilson, 2008; Root et al., 2008) and the biosynthetic enzymes that produce GABA can be found in olfactory tissues (Matthews et al., 2016), but GABA signaling from OSNs has not been described. A GABA-gated chloride receptor (*Rdl*, AAEL008354) was detected at similar levels in the maxillary palps of nbf and bf females (**Figure 7E**). Ten neurotransmitter receptor genes were expressed in the maxillary palps (**Figure 7E**). The predominant type of receptor detected had glutamate as its cognate ligand, the transcripts of which either did not differ in abundance (*NMDAR1*, *clumsy02506*, *clumsy02518*) or were more abundant in the maxillary palps of nbf females ($GluRII_{D/E}$, 1.8-fold, $FDR\ P < 0.0001$; *clumsy02538*, 2.6-fold, $FDR\ P < 0.0001$). Two other neurotransmitter receptors were detected in the maxillary palps, with one acetylcholine receptor being more abundant in nbf females (*nAChR_2*; AAEL018308, 3.6-fold, $FDR\ P = 0.0064$), and one glycine receptor being more abundant in bf females (*GlycR*; AAEL001568, 2.1-fold, $FDR\ P = 0.0012$), and commonly mediating synaptic excitation and inhibition, respectively. The one neuromodulator transporter that was detected in the maxillary palps, a sodium- and chloride-dependent GABA transporter (*ine*, AAEL006412), was not differentially regulated (**Figure 7E**), and lends support to the importance of GABA signaling in this chemosensory tissue.

Conclusions

In this study, chemosensory, neuromodulatory, and signal transduction-related genes in the maxillary palps of *Ae. aegypti* females have been shown to be regulated post-blood meal. Differentially regulated genes in the maxillary palps between nbf and bf females are highlighted. Additional screens for gene expression in the future may consider adding a non-blood membrane feeding condition to clarify the role of the experience of other sensory modalities in producing differential gene expression. The future functional characterization of the proteins generated by the genes-of-interest identified in this study may offer important insights in the regulation of vector-related odor coding in female *Ae. aegypti*, and its impact on gonotrophic behaviors that may in turn lead to new innovations in vector control.

DATA AVAILABILITY

The datasets generated for this study can be found in the NCBI project database, BioProject, with the ID PRJNA545481.

AUTHOR CONTRIBUTIONS

SH and RI conceived. SH designed the gene expression study and conducted the analyses and drafted the manuscript. RI conceived and designed the electrophysiological study. SH conducted all the experiments, except the physiological single sensillum recordings, which were conducted, and analyzed by MG. All authors provided constructive input toward the manuscript and participated in revising it critically for important intellectual content.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00336/full#supplementary-material

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