Microbiome-Gut-Brain-Axis communication influences metabolic switch in the mosquito Anopheles culicifacies

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Research

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Abstract

Background: Periodic ingestion of a protein-rich blood meal by adult female mosquitoes causes a drastic metabolic change in their innate physiological status, which is referred to as ‘metabolic switch. Although the down-regulation of olfactory factors is key to restrain host-attraction, how the gut ‘metabolic switch’ modulates brain functions, and resilience physiological homeostasis remains unexplored.

Methods: To uncover a possible correlation of gut metabolic switching and brain function, we carried out a comparative RNAseq analysis of naïve and blood-fed mosquito’s brain. Spatio-temporal expression of neuro-signaling and neuro-modulatory genes was monitored through Real-Time PCR. To establish a proof-of-concept, we followed LC/MS-based absolute quantification of different neurotransmitters (NT) and compared their levels in the brain as well as in the gut of the mosquitoes. To correlate how microbiome influences gut-brain-axis communication, we performed a comparative gut metagenomic analysis.

Results: Our findings demonstrate that the protein-rich diet induces the expression of brain transcripts related to mitochondrial function and energy metabolism, possibly to cause a shift of the brain’s engagement to manage organismal homeostasis. A dynamic expression pattern of neuro-signaling and neuro-modulatory genes in both gut and brain, presumably a key to establish an active brain-distant organ communication. Disruption of this comunication through decapitation, does not affect the modulation of the neuro-modulator receptor genes in the gut. In parallel, an unusual and paramount shift in the level of the Neurotransmitters (NTs), from the brain to the gut after blood feeding, further supports the idea of the gut’s ability to serve as a ‘second brain’. Finally, a comparative metagenomics evaluation of gut microbiome population dynamics, highlighted that blood-feeding not only suppresses Enterobacteriaceae family member by 50%, but favors rapid proliferation of Pseudomonadales to 46% of the total community. Notable obesrvation of a rapid proliferation of Pseudomonas bacterial sp. in the gut correlates a possible cause for the suppression of appetite after blood-feeding. Additionally, an altered NTs dynamics of naïve and aseptic mosquitoes provide the initial evidence that gut-endosymbionts are key modulators for the synthesis of major neuroactive molecules.

Conclusion: Our data establish a new conceptual understanding of microbiome-gut-brain-axis communication in mosquitoes.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the latest manuscript can be downloaded and accessed as a PDF.

Figures

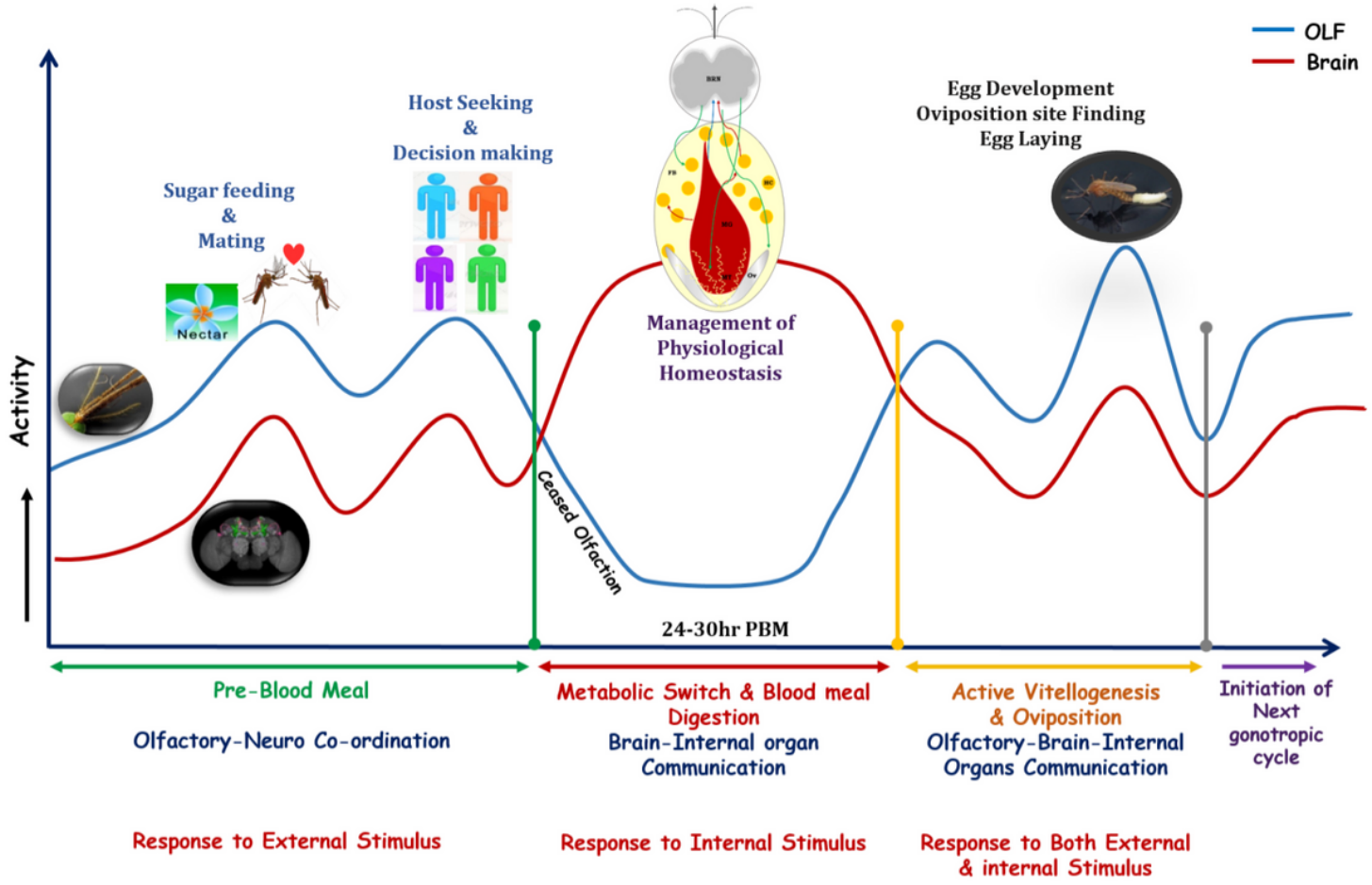

Figure 1

A proposed working hypothesis to establish the correlation between the gut metabolic switch and brain functions in adult female mosquitoes. The behavior of any organism is a very complex event that needs tight coordination between the sensory and neuronal systems. After emergence from pupae, the dynamic changes in the neuro-olfactory system coordinate and regulate different behavioral activities such as mating, sugar feeding, and vertebrate host-seeking, etc. These pre-blood meal-associated behaviors are guided by external stimuli, followed by neuronal decision making. Once the female mosquitoes take a blood meal, their olfactory responses are temporarily arrested to minimize brain and environmental (external) communication. But blood-feeding causes a global change in the physiological homeostasis, and drives multiple tissues (midgut, Malpighian tubule, ovary, and fat body) engagement to manage the systemic equilibrium. Here, we hypothesize that an ‘internal stimulus’ of gut-metabolic-switch may modulate brain functions to ensure optimal inter-organ communication, at least for the first 30h until blood meal digestion is completed in the gut. However, after 30-40h of blood-feeding reactivation of the olfactory system, restores olfactory-neuro co-ordination to perform the next level of behavioral activities, such as oviposition and initiation of the second gonotrophic cycle. Blue and red lines indicate the possible functional patterns of the olfactory system (OLF) and the brain, respectively.

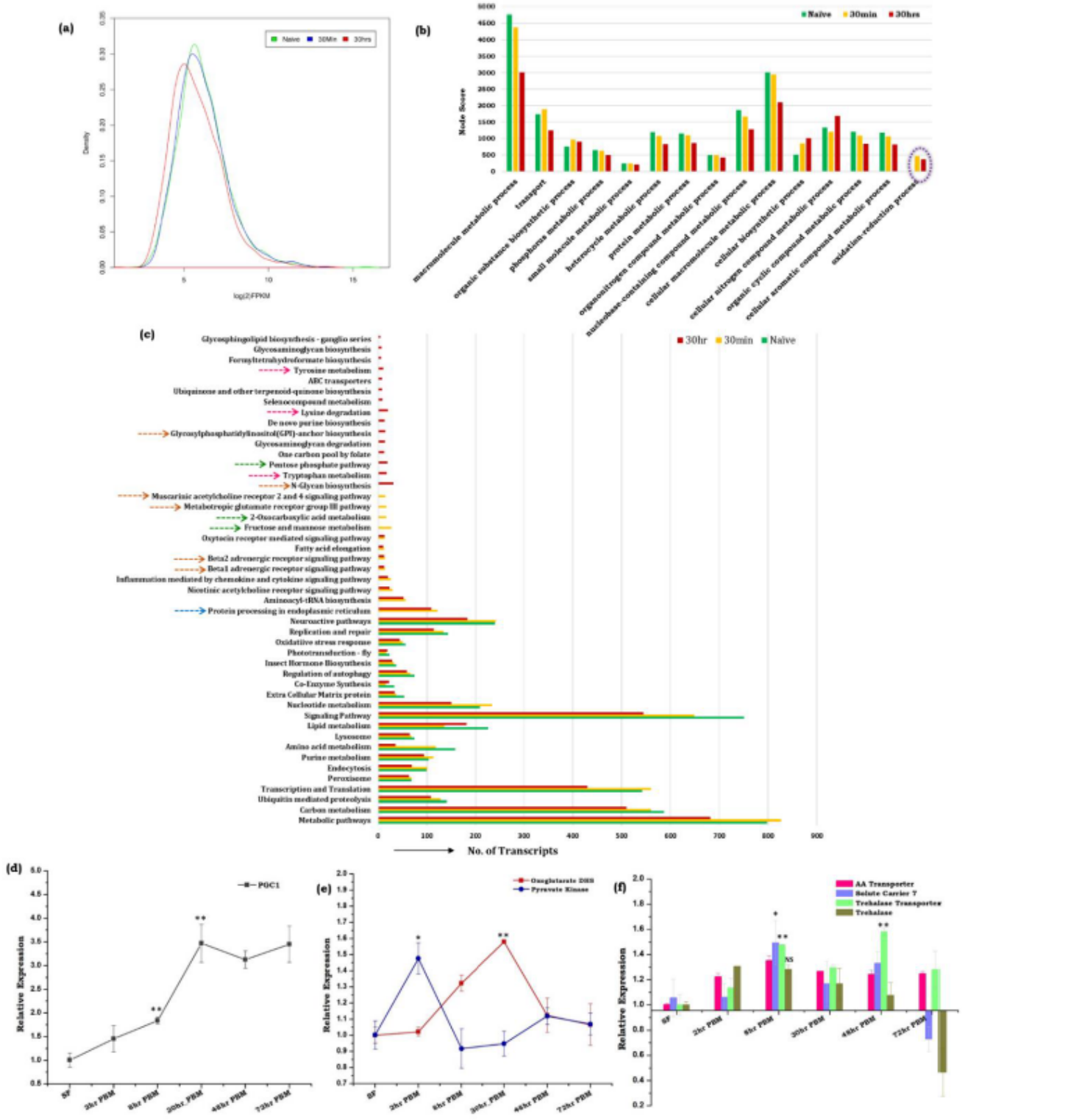

Figure 2

Blood meal causes notable changes in the molecular architecture of the brain tissue. (a) Comparison of the read density map of the naïve, 30min, and 30h post blood meal (PBM) transcriptomic data of brain tissue (n=25); (b) Functional annotation and molecular cataloging of brain transcriptome (Biological Process/Level4/Node score). Purple circle highlighted the unique category of genes that appeared in the brain tissue after blood meal intake; (c) KOBAS 3.0 software mediated gene list enrichment and

comparative pathway analysis of naïve and blood-fed brain tissues. Green arrow links to energy metabolic pathways, the pink arrow links to neurotransmitter synthesis pathway, and the brown arrow indicate neurite out-growth and synaptic transmission; (d) Relative expression profiling of PGC-1 gene in the brain of naïve and blood-fed mosquitoes (n = 25, N = 3); (e) Transcriptional profiling of transcripts related to energy metabolism in the brain tissue of naïve and blood-fed mosquitoes at different time points; (f) Comparative transcriptional response of amino acid transporters and trehalose transporter along with trehalase enzyme in the brain tissue after the metabolic switch (n = 25, N = 3). Statistically significant variation in the expression of the respective genes was tested by the t-test and compared with the sugar-fed control brain. (n = number of mosquitoes from, which the respective tissue was dissected and pooled for each independent experiment; N = number of biological replicates). SF = naïve sugar-fed, 2hr-PBM (Post-Blood-Meal); 8hr-PBM; 30hr-PBM; 48hr-PBM; 72hr-PBM.

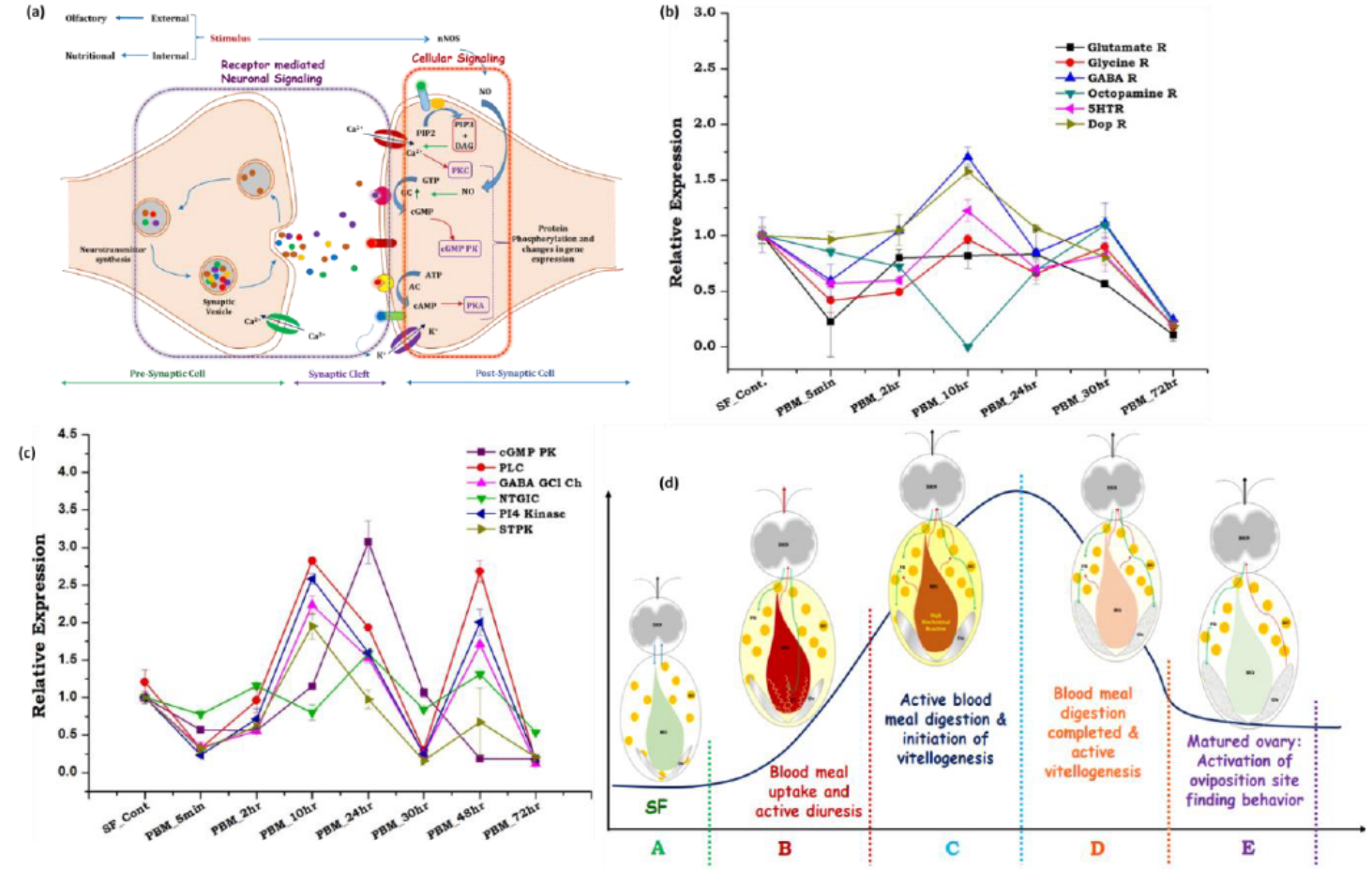

Figure 3

Metabolic switch influences neuro-signaling modulation and inter-organ communication. (a) Synaptic signal transmission and probable mechanism of neuro-signaling. After activation by external and/or internal stimuli, the synaptic vesicles of the presynaptic neuron, containing the neurotransmitters, are released into the synaptic cleft. Binding of the respective neurotransmitter with their cognate receptors activates the downstream signal transduction process in the postsynaptic neuron and thus activates and transmits the initial signal through the interconnecting neurons; (b) Transcriptional response of

neurotransmitter receptor genes as per the designed blood meal time-series experiment. Brain tissues were collected from 5-6day old naïve sugar-fed adult female mosquitoes. Then, mosquitoes were provided with blood meal, and the brain tissues were collected at different time points after blood feeding viz. 5min post blood meal (PBM-5min), PBM-2h, PBM-10h, PBM-24h, PBM 30h, and PBM-72h. Glutamate R: Glutamate Receptor; Glycine R: Glycine Receptor; GABA R: Gamma-Aminobutyric Acid Receptor; Octopamine R: Octopamine Receptor; 5HTR: Serotonin Receptor; Dop R: Dopamine Receptor. Statistical analysis using two-way ANOVA has implied at 0.05 level the expression pattern of the respective genes was not statistically significant at $p \leq 0.2$ at different time points after blood feeding (n = 25, N = 4); (c) Relative expression profiling of the genes involved in signal transduction molecules according to the detailed blood meal time-series experiment. cGMP PK: Cyclic GMP Protein Kinase; PLC: Phospholipase C; GABA GClCh: GABA Gated Chloride Channel; NTGIC: Neurotransmitter Gated Ion Channel; PI4 Kinase: Phosphatidyl-inositol-4-Kinase; STPK: Serine Threonine Protein Kinase. Statistical analysis using two-way ANOVA stated that the expression change of the respective genes is statistically significant $p \leq 0.005$(n = 25, N = 4); (d) Schematic representation of the brain's possible engagement during inter-organ communication after the gut-metabolic switch. Brain actions are subdivided into different phases. We proposed, while in sugar-fed status, the brain manages daily behavioral activities, but as soon as mosquitoes take blood-meal, rapid modulation of the brain's intracellular signaling occurs to manage multiple physiological events such as diuresis process for osmotic regulation, active blood meal digestion in the midgut, co-ordinate with MG, FB, and ovary for egg maturation and vitellogenesis. After 30-40h of blood-feeding, brain function restores to coordinate the reactivated olfactory system. (n = number of mosquitoes from which the respective tissue was dissected and pooled for each independent experiment; N = number of biological replicates)

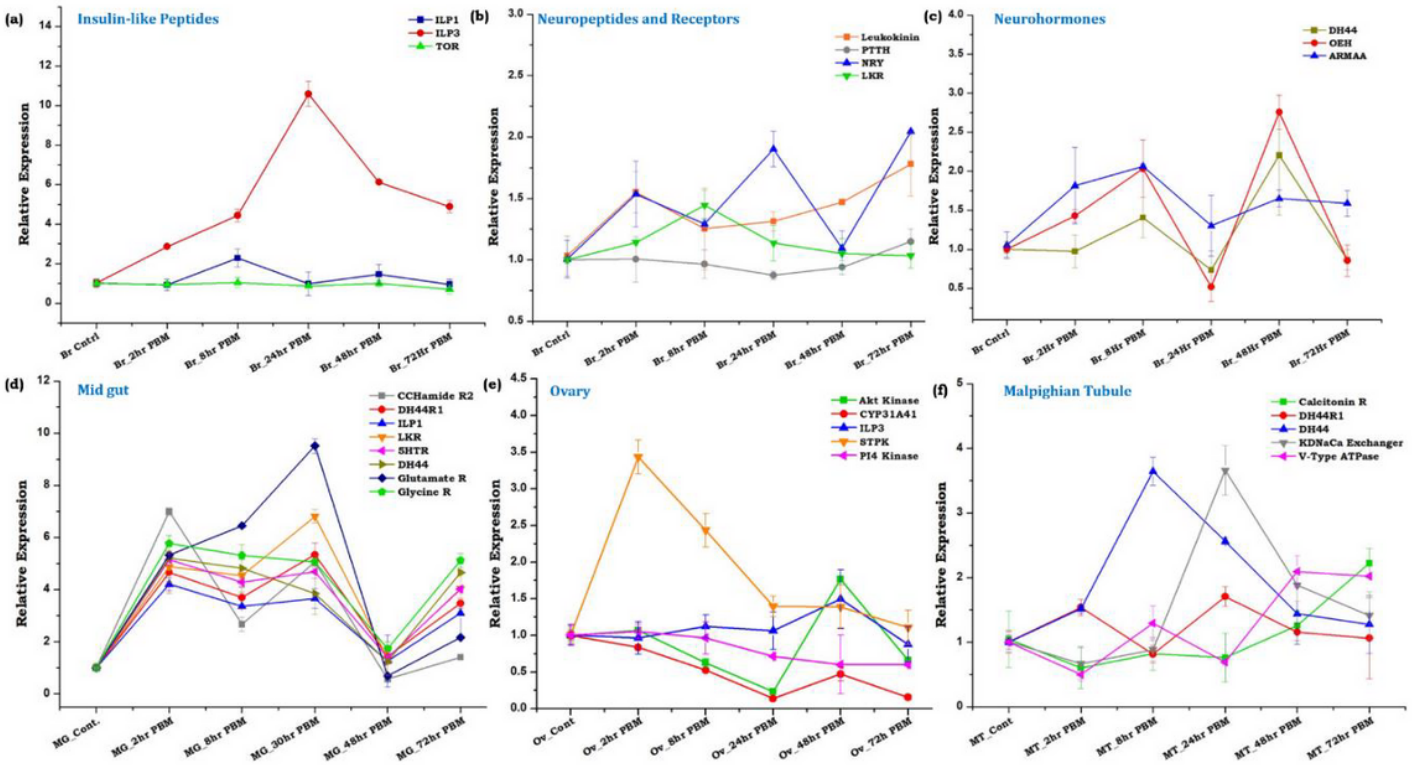

Figure 4

Metabolic switch modulates tissue-specific neuro-modulator transcripts expression. (a-c) Transcriptional expression profiling of Insulin-like-peptides, neuropeptides, neurohormones, and receptor genes in the brain tissue during the metabolic switch. Statistical analysis using two-way ANOVA and Tukey's test implied that the expression change of the respective genes is statistically significant for insulin-like-peptides $p \leq 0.007$; neuropeptides and receptors $p \leq 0.009$, but for neuro-hormones, it was non-significant $p \leq 0.2$ (n = 25, N = 4); (d) Relative expression profiling of a subset of neuromodulator genes in the midgut of naïve and blood-fed mosquitoes at the same time point described above. Statistical analysis using two-way ANOVA implied that the expression change of the respective genes is statistically significant $p \leq 0.005$ (n = 12, N = 4); (e) Transcriptional profiling of genes involved in signal transduction during vitellogenesis in the ovary. Statistical analysis using two-way ANOVA and Tukey's test indicated that the expression change of the respective genes was statistically significant at $p \leq 0.002$ (n = 12, N = 4); (f) Relative gene expression analysis of diuresis-related genes in the Malpighian tubule of naïve and blood-fed mosquitoes. Statistical analysis using two-way ANOVA and Tukey's test indicates that the expression change of the respective genes is non-significant at $p \leq 0.4$ (n = 25, N = 4). (n = number of mosquitoes from which the respective tissue was dissected and pooled for each independent experiment; N = number of biological replicates)

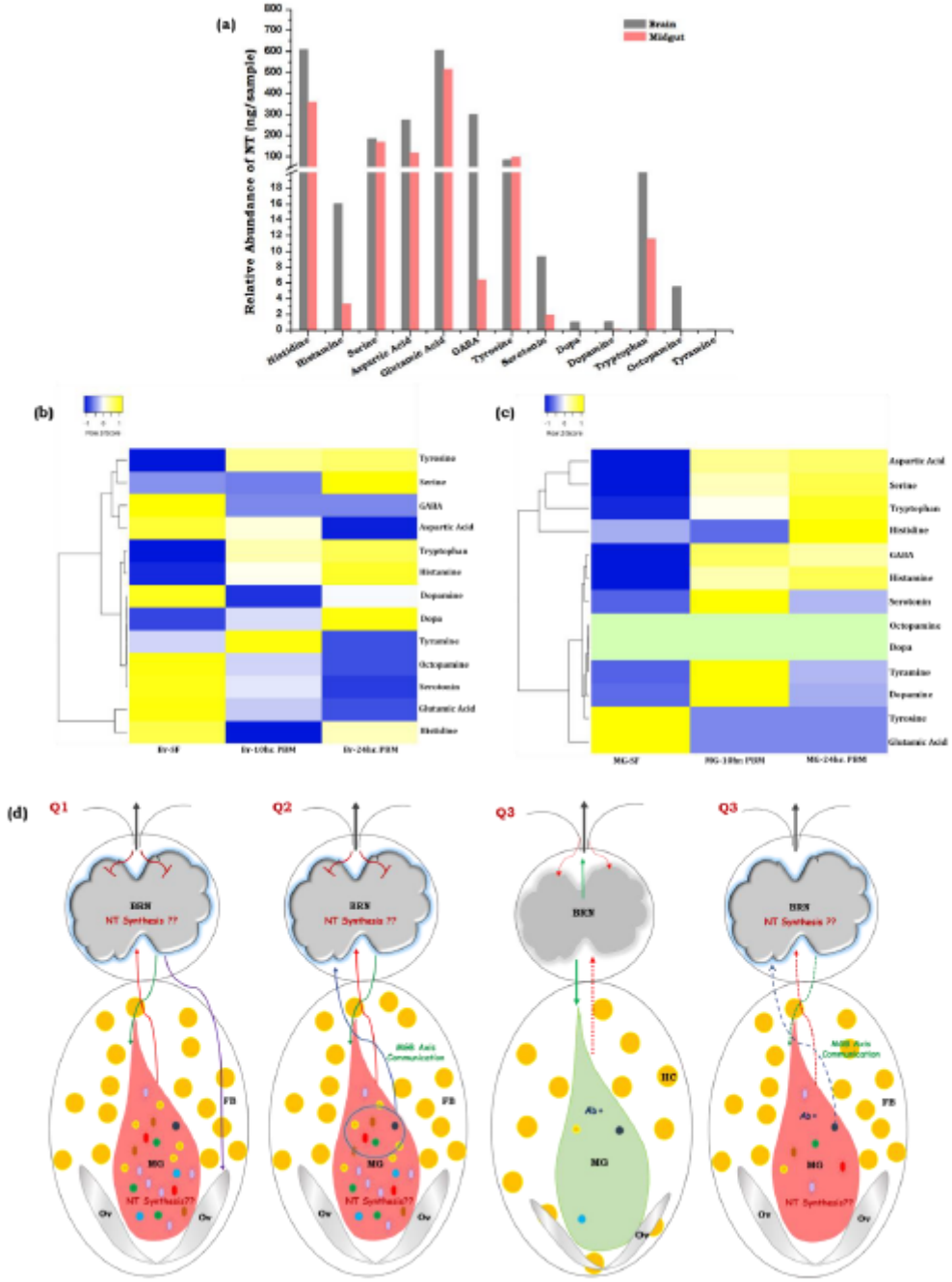

Figure 5

Gut-Brain-Axis (GBA) communication and neurotransmitter (NT) estimation in mosquito An. culicifacies. (a) Comparative analysis of NT abundance in the naïve mosquitoes' brain and midgut; (b) Heatmap showing the alteration of neurotransmitters level in mosquito brain tissue. NT levels were measured by LC-MS from the brains of naïve (sugar-fed) and blood-fed females (10 and 24 h PBM) (n = 65, N = 2). Statistically significant differences in the amount of metabolites were tested by p-values ($p \leq 0.005$) that are deduced by two-way ANOVA and Tukey's test; (c) Heatmap of neurotransmitters levels of mosquito gut tissue that vary during the metabolic switch. NT levels were measured by LC-MS from the gut of naïve (sugar-fed) and blood-fed females (10 and 24 h PBM) (n = 50, N = 2). Statistically significant differences

in the amount of metabolites were tested by p-values ($p \leq 0.005$) that are deduced by two-way ANOVA and Tukey's test. (n = number of mosquitoes from which the respective tissue was dissected and pooled for each independent experiment; N = number of biological replicates); (d) Pictorial presentation demonstrating GBA communication in response to gut-metabolic switch in mosquitoes. Q1: Blood-feeding pauses external stimulus-guided neuro-olfactory responses, but may shift brain engagement through the vagus pathway (red arrow) to regulate actions in the distant organs such as the midgut (green arrow) and ovary (purple arrow). Here, we questioned whether increased levels of amino acids in the gut during blood meal digestion may act like an NT. Q2: Do blood-meal-induced gut flora proliferation (different colored shapes indicate diverse microbial flora) influence GBA communication in mosquitoes. Q3: Whether gut-bacterial removal by antibiotic treatment confers the establishment of microbiome-gut-brain axis (MGB) communication. BRN: Brain, MG: midgut, FB: Fat body, Ov: Ovary, Ab+: Antibiotic positive/treated.

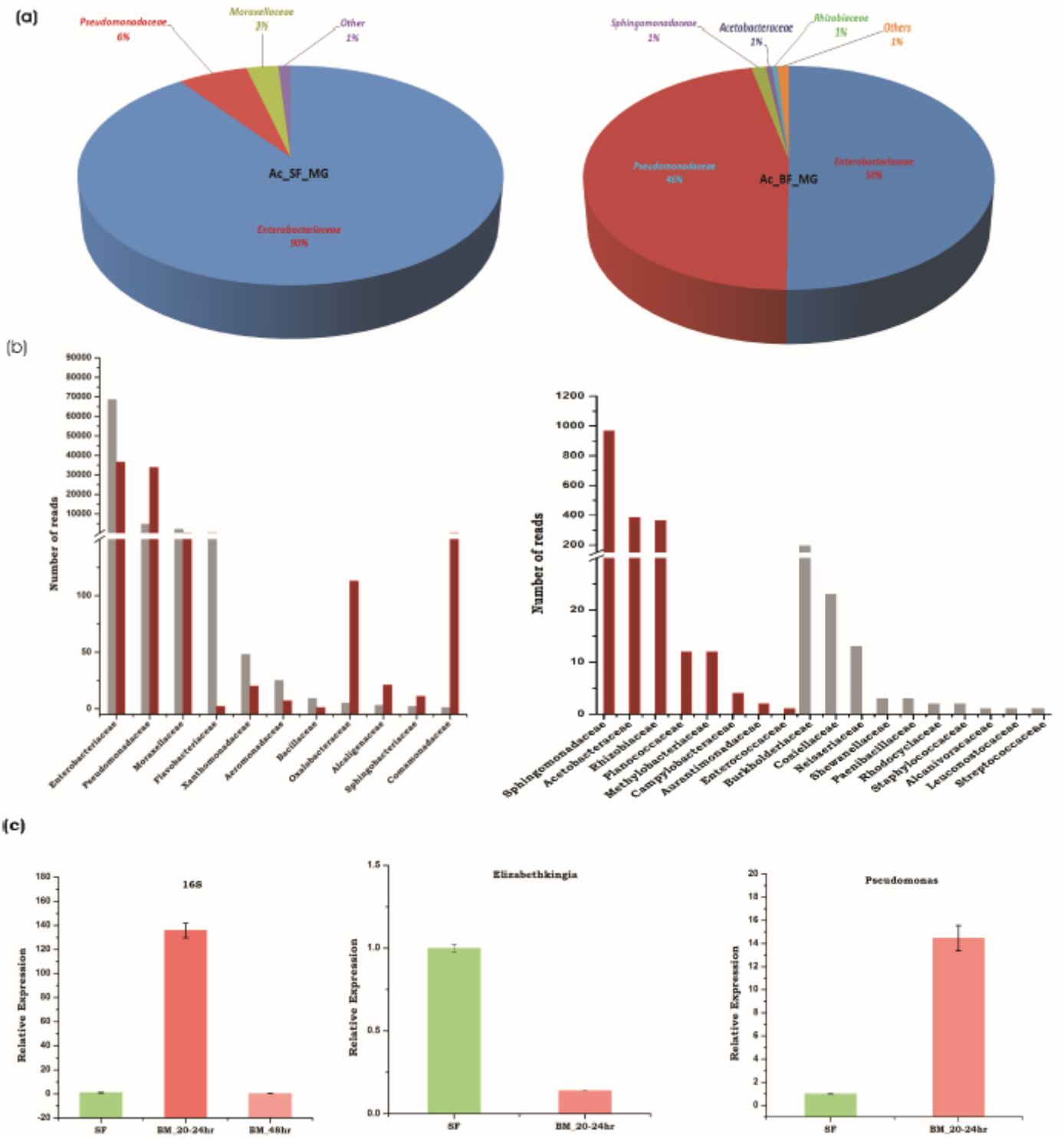

Figure 6

Comparison of gut-metagenomes in the naïve sugar-fed and blood-fed mosquito Anopheles culicifacies: (a) Pie charts representing the major bacterial families under the two feeding status (b) Number of reads based comparative bar graphs showing common and unique families microbes (c) Relative quantitative distribution of microbiota based on 16SrRNA based expression in the midgut of An. culicifacies in

response to sugar and post blood feeding (20-24hrPBM, 48hr PBM); Relative abundance of Elizabethkingia and Pseudomonas sp. bacteria in sugar-fed and blood-fed (20-24hr PBM) condition.

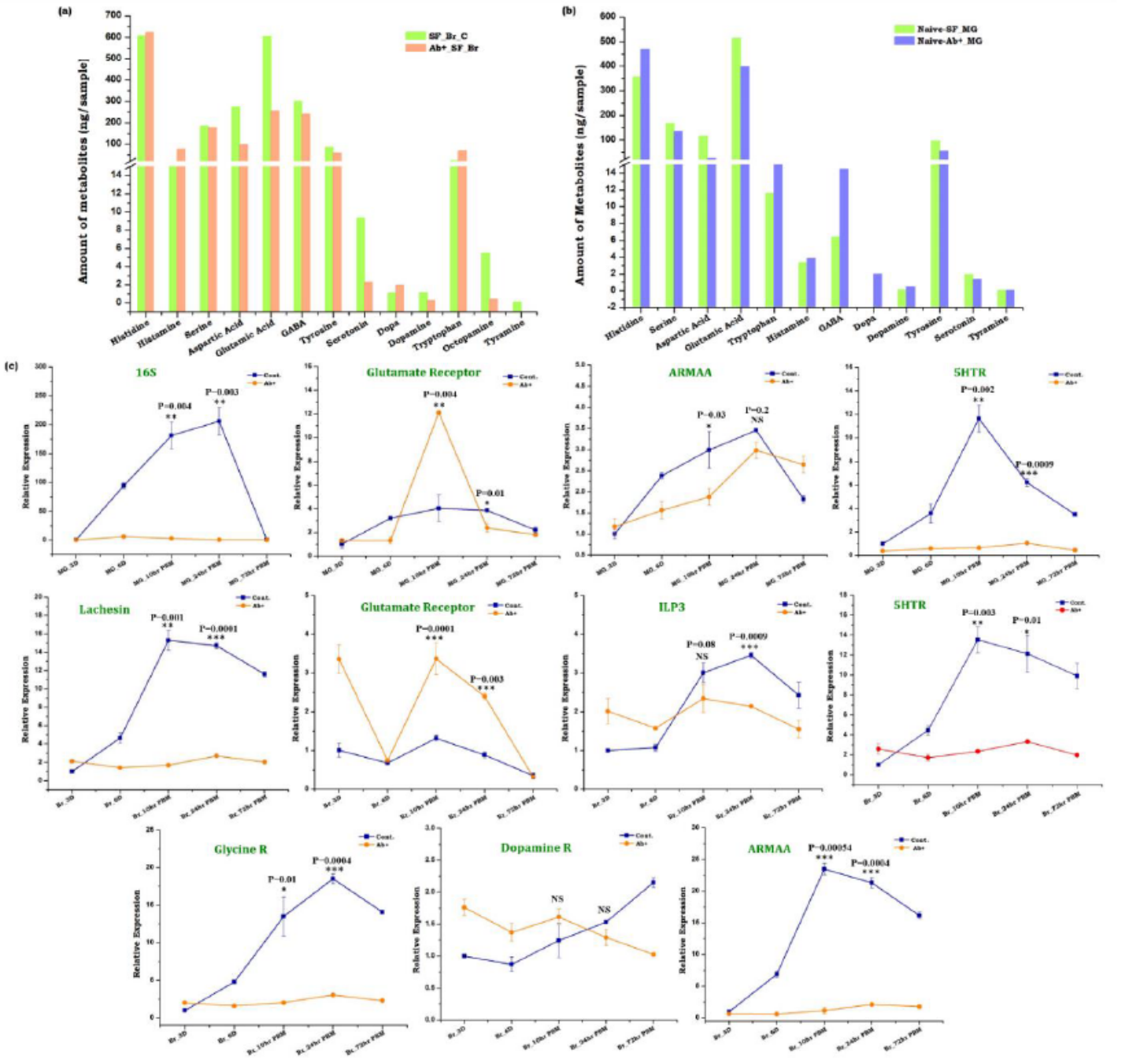

Figure 7

Establishing Microbiome-Gut-Brain-Axis (MGB) communication in mosquitoes. (a) Absolute quantification of the neurotransmitters (NT) in the brain tissues of naïve sugar-fed and antibiotic-treated mosquitoes (n = 65, N = 2); (b) Quantitative estimation of the neurotransmitters (NT) in the gut tissues of naïve sugar-fed and antibiotic-treated mosquitoes. Statistically significant differences in the amount of metabolites were tested by p-values ($p \leq 0.005$) that are deduced by two-way ANOVA and Tukey's test, (n

= 50, N = 2); (c) Relative expression profiling of the 16S gene to show the population of microbial flora and other neuro-transcripts in the gut and brain of naïve and antibiotic-treated mosquitoes undergoing metabolic switch. Statistical significance of differences of the respective genes in control (without antibiotic) and aseptic mosquitoes (antibiotic-treated) were tested by the t-test. (n = number of mosquitoes from which the respective tissue was dissected and pooled for each independent experiment; N = number of biological replicates).

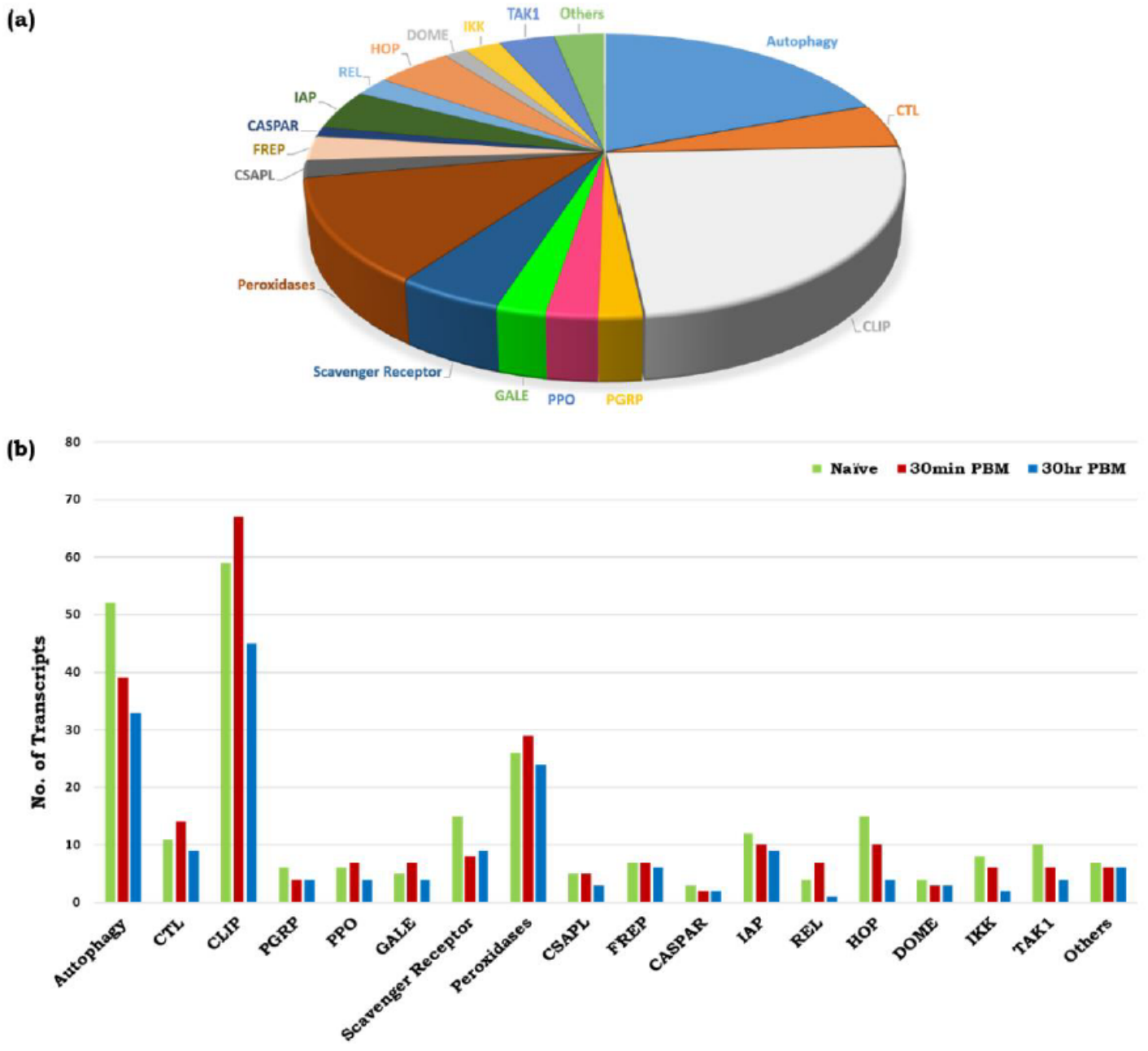

Figure 8

Molecular catalog of brain-specific immune transcripts. (a) Molecular catalog of the different classes of immune genes expressed in brain tissue; (b) Differential expression patterns of the brain immunome as

determined by the number of sequences that appeared in each RNA-Seq data of naïve and blood-fed mosquito brains.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ACBRAnnotation.xls
- SupportingInformationBrainPaper.pdf