

Full length article

Comparative transcriptomics reveals eyestalk ablation induced responses of the neuroendocrine-immune system in the Pacific white shrimp *Litopenaeus vannamei*

Jiahui Liu ^a, Tingting Zhou ^a, Chenggui Wang ^a, Wei Wang ^{a,b,*}, Siuming Chan ^{a,b,**}^a College of Fisheries, Guangdong Ocean University, Zhanjiang, Guangdong, PR China^b Guangdong Provincial Engineering Laboratory for Mariculture Organism Breeding, Guangdong Ocean University, Zhanjiang, Guangdong, PR China

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ABSTRACT

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In decapod crustaceans, eyestalk ablation is widely used to expedite ovarian maturation and spawning because of the removal of a gonad inhibiting hormone produced by the X-organ sinus gland. However, eyestalk ablation also results in negative impacts on the immunocompetence of the eyestalk-ablated females. In the current study, we investigated the impact of eyestalk ablation on the transcriptomic responses of three major nervous organs of shrimp, including the eyestalk ganglion, brain and thoracic ganglion, using the Illumina HiSeq™ 4000 platform. A total of 48,249 unigenes with an average length of 1253 bp and a N50 value of 2482 bp were obtained. Following eyestalk ablation treatment, a total of 2,983, 6325 and 6575 unigenes were detected as differentially expressed ($\log_2\text{Ratio} > 1$ and $\text{FDR} < 0.05$) from the eyestalk, brain and thoracic ganglia, respectively. Functional GO and KEGG analysis of these differential expression genes (DEGs) showed that these DEGs were associated with a wide variety of biological processes and pathways. The distribution of DEGs among three comparison groups was similar, and many DEGs were mapped to the phagosome pathway, indicating that eyestalk ablation triggers activation of the neuroendocrine-immune (NEI) system. Interestingly, several important pathways were uniquely enriched in the brain tissue, suggesting that the brain may play a crucial role in the NEI system in response to eyestalk ablation. This is the first report on the transcriptomic regulation of the nervous system in response to eyestalk ablation in *L. vannamei*. The genes and pathways identified in this study will help to elucidate the molecular mechanisms of neuroendocrine-immune responses to eyestalk ablation in penaeid shrimp.

1. Introduction

The neuroendocrine-immune (NEI) regulatory network is a complex system consisting of the nervous, endocrine and immune system, and modulates the immune defense against the infections of various pathogenic microorganisms [1]. When threatened by extrinsic stressors, the NEI system can release neurotransmitters and neurohormones to modulate the immune system. The immune system can also activate the NEI system through cytokines. These systems interact with each other and form a multi-directional regulation network to maintain physiological homeostasis [2]. The NEI regulatory network is known to exist in vertebrates [3], and increasing evidence has suggested that NEI regulatory network also plays important roles in response to adverse

environments in invertebrates [4,5].

The X-organ/sinus gland (XOSG) complex is the principal neuroendocrine organ of crustaceans, similar to the hypothalamus pituitary system of vertebrates [6]. It can secrete a variety of chemical messengers including many neuropeptide groups that regulate many important physiological processes to the hemolymph. The crustacean hyperglycemic hormone (CHH) is a member of the CHH/MIH/GIH neuropeptide family. It is the most abundant neuropeptide produced by the XOSG. CHH is considered as a stress hormone of the immune system in addition to its energy regulatory role. Previous studies have shown that injection of recombinant CHH protein to *Litopenaeus vannamei* significantly up-regulated exocytosis-related genes and phagocytosis-related genes [7,8]. CHH can contribute to the activation of prophenoloxidase system

* Corresponding author. College of Fisheries, Guangdong Ocean University, Zhanjiang, Guangdong, PR China.

** Corresponding author. College of Fisheries, Guangdong Ocean University, Zhanjiang, Guangdong, PR China.

E-mail addresses: wangwei.hg@outlook.com (W. Wang), siuming573@sina.com (S. Chan).

and phagocytosis. Furthermore, available evidence has demonstrated that biogenic amines such as dopamine, noradrenaline and 5-hydroxytryptamine act as key neurotransmitters to bridge the nervous and immune systems in crustaceans including *L. vannamei* [6,9], *Penaeus monodon* [10] and *Macrobrachium rosenbergii* [11]. Despite that research on the NEI regulatory network in model organisms has rapidly increased in recent years, our understanding of the NEI regulatory network in shrimp is still limited.

Eyestalk ablation is extensively practiced as a means to stimulate ovarian maturation and spawning in female crustaceans [12]. The terminal end of the optic nerve of eyestalk consists of the XOSG complex. Although unilateral eyestalk ablation can promote gonad maturation and reproduction, many studies have reported that eyestalk ablation can result in negative impacts on the immunocompetence of female shrimp, including decreased total hemocyte count and total serum protein concentration [13], subacute neurodegeneration [14], and even increased mortality [15]. Understanding the host immune responses to eyestalk ablation is the foundation to develop effective strategies for disease control and prevention. However, studies on the physiological responses of the neuroendocrine-immune system to eyestalk ablation are quite scarce.

The Pacific white shrimp, *L. vannamei*, is the most popular cultured shrimp species in the world. Using this species as a model, we conducted a comparative transcriptomic analysis of three major nervous organs of shrimp, including the eyestalk ganglion, brain and thoracic ganglia, after unilateral eyestalk ablation (UEA) treatment. A large number of genes were identified from these nervous organs, and differentially expressed genes as well as enriched gene pathways in the nervous system following eyestalk ablation were analyzed, with an aim of gaining more insights into the molecular mechanisms of neuroendocrine-immune responses to eyestalk ablation stress in penaeid shrimp.

2. Materials and methods

2.1. Sample collection and RNA isolation

Healthy adult female (35.58 ± 3.5 g) were obtained from the Marine Biological Research Center of Guangdong Ocean University (GDOU). Prior to the experiments, the shrimp were cultured in a circulated pond (16 m^2) for one month, and maintained at 28°C with a salinity of 30% and a 12 L: 12 D photoperiod. The diet was composed of 50% squid, 25% polychaetes and 25% clams. Feeding was divided into six equal daily rations accounting for a total daily supply of 17% of wet weight biomass. Shrimp (two groups of 30 individuals) with uniform ovarian condition (immature) were selected for the experiment. The first group was subjected to unilateral eyestalk ablation (UEA) and the control group was intact animal (INT). 48 h after UEA procedure, three shrimp from each group were dissected, and the eyestalk ganglion, brain, and thoracic ganglion were dissected and frozen in liquid nitrogen. The tissues were then stored at -80°C until RNA extraction.

2.2. RNA extraction, library construction and Illumina sequencing

Total RNAs from the eyestalk, brain, and thoracic ganglia tissues were extracted using Trizol reagent (Invitrogen Life Technologies, CA, USA) according to the manufacturer's instructions. RNA concentration and integrity of each sample was measured using NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) and Agilent 2100 Bio-analyzer (Agilent Technologies, Santa Clara, CA, USA), and the RNA quality of each sample was checked by RNase-free agarose gel electrophoresis. Each shrimp was considered a separate sample and three samples were taken as biological replicates for each group, and an equal amount of RNA from three individuals was pooled to make a sample for library construction.

RNA library construction was carried out at the Gene Denovo Biotechnology Company (Guangzhou, China) using Illumina HiSeq™

4000 platform. The main procedure was as follows: mRNA was enriched by Oligo(dT) beads, and then enriched mRNA was fragmented into short fragments using fragmentation buffer and reverse transcribed into cDNA with random primers. Second-strand cDNA was synthesized by DNA polymerase I, RNase H, dNTP and reaction buffer. Then the cDNA fragments were purified with QiaQuick PCR extraction kit (Qiagen, Venlo, Netherlands), end repaired, poly(A) added, and ligated to Illumina sequencing adapters. Following agarose gel electrophoresis and extraction of the cDNA from gels, the cDNA fragments were purified and enriched by PCR to construct the final cDNA library.

2.3. Assembly of sequencing data and gene function annotation

Raw sequence data were transformed by base calling into sequence data and stored in fastq format. Raw reads were cleaned by removing the adapter sequences, ambiguous sequences ("N" larger than 10%), or low-quality sequences (quality scores lower than 20). The clean reads were then assembled using the Trinity software [16], and further processed by the software TGICL V2.1 [17] to delete redundant sequences and to produce unique transcripts (unigenes).

For annotation analysis, unigenes were BLASTX-searched against five major gene/protein databases, including the National Center for Biotechnology Information (NCBI) non-redundant protein sequence (NR) database, SwissProt, Gene Ontology (GO), Clusters of Orthologous Groups of Proteins (COG), and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. The cutoff E-value was set at 10^{-5} and only the top hit was used to annotate each unigene. For unigenes with unambiguous annotation, information on their coding regions was inferred from their homologous sequences. For unigenes without BLAST hits, the software ESTScan version 3.0.2 was used to predict potential coding regions and sequence directions. The GO annotation was performed with Blast2GO version 2.5.0 [18], based on the NR BLAST results, and categorized according to Biological Process, Molecular Function, and Cellular Component. Further, COG classification and signal pathway annotation of unigenes was performed by BLASTx searching against the COG database and KEGG database respectively.

2.4. Analysis of differential expression genes and functional enrichment

To eliminate the influence of different gene lengths and sequence discrepancies on expression calculations, clean reads were mapped back to the assembled unigenes, and the expression level of unigenes in each sample was calculated and normalized to Reads Per kb per Million reads (RPKM) value [19]. The edgeR package (<http://www.r-project.org/>) was used for differentially expressed genes analysis between samples [20]. The p-value was assigned to each unigene and adjusted by the Benjamini and Hochberg's approach as q-value for controlling the false discovery rate (FDR). Unigenes with $q < 0.05$ and $|\log_2\text{Ratio}| > 1$ in a comparison were identified as differentially expressed genes (DEGs). DEGs were then subjected to enrichment analysis of GO functions and KEGG pathways. The GO terms and KEGG pathways enrichment of DEGs were defined by a hypergeometric test comparing to the genome background, in which the p-value was calculated and adjusted as q-value. GO terms or KEGG pathways with $q \leq 0.05$ were defined as significantly enriched.

2.5. Validation of RNA-Seq data by Real-time PCR

To verify the accuracy of RNA-seq data, eight unigenes were selected to validate their expression levels by Real-time PCR (RT-PCR). The selected genes, including four up-regulated and four down-regulated genes, were related to the significantly enriched pathways in DEGs of the brain. The elongation factor 1- α gene (EF1- α) was used as internal standard, and specific primers used for RT-PCR amplification were designed using the Primer 5.0 software (Table 1). RT-PCR was performed in a volume of 20 μL containing 10 μL of 2 \times SYBR Green PCR

Table 1
Primers used in the study.

ID	Gene description	Forward primer	Reverse primer
Unigene0003574	Integrin beta subunit	GACGAGCCGCTGAGTGAT	GCTTGAGTCGGAGTCTTTGT
Unigene0013393	Thrombospondin	CTCAGTTCACTGTCTGGTAG	CAAGTCGCGTGGTGCT
Unigene0022795	Chitooligosaccharidolytic beta-N-acetylglucosaminidase	CCCATCATTACTCTCCCTC	AGTCTCCGCCTTGCTC
Unigene0047429	Trypsin	AATCCTGGGCATCAACTT	TGCTCGTCACCGTCATC
Unigene0028801	5-hydroxytryptamine receptor 1	TTCACCAACCGTAAACAGC	TTCTTGGCGACGATCTC
Unigene0047101	5-hydroxytryptamine receptor 7	GAGTACGGCGTGAAGCG	TGACGGTGAGCGGGAT
Unigene0047520	Substance-K receptor	TTCGTAAACCTCGCTGTG	CCACCAGGACATTACATCTT
Unigene0012875	Relaxin family peptide receptor 1	TCTTGGCTGTCTTCTG	ATGCTGAGCGTGTGATAC

Table 2
Summary of transcriptome sequencing data.

Sample	Raw Data (bp)	Clean Data (bp)	Q20(%)	Clean reads	Mapped Reads	Mapping Ratio (%)
INT-ES	6,253,330,500	5,992,154,964	98.61	40,712,220	37,514,105	92.15
INT-BR	7,545,988,500	7,194,735,972	98.48	49,071,966	42,774,672	87.17
INT-TG	7,637,311,800	7,272,959,499	98.42	49,608,332	45,287,816	91.29
UEA-ES	7,454,836,500	7,119,259,085	98.50	48,475,020	45,037,808	92.91
UEA-BR	8,246,188,200	7,893,013,457	98.49	53,719,480	47,396,371	88.23
UEA-TG	6,993,856,200	6,678,319,755	98.49	45,492,796	40,601,165	89.25
Total	44,131,511,700	42,150,442,732	/	287,079,814	258,611,937	/
Mean	7,355,251,950	7,025,073,789	98.50	47,846,636	43,101,990	90.16

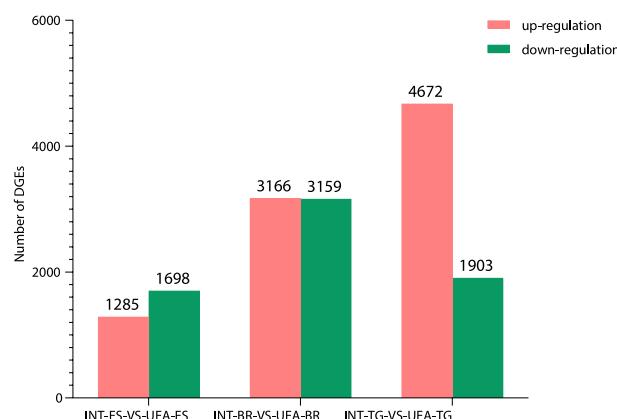


Fig. 1. Summary of up- and down-regulated genes in the eyestalk ganglion (ES), brain (BR), and thoracic ganglion (TG). X-axis represents pairs of samples; Y-axis represents number of differentially expressed genes; red column shows up-regulated genes, and green column shows down-regulated genes. INT, intact; UEA, unilateral eyestalk ablation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

buffer, 1 μ L of each primer (10 μ M), 1 μ L cDNA (50 ng), and 7 μ L of ddH₂O, using the SYBR® Premix EX Taq™ II (TaKaRa, Japan) kit. The amplification condition was as follows: 95 °C for 3 min, followed by 39 cycles of 95 °C for 10 s and 55 °C for 30 s. Gene relative expression levels were calculated using the comparative Ct method with formula $2^{-\Delta\Delta Ct}$, and the results were then compared with transcriptome data (RPKM) to evaluate the expression correlation.

3. Results

3.1. RNA-seq and de novo assembly

To build a reference transcriptome and identify genes from *L. vannamei*, six cDNA libraries were generated from the eyestalk, brain, and thoracic ganglia tissues. A total of 44,131,511,700 raw reads were produced by paired-end sequencing. After removal of adapter sequences, low quality reads or ambiguous sequences, more than 95% of the raw reads were filtered as clean reads, with an average Q20% of

98.50%. The clean reads were subjected to do novo assembly using the Trinity package. Using Bowtie2 mapping software, 89.3% of the high-quality clean reads were mapped to the reference assembly, indicating adequate representation of the sequenced genes (Table 2). A total of 48,249 unique transcripts (unigenes) were obtained, with a N50 value of 2482 bp and an average length of 1253 bp. The length distribution of assembled unigenes was shown in Supplemental Data S1.

3.2. Analysis of differential gene expression

We identified differentially expressed unigenes with a cutoff *q*-value <0.05 and an absolute value of log₂Ratio>1, to compare the differential gene expression levels between the INT group and UEA group. The direction of the expression changes (up- or down-regulation) of the DEGs were shown in Fig. 1. A total of 2983 DEGs were detected for the eyestalk samples INT-ES and UEA-ES, of which 1285 and 1698 unigenes were up-regulated and down-regulated, respectively. For comparison between the brain samples INT-BR and UEA-BR, 6325 DEGs were detected, of which 3166 and 3159 unigenes were induced and repressed, respectively. The largest number of DEGs was detected between the thoracic ganglion samples INT-TG and UEA-TG. Of the 6575 DEGs, 4672 and 1903 unigenes were up-regulated and down-regulated, respectively.

3.3. Global analysis of functional enrichment

In GO enrichment analysis, 368 GO terms were further divided into 41 functional subgroups (biological process 19; molecular function 10; cellular component 12). As shown in Fig. 2, the classification patterns of all DEGs among three pairwise comparisons were similar. In the category of biological process, more unigenes were assigned to “single-organism process”, “metabolic process”, and “cellular process” than other subgroups. In the category of molecular function, “catalytic activity” and “binding” were the most enriched GO terms. In the category of cellular component, “cell”, “cell part” and “membrane” were the three most abundant GO terms. Of the 368 GO terms, only 5 significantly enriched terms were identified with a *q*-value < 0.05 (Supplemental Data S2), including extracellular region (GO:0005576), extracellular region part (GO:0044421), hormone activity (GO:0005179), peptidase activity (GO:0008233), and hydrolase activity (GO:0016787).

To analyze the involved signal pathways, we further conducted the KEGG pathway enrichment analysis. The DEGs were mapped to 202

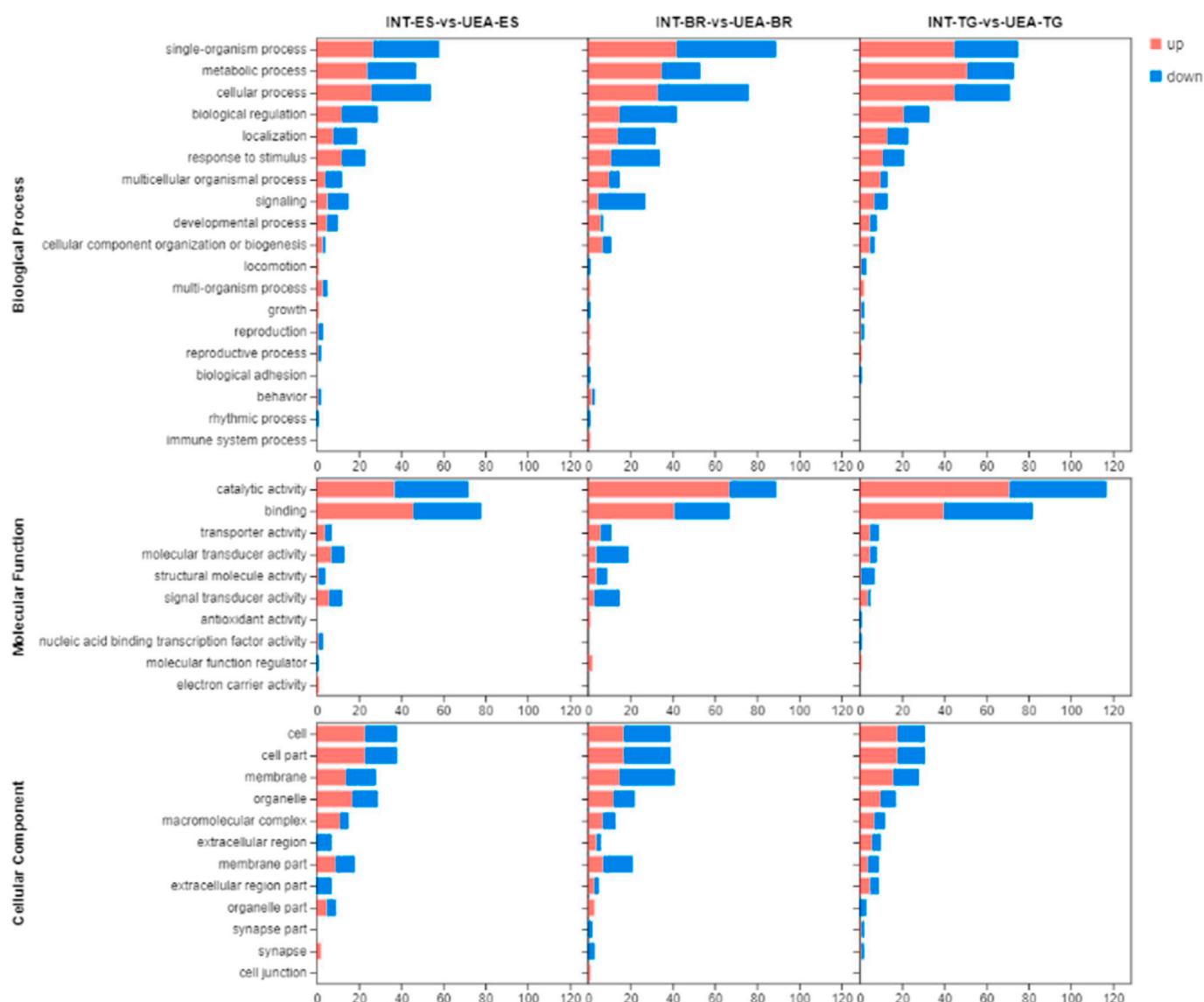


Fig. 2. GO classification of DEGs in the eyestalk ganglion (ES), brain (BR), and thoracic ganglion (TG). The DEGs were assigned to different terms in three major categories of the GO database, namely biological process, cellular component, and molecular function. Red bars indicate up-regulated genes and blue bars indicate down-regulated genes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

pathways within 33 categories, and the corresponding gene counts among three pairwise comparisons were shown in Fig. 3A. Pathways with the largest numbers of DEGs were closely associated with “global and overview maps in metabolism”, followed by “transport and catabolism in cellular processes”. To further understand immune response of the NEI system against eyestalk ablation challenge, we summarized pathways closely related to organismal systems and environmental information processing. These selected pathways were categorized into four groups, including immune system, endocrine system, nervous system, and signal transduction (Supplemental Data S3). Among them, some well-characterized genes implicated in immune defense, such as C-type lectin, glucose-6-phosphatase, cytochrome P450, integrin, caspase 3 and activin receptor type-1, were identified. In addition, KEGG enrichment analysis was performed to identify significantly enriched pathways in the DEGs for the INT-BR-vs-UEA-BR, INT-TG-vs-UEA-TG and INT-ES-vs-UEA-ES comparisons. As Fig. 3B showed, the phagosome (ko04145) pathway was significantly enriched in all three comparison groups. Moreover, many significantly enriched pathways, such as neuroactive ligand-receptor interaction (ko04080, Fig. 4), phototransduction-fly (ko04745), glycosaminoglycan degradation

(ko00531), ECM-receptor interaction (ko04512, Fig. 5), and gap junction (ko04540), were only detected in the INT-BR-vs-UEA-BR comparison, suggesting that the brain tissue may play an important role in NEI regulation.

3.4. Validation of RNA-Seq data by Real-time PCR

To verify the RNA-seq differential expression data, eight differentially expressed genes were selected for RT-PCR assay. The expression patterns of the selected genes determined by RT-PCR exhibited a good consistency with the differential expression data of transcriptome analysis (Fig. 6A). The positive correlation ($R^2 = 0.7098$, Fig. 6B) between RT-PCR results and RNA-seq data confirmed that gene expression profiles derived from RNA-Seq were reliable.

4. Discussion

In recent years, research on the communication between the neuroendocrine system and the immune system in invertebrates has attracted much attention [21]. It is now recognized that the

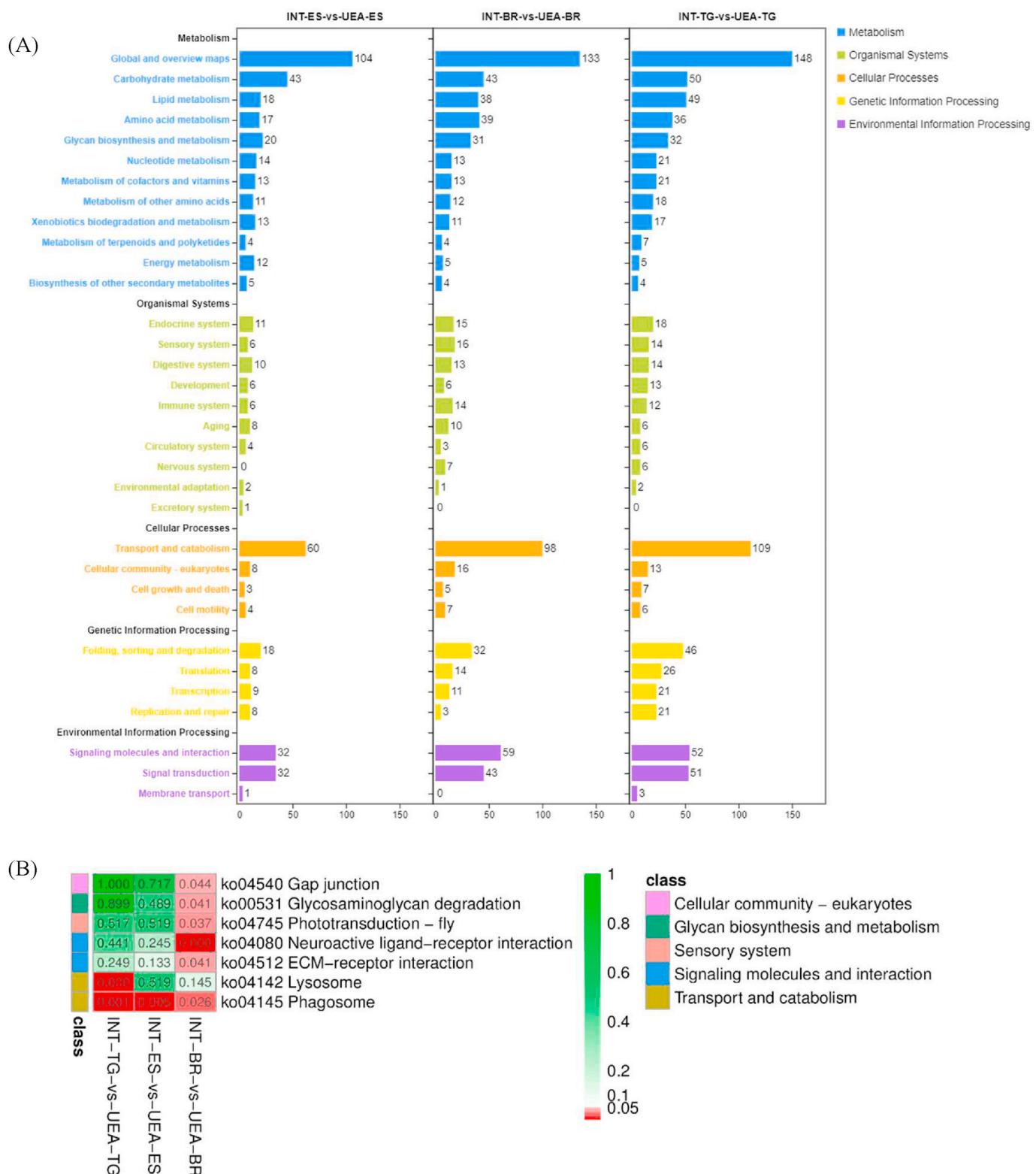


Fig. 3. KEGG pathway classification and enrichment analysis of DEGs in the eyestalk ganglion (ES), brain (BR), and thoracic ganglion (TG). (A) KEGG classification of all DEGs. Each column represents a pathway, and the number of DEGs in each pathway is shown at the end. (B) Significantly enriched KEGG pathways with a *q*-value < 0.05 in three tissues. The color from green to red represents a *q*-value from 1 to 0. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

neuroendocrine and immune systems form a bidirectional regulation network to maintain the physiological homeostasis [4]. UEA can cause degeneration of the distal stump of the protocerebral tract and inflammatory reaction, and triggers immune cells recruited to the injury site in

the crab *Ucides cordatus* [14] and the crayfish *Procambarus clarkii* [22]. Although UEA has been extensively practiced in commercial shrimp culture as a technique to accelerate maturation of female gonad, the response mechanism of the nervous system to UEA is still not fully

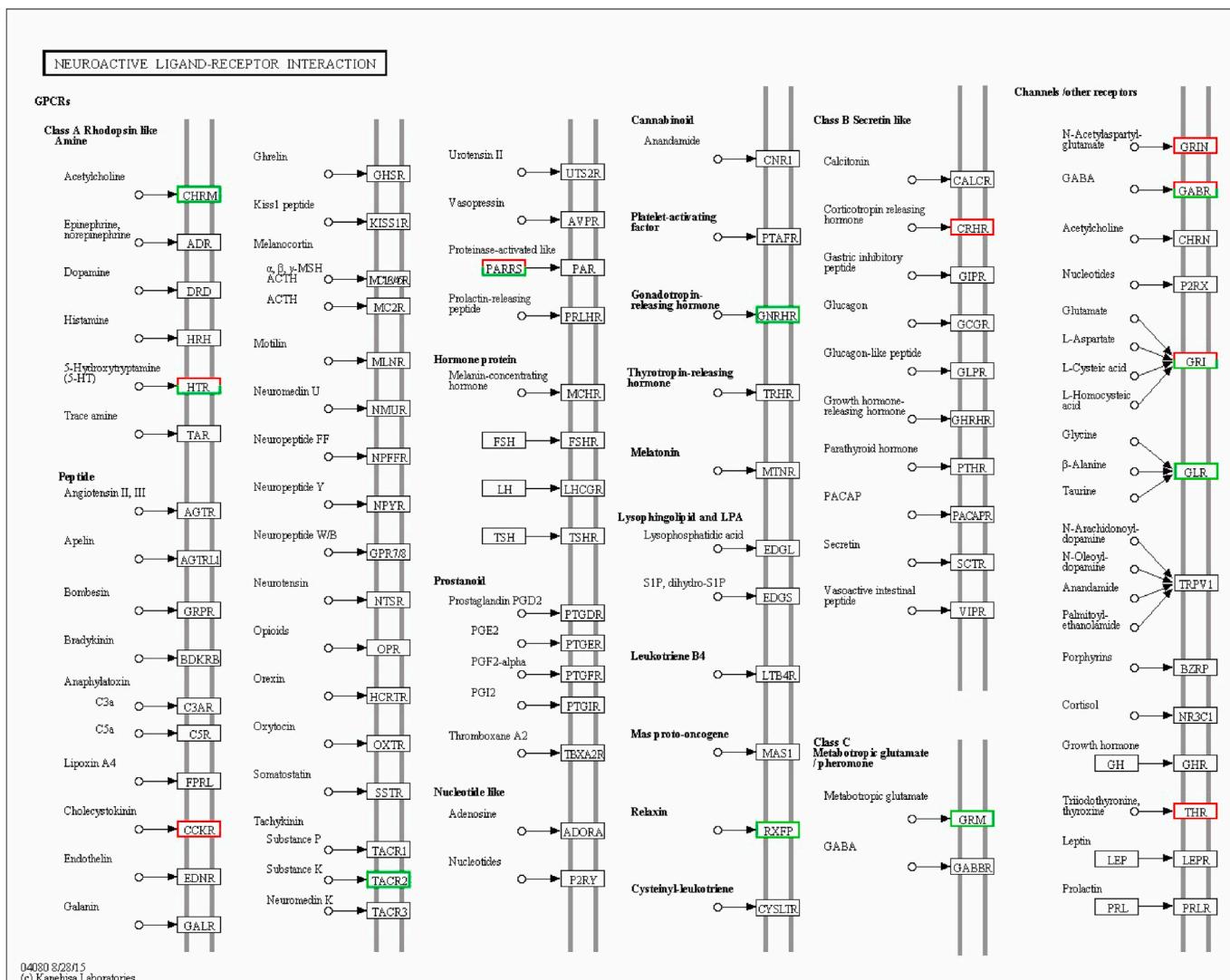


Fig. 4. Pathway of neuroactive ligand-receptor interaction (ko04080) enriched in the brain. Up-regulated genes are labeled in red, and down-regulated genes are labeled in green. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

understood in shrimp.

In this study, we took advantage of the RNA-Seq platform to investigate eyestalk ablation induced transcriptomic changes of three major nervous organs of shrimp, including the eyestalk ganglion, brain and thoracic ganglion. Through comparative transcriptomic analysis, a variety of genes were identified as differentially expressed after UEA in the three nervous organs. From DEGs comparisons of INT-ES vs. UEA-ES, INT-BR vs. UEA-BR, and INT-TG vs. UEA-TG, 2,983, 6325 and 6575 genes were detected as differentially expressed, respectively. GO and KEGG enrichment analysis further revealed that these DEGs were related to a wide range of biological processes and pathways.

Noticeably, the phagosome pathway was detected as significantly enriched in all the three nervous organs, indicating that this pathway is likely to be involved in the immuno-responses of the nervous system to eyestalk ablation. Moreover, several important pathways were only significantly enriched in the brain tissue. These pathways included those for neuroactive ligand-receptor interaction (ko04080, Fig. 4), phototransduction-fly (ko04745), glycosaminoglycan degradation (ko00531), ECM-receptor interaction (ko04512, Fig. 5), and gap junction (ko04540), suggesting that the brain may play a crucial role in the neuroendocrine immune response of *L. vannamei* to eyestalk ablation stress. Previous studies have revealed that hematopoietic tissue extends forward and connects to the brain, and hemocytes can be a source of

neuronal precursors in adult brain [23,24]. In addition, there is evidence that the hemocytes also function as neuroendocrine system [5]. For example, in *L. vannamei*, several neuroendocrine factors had been identified and characterized in hemocytes [8,25–27]. In the scallop *Chlamys farreri*, alpha-adrenergic receptor and opioid growth factor receptor modulate the hemocyte immune response [28,29]. These studies support a functional communication between the hematopoietic tissue and the brain. However, little information is available in relation to the underlying communication mechanisms between the brain and the immune system in shrimp.

4.1. Phagosome pathway

Like other invertebrates, shrimp mainly rely on innate immune system for defense. It consists of cellular and humoral immunity. Phagocytosis is a highly conserved process that can remove pathogens and cell debris. It is an important component of the cellular immunity [30]. In shrimp, phagocytosis is essential for host antiviral responses, and plays a part in cellular defense against pathogen infection [5,31]. In the KEGG enrichment analysis, significantly enriched phagosome pathway (ko04145) was identified in all the three groups, indicating that cellular immune phagocytosis may be the major innate immune response to eyestalk removal in the brain, eyestalk and thoracic ganglia of

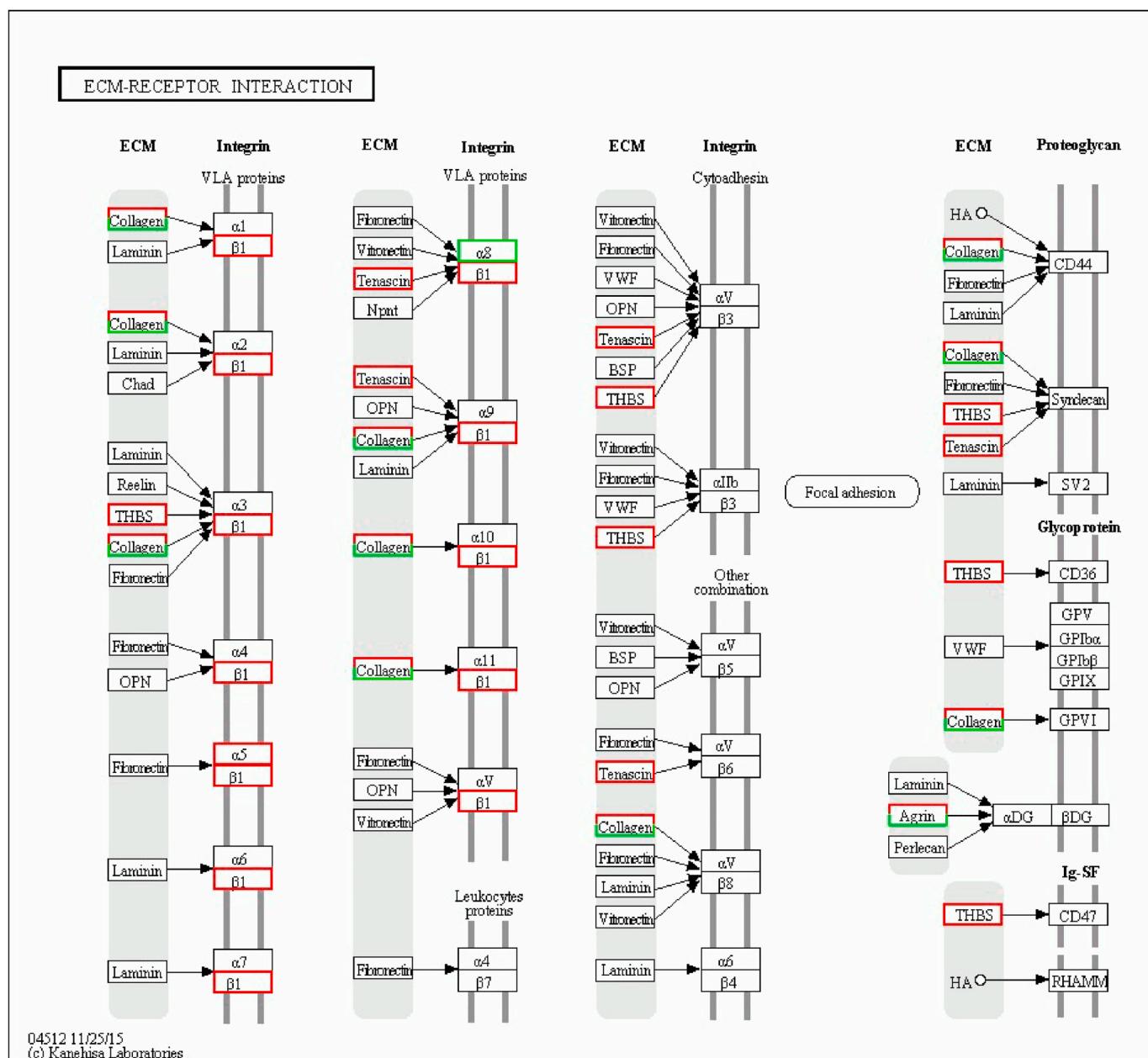


Fig. 5. ECM-receptor interaction pathway (ko04512) enriched in the brain. Up-regulated genes are labeled in red, and down-regulated genes are labeled in green. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

L. vannamei. It was speculated that the phagocytosis pathway may be involved in the NEI regulatory network of *L. vannamei*.

4.2. Neuroactive ligand-receptor interaction pathway

In this study, many genes in the neuroactive ligand-receptor interaction pathway showed significant up- or down-regulation in the brain. It has been reported that low salinity stress [32] and pathogen defense in arthropods [33] are associated with the neuroactive ligand-receptor interaction pathway. In the neuroactive ligand-receptor interaction pathway, 27 identified transcripts encoded neuropeptide receptors and neurotransmitter receptors. These receptors included cholecystokinin-like receptor, tachykinin receptor 2, thyroid hormone receptor α, gonadotropin-releasing hormone receptor, relaxin receptor, gamma-aminobutyric acid receptor, glycine receptor α-1, glutamate receptor 3, muscarinic acetylcholine receptor DM1, and 5-hydroxytryptamine receptors (5-HT1 and 5-HT7 receptors). The RT-PCR results

verified that the expression levels of substance K (SK) receptor, relaxin family peptide receptor 1 (RXFP1 receptor), 5-HT1 and 5-HT7 receptors were significantly down-regulated in the brain. In mammals, the tachykinin SK (Neurokinin A, NKA) is a neurotransmitter distributed in the central and peripheral nervous system. It is a potential mediator in human airway and gastrointestinal tissues, and can induce primary antibody response in rats by intracellular signaling through its receptor (NK-2 receptor) [34,35]. Kitamura et al. [36] also found that the NKA-NK2R signaling activates murine and human dendritic cell-mediated CD4⁺ T and CD8⁺ T cell responses. The RXFP1 ligand-receptor system has important functions in tumor growth and tissue invasion [37]. In mouse, serotonin (5-HT) can upregulates the activity of phagocytosis through 5-HT1A receptors [38]. In some crustacean, 5-HT and its receptors are involved in the modulation of the hemocyte count, prophenoloxidase exocytosis, and cheliped regeneration [7,39,40]. However, there are few reports on the immune response function of SK-SKR signaling and RXFP1 ligand-receptor signaling in

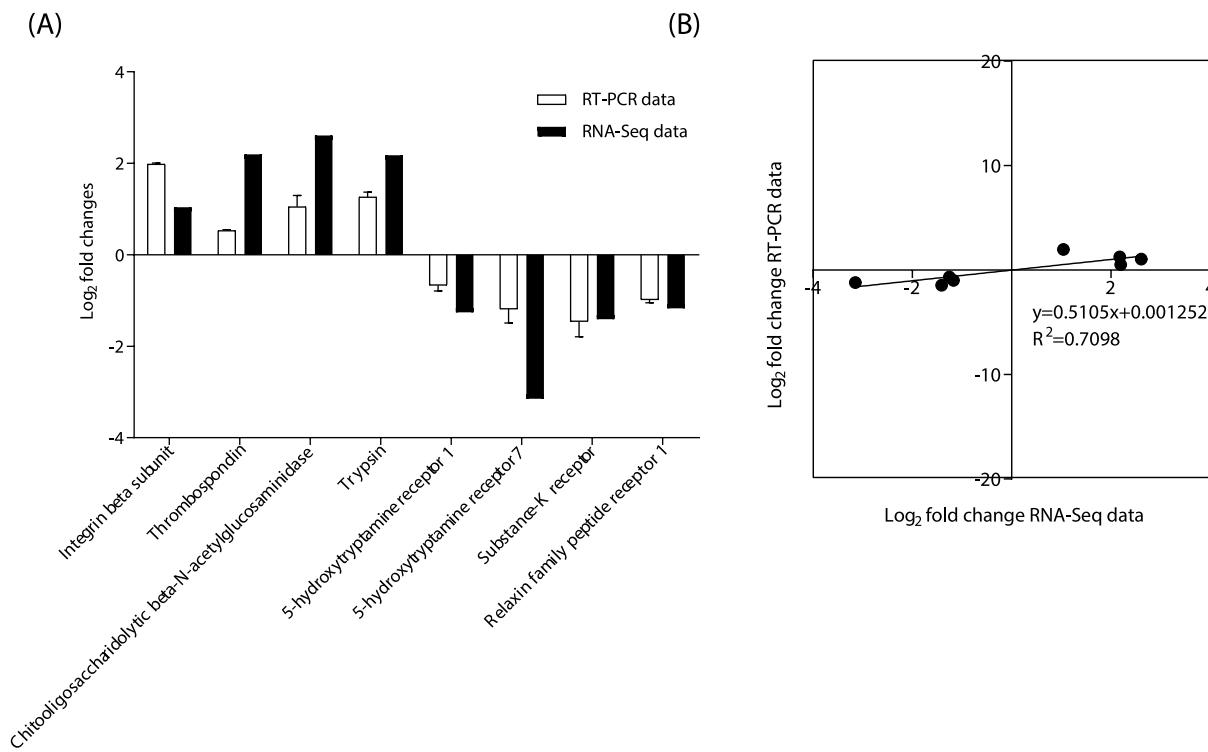


Fig. 6. Comparison (A) and correlation (B) of gene expression data between RNA-Seq and RT-PCR analysis. The eight selected genes showed consistent expression patterns when the two different methods were used.

shrimp. Brain trypsin has been shown to regulate long-term memory formation [41], synapses reorganization [42] and neuroregeneration [43]. In the current study, RT-PCR results confirmed that the expression of brain trypsin was significantly up-regulated after UEA, suggesting that brain trypsin might be involved in the recovery of neurodegeneration caused by eyestalk-ablation. These data indicated that neuroactive ligand-receptor interaction pathway may participate in the signal transmission between the immune and the nervous system. However, further research is needed to fully clarify the role of this pathway in aquatic invertebrate immune responses.

4.3. ECM-receptor interaction pathway

The extracellular matrix (ECM) consists of a complex mixture of structural and functional macromolecules and plays important roles in connecting different parts of the immune response [44]. In crustacean, several reports have shown that ECM-receptor interaction pathway may participate in the immune response against bacterial infection [45], white spot syndrome virus (WSSV) infection [46] and heavy metal exposure [47]. Based on KEGG pathway analysis, we have identified some unigenes encoding thrombospondin (TSP), agrin, collagen, integrin α ($\alpha 5$ and $\alpha 8$), and integrin β subunit in ECM-receptor interaction pathway. RT-PCR results confirmed that integrin β and TSP were significantly up-regulated after UEA treatment. Integrin, a member of cell adhesion receptors superfamily, is composed of α and β chain heterodimers of type I transmembrane glycoproteins with a short cytoplasmic tail. It mediates cell to cell, cell to ECM, and cell to pathogen interactions, and therefore has important roles in phagocytosis and adhesion strengthening, and transmission of signals [48]. TSPs are a family of ECM proteins expressed throughout the nervous system that promote rat neurite outgrowth *in vitro* by binding to integrin $\alpha 3\beta 1$ [49]. In *L. vannamei*, the expression level of integrin β was significantly up-regulated after *Listonella anguillarum* challenge [50] and long-term low salinity exposure [51]. In another shrimp *Fenneropenaeus chinensis*, TSP was up-regulated in various tissues of shrimp challenged with

microorganisms [52]. These reports were similar to our study, indicating that ECM-receptor interaction pathway may be involved in the neuroendocrine-immune response of *L. vannamei*.

5. Conclusion

This study presents the first transcriptome analysis of the nervous system in response to eyestalk ablation in the Pacific white shrimp *L. vannamei*. A large number of immune-related genes were identified as differentially expressed after eyestalk ablation. Based on GO and KEGG enrichment analysis, many enriched genes were mapped to phagosome pathway, indicating that innate immune reaction of phagosome may play an important role in NEI regulation of shrimp following eyestalk ablation. Additionally, the brain tissue had the largest number of uniquely enriched pathways, suggesting that the brain may play a pivotal role in nervous system response to eyestalk ablation. The results obtained would contribute to a deeper understanding of the molecular mechanisms of neuroendocrine-immune responses to eyestalk ablation in penaeid shrimp.

CRediT authorship contribution statement

Jiahui Liu: Experimentation, Formal analysis, Methodology, Writing - original draft, preparation. **Tingting Zhou:** Data curation, Formal analysis, Methodology, Investigation. **Chenggui Wang:** Data curation, Formal analysis, Methodology. **Wei Wang:** Conceptualization, Formal analysis, Funding acquisition, Supervision, Writing - review & editing. **Siuming Chan:** Conceptualization, Funding acquisition, Supervision, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fsi.2020.08.029>.

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