Molecular Cloning and Expression Analysis of 5-hydroxytryptamine Receptor 7 in Ant Polyrhachis vicina Roger (Hymenoptera: Formicidae)

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Abstract

Serotonin (5-hydroxytryptamine [5-HT]) is a monoamine neurotransmitter that plays an important role in regulating a variety of physiological and behavioral activities. In this study, the 5-HT receptor gene was cloned from the ant Polyrhachis vicina Roger (1863). The complete Pv5-HT receptor cDNA is 3054 bp, including a 5′-untranslated region (UTR) of 790 bp, a 3′-UTR of 752 bp and an open reading frame of 1512 bp encoding polypeptide of 503 amino acids. Hydrophobic analysis suggests that seven trans-membrane domains are the major sequence characteristic of the Pv5-HT receptor. In addition, the Pv5-HT receptor has three potential N-glycosylation sites, a palmitoylation site, three protein kinase A phosphorylation sites, and four protein kinase C phosphorylation sites. Phylogenetic analysis revealed that the deduced Pv5-HT receptor sequence shared a high homology with 5-HT receptor sequences of other species, such as a 78% similarity with the Am5-HT7 receptor (Apis mellifera). Real-time quantitative PCR (qRT-PCR) results showed that the expression level of the Pv5-HT7 receptor was low in the eggs and 1st-4th larval stages, but it was increased in the pupae stage and reached its peak in the adult workers. Western blot results showed that the highest protein expression was in the male body, head, and thorax. These results suggest that the Pv5-HT7 receptor may have specific functions in regulating the development of P. vicina, especially in adult formation and caste differentiation, feeding and caring behaviors of workers in the nest, and in the development of motor organs and mating behaviors in males.

Key words: 5-hydroxytryptamine receptor 7 (5-HT7 receptor), molecular cloning, real-time quantitative PCR, western blot

Serotonin (5-hydroxytryptamine [5-HT]) is one of the most important monoamine neurotransmitters that is widely present in both animal phyla and humans, and modulates physiological and behavioral activities (Weiger 1997, Hoyer et al. 2002, Schlenstedt et al. 2006). In humans, many mental diseases, including anxiety, depression, social phobia, and premenstrual dysphoria are accompanied by 5-HT functional disorders (Weiger 1997, Hoyer et al. 2002). In insects, 5-HT is involved in the modulation of feeding, the circadian rhythm, the heart rate, controlling secretory processes, development, aggressive behavior, circadian behavior, enhancing solitariness in phase transition of the migratory locust, as well as contributing to learning and memory (Watanabe et al. 2011, Falibene et al. 2012, Guo et al. 2013, Alice et al. 2014, Qi et al. 2014a, 2017, Blenau et al. 2017). Hoyer et al. (2005) reported that serotonergic neurons make differences between males and females of ant Harpagognathos saltator, which implies 5-HT roles in regulating physiological activities of different sexes including behaviors. Usually, 5-HT functions are mediated through its receptors. To date, 14 5-HT receptor subtypes have been identified in vertebrates (Schlenstedt et al. 2006, Zmudzka et al. 2018), which can be further divided into 7 distinct classes (5-HT1 to 5-HT7), based on structural, transductional, and operational features (Hannon and Hoyer 2008). With the exception of the 5-HT7 receptor, which belongs to the ligand-gated ion channel receptor family, the other 5-HT receptors belong to the G-protein-coupled receptor (GPCR) family (Eglen et al. 1997, Barnes and Sharp 1999, Hoyer et al. 2002, Schlenstedt et al. 2006). Qi et al. (2014b) found a novel serotonin Pr5-HT5 receptor from larval Pieris rapae, which shares relatively low similarity to known 5-HT receptor classes and has a unique pharmacological profile.

High sequence similarity between 5-HT receptors has been observed in some invertebrate species, including in the Aa5-HT receptor (Aedes aegypti) (Pietrantonio et al. 2001), the Dm5-HT receptor (Drosophila melanogaster) (Witz et al. 1990), the Ce5-HT receptor (Caenorhabditis elegans) (Hobson et al. 2003), and the Am5-HT receptor (Apis mellifera) (Schlenstedt et al. 2006). These invertebrate species 5-HT receptors share a similarity with...
mammalian 5-HT receptors. However, the role of the 5-HT receptor in *Polyrhachis vicina* is unknown. Therefore, we cloned the full-length cDNA of the 5-HT receptor (3054 nucleotides) from the ant *P. vicina* for the first time, named the *Pv5-HT7* receptor, and established a phylogenetic tree of 5-HT receptors in some animal species using MEGA 6.0. The expression patterns of *Pv5-HT7* receptor mRNA and protein in different developmental stages and castes were determined using real-time quantitative polymerase chain reaction (PCR) and western blot. These results may provide a valuable foundation for understanding the structure and biological functions of the *Pv5-HT7* receptors and their role in regulating ant development and caste differentiation.

Materials and Methods

Experimental Insects

*Polyrhachis vicina* Roger (1863) is a typical social insect, with the adults having the typical characteristics of caste differentiation in females, males, and workers. *Polyrhachis vicina* is distributed mainly in the areas of eastern and southern China, Burma, Cambodia, Japan, Australia, and Papua New Guinea. The colonies of *P. vicina* used in this study were purchased from Ruian of Zhejiang Province, China. The ants were raised in chambers and supplied with fruit, fish food, and honeydew under standard laboratory conditions at 25 ± 2°C, 50% relative humidity, and under a natural light–dark cycle. Eggs (5 d), first to fourth larvae (5 d), pupae (5 d), and adults (workers, males, and reproductive females) were collected from the three colonies (a total of 10 individuals from each colony), immersed in liquid nitrogen, and stored at −80°C until needed for RNA extraction (Lü et al. 2008, Ouyang et al. 2009).

RNA Preparation and cDNA Synthesis

Total RNA was extracted from the ant samples using RNAiso Plus (Takara, Shiga, Japan) according to the manufacturer’s instructions. The integrity and concentration of the total RNA was examined using 1.2% agarose gel electrophoresis and by means of spectrophotometer analysis. Then, cDNA was synthesized from 2 µg of total RNA using the First Strand cDNA Synthesis Kit with oligo (dT) primer (Fermentas Life Sciences, Burlington, Ontario, Canada). The integrity of the total RNA was examined using 1.2% agarose gel electrophoresis and by means of spectrophotometer analysis. Then, cDNA was synthesized from 2 µg of total RNA using the First Strand cDNA Synthesis Kit with oligo (dT) primer (Fermentas Life Sciences, Burlington, Ontario, Canada).

Table 1. Primers used for cDNA cloning and real-time quantitative polymerase chain reaction (qRT-PCR)

| Primer type       | Name            | primer sequences (5′-3′)                                      | Length (bp) | Efficiency (%) |
|-------------------|-----------------|--------------------------------------------------------------|-------------|---------------|
| Degenerate primers| *Pv5-HT*,DF1    | TAYGGSCTSAARAGDA                                             | 664         |                |
|                   | *Pv5-HT*,DF2    | AARAGDACCCVCVGDAVGAT                                         |             |                |
|                   | *Pv5-HT*,DR1    | TCRCGCTAYTGRCTSTG                                            |             |                |
|                   | *Pv5-HT*,DR2    | GTGVTSTCTNGYTBCCGYTCYTT                                      |             |                |
| 5′ RACE            | *Pv5-HT*,outer primer | CATGGCTACATGCTAGACGCTA                                       | 1336        |                |
|                   | *Pv5-HT*,inner primer | CCGGGATCCACACGCTACTGAGTACATCGATG                            |             |                |
|                   | *Pv5-HT*,R1     | GACGAACACGGGGGATGTGAAAG                                      |             |                |
|                   | *Pv5-HT*,R2     | CTGATAACACGGCTCTAACC                                         |             |                |
| 3′ RACE            | *Pv5-HT*,Outer Primer | TACCCTGCTTCCACTAGTGATT                                       | 1252        |                |
|                   | *Pv5-HT*,Inner Primer | CGCGGATCCACACGCTACTGAGTACATCGATG                            |             |                |
|                   | *Pv5-HT*,F1     | AGAAGTAACACGGCTCTAACC                                       |             |                |
|                   | *Pv5-HT*,F2     | CTGCGCCACACGGCGACGAAAG                                      |             |                |
| Real-time PCR      | *Pv5-HT*,RTF    | TATTTTACTATGCCACACCAACC                                     | 168         | 93.4           |
|                   | *Pv5-HT*,RTR    | AGCAAGGACGATTTCCTCC                                          |             |                |
|                   | *Pvβ-actinF     | CTCCTTTCAACCCCTCGTTC                                        | 250         | 96.7           |
|                   | *Pvβ-actinR     | CCACCGATCCCATACGGAGTA                                       |             |                |

Sequence Analysis

All sequence outputs (ABI files) were analyzed with the ContigExpress software. The molecular weights and isoelectric point of deduced protein sequences were predicted by ProtParam (http://web.expasy.org/protparam/). The homology of protein sequences was performed using BLAST and the function sites of the deduced amino acids were analyzed by PROSITE (http://prosite.expasy.org/). Glycosylation sites and transmembrane helices were analyzed using NetNGlyc (http://www.cbs.dtu.dk/services/NetNGlyc/) and TMHMM (http://www.cbs.dtu.dk/services/TMHMM/), respectively. The signal peptide was predicted by Signal P4.0. 5-HT, gene sequence methylation sites were predicted by MethPrimer software (http://www.urogene.org/methprimer/). 5-HT, protein 3-dimensional structure was calculated by SWISSMODEL (http://swissmodel.expasy.org/interactive).

Multiple Sequence Alignment and Phylogenetic Analysis

Multiple alignments of amino acid sequences were performed using ClustalX. The phylogenetic trees were constructed using the Neighborhood method in MEGA 6.0. Alignment sequences were obtained from the conserved nucleotide sequences of *A. mellifera*, *Anopheles gambiae*, *Nasonia vitripennis*, and *A. aegypti* from GenBank. The initial PCR was performed in a total volume of 20 µL, containing 1 µL of cDNA, 1 µL of each primer (20 µM), 2 µL of dNTP (2.5 mM each), and 2 µL 10x PCR buffer (Mg2+ plus). PCR was performed using a Thermal Cycler under the following conditions: 94°C for 3 min, followed by 35 cycles of 94°C for 45 s, 56°C for 55 s, and 72°C for 1 min, with a final extension of 72°C for 10 min. The second PCR used a 10-fold dilution of the initial PCR products as templates and was carried out under the same conditions used for the initial PCR. The PCR products were purified from agarose gels using the BioTeke kit (Beijing, China) and cloned into the pMD18-T Simple Vector (Takara, Dalian, Liaoning, China), then were transformed into *Escherichia coli* DH5α and incubated overnight at 37°C. Positive clones were identified and sequenced by Sangon Biotech (Shanghai, China). The whole length of the *Pv5-HT₇* receptor was amplified with the Takara 5′ and 3′ RACE kit. Gene-specific primers (shown in Table 1) were designed using known sequences that were previously obtained. 5′ and 3′ RACE PCR was performed in two rounds. PCR products were sequenced in both directions as described above.
bootstrapped 1,000 times to calculate the percentage of replicate trees in which sequences were clustered together. The sequence of *Caenorhabditis elegans* was used as an out-group. The accession numbers of sequences used in the phylogenetic analysis are shown in Table 2.

**Real-Time Quantitative PCR Analysis**

To determine the *Pv5-HT* receptor expression pattern in different developmental stages and castes, we isolated total RNA from eggs (5 d), first to fourth larvae (5 d), pupae (5 d), and adults (winged females, winged males, and workers). Samples were selected from three colonies. Each sample was run in triplicate along with the internal control gene β-actin. The primers and probe sequences are listed in Table 1. qRT-PCR was performed using a Bioread system in a total volume of 25 µL, containing 12.5 µL of SYBR Premix Ex Taq II, 1 µL of PCR each primer (10 µM), 1 µL of cDNA template, and 9.5 µL of sterile water. The reaction procedure was as follows: denaturation at 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. A dissociation curve analysis (from 65 to 90°C with increments of 0.5°C every 15 s) was conducted to ensure that only one PCR product was amplified and detected. The standard curve was obtained by the determining Ct (cycle threshold) values of a series of 10-fold diluted samples. The slope of the standard curve and the PCR efficiency of the genes were calculated. A negative control was carried out using sterile water instead of cDNA template. The relative expression value was calculated using the 2^ΔΔCt method.

**Western Blot**

Entire ants and the different parts of the body (including head, thorax, and abdomen) were homogenized and lysed with RIPA. The

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**Table 2. BLAST analysis of Pv5-HT, receptors amino acid sequence with other homology proteins**

| No. | Accession No. | Origin       | Coverage (%) | Similarity (%) | Amino acids |
|-----|---------------|--------------|--------------|----------------|-------------|
| 1   | NP_001071289.1| *Apis mellifera* L. | 99           | 78             | 501         |
| 2   | AIZ66402.1    | *Bombyx mori* L.  | 65           | 69             | 498         |
| 3   | AMQ67549.1    | *Pieris rapae* L. | 94           | 55             | 559         |
| 4   | AAG49292.1    | *Aedes aegypti* L. | 91           | 59             | 464         |
| 5   | XP_966777.2   | *Tribolium castaneum* Herbst | 86          | 60             | 481         |
| 6   | CCF77367.2    | *Calliphora vicina* Robineau-Desoody | 88        | 54             | 666         |
| 7   | NP_001263131.1| *Drosophila melanogaster* (Meigen) | 68          | 68             | 564         |
| 8   | ALC48019.1    | *Drosophila buscki* Coquillet | 86          | 57             | 569         |
| 9   | EAA08540.3    | *Anopheles gambiae* Giles | 74          | 59             | 496         |
| 10  | XPIP21835.1   | *Acyrthosiphon pisum* Harris | 73          | 58             | 385         |
| 11  | AGI46976.1    | *Manduca sexta* L. | 89           | 57             | 563         |
| 12  | BAJ63482.1    | *Gryllus bimaculatus* DeGeer | 41          | 76             | 230         |
| 13  | AAC15827.1    | *Caenorhabditis elegans* Dougherty | 85         | 28             | 683         |
| 14  | NP_001079253.1| *Xenopus laevis* Daudin | 55           | 43             | 425         |
| 15  | NP_000863.1   | *Homo sapiens* L. | 54           | 45             | 445         |
| 16  | AAY34776.1    | *Mesocricetus auratus* Waterhouse | 54          | 46             | 448         |
| 17  | NP_00166435.1 | *Cavia porcellus* L. | 54           | 45             | 446         |
| 18  | NP_075227.1   | *Rattus norvegicus* Berkenhout | 54         | 45             | 435         |
| 19  | NP_032341.2   | *Mus musculus* L. | 54           | 45             | 448         |

**Fig. 1.** The electrophoretogram of polymerase chain reaction amplification of full-length cDNA sequences of *P. vicina*. M1, 100bp ladder DNA Marker; M2, DL2000 DNA Marker; 1, Intermediate sequence; 2, 5′ RACE product; 3, 3′ RACE product.
tissue debris was removed by centrifugation at 12,000×g for 15 min and the supernatant was collected. The protein concentration was determined using BCA protein assay. A total of 30 µg protein per lane was loaded on a 12% sodium dodecyl sulfate-polyacrylamide gel and the separated samples were then transferred onto a polyvinylidene difluoride (PVDF) membrane for 2 h at 100 volts.
PVDF membrane was blocked with 5% dry milk in Tris-buffered saline with Tween 20 (TBST: 20 mM Tris-HCl pH 7.6, 200 mM NaCl, 0.1% Tween 20) for 60 min at room temperature. The PVDF membrane was incubated in polyclonal antiserum (1:1,000 dilution). After washing, the PVDF membrane was incubated with a goat anti-mouse IgG conjugated with horseradish peroxidase secondary antibody (1:5000 dilution) and finally visualized using enhanced chemiluminescence. The ratio of 5-HT7 to β-actin was calculated by Gel-Pro analyzer software.

Results
Cloning the Full-Length cDNA of Pv5-HT, Receptor
The full-length cDNA sequence of the Pv5-HT, receptor was obtained using nested-PCR and RACE. The amplification products are shown in Fig. 1. The degenerate primers were used to amplify a 664 bp partial cDNA fragment. Subsequently, the 5′ and 3′ RACE resulted in amplification of a 1336 bp and a 1252 bp fragment using two pairs of gene-specific primers designed using sequences from the P. vicina partial cDNA fragment. The full-length cDNA of Pv5-HT, receptor was obtained by overlapping the partial cDNA and RACE fragments. Sequence analysis showed that the full-length cDNA of the Pv5-HT, receptor (NCBI GenBank accession number KF297572.1) is 3054 bp and contains a 5′ untranslated region (UTR) of 790 bp and a 3′ UTR of 752 bp. The open reading frame of the Pv5-HT, receptor is 1512 bp and encodes 503 amino acid residues.

Sequence Characteristics of Pv5-HT, Receptor
The nucleotide and deduced amino acid sequences of the Pv5-HT, receptor are shown in Fig. 2. The predicted molecular weight and isoelectric point were 55.75 kDa and 8.44, respectively. Hydrophobic analysis showed that there are seven hydrophobic regions corresponding to the seven trans-membrane domains in Pv5-HT, receptor. Consensus sites for N-linked glycosylation are located at position Asn234, Asn290, and Asn333. Three consensus sites for phosphorylation by protein kinase A are located at positions Ser138, Ser236, and Ser253. Four consensus sites for phosphorylation by protein kinase C are located at positions Thr20, Thr172, Thr240, and Thr335. The tripeptide D-R-F are located at positions Asp₁₀⁻Arg₁₀⁴⁻Phe₁₀⁵, and the N-P-x-x-Y motif are located at positions Asn₁₄⁸⁻Pro₁₄₁⁻Tyr₁₄₄. Pv5-HT, receptor contains a PDZ binding domain (Glu₅₀₀⁻Ser₅₀₁⁻Phe₅₀₂⁻Leu₅₀₃) at its C-terminus. A putative polyadenylation signal ‘AATAAA’ was found at 2697 bp.

MethPrimer Result and Predicted 3D Structures
The prediction of the Pv5-HT, receptor gene methylation sites is shown in Fig. 3A. CpG islands were enriched in the 5′ upstream region. The predicted 3D structural model of the Pv5-HT, receptor protein was similar to the structure of A. mellifera 5-HT, receptor. The seven trans-membrane domain segments are flanked by an extracellular N-terminus of 9 residues and an intracellular C-terminus of 86 residues (Fig. 3B). The results of secondary structure prediction indicated that Pv5-HT, receptor of P. vicina contained 21 alpha helices, 33 beta helices, 26 turns, and 19 random coils (Fig. 3C).

Multiple Sequence Alignment and Phylogenetic Analysis
We compared nine species of insects to ensure the reliability of the alignments in Fig. 4. Multiple sequence alignments of 5-HT, receptor amino acid sequences demonstrated a high degree of conservatism. The Pv5-HT, receptor shared 78% identity with the Am5-HT, receptor (A. mellifera) and 69% with the Bm5-HT, receptor (Bombyx mori), followed by a 68% identity with the Dm5-HT, receptor (D. melanogaster), 60% with the Trica5-HT, receptor (Tribolium castaneum), 59% with the Aae5HT, receptor (A. aegypti), 55% with the Pr5-HT, receptor (P. rapae), 54% with the Cv5-HT, receptor...
(C. vicina), and 57% with the Dm5-HT7 receptor (Drosophila buseckii). The phylogenetic tree was constructed from these selected 5-HT7 receptors (Fig. 5). These receptors can be divided into two distinct groups of vertebrates and invertebrates.

mRNA Expression of the Pv5-HT7 Receptor
The mRNA expression patterns of the Pv5-HT7 receptor in the entire body during different developmental stages (eggs, first to fourth larvae, pupae, and adults) were examined by using qRT-PCR.

The results showed that Pv5-HT7 receptor mRNA was expressed weakly in eggs and in the first to fourth larval stages. The expression increased in later stages, with little change during the pupae and adult stages in females, finally reaching a peak level in the adult stage in workers, followed by that of males (Fig. 6A).

Western Blot Analysis
To investigate the expression of the Pv5-HT7 receptor protein, the protein contained in the entire body was extracted from individuals
of different developmental periods and castes. Western blot analysis showed that Pv5-HT7 receptor protein expression did not emerge in the egg and first to fourth larvae stages, became obvious in the pupae stage and reached the highest level in the adult males, followed by the adult females and workers (Fig. 6B). The expression of Pv5-HT7 receptor protein was detected in different body segments of adult ants. In the females, the protein expression in the thorax was highest. And the male protein expression is the highest in the thorax, the lowest in the abdomen. The worker expression profile was different from that of males, in that receptor expression was lowest in the thorax, the lowest in the abdomen. The worker expression profile was different from that of males, in that receptor expression was lowest in the thorax, and highest in the head (Fig. 7). There was a significant difference in Pv5-HT7 receptor protein expression in the head, thorax, and abdomen of the three ant castes. In the head, the most abundant expression appears in the males, followed by the workers, with the minimum quantity of expression in the females. In the thorax, expression from highest to lowest was the males to the females to the workers, and in the abdomen expression increased little in the males, and females as well as workers did not have any obvious difference in expression among adults (Fig. 7).

**Discussion**

In this study, the full-length cDNA of Pv5-HT7 receptor is obtained from *P. vicina* for the first time, which is identified as one member of the GPCR family (Ruat et al. 1993, Barnes and Sharp 1999). Pv5-HT7 receptor gene own sequence motifs which are essential for ligand-binding (Barak et al. 1994), and signal transduction and three-dimensional structure are kept in the different species 5-HT receptors including vertebrates and invertebrates. Pv5-HT7 receptor has PDZ binding domain at the C-terminus which is contained in all insect 5-HT receptors (Schlenstedt et al. 2006). These results exhibit the stability and conservatism of Pv5-HT7 receptor gene structures.

The multiple sequence alignment and phylogenetic analysis in Fig. 4 and Table 2 show that the deduced amino acid sequence of the Pv5-HT7 receptor was very similar to some insect species. Schlenstedt et al. (2006) reported that the Am5-HT7 receptor (*A. mellifera*) was highly similar to members of the insect 5-HT7 receptor family (Schlenstedt et al. 2006), Vleugels et al. (2014) showed that the Trica5-HT7 receptor (*T. castaneum*) has a 74.4% similarity to the Am5-HT7 receptor, 70.0% to the Dm5-HT7 receptor (*D. melanogaster*), and 66.3% to the Aae5-HT7 receptor (*A. aegypti*) (Vleugels et al. 2014). The phylogenetic tree analysis indicated that *P. vicina* had the closest relationship with *A. mellifera*, both of which belong to Hymenoptera. Our data confirm again those observations and verify the conservatism of 5-HT receptor gene family.

The degree of Pv5-HT7 receptor mRNA and protein expression in the different developmental stages of the ant may infer its significant role in regulating the ant’s development and physiological functions, which also coincide with the conclusion about serotonin modulating development of insects reported by Blenau et al. (2017) and Qi et al. (2017). Hoyer et al. (2005) pointed out that serotonergic neurons make differences between males and females, which imply 5-HT roles regulating physiological activities of different sexes including behaviors. Giraldo et al. (2013) reported serotonin titer
increases in the brain with age, modulating some behaviors characteristic of mature individuals in the ant *Pheidole dentate* (Giraldo et al. 2013). Pv5-HT7 receptor mRNA and protein expression in the pupae is not much higher than in the eggs and larvae stages (Fig. 6). As we know, the pupae stage of holometabolic insects is a critical period for adult formation. The state of Pv5-HT7 receptor expression in pupae may be deduced its important role in modulating adult formation and caste differentiation in *P. vicina*.

Falibene et al. (2012) reported that 5-HT modulates feeding-related processes in ants (Falibene et al. 2012). Generally, worker ants take on the task of feeding and caring for females as well as larvae in the nest (Wang et al. 2016). Schlenstedt et al. (2006) inferred that Am5-HT7 receptor acts in processing sensory information and possibly higher-order brain activities by reason of the higher mRNA expression on optic lobes, central brain, and flight muscle in honeybee *A. mellifera* (Schlenstedt et al. 2006). Although we did not examine Pv5-HT7 receptor expression in the brain and salivary gland of the worker ants, but highest mRNA expression level of the Pv5-HT7 receptor was detected in the worker body (Fig. 6A), highest protein expression level of the Pv5-HT7 receptor was detected in the worker head compared with its thorax and abdomen (Fig. 7A). And those results may suppose a possible correlation with the worker ants in feeding and caring behaviors.

Röser et al. (2012) found that Cv5-HT7 receptors mRNA expressed in the brain and salivary glands and flight muscles of blowfly *Calliphora vicina*, especially the highest expression in brain. Watanabe et al. (2011) reported that 5-HT7 receptors mRNA expressed in the central brain, suboesophageal ganglion, optic lobe, thoracic muscles, and salivary gland in the field cricket *Gryllus bimaculatus* (Watanabe et al. 2011). They suggest that 5-HT mediated the skeletal muscle activity and modulates aggressive behavior in the cricket *G. bimaculatus*. Although we did not check Pv5-HT7 receptor expression in the muscle and appendages of the ants, the highest protein expression levels in the female (Fig. 7A), the male body, head, and thorax (Fig. 6B, Fig. 7A and B) were detected, which may conclude to the Pv5-HT7 receptor roles in corresponding parts of male ants.

**Fig. 6.** Relative expression mRNA and protein of Pv5-HT7 receptor in different developmental stages and castes. Eggs (E), the first-instar (L1), second-instar (L2), third-instar (L3), fourth-instar (L4) larvae, pupae (P), females (F), males (M), and workers (W). Different letters indicate statistically significant differences according to Duncan’s test (*P* < 0.05). (A) The relative expression mRNA of Pv5-HT7 receptor. The expression value was obtained by quantitative real-time polymerase chain reaction and normalized using β-actin as an reference gene. The relative expression value of Pv5-HT7 receptor at first-instar larva was arbitrarily set to 1. (B) Relative expression protein of Pv5-HT7 receptor by western blot analysis. The anti-Pv5-HT7 receptor antibody (1: 800) recognized a single band of about 55 kDa.

**Fig. 7.** (A) western blot analysis of Pv5-HT7 receptor in different parts of *P. vicina*. The anti-Pv5-HT7 receptor antibody (1:800) recognized a single band of about 55 kDa. FH, female head; FT, female thorax; FA, female abdomen; MH, male head; MT, male thorax; MA, male abdomen; WH, worker head; WT, worker thorax; WA, worker abdomen. Different letters indicate statistically significant differences according to Duncan’s test (*P* < 0.05). (B) Western blot analysis of Pv5-HT7 receptor in different parts of *P. vicina*. The anti-Pv5-HT7 receptor antibody (1:800) recognized a single band of about 55 kDa. Different letters indicate statistically significant differences according to Duncan’s test (*P* < 0.05).
Johnson et al. (2009) show that the *Drosophila melanogaster* 5-HT, (Dro5-HT) receptor is involved in aggressive behaviors, including acquisition of food, territory, and mates. Becnel et al. (2011) report that the strong expression of Dro5-HT receptor in the adult brain appears to mediate normal courtship and mating behaviors in the fly. As a type of social insect, the main duty of male ants is mating with the female ants (Wang et al. 2016). In view of our works and combining with the other person research data, we speculate that protein expression profile of Pv5-HT, receptor in the the female (Fig. 7A) and male ants (Figs. 6B and 7B), may contribute to specific physiological activities and behaviors in *P. vicina*, especially modulate the male courtship and mating behaviors with the female.

**Conclusion**

The full-length cDNA of the Pv5-HT7 receptor gene is 3054 bp (GenBank accession no. KF297572.1). mRNA and protein expression of the Pv5-HT7 receptor are examined in different developmental stages and adult castes in *P. vicina*. The higher expression of the Pv5-HT7 receptor mRNA and protein in the pupae implies that it plays a role in the adult formation and caste differentiation of *P. vicina*. The highest mRNA expression in the worker body, highest protein expression in the worker head speculate that the Pv5-HT7 receptor may modulate the workers' feeding activities and caring behaviors. The higher protein expression in the female thorax, the male body, head, and thorax speculate that the Pv5-HT7 receptor may modulate the female and male ants’ reproductive-related physiological activities and behaviors. These results may provide a foundation for the investigation of the diverse functions of 5-HT receptors, and demonstrate that further study of the regulatory mechanisms of the Pv5-HT7 receptor in *P. vicina* is necessary.

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