Manuscript #: IMB-OA-11-021.R2

Identification and expression analysis of the genes involved in serotonin biosynthesis and transduction in the field cricket *Gryllus bimaculatus*

Takayuki Watanabe[1], Hisayo Sadamoto[2], and Hitoshi Aonuma[1]*

[1]Laboratory of Neuro-Cybernetics, Research Institute for Electronic Science, Hokkaido University, Sapporo, Japan

[2]Laboratory of Functional Biology, Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, Sanuki, Japan

Running title: 5-HT-related genes in the cricket

*Corresponding author. Hitoshi Aonuma, D.Sc., Laboratory of Neuro-Cybernetics, Research Institute for Electronic Science, Hokkaido University, Nishi 7 Chome, Kita 12 Jo, Kitaku, Sapporo, Hokkaido 060-0812, Japan. Tel/Fax: +81-11-706-3832. Email: aon@es.hokudai.ac.jp

ABSTRACT

Serotonin (5-HT) modulates various aspects of behaviors such as aggressive behavior and circadian behavior in the cricket. To elucidate the molecular basis of the cricket 5-HT system, we identified 5-HT-related genes in the field cricket *Gryllus bimaculatus* DeGeer. Complementary DNA of tryptophan hydroxylase (TRH) and phenylalanine-tryptophan hydroxylase (TPH) which convert tryptophan into 5-hydroxy-L-tryptophan (5-HTP), and that of aromatic L-amino acid decarboxylase (AADC) which converts 5-HTP into 5-HT were isolated from a cricket brain cDNA library. In addition, four 5-HT receptor genes (5-HT_{1A}, 5-HT_{1B}, 5-$HT_{2\alpha}$, and 5-HT_{7}) were identified. Expression analysis of *TRH* and *TPH*, which are selectively involved in neuronal and peripheral 5-HT synthesis in *Drosophila*, suggested that two 5-HT synthesis pathways co-exist in the cricket neuronal tissues. The four of 5-HT receptor genes were expressed in various tissues at differential expression levels, suggesting that the 5-HT system is widely distributed in the cricket.

Keywords: serotonin, tryptophan hydroxylase, phenylalanine-tryptophan hydroxylase, aromatic L-amino acid decarboxylase, serotonin receptors, *Gryllus bimaculatus*.

INTRODUCTION

Crickets show various behaviors regulated by biogenic amines. Pharmacological and behavioral studies have revealed the involvement of specific biogenic amines in modulating various aspects of behavior. For example, the octopaminergic and dopaminergic systems respectively mediate reward and punishment signals in olfactory and visual learning (Unoki et al., 2005; Unoki et al., 2006). In the optic lobes, serotonergic neurons regulate the circadian pacemakers to set the day state (Saifullah and Tomioka, 2002). Pharmacological inhibition of aminergic neurotransmitter biosynthesis revealed that depletion of serotonin (5-HT) and dopamine/octopamine had differential effects on aggressive and escape behaviors of male crickets (Stevenson et al., 2000). Furthermore, hormonally released octopamine and 5-HT reciprocally control the length of the mating interval in male crickets (Nagao et al., 1991). Although physiological and behavioral aspects of the aminergic system have been extensively studied, the molecular basis of the cricket aminergic system has not been investigated yet. In the present study, we investigated the molecular basis of the 5-HT system in the cricket *Gryllus bimaculatus* DeGeer.

The biogenic monoamine 5-HT is an ancient intracellular signaling molecule found in all phyla that possess nervous systems. In the nervous system of both vertebrates and invertebrates, 5-HT functions as a neurotransmitter/modulator, or as a neurohormone that modulates various principal behaviors, such as feeding, circadian behavior, sleep, sexual behavior, and social behavior.

5-HT synthesis requires two enzymes, tryptophan hydroxylase and aromatic L-amino acid decarboxylase (AADC) (Figure 1). As first step of 5-HT synthesis, tryptophan

hydroxylase converts L-tryptophan into 5-hydroxy-L-tryptophan (5-HTP). Then, AADC converts 5-HTP into 5-HT. In the fruit fly *Drosophila melanogaster*, tryptophan hydroxylase is encoded by two genes, *dTPH* and *dTRH* (Coleman and Neckameyer, 2005). Both gene products have tryptophan hydroxylase activity *in vivo*, but the *dTPH* gene is expressed in non-neural tissues, and the *dTRH* gene is expressed in the neural tissues (Neckameyer et al., 2007). Homologues of these genes were found in the genome of other insects such as bees, beetles, and moths, but their tissue-specific expression patterns have not been examined. AADC is not only involved in the biosynthesis of serotonin but also dopamine, and thus, AADC is expressed in serotonergic and dopaminergic neurons in the central nervous system (CNS).

All of the known insect 5-HT receptors belong to the seven-transmembrane segments (7TM)-containing G protein-coupled receptor (GPCR) superfamily. In *Drosophila,* five 5-HT receptors (5-HT_{1A}, 5-HT_{1B}, 5-$HT_{2\alpha}$, 5-$HT_{2\beta}$, and 5-HT_{7}) were identified (Brody and Cravchik, 2000), and their homologues were found in some other insects. The 5-$HT_{2\beta}$ receptor has not yet been characterized in any insect (Blenau and Thamm, 2011). The other four 5-HT receptor subtypes are known to be coupled with subtype-specific signal transduction mechanisms (Tierney, 2001). Behavioral pharmacology and genetic studies on the insect 5-HT receptors revealed the specific involvement of the receptor subtypes in modulating behaviors such as circadian behavior and aggressive behavior (Yuan et al., 2005; Yuan et al., 2006; Nichols 2007; Johnson et al., 2009).

To elucidate the molecular basis of the cricket 5-HT system, we identified seven genes involved in synthesis and transduction of 5-HT expressed in the brain of the cricket, *G. bimaculatus*. We obtained the full-length open reading frame (ORF) clones of the *TPH, TRH,* and *AADC* genes, as well as the partial ORF clones of four 5-HT receptor genes (*5-HT_{1A}*, *5-HT_{1B}*,

5-HT$_{2\alpha}$, and *5-HT$_7$*). Expression analysis of the 5-HT-related genes revealed that the 5-HT system is widespread in the CNS and in peripheral tissues of the cricket.

RESULTS

Nucleotide Sequence and Structural Features of *Gryllus TRH* cDNA

We identified a tryptophan hydroxylase gene, *TRH*, expressed in the cricket brain. We performed RT-PCR with the gene-specific primers (GSPs) designed at the 5' and 3' UTRs of the gene, and obtained 1706-bp and 1823-bp cDNA fragments of *TRH* (GenBank accession numbers: AB618095 and AB626809, respectively). The short variant contains an ORF spanning 13–1629 bp, resulting in a protein product of 538 amino acids. The long variant contains an ORF spanning 13–1746 bp, resulting in a protein product of 577 amino acids. Several functional domains and residues, such as the N-terminal regulatory (ACT) domain, the catalytic domain, the C-terminal coiled-coil region involved in tetramerization, tetrahydrobiopterin (BH4)-binding sites, and iron binding sites, are conserved in *Gryllus* TRH (Fig. 2(A)). The long variant contains the 39 amino acid insertion in the N-terminal region of the catalytic domain (between amino acids 179 and 180 of the short variant) (supplementary figure 1).

Comparison of the deduced amino acid sequence of *Gryllus* TRH with those of other TRH, cloned from both vertebrates and invertebrates, indicated that *Gryllus* TRH is structurally most similar to the other known insect TRH (64% identical to *Drosophila* TRH, 66.5% identical to *Tribolium* TRH, and 74.9% identical to *Apis* TRH). The molecular phylogenetic analysis of aromatic amino acid hydroxylases using the full-length of the deduced amino acid sequence of the *Gryllus TRH* gene and those of various aromatic amino acid hydroxylases also indicated that *Gryllus* TRH is closely related to the insect TRH proteins (Fig. 3).

Nucleotide Sequence and Structural Features of *Gryllus TPH* cDNA

We identified a phenylalanine-tryptophan hydroxylase gene, *TPH*, expressed in the cricket brain. We performed RT-PCR with the GSPs designed at the 5' and 3' UTRs of the gene, and obtained a 1611-bp cDNA fragment of *TPH* (GenBank accession number: AB618096). This clone contains an ORF spanning 68–1435 bp, resulting in a protein product of 455 amino acids. Several functional domains and residues, such as the N-terminal regulatory (ACT) domain, the catalytic domain, the C-terminal coiled-coil region involved in BH4-binding sites, and iron binding sites, are conserved in *Gryllus* TPH (Fig. 2(B)).

Comparison of the deduced amino acid sequence of *Gryllus* TPH with those of other invertebrate TPH and vertebrate phenylalanine-4-hydroxylase indicated that *Gryllus* TPH is structurally most similar to the other known insect TPH (75.9% identical to *Apis* TPH isoform 2, 75.5% identical to *Papilio* TPH, and 71.6% identical to *Drosophila* TPH isoform A). The molecular phylogenetic analysis of aromatic amino acid hydroxylases using the full-length of the deduced amino acid sequence of the *Gryllus TPH* gene and those of various aromatic amino acid hydroxylases also indicated that *Gryllus* TPH is closely related to the insect TPH proteins (Fig. 3).

Nucleotide Sequence and Structural Features of *Gryllus AADC* cDNA

We identified an aromatic L-amino acid decarboxylase gene, *AADC*, expressed in the cricket brain. We performed RT-PCR with the GSPs designed at 5' and 3' UTRs of the *Gryllus AADC* gene, and obtained a 1522-bp cDNA fragment (GenBank accession number: AB618097). This clone contains an ORF spanning 12–1454 bp, resulting in a protein product of 480 amino acids. The catalytic domain and several functional residues, such as the pyridoxal 5'-phosphate binding sites and the catalytic loop, are conserved in *Gryllus* AADC (Fig. 4(A)).

Comparison of the deduced amino acid sequence of *Gryllus* AADC with those of other AADC indicated that *Gryllus* AADC is structurally most similar to the other known insect AADC (75.2% identical to *Tribolium* AADC, 75.3% identical to *Bombyx* AADC, and 74.2% identical to *Drosophila* AADC). The molecular phylogenetic analysis of AADC proteins using the full-length of the deduced amino acid sequence of the *Gryllus AADC* gene and those of various amino acid decarboxylases also indicated that *Gryllus* AADC is closely related to the insect AADC proteins (Fig. 4(B)).

Nucleotide Sequence and Structural Features of cDNAs of the *Gryllus* 5-HT receptor genes

We identified four 5-HT receptor genes (*5-HT$_{1A}$*, *5-HT$_{1B}$*, *5-HT$_{2\alpha}$*, and *5-HT$_{7}$*) expressed in the cricket brain. We performed RT-PCR to amplify the partial ORF of *Gryllus 5-HT$_{1A}$*, *5-HT$_{1B}$*, *5-HT$_{2\alpha}$*, and *5-HT$_{7}$*, and obtained a 1386-bp cDNA fragment of *5-HT$_{1A}$,* a 1038-bp cDNA fragment of *5-HT$_{1B}$*, a 420-bp cDNA fragment of *5-HT$_{2\alpha}$*, and a 706-bp cDNA fragment of *5-HT$_{7}$* (GenBank accession numbers: AB618098, AB618099, AB618100, and AB618101, respectively). These genes have the following properties: *5-HT$_{1A}$*, partial ORF = 1–1332 bp, protein product = 443 amino acids; *5-HT$_{1B}$*, partial ORF = 1–991 bp, protein product = 329 amino acids; *5-HT$_{2\alpha}$*, partial ORF = 1–420 bp, protein product = 140 amino acids; *5-HT$_{7}$*, partial ORF = 17–706 bp, protein product = 230 amino acids (Fig. 5(A), 5(B), 6(A), and 7(A)).

5-HT$_{1A}$ and 5-HT$_{1B}$: The partial cDNA fragment of both *Gryllus* 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors encoded the five transmembrane (TM) segments corresponding to the third to seventh TM segments of GPCR (TM3, TM4, TM5, TM6, and TM7 in Fig. 5(A) and 5(B)). The third cytoplasmic loop (the region between TM5 and TM6) of the human 5-HT$_{1A}$ receptor contains two calmodulin (CaM)-binding sites (Turner *et al*., 2004). We compared the amino acid

sequence of the third cytoplasmic loop of *Gryllus* 5-HT_{1A} and 5-HT_{1B} receptors and the human 5-HT_{1A} receptor, and found two putative CaM-binding sites in the third cytoplasmic loop of both *Gryllus* 5-HT_{1A} and 5-HT_{1B} receptors (Fig. 5(A) and 5(B)).

Comparison of the deduced amino acid sequence of *Gryllus* 5-HT_{1A} with those of the corresponding part of other 5-HT_1 receptors indicated that *Gryllus* 5-HT_{1A} is closely related to the other known insect 5-HT_{1A} receptors (52.2% identical to *Antheraea* 5-HT_{1A}, 59.3% identical to *Tribolium* 5-HT_{1A}, and 66.8% identical to *Periplaneta* 5-HT_{1A} (Troppmann et al., 2010)). Comparison of the deduced amino acid sequence of *Gryllus* 5-HT_{1B} with those of other 5-HT_1 receptors indicated that *Gryllus* 5-HT_{1B} is structurally most similar to the other known insect 5-HT_{1A} (67.8% identical to *Antheraea* 5-HT_{1B}, 68.0% identical to *Tribolium* 5-$HT_{2\alpha}$, and 64.1% identical to *Nasonia* 5-HT_{1B}). The molecular phylogenetic analysis of 5-HT_1 receptors using the deduced amino acid sequence of the partial cDNA of *Gryllus* 5-HT_1 receptors and those of the corresponding part of various 5-HT receptors also indicated that *Gryllus* 5-HT_{1A} and 5-HT_{1B} are closely related to the insect 5-HT_{1A} and 5-HT_{1B} receptors, respectively (Fig. 6).

5-$HT_{2\alpha}$: The cDNA fragment of *Gryllus 5-$HT_{2\alpha}$* encoded the four TM segments corresponding to the first to fourth TM segments of GPCR (TM1, TM2, TM3, and TM4 in Fig. 7(A)). Comparison of the deduced amino acid sequence of *Gryllus* 5-$HT_{2\alpha}$ with those of other 5-HT_2 receptors indicated that *Gryllus* 5-$HT_{2\alpha}$ is structurally most similar to the other known insect 5-$HT_{2\alpha}$ (80.7% identical to *Drosophila* 5-$HT_{2\alpha}$ (Colas et al., 1995), 87.1% identical to *Tribolium* 5-$HT_{2\alpha}$, and 87.9% identical to *Apis* 5-$HT_{2\alpha}$ (Thamm et al., 2010)). The molecular phylogenetic analysis of 5-HT_2 receptors using the deduced amino acid sequence of the partial cDNA of *Gryllus* 5-$HT_{2\alpha}$ and those of the corresponding part of various 5-HT receptors also indicated that *Gryllus* 5-$HT_{2\alpha}$ is closely related to the insect 5-$HT_{2\alpha}$ proteins (Fig. 7(B)).

5-HT$_7$: The cDNA fragment of *Gryllus* 5-HT$_7$ encoded the five TM segments corresponding to the first to fifth TM segments of GPCR (TM1, TM2, TM3, TM4, and TM5 in Fig. 8(A)). Comparison of the deduced amino acid sequence of *Gryllus* 5-HT$_7$ with those of other 5-HT$_7$ receptors, cloned from both vertebrates and invertebrates, indicated that *Gryllus* 5-HT$_7$ is structurally most similar to the other known insect 5-HT$_7$ (55.4% identical to *Aedes* 5-HT$_7$ (Pietrantonio et al., 2001), 70.6% identical to *Tribolium* 5-HT$_7$, and 66.7% identical to *Apis* 5-HT$_7$ (Schlenstedt et al., 2006)). The molecular phylogenetic analysis of 5-HT$_7$ receptors using the partial deduced amino acid sequence of *Gryllus* 5-HT$_7$ and those of the corresponding part of various 5-HT receptors also indicated that *Gryllus* 5-HT$_7$ is closely related to the other insect 5-HT$_7$ receptors (Fig. 8(B)).

Tissue-specific expression patterns of 5-HT-related genes

Tissue-specific expression patterns of 5-HT-related genes were investigated by using RT-PCR analysis. The following ten tissues were subjected to RT-PCR analyses: the central brain, suboesophageal ganglion (SOG), optic lobe (lamina + medulla) + retina, corpus cardiacum-corpora allata complex (CC + CA), thoracic muscles, salivary gland, midgut, Malpighian tubules, testicle, and ovary.

First, we examined tissue-specific expression patterns of genes involved in the 5-HT synthesis (Fig. 9(A)). *Gryllus TRH*, *TPH*, and *AADC* genes showed differential expression levels in different tissues. An intensely stained band of PCR product of *Gryllus TRH* was detected in the lanes of the central brain, SOG, CC + CA, and ovary, whereas that of *Gryllus TPH* was detected in the lanes of the central brain, SOG, CC + CA, thoracic muscle, and salivary gland. A weaker stained band of PCR product of *Gryllus TRH* was detected in the lanes of the

ovary, and that of *Gryllus TPH* was detected in the lanes of the Malpighian tubules, testicle, and ovary. In the lane of the central brain, a 117 bp-longer PCR product corresponding to the *Gryllus TRH* long variant was detected. After extended amplification, PCR product of *TRH* was detected in the lanes of the optic lobe + retina, midgut, and testicle, and that of *TPH* was detected in the lanes of all of the examined tissues (supplementary figure 3). PCR product of *Gryllus AADC* was detected in the lanes of all of the examined tissues.

Gryllus 5-HT receptor genes exhibited receptor subtype-specific distributions (Fig. 9(B)). *Gryllus 5-HT$_{1A}$* and *5-HT$_{1B}$,* and *5-HT$_{7}$* genes were almost ubiquitously expressed in all of the examined tissues. An intensely stained band of PCR product of *5-HT$_{1A}$* was detected in the lanes of the central brain, SOG, CC + CA, testicle, and ovary. A weaker stained band of PCR product of *5-HT$_{1A}$* was detected in the lane of the optic lobe + retina, thoracic muscle, salivary gland, midgut, and Malpighian tubules. An intensely stained band of PCR product of *5-HT$_{1B}$* was detected in the lanes of the central brain, SOG, CC + CA, testicle, and ovary. A weaker stained band of PCR product of *5-HT$_{1B}$* was detected in the lane of the optic lobe + retina, thoracic muscle, salivary gland, and midgut. An intensely stained band of PCR product of *5-HT$_{7}$* was detected in the lanes of the salivary gland and midgut, and a weaker stained band was detected in the lanes of the other tissues.

The *Gryllus 5-HT$_{2}$* gene exhibited a tissue-specific expression pattern. An intensely stained band of PCR product of *5-HT$_{2}$* was detected in the lane of the salivary gland, and a weaker stained band was detected in the lanes of the central brain and SOG. In the lanes of the testicle and ovary, slightly stained bands were detected. PCR product of *5-HT$_{2}$* was detected in the lane of all examined tissues after extended amplification (supplementary figure 3).

DISCUSSION

5-HT functions as a neurotransmitter/neuromodulator, as a hormone, and as a signaling molecule that regulates segmentation during embryogenesis in insects (Nässel, 1988; Colas et al., 1999). To investigate the molecular basis of the 5-HT system in the cricket, we recently identified three genes involved in 5-HT synthesis (TRH, TPH, and AADC), as well as two 5-HT_1 receptors (5-HT_{1A} and 5-HT_{1B}), one 5-HT_2 receptor (5-$HT_{2\alpha}$), and one 5-HT_7 receptors in the cricket. We failed to identify the cricket homologue of the β type 5-HT_2 receptor (5-$HT_{2\beta}$). In a further study, we plan to identify and characterize the cricket homologue of the 5-$HT_{2\beta}$ receptor.

In insects, genes involved in the 5-HT synthesis were mainly characterized in *Drosophila melanogaster* (Livingstone and Tempel, 1983; Coleman and Neckameyer, 2005; Neckameyer et al., 2007). In *Drosophila*, the first and rate-limiting step of 5-HT biosynthesis is catalyzed by two tryptophan hydroxylases, *d*TRH and *d*TPH, which hydroxylate tryptophan to generate 5-HTP. *dTRH* is primarily expressed in neuronal tissues, and neuronal 5-HT was diminished by null-mutation of *dTRH* (Neckameyer and White, 1992). On the other hand, *dTPH* is thought to be involved in 5-HT biosynthesis in non-neuronal tissues. In the present study, we identified the cricket homologue of *dTRH* and *dTPH*. Interestingly, both *Gryllus TRH* and *TPH* genes were expressed in the CNS, although *Gryllus TRH* is primarily expressed in neuronal tissues while *Gryllus TPH* is more ubiquitously expressed. Our data suggest that TRH- and TPH-mediated 5-HT biosynthesis pathways are not compartmentalized into neuronal and peripheral tissues in the cricket, and the cricket CNS has two distinct mechanisms of the regulation of 5-HT synthesis.

Our expression analysis revealed that mRNA of AADC, which catalyze the second step of 5-HT biosynthesis, was ubiquitously distributed in the cricket. In addition to 5-HT synthesis,

AADC also catalyzes the formation of dopamine, which functions as a neurotransmitter/neuromodulator and is metabolized to produce melanine. Melanin plays essential roles in cuticular sclerotization and the cellular immune response in insects, and *AADC* is up-regulated during the innate immune response in *Drosophila* (Davis et al., 2007). Ubiquitous expression of *AADC* might reflect its functional importance in various biochemical pathways.

With the exception of the 5-HT_3 receptor and the *Caenorhabditis elegans* 5-HT-gated Cl^- channels (MOD-1 and LGC-40) (Ranganathan et al., 2000; Ringstad et al., 2009), all of the 5-HT receptors belong to the G-protein-coupled receptor (GPCR) superfamily. GPCR-type 5-HT receptors are one of the most ancient and diversified groups of the ligand-dependent GPCR in animals, and are classified into three subtypes (5-HT_1/5-HT_4/5-HT_5/5-HT_7, 5-HT_2, and 5-HT_6). All known arthropod 5-HT receptors belong to the 5-HT_1/5-HT_7 and 5-HT_2 receptor subtypes. Each 5-HT receptor is coupled with receptor subtype-specific signal transduction mechanisms: Activation of *Drosophila* 5-HT_{1A} and 5-HT_{1B} receptors leads to decrease in cAMP formation and increase in inositol 1,4,5- triphosphate (IP_3) (Saudou et al., 1992). Activation of the *Drosophila* 5-HT_7 receptor leads to increase in cAMP formation. Since the downstream signal transduction of the *Drosophila* 5-HT_2 receptor has not yet been characterized, activation of the receptor might stimulate phospholipase C, resulting in increase in IP_3.

Basically, insects have two 5-HT_1 receptors (5-HT_{1A} and 5-HT_{1B}). Our phylogenetic analysis of 5-HT_1 receptors indicated that the diversification of two insect 5-HT_1 receptor subtypes occurred after the arthropod-nematode divergence (Fig. 6). Interestingly, our data indicated that two *Drosophila* 5-HT_1 receptors structurally resemble each other, and both receptors were classified into the insect 5-HT_{1A} receptor. Two *Drosophila* 5-HT_1 receptor

genes are mapped in adjacent loci (56B1-56B5 on chromosome 2R), and are present in an opposite orientation. Genomic organization of the genes suggests that the *Drosophila* 5-HT_{1A} and 5-HT_{1B} genes were produced by inverted tandem duplication. Moreover, the *Drosophila* genome lacks gene(s) that encode homologue of the insect 5-HT_{1B} receptor subtypes. These data suggest a rapid evolution of the insect 5-HT receptor family in *Drosophila*. Comparative studies of the expression and function of 5-HT receptors may provide insights into the evolutionary modification of the insect 5-HT system.

5-HT is widely distributed in the CNS and in peripheral tissues of both vertebrates and invertebrates. The 5-HT system is involved in modulating various behaviors in the insect CNS, and in the peripheral tissue, 5-HT controls various physiological phenomena such as heart beat, salivary gland secretion, diuresis in the Malpighian tubules (Berridge and Patel, 1968; Collins and Miller, 1977; Maddrell et al., 1991). To elucidate the sites of 5-HT action in the cricket, we investigated the tissue-specific expression patterns of four 5-HT receptor genes in the cricket.

Central nerve system: Our expression analysis revealed that four 5-HT receptors were expressed in the cricket CNS. In the insect CNS, the 5-HT system regulates various behaviors via distinct 5-HT receptor pathways. In *Drosophila*, mutants of the 5-HT_{1A} receptor show a sleep defect, whereas the 5-HT_{1B} receptor is involved in the entrainment of circadian rhythms (Yuan et al., 2005; Yuan et al., 2006). In the *Drosophila* CNS, the 5-HT_2 receptor is expressed in the protocerebrum and ellipsoid body, and is involved in modulating circadian behaviors (Nichols 2007). Moreover, the inhibition of 5-HT_1 receptors and the 5-HT_2 receptor had differential effects on aggressive behaviors (Johnson et al., 2009). In the cricket optic lobe, the 5-HT_7-like receptor is involved in the regulation of circadian pacemaker neurons (Saifullah and

Tomioka, 2003). Functional analysis of each 5-HT receptor in the cricket CNS will reveal the differential involvement of specific 5-HT receptor subtypes to behavior.

Recently, the distribution of 5-HT_{1A} and 5-HT_{1B} receptor proteins were investigated in two cricket species (*Dianemobius nigrofasciatus* and *Allonemobius allardi*) using antibodies produced against the C-terminal Arg/Lys-rich region of the silk moth *Antheraea pernyi* 5-HT_1 receptors (5-HT_{1A} epitope peptide, AFARILFGTHRRGRNKKF; 5-HT_{1B} epitope peptide, RHAFQRLLCGRRVRRRRAPP) (Shao et al., 2010). However, the amino acid sequences of the epitope peptides were quite different from the corresponding parts of *Gryllus* 5-HT_{1A} and 5-HT_{1B} receptors (5-HT_{1A}, AFKRILCGSGRRSRSRKMR; 5-HT_{1B}, RHAFKRILCGRRSARRRNRHFGVRYLQ; differential residues are indicated by underlines). Furthermore, the molecular mass of the proteins detected with the anti-*Antheraea* 5-HT_{1A} and anti-*Antheraea* 5-HT_{1B} antibodies were approximately equal to or even smaller than the calculated molecular mass of the partial sequence of *Gryllus* 5-HT_1 receptors which lack the N-terminal extracellular region and first two transmembrane segments (TM1 and TM2). These data raise a question about the reliability of the immunohistochemical assays for the cricket 5-HT_1 receptors in the previous study. Detailed histochemical expression analyses at mRNA and protein levels are needed in further studies.

Corpus cardiacum-corpora allata complex: The corpus cardiacum-corpora allata complex (CC + CA) of crickets contains biogenic amines including 5-HT (Iba et al., 1996). In the cricket *Teleogryllus commodus*, the 5-HT-immunoreactive axons, which presumably originate from pars intercerebralis and pars lateralis of the brain, innervate the CC + CA (Pipa and Moore, 1988). Our data demonstrated that the CC + CA of the cricket express four 5-HT receptor genes, suggesting that the 5-HT system regulates the activity of CC + CA in the cricket. The

amount of biogenic amines in the cricket CC + CA changes in relation to population density (Iba et al., 1995). In the cricket *G. rubens*, a population density-dependent wing polymorphism is regulated by juvenile hormone, and juvenile hormone production in the corpora allata of the larval honeybee is stimulated by octopamine and 5-HT (Rachinsky, 1994). These data allow us to hypothesize that environmental cues (i.e. population density) alter the activity of aminergic systems that affect the activity of the corpus cardiacum-corpora allata complex in the cricket.

Thoracic muscle: Neuromuscular transmission is modulated by 5-HT in both vertebrates and invertebrates. In the lobster neuromuscular junction, 5-HT acts on both presynaptic sites and muscle fibers to facilitate transmitter release, to produce a contracture, and to induce calcium action potentials (Kraits et al., 1980). Expression of 5-HT receptors in the cricket thoracic muscles suggests that the 5-HT-mediated modulation of the skeletal muscle activity is present in the cricket.

Salivary gland: Our expression analysis revealed that the salivary gland of the cricket expresses all four types of 5-HT receptors. In insects, salivation is stimulated by 5-HT, and 5-HT induced secretion is mediated by an increase of intracellular cAMP (Berridge and Patel, 1968; Rietdorf et al., 2005; Troppmann et al., 2007). Of the four 5-HT receptor subtypes, the 5-HT_7 receptor is positively coupled to adenylyl cyclase. Therefore, 5-HT-mediated stimulation of salivation might be mainly mediated by the 5-HT_7 receptor. Further studies are necessary to reveal functional roles of the other three 5-HT receptor subtypes expressed in the salivary gland.

Midgut: A pharmacological study on the locust midgut showed that application of 5-HT leads to relaxation of the midgut circular muscle (Molaei and Lange, 2003). In the midgut of the blood sucking bug *Rhodnius prolixus*, intracellular cAMP was elevated in response to 5-HT (Barrett et al., 1993). Although the pharmacological profile of the 5-HT receptor of the *Rhodnius* midgut

resembled the mammalian 5-HT_2 receptor, the 5-HT_2 receptor is not positively coupled to adenylyl cyclase activation. Our study revealed that the 5-HT_7 receptor, which is positively coupled to adenylyl cyclase, is expressed in the cricket midgut. Therefore, 5-HT-induced elevation of cAMP in the midgut might be mediated by the 5-HT_7 receptor. In mammals, the 5-HT_7 receptor also mediates smooth muscle relaxation in the gastrointestinal tract (Vanhoenacker et al., 2000). 5-HT_7 receptor-mediated regulation of smooth muscle activity in the gastrointestinal tract might thus be evolutionary conserved between vertebrates and invertebrates.

Malpighian tubules: 5-HT acts on the Malpighian tubules as a diuretic hormone in insects (Maddrell et al., 1991). Our expression analysis revealed that the Malpighian tubules mainly expressed 5-HT_{1A} and 5-HT_7 genes. Expression of 5-HT_1 receptors in the Malpighian tubules have also been shown in *Periplaneta* (Troppmann et al., 2010) and *Apis* (Thamm et al., 2010), and expression of 5-HT_7 receptor in the Malpighian tubules have been shown in *Aedes* (Pietrantonio et al., 2001) and *Apis* (Schlenstedt et al., 2006). In the larvae of the mosquito *Aedes aegypti*, 5-HT is released into the hemolymph in response to increased salinity, and stimulates fluid secretion activity of the Malpighian tubules (Clark et al., 1998). In the Malpighian tubules of *Locusta migratoria*, application of 5-HT neither lead to an increase of the intracellular cAMP level nor to an activation of adenylyl cyclase (Morgan and Mordue, 1984). Therefore, 5-HT_{1A}, which is negatively coupled to adenylyl cyclase, might be involved in the 5-HT-mediated stimulation of fluid secretion in the Malpighian tubules.

Testicle and ovary: Almost all of the genes involved in 5-HT biosynthesis and 5-HT receptors were expressed in the testicle and ovary. Serotonergic regulation of the oviduct contraction was reported in several insects (Bamji and Orchard, 1995; Lange, 2004). A pharmacological study on cockroaches revealed that the pharmacological property of the oviduct 5-HT receptor

resembles the vertebrate $5HT_2$ receptor (Bamji and Orchard, 1995). Our expression analysis indicated that the cricket ovary expresses not only 5-$HT_{2\alpha}$ but also 5-HT_1 and 5-HT_7 receptors. Our data suggest that 5-HT regulates not only oviduct contraction but also other ovarian activities mediated by the other subtypes of the 5-HT receptors. Moreover, to our knowledge, our study is the first report on expression of 5-HT receptors in the insect testicle. Further studies are necessary to reveal physiological roles of 5-HT on gonadal functions in insects.

In summary, we identified seven genes involved in the synthesis and transduction of 5-HT in the cricket *G. bimaculatus*. Two tryptophan hydroxylase genes, *TRH* and *TPH*, were co-expressed in the cricket CNS, suggesting that the two 5-HT synthesis pathways were not compartmentalized in neuronal and peripheral tissues. Tissue-specific expression analysis of 5-HT receptors showed that the 5-HT system is widely distributed in the cricket, and that the 5-HT system might regulate various aspects of physiological phenomena via distinct 5-HT receptor pathways.

EXPERIMENTAL PROCEDURES

Animals

Crickets *Gryllus bimaculatus* DeGeer were reared on a 14-h:10-h light/dark cycle at 27°C. They were fed a diet of insect food pellet (Oriental Yeast Co., Tokyo, Japan), chopped carrot and water *ad libitum*.

Complementary DNA Cloning of *AADC*, *TPH*, *TRH*, and four 5-HT receptor genes

First, we cloned partial cDNAs of *Gryllus AADC*, *TPH*, *TRH*, and four 5-HT receptor genes (*5-HT$_{1A}$*, *5-HT$_{1B}$*, *5-HT$_{2\alpha}$*, and *5-HT$_{7}$*) by reverse transcriptase-polymerase chain reaction (RT-PCR) techniques. Next, we performed the 5' and 3' rapid amplification of cDNA ends (RACE) using the FirstChoice RLM-RACE kit (Ambion, Austin, TX, USA). Finally, we performed RT-PCR to amplify the cDNA fragments containing the full-length or partial open reading frame (ORF) of the genes and registered their sequences in GenBank.

Complementary DNA preparation for partial cDNA cloning

The cricket brain was isolated in ice-cold phosphate-buffered saline (PBS, pH 7.4). Total RNA was isolated from the brain with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and treated with DNase I (Takara, Shiga, Japan) at 37^{o}C for 1 h. Total RNA was reverse-transcribed using Superscript III reverse transcriptase (Invitrogen) with a random decamer.

Partial cDNA cloning of *TRH*, *TPH*, *AADC*, and four 5-HT receptor genes

***TRH*:** Partial cDNA of *TRH* was amplified using degenerate primers. Degenerate primers were designed on the basis of conserved amino acid sequences (GLAFR and YTPEPD) among the insect TPH proteins. Sequences of the degenerate primers are listed in Table 1.

***TPH*:** Partial cDNA of *TPH* was amplified using GSPs designed on the basis of the *G. bimaculatus* EST clones corresponding to *TRH* (GenBank accession numbers; AK264489, AK268330, and AK270171).

***AADC*:** Partial cDNA of *AADC* was amplified using GSPs designed on the basis of the *G. bimaculatus* EST clones corresponding to *AADC* (GenBank accession numbers; DC443272 and AK278380).

***5-HT$_{1A}$*:** Partial cDNA of *5-HT$_{1A}$* was amplified using degenerate primers. Degenerate primers were designed on the basis of conserved amino acid sequences (TLAIITGAFV and WLGYFNST) among the insect 5-HT$_{1A}$ receptor proteins. Sequences of the degenerate primers are listed in Table 1.

***5-HT$_{1B}$*:** Partial cDNA of *5-HT$_{1B}$* was amplified using degenerate primers. Degenerate primers were designed on the basis of conserved amino acid sequences (LAVADLM and DRYWAVT) among insect 5-HT$_{1B}$ proteins. Sequences of the degenerate primers are listed in Table 1.

***5-HT$_{2\alpha}$*:** Partial cDNA of *5-HT$_{2\alpha}$* was amplified using degenerate primers. Degenerate primers were designed on the basis of conserved amino acid sequences (KLQNVTNYFL and LHMCFIGL) among the insect 5-HT$_2$ receptor proteins. Sequences of the degenerate primers are listed in Table 1.

***5-HT$_7$*:** Partial cDNA of *5-HT$_7$* was amplified using degenerate primers. Degenerate primers were designed on the basis of conserved amino acid sequences (LVMPMA and CMISVDR)

among the insect 5-HT_7 receptor proteins. Sequences of the degenerate primers are listed in Table 1.

5' RACE and 3' RACE

To extend partial cDNA clones by amplifying the 5' and 3' sequences of the corresponding mRNAs, we performed 5' and 3' RACEs using the FirstChoice RLM-RACE kit. For the 5' RACE, the cricket brain total RNA was modified using the kit, and was reverse-transcribed using Superscript III reverse transcriptase (Invitrogen) with a random decamer as a primer. For the 3' RACE, the cricket brain total RNA was reverse-transcribed using Superscript III reverse transcriptase (Invitrogen) with a 3' RACE adapter packaged in the kit as a primer. The RACE PCR was carried out using the GSPs designed on the basis of the sequence obtained by the partial cDNA cloning described above.

Complementary DNAs containing the full-length or partial ORF were amplified using the GSPs designed at the 5' and 3' end of the RACE fragments. The primers used to amplify the cDNAs containing the full-length or partial ORF are listed in Table 1.

All PCRs were performed using *LA Taq* polymerase (TaKaRa), *Taq* Blend HighFidelity polymerase (Greiner Bio-One, Tokyo, Japan), *Pfu*-X polymerase (Greiner Bio-One), or Phusion High-Fidelity DNA polymerase (Roche Applied Science, Tokyo, Japan). The amplified DNA fragments were subcloned into the plasmid vector pGEM-T easy (Promega, Madison, WI, USA), and the nucleotide sequences were determined.

Sequence comparison, prediction of transmembrane regions, and phylogenetic analysis

The deduced full amino acids sequences of *Gryllus* TRH short variant, TPH, and AADC, and the deduced partial amino acids sequences of four *Gryllus* 5-HT receptors were compared with those of the corresponding parts of the known homologous genes of other species by using the MAFFT or MUSCLE algorisms on the Geneious 5.3 program (Drummond et al., 2010). Transmembrane regions of 5-HT receptors were predicted by the TMHMM v. 2.0 program (Sonnhammer *et al*., 1998; http://www.cbs.dtu.dk/services/TMHMM/). Phylogenetic trees were constructed with the aligned sequences by the bootstrap neighbor-joining algorithms on the Geneious 5.3 program. The obtained tree was visualized with the Geneious 5.3 program. The GenBank accession numbers of the proteins used for the comparison are listed in Table 3.

Tissue-specific expression analysis

RT-PCR analysis was performed to assess the tissue-specific expression of the *Gryllus TRH*, *TPH*, *AADC*, and four 5-HT receptor genes. The following tissues were subjected to expression analysis: (1) the central brain, (2) suboesophageal ganglion (SOG), (3) optic lobe (lamina + medulla) and retina, (4) corpus cardiacum-corpora allata complex (CC + CA), (5) thoracic muscles, (6) salivary glands, (7) midgut, (8) Malpighian tubules, (9) testis, and (10) ovary. Seven days after emergence, adult crickets were dissected in ice cold PBS, and total RNA of each tissue was extracted using the TRIzol reagent (Invitrogen). Then, total RNAs were treated with DNase I (TaKaRa) at 37^{o}C for 1 h. 1 μg of each total RNA was reverse-transcribed in a 20 μl reaction using the Transcriptor First Strand cDNA Synthesis Kit (Roche Applied Science, Tokyo, Japan). A random hexamer and an anchored oligo(dT)$_{18}$ primer were

used as primers. PCR was carried out using *Ex* taq polymerase (TaKaRa). For first PCR amplification, 0.2 µl of cDNA solution was added to a 10 µl PCR reaction. For extended amplification (secondary PCR), 0.4 µl of the first PCR product was added to a 10 µl PCR reaction. PCR amplification of target genes was performed for 35 cycles at 96°C for 15 s, 58 ºC for 15 s, and 72°C for 60 s, followed by a final extension at 72°C for 5 min. PCR products were run through a 1.5% agarose gel and visualized by ethidium bromide. *Ef1alpha*, *ß-actin*, and *rp49* genes were amplified as internal control genes. To confirm that genomic DNA was not present in cDNA solutions, we amplified *Ef1alpha*, *ß-actin*, and *rp49* genes and checked the absence of an amplification product in the RT negative controls (RT(-)). Primers used for the RT-PCR analysis are listed in Table 1.

ACKNOWLEDGEMENTS

We thank Dr. Miriam Henze (Lunds Universitet, Sweden) for reviewing. This research was funded in part by grants-in-aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology, Scientific Research on Priority Areas (Area No. 454) to H. Aonuma (17075001).

REFERENCES

Bamji, S.X., Orchard, I. (1995) Pharmacological profile of octopamine and 5HT receptor on the lateral oviducts of the cockroach, *Periplaneta americana*. *Arch Ins Bioch Phys* **28**:49–62.

Barrett, F.M., Orchard, I., Tebrugge, V. (1993) Characteristics of serotonin-induced cyclic AMP elevation in the integument and anterior midgut of the blood-feeding bug, *Rhodnius prolixus.* *J Insect Physiol* **39**:581–587.

Baumann, O., Dames, P., Kühnel, D., Walz, B. (2002) Distribution of serotonergic and dopaminergic nerve fibers in the salivary gland complex of the cockroach *Periplaneta americana*. *BMC Physiol* **2**:9.

Baumann, O., Kühnel, D., Dames, P., Walz, B. (2004) Dopaminergic and serotonergic innervation of cockroach salivary glands: distribution and morphology of synapses and release sites. *J Exp Biol* **207**:2565–2575.

Berridge M.J., Patel N.G. (1968) Insect salivary glands: stimulation of fluid secretion by 5-hydroxytryptamine and adenosine-3',5'-monophosphate. *Science* **162**:462-463.

Berridge, M.J. (1970) The role of 5-hydroxytryptamine and cyclic AMP in the control of fluid secretion by isolated salivary glands. *J Exp Biol* **53**:171–186.

Blenau, W., Thamm, M. (2011) Distribution of serotonin (5-HT) and its receptors in the insect brain with focus on the mushroom bodies. Lessons from *Drosophila melanogaster* and *Apis mellifera*. *Arthropod Struct Dev*, in press.

Brody, T., Cravchik, A. (2000) *Drosophila melanogaster* G protein-coupled receptors. *J Cell Biol* **150**:F83–88.

Clark, T.M., Koch, A.R., Moffett, D.F. (1998) Malpighian tubules of larval *Aedes aegypti* are hormonally stimulated by 5-hydroxytryptamine in response to increased salinity. *Arch Insect Biochem Physiol* **34**:123–141.

Colas, J.F., Launay, J.M., Kellermann, O., Rosay, P., Maroteaux, L. (1995) *Drosophila* 5-HT_2 serotonin receptor: coexpression with fushi-tarazu during segmentation. *Proc Natl Acad Sci U S A* **92**:5441-5445.

Colas, J.F., Launay, J.M., Vonesch, J.L., Hickel, P., Maroteaux, L. (1999) Serotonin synchronises convergent extension of ectoderm with morphogenetic gastrulation movements in *Drosophila*. *Mech Dev* **87**:77–91.

Coleman, C.M., Neckameyer, W.S. (2005) Serotonin synthesis by two distinct enzymes in *Drosophila melanogaster*. *Arch Insect Biochem Physiol* **59**:12–31.

Collins, C., Miller, T. (1977) Studies on the action of biogenic amines on cockroach heart. *J Exp Biol* **67**:1–15.

Davis, M.M., Primrose, D.A., Hodgetts, R.B. (2008) A member of the p38 mitogen-activated protein kinase family is responsible for transcriptional induction of Dopa decarboxylase in the epidermis of *Drosophila melanogaster* during the innate immune response. *Mol Cell Biol* **28**:4883–4895.

Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Heled, J., Kearse, M., Moir, R., Stones-Havas, S., Sturrock, S., Thierer, T., Wilson, A. (2010) Geneious v5.1, Available from http://www.geneious.com

Iba, M., Nagao, T., Urano, A. (1995) Effects of Population Density on Growth, Behavior and Levels of Biogenic Amines in the Cricket, *Gryllus bimaculatus*. *Zool Sci* **12**:695–702.

Iba, M., Nagao, T., Urano, A. (1996) The levels of biogenic amines in the corpora allata, corpora cardiaca and frontal ganglion in the cricket, *Gryllus bimaculatus*. *Zool Sci* **13**:213–217.

Johnson, O., Becnel, J., Nichols, C.D. (2009) Serotonin 5-HT_2 and 5-HT_{1A}-like receptors differentially modulate aggressive behaviors in *Drosophila melanogaster*. *Neuroscience* **158**:1292–1300.

Kravitz, E.A., Glusman, S., Harris-Warrick, R.M., Livingstone, M.S., Schwarz, T., Goy, M.F. (1980) Amines and a peptide as neurohormones in lobsters: actions on neuromuscular preparations and preliminary behavioural studies. *J Exp Biol* **89**:159–175.

Lange, A.B. (2004) A neurohormonal role for serotonin in the control of locust oviducts. *Arch Insect Biochem Physiol* **56**:179–190.

Livingstone, M.S., Tempel, B.L. (1983) Genetic dissection of monoamine neurotransmitter synthesis in *Drosophila*. *Nature* **303**:67–70.

Maddrell, S.H.P., Herman, W.S., Mooney, R.L., Overton, J.A. (1991) 5-hydroxytryptamine: a second diuretic hormone in *Rhodnius prolixus*. *J Exp Biol* **156**:557–566.

Molaei, G., Lange, A.B. (2003) The association of serotonin with the alimentary canal of the African migratory locust, *Locusta migratoria*: distribution, physiology and pharmacological profile. *J Insect Physiol* **49**:1073–1082.

Morgan, P.J., Mordue, W. (1984) 5-Hydroxytryptamine stimulates fluid secretion in locust malpighian tubules independently of cAMP. *Comp Biochem Physiol C* **79**:305–310.

Nagao, T., Tanimura, T. Shimozawa, T. (1991) Neurohormonal control of the mating interval in the male cricket, *Gryllus bimaculatus* DeGeer. *J Comp Physiol* **168**:159–164.

Nässel, D.R. (1988) Serotonin and serotonin-immunoreactive neurons in the nervous system of insects. *Prog Neurobiol* **30**:1-85.

Neckameyer, W.S., White, K. (1992) A single locus encodes both phenylalanine hydroxylase and tryptophan hydroxylase activities in *Drosophila*. *J Biol Chem* **267**:4199–4206.

Neckameyer, W.S., Coleman, C.M., Eadie, S., Goodwin, S.F. (2007) Compartmentalization of neuronal and peripheral serotonin synthesis in *Drosophila melanogaster*. *Genes Brain Behav* **6**:756–769.

Nichols, C.D. (2007) 5-HT2 receptors in *Drosophila* are expressed in the brain and modulate aspects of circadian behaviors. *Dev Neurobiol* **67**:752–763.

Pietrantonio P.V., Jagge C., McDowell C. (2001) Cloning and expression analysis of a $5HT_7$-like serotonin receptor cDNA from mosquito *Aedes aegypti* female excretory and respiratory systems. *Insect Mol Biol*. **10**:357–369.

Pipa, R., Moore, D. (1988) Serotonin-immunoreactive neurons in the retrocerebral neuroendocrine complex of the cricket *Teleogryllus commodus* (Walker) (Orthoptera : Gryllidae) and cockroach *Periplaneta americana* (L.) (Dictyoptera : Blattidae). *Int J Insect Morphol Embryol* **17**:303–311.

Rachinsky, A. (1994) Octopamine and serotonin influence on corpora allata activity in honey bee (*Apis mellifera*) larvae. *J Insect Physiol* **40**:549–554.

Ranganathan, R., Cannon, S.C., Horvitz, H.R. (2000) MOD-1 is a serotonin-gated chloride channel that modulates locomotory behaviour in *C. elegans*. *Nature* **408**:470–475.

Rietdorf, K., Blenau, W., Walz, B. (2005) Protein secretion in cockroach salivary glands requires an increase in intracellular cAMP and Ca^{2+} concentrations. *J Insect Physiol* **51**:1083–1091.

Ringstad, N., Abe, N., Horvitz, H.R. (2009) Ligand-gated chloride channels are receptors for biogenic amines in *C. elegans*. *Science* **325**:96–100.

Saifullah, A.S., Tomioka, K. (2002) Serotonin sets the day state in the neurons that control coupling between the optic lobe circadian pacemakers in the cricket *Gryllus bimaculatus*. *J Exp Biol* **205**:1305–1314.

Saifullah, A.S., Tomioka, K. (2003) 5-HT(7)-like receptors mediate serotonergic modulation of photo-responsiveness of the medulla bilateral neurons in the cricket, *Gryllus bimaculatus*. *Zoolog Sci* **20**:303–309.

Saudou, F., Boschert, U., Amlaiky, N., Plassat, J.L., Hen, R. (1992) A family of *Drosophila* serotonin receptors with distinct intracellular signalling properties and expression patterns. *EMBO J* **11**:7–17.

Schlenstedt J., Balfanz S., Baumann A., Blenau W. (2006) Am5-HT_7: molecular and pharmacological characterization of the first serotonin receptor of the honeybee (*Apis mellifera*). *J Neurochem*. **98**:1985-1998.

Shao, Q.M., Fouda, M.M., Takeda, M. (2010) Serotonin- and two putative serotonin receptors-like immunohistochemical reactivities in the ground crickets *Dianemobius nigrofasciatus* and *Allonemobius allardi*. *J Insect Physiol* **56**:1576–1586.

Sonnhammer, E.L., von Heijne, G., Krogh, A. (1998) A hidden Markov model for predicting transmembrane helices in protein sequences. *Proc Int Conf Intell Syst Mol Biol* **6**:175–182.

Stevenson, P.A., Hofmann, H.A., Schoch, K., Schildberger, K. (2000) The fight and flight responses of crickets depleted of biogenic amines. *J Neurobiol* **43**:107–120.

Stevenson, P.A., Dyakonova, V., Rillich, J., Schildberger, K. (2005) Octopamine and experience-dependent modulation of aggression in crickets. *J Neurosci* **25**:1431–1441.

Thamm, M., Balfanz, S., Scheiner, R., Baumann, A., Blenau, W. (2010) Characterization of the 5-HT_{1A} receptor of the honeybee (*Apis mellifera*) and involvement of serotonin in phototactic behavior. *Cell Mol Life Sci* **67**:2467–2479.

Tierney, A.J. (2001) Structure and function of invertebrate 5-HT receptors: a review. *Comp Biochem Physiol A Mol Integr Physiol* **128**:791–804.

Troppmann, B., Walz, B., Blenau, W. (2007) Pharmacology of serotonin-induced salivary secretion in *Periplaneta americana*. *J Insect Physiol* **53**:774–781.

Troppmann, B., Balfanz, S., Baumann, A., Blenau, W. (2010) Inverse agonist and neutral antagonist actions of synthetic compounds at an insect 5-HT_1 receptor. *Br J Pharmacol* **159**:1450–1462.

Turner, J.H., Gelasco, A.K., Raymond, J.R. (2004) Calmodulin interacts with the third intracellular loop of the serotonin 5-hydroxytryptamine1A receptor at two distinct sites: putative role in receptor phosphorylation by protein kinase C. *J Biol Chem* **279**:17027–17037.

Unoki, S., Matsumoto, Y., Mizunami, M. (2005) Participation of octopaminergic reward system and dopaminergic punishment system in insect olfactory learning revealed by pharmacological study. *Eur J Neurosci* **22**:1409–1416.

Unoki, S., Matsumoto, Y., Mizunami, M. (2006) Roles of octopaminergic and dopaminergic neurons in mediating reward and punishment signals in insect visual learning. *Eur J Neurosci* **24**: 2031–2038.

Vanhoenacker, P., Haegeman, G., Leysen, J.E. (2000) 5-HT7 receptors: current knowledge and future prospects. *Trends Pharmacol Sci* **21**:70–77.

Walz, B., Baumann, O., Krach, C., Baumann, A., Blenau, W. (2006) The aminergic control of cockroach salivary glands. *Arch Insect Biochem Physiol* **62**:141–152.

Yuan, Q., Lin, F., Zheng, X., Sehgal, A. (2005) Serotonin modulates circadian entrainment in *Drosophila*. *Neuron* **47**:115–127.

Yuan, Q., Joiner, W.J., Sehgal, A. (2006) A sleep-promoting role for the *Drosophila* serotonin receptor 1A. *Curr Biol* **16**:1051–1062.

FIGURES AND TABLES

Figure 1

5-HT synthesis pathway in insects. 5-HT is synthesized from L-tryptophan in two reaction steps. Tryptophan hydroxylase (*TRH*) and phenylalanine-tryptophan hydroxylase (*TPH*) convert L-tryptophan to 5-hydroxy-L-tryptophan (5-HTP), and aromatic L-amino acid decarboxylase (AADC) converts 5-HTP to 5-HT. The TRH pathway and TPH pathway are compartmentalized in neuronal and peripheral tissues in *Drosophila* (Neckameyer et al., 2007)**.**

Figure 2

Comparison of amino acid sequences of *Gryllus* TRH and TPH with their *Drosophila* homologues. (A) Alignment of the deduced amino acid sequence of *Gryllus* TRH short variant with that of *Drosophila* TRH. (B) Alignment of the deduced amino acid sequence of *Gryllus* TPH with that of *Drosophila* TPH. Identical amino acids are printed in white letters on a black background. The catalytic domain is surrounded by a gray box. Black lines indicate the N-terminal regulatory (ACT) domain and the C-terminal coiled-coil region. The tetrahydrobiopterin (BH4)-binding sites and iron binding sites are indicated by white and black arrowheads, respectively.

Figure 3

Molecular phylogenetic tree of *Gryllus* TRH and TPH with other aromatic amino acid hydroxylases. Insect and vertebrate tyrosine hydroxylases (TH) are used as an outgroup. The

scale bar indicates 0.06 substitutions per site. The GenBank accession numbers of the proteins used for phylogenetic analysis are listed in Table 2.

Figure 4

Comparison of amino acid sequences and phylogenetic tree of *Gryllus* AADC including other AADC proteins. (A) Alignment of the deduced amino acid sequence of *Gryllus* AADC with that of *Drosophila* AADC. Identical amino acids are printed in white letters on a black background. The catalytic domain is surrounded by a gray box. Black lines indicate the putative catalytic loop. The pyridoxal 5'-phosphate binding sites are indicated by black arrowheads. (B) Molecular phylogenetic tree of *Gryllus* AADC and other amino acid decarboxylases. *Drosophila* glutamate decarboxylases (GAD1 and GAD2) are used as an outgroup. The scale bar indicates 0.2 substitutions per site. The scale bar indicates 0.09 substitutions per site. The GenBank accession numbers of the proteins used for phylogenetic analysis are listed in Table 2.

Figure 5

Comparison of amino acid sequences of two *Gryllus* 5-HT_1 receptors (5-HT_{1A} and 5-HT_{1B}) with other 5-HT_1 receptors. (A) Alignment of the deduced amino acid sequence of *Gryllus* 5-HT_{1A} with that of *Antheraea* 5-HT_{1A}. (B) Alignment of the deduced amino acid sequence of *Gryllus* 5-HT_{1B} with that of *Antheraea* 5-HT_{1B}. Identical amino acids are printed in white letters on a black background. Black lines indicate the transmembrane segments (TM). Putative calmodulin (CaM)-binding sites are surrounded by a gray box. The GenBank accession numbers of *Antheraea* 5-HT_1 receptors are listed in Table 2.

Figure 6

Molecular phylogenetic tree of *Gryllus* 5-HT_1 receptors and other 5-HT_1 receptors. *Drosophila* 5-HT_2 and 5-HT_7 receptors are used as an outgroup. The scale bar indicates 0.08 substitutions per site. The GenBank accession numbers of the proteins used for phylogenetic analysis are listed in Table 2.

Figure 7

Comparison of amino acid sequences and phylogenetic tree of *Gryllus* 5-$HT_{2\alpha}$ receptor including other 5-HT_2 receptors. (A) Alignment of the deduced amino acid sequence of *Gryllus* 5-$HT_{2\alpha}$ with that of *Drosophila* 5-$HT_{2\alpha}$. Identical amino acids are printed in white letters on a black background. Black lines indicate the transmembrane segments (TM). (B) Molecular phylogenetic tree of *Gryllus* 5-$HT_{2\alpha}$ receptor and other 5-HT_2 receptors. *Drosophila* 5-HT_1 and 5-HT_7 receptors are used as an outgroup. The scale bar indicates 0.08 substitutions per site. The GenBank accession numbers of the proteins used for phylogenetic analysis are listed in Table 2.

Figure 8

Comparison of amino acid sequences and phylogenetic tree of *Gryllus* 5-HT_7 receptor including other 5-HT_7 receptors. (A) Alignment of the deduced amino acid sequence of *Gryllus* 5-HT_7 with that of *Aedes* 5-HT_7. Identical amino acids are printed in white letters on a black background. Black lines indicate the transmembrane segments (TM). The putative calmodulin (CaM)-binding site is surrounded by a gray box. (B) Molecular phylogenetic tree

of *Gryllus* 5-HT_7 receptor and other 5-HT_7 receptors. *Drosophila* 5-HT_1 and 5-HT_2 receptors are used as an outgroup. The scale bar indicates 0.09 substitutions per site. The GenBank accession numbers of the proteins used for phylogenetic analysis are listed in Table 2.

Figure 9

Tissue-specific expression patterns of *Gryllus TRH*, *TPH*, *AADC*, and four 5-HT receptor genes. (A) RT-PCR analysis for *Gryllus TRH*, *TPH*, and *AADC* genes. (B) RT-PCR analysis for *Gryllus 5-HT_{1A}*, *5-HT_{1B}*, *5-$HT_{2\alpha}$*, and *5-HT_7* genes. *Gryllus Ef1alpha* gene was used as a internal control gene (see supplementary figure 2). SOG = suboesophageal ganglion; CC + CA = corpus cardiacum-corpora allata complex.

Table 1. List of primers used in this study

Degenerate primers		
#	Forward primer	Reverse primer
TRH	5'-GGVYTVGCNTTCCG-3'	5'-CBGGTTCNGGHGTGTA-3'
5-HT$_{1A}$	5'-GCNATNATYACNGGNGCNTTCG-3'	5'-GTSGAGTTRAARTANCCNARCCA-3'
5-HT$_{1B}$	5'-GYTNGCCGTNGCNGACYTGATGGT-3'	5'-GTNACNGCCCAGTANCKGTC-3'
5-HT$_{2\alpha}$	5'-RGCTGCASAAYGTSACYAAYTACTTYCT-3'	5'-AGGCTGATGAARCACATGTGC-3'
5-HT$_{7}$	5'-CTNGTNATGCCVATGGC-3'	5'-GGTCSACRSWKATCATGC-3'
Primers for full-length/partial ORF amplification		
#	Forward primer	Reverse primer
TRH	5'-GGGCCAGCAGCGATGAGC-3'	5'-GCTCCCGCAGAGAAGC-3'
TPH	5'-CCGCGATTGTTGGGAGAG-3'	5'-GGTGAACAGAGTAGAGAATG-3'
AADC	5'-CCGACCCCGTCATGGAG-3'	5'-CCTCTGCAACAGTGATGC-3'
5-HT$_{1A}$	5'-GGATGGATCCTGGGGCCGGAG-3'	5'-CTGCTACACATATTTATGTTAAATTGTTTATTG-3'
5-HT$_{1B}$	5'-GCTGGGTGCAGTGTACGAG-3'	5'-CCCAGCTGGAACCAGAG-3'
5-HT$_{2\alpha}$	5'-TACGACTGGAGCTTCCTC-3'	5'-GAGCACGGTGATGGACG-3'
5-HT$_{7}$	5'-CCACCCCTCCACGCGTATG-3'	5'-GCCGTTGCGCACGCTGATC-3'
Primers for tissue-specific expression analysis		
#	Forward primer	Reverse primer
TRH	5'-CAGCGGGTACTGATGTATGG-3'	5'-GCCCCACGTCTTGATCTC-3'
TPH	5'-GAGGCCGTTTGAACCAGC-3'	5'-GGGCGAGGTATTGTATGTGC-3'
AADC	5'-CCCGATTACAGACATTGGCAG-3'	5'-GGCCCATGGTAACTTCTCC-3'
5-HT$_{1A}$	5'-CTCGCCACCTCCAACAACG-3'	5'-GATGACGGCGTCCTTCTG-3'

5-HT$_{1B}$	5’-GCCGTCGTCACCGTCATCG-3’	5’-CGAGTCCGTTGGCGAAG-3’
5-HT$_{2\alpha}$	5’-GGCTTCCTGGGGTACTG-3’	5’-GCCGCTTGGTGCTGTAG-3’
5-HT$_{7}$	5’-GCTGGAGTACGGCGTGAAG-3’	5’-GGTGAGCGGGATGTAGAAG-3’
β-actin	5’-GGGCCAGAAGGACAGCTATG-3’	5’-CTCCTCAGGGGCAACTC-3’
Ef1alpha	5’-GTGTTCTGAAGCCAGGTATGG-3’	5’-CTCCAGCAACATAACCACGAC-3’
rp49	5’-GGAAGTGTTGATGATGCAGAATCG-3’	5’-CTTCACTGCGTAATCTTGCATTAGC-3’

Table 2. GenBank accession number of protein sequences used for structural comparison and phylogenetic analyses

		Genes	Species	GenBank accession number
TRH	Insect tryptophan hydroxylase	*Drosophila* TRH	*Drosophila melanogaster*	NP_612080
		Apis TRH	*Apis mellifera*	XP_394674
		Tribolium TRH	*Tribolium castaneum*	XP_967413
	Nematoda tryptophan hydroxylase	*Caenorhabditis* tph-1	*Caenorhabditis elegans*	NP_495584
	Mollusc tryptophan hydroxylase	*Aplysia* TPH	*Aplysia californica*	ABF18968
	Vertabrate tryptophan hydroxylase 1	*Mus* TpH1	*Mus musculus*	NP_033440
		Danio TpH1	*Danio rerio*	NP_840091
	Vertabrate tryptophan hydroxylase 2	*Mus* TpH2	*Mus musculus*	NP_775567
		Danio TpH2	*Danio rerio*	NP_999960
		Danio tphd2		AAT38217
TPH	Insect phenylalanin/tryptophan hydroxylase	*Drosophila* TPH (Henna isoform A)	*Drosophila melanogaster*	NP_523963
		Apis TPH isoform 1	*Apis mellifera*	XP_392163
		Tribolium TPH	*Tribolium castaneum*	XP_967025
	Nematoda phenylalanin hydroxylase	*Caenorhabditis* pah-1	*Caenorhabditis elegans*	NP_495863
	Vertabrate phenylalanin hydroxylase	*Mus* TPH	*Mus musculus*	NP_032803
		Danio TPH	*Danio rerio*	NP_956845
TH	Insect tyrosine hydroxylase	*Drosophila* TH	*Drosophila melanogaster*	NP_476897
		Bombyx TH	*Bombyx mori*	ADV56718
		Apis TH	*Apis mellifera*	NP_001011633
		Tribolium TH	*Tribolium castaneum*	NP_001092299
	Nematoda tyrosine hydroxylase	*Caenorhabditis* TH	*Caenorhabditis elegans*	ADZ54165
	Vertabrate tyrosine hydroxylase#	*Mus* TH	*Mus musculus*	NP_033403
		Danio TH	*Danio rerio*	NP_571224
		Danio TH2		NP_001001829
AADC	Insect aromatic L-amino acid decarboxylase	*Drosophila* AADC	*Drosophila melanogaster*	AAO16853

		Aedes AADC	*Aedes aegypti*	XP_001648263
		Bombyx AADC	*Bombyx mori*	NP_001037174
		Apis AADC	*Apis mellifera*	XP_394115
		Tribolium AADC	*Tribolium castaneum*	NP_001096056
	Nematoda aromatic L-amino acid decarboxylase	*Caenorhabditis* bas-1	*Caenorhabditis elegans*	NP_001021150
	Vertabrate aromatic L-amino acid decarboxylase	*Mus* AADC	*Mus musculus*	NP_057881
		Danio AADC	*Danio rerio*	NP_998507
HDC	Histidine decarboxylase	*Drosophila* HDC	*Drosophila melanogaster*	NP_523679
TDC	Tyrosine decarboxylase	*Drosophila* TDC1	*Drosophila melanogaster*	NP_610226
		Drosophila TDC2		NP_724489
Amd	Alpha methyl dopa-resistant	*Drosophila* Amd	*Drosophila melanogaster*	NP_724162
GAD	Glutamic acid decarboxylase	*Drosophila* GAD1	*Drosophila melanogaster*	NP_523914
		Drosophila GAD2		NP_476788
5-HT_1	Insect 5-HT_{1A}	*Drosophila* 5-HT_{1A}	*Drosophila melanogaster*	NP_476802
		Antheraea 5-HT_{1A}	*Antheraea pernyi*	ABY85410
		Tribolium 5-HT_{1A}	*Tribolium castaneum*	XP_967449
		Periplaneta 5-HT_{1A}	*Periplaneta americana*	CAX65666
	Insect 5-HT_{1B}	*Drosophila* 5-HT_{1B}	*Drosophila melanogaster*	NP_523789
		Antheraea 5-HT_{1B}	*Antheraea pernyi*	ABY85411
		Tribolium 5-HT_{1B}	*Tribolium castaneum*	XP_972856
	Crustacea 5-HT_1	*Procambarus* 5-HT_1	*Procambarus clarkii*	ABX10973
		Penaeus 5-HT_1	*Penaeus monodon*	AAV48573
	Nematoda 5-HT_1	*Caenorhabditis* Ser-4	*Caenorhabditis elegans*	NP_497452
		Haemonchus 5-HT_1	*Haemonchus contortus*	AAO45883
	Mollusc 5-HT_1	*Aplysia* 5-HT_{ap1}	*Aplysia californica*	AAC28786
		Aplysia 5-HT_{ap2}		AF372526
		Lymnaea 5-HT_1	*Lymnaea stagnalis*	Q25414
		Mizuhopecten 5-HT_1	*Mizuhopecten yessoensis*	BAE72141

	Vertabrate 5-HT_1	*Mus* 5-HT_{1A}	*Mus musculus*	NP_032334
		Danio 5-HT_{1A}	*Danio rerio*	NP_001116793
5-HT_2	Insect 5-HT_{2A}	*Drosophila* 5-HT_{2A}	*Drosophila melanogaster*	NP_730859
		Apis 5-HT_{2A}	*Apis mellifera*	NP_001189389
		Tribolium 5-HT_{2A}	*Tribolium castaneum*	XP_972327
	Insect 5-HT_{2B}	*Drosophila* 5-HT_{2B} (CG42796)	*Drosophila melanogaster*	NP_649806
		Apis 5-HT_{2B}	*Apis mellifera*	NP_001191178
		Tribolium 5-HT_{2B}	*Tribolium castaneum*	EFA04642
	Crustacea 5-HT_2	*Procambarus* 5-HT_2	*Procambarus clarkii*	ABX10972
	Nematoda 5-HT_2	*Caenorhabditis* 5-HT_2 (Ser-1)	*Caenorhabditis elegans*	NP_001024728
	Mollusc 5-HT_2	*Lymnaea* 5-HT_2	*Lymnaea stagnalis*	AAC16969
	Vertabrate 5-HT_2	*Mus* 5-HT_{2A}	*Mus musculus*	NP_766400
		Mus 5-HT_{2B}		NP_032337
		Danio 5-HT_{2A}	*Danio rerio*	CAQ15355
		Danio 5-HT_{2A}-like		XP_688270
		Danio 5-HT_{2B}		NP_001038208
		Danio 5-HT_{2C}		NP_001123365
5-HT_7	Insect 5-HT_7	*Drosophila* 5-HT_7	*Drosophila melanogaster*	NP_524599
		Aedes 5-HT_7	*Aedes aegypti*	AF296125
		Apis 5-HT_7	*Apis mellifera*	NP_001071289
		Tribolium 5-HT_7	*Tribolium castaneum*	XP_966577
	Nematoda 5-HT_7	*Caenorhabditis* 5-HT_7 (Ser-7)	*Caenorhabditis elegans*	NP_741730
	Mollusc 5-HT_7	*Aplysia* 5-HT_7	*Aplysia californica*	ACJ63458
	Vertabrate 5-HT_7	*Mus* 5-HT_7	*Mus musculus*	CAQ76701
		Danio 5-HT_7-like 1	*Danio rerio*	XP_690599
		Danio 5-HT_7-like 2		XP_693673

SUPPORTING INFORMATION

Supplementary figure 1

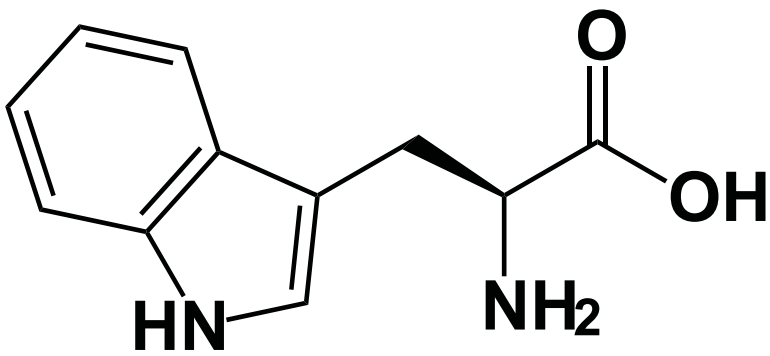
Schematic representation of protein structure of two *Gryllus* TRH isoforms. *Gryllus* TRH long variant contains a 39 amino acid (117 bp) residue insertion within the N-terminal region of the catalytic domain.

Supplementary figure 2

Tissue-specific expression patterns of three house keeping genes (*Gryllus ß-actin*, *Ef1alpha*, and *rp49*). *Ef1alpha* expression was the one that was most stable in the different tissues.

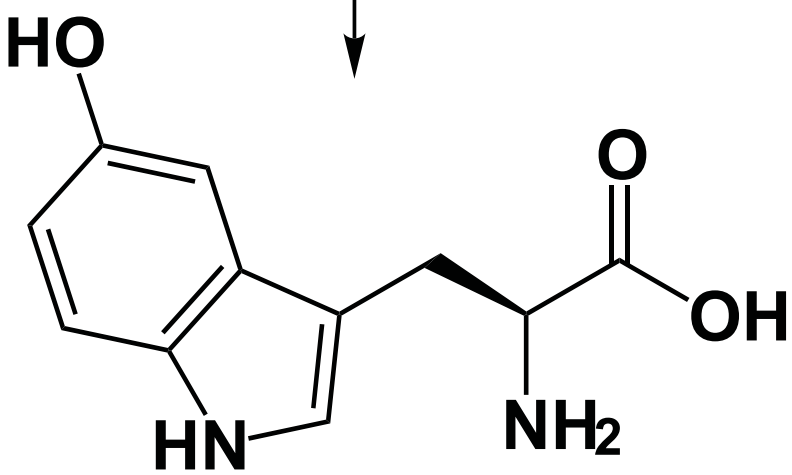
Supplementary figure 3

Tissue-specific expression patterns of *Gryllus TRH*, *TPH*, *5-HT$_{2\alpha}$, and 5-HT$_7$* genes. The PCR products of a secondary PCR were run through a 1.5% agarose gel and visualized by ethidium bromide. *Gryllus Ef1alpha* gene was used as a internal control gene.



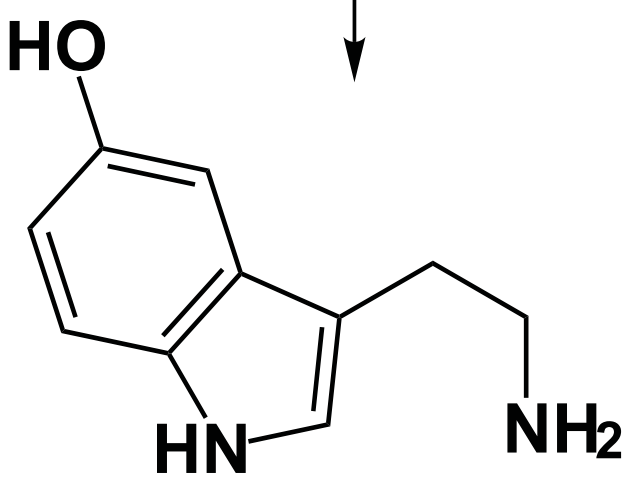
L-Tryptophan

TRH, TPH

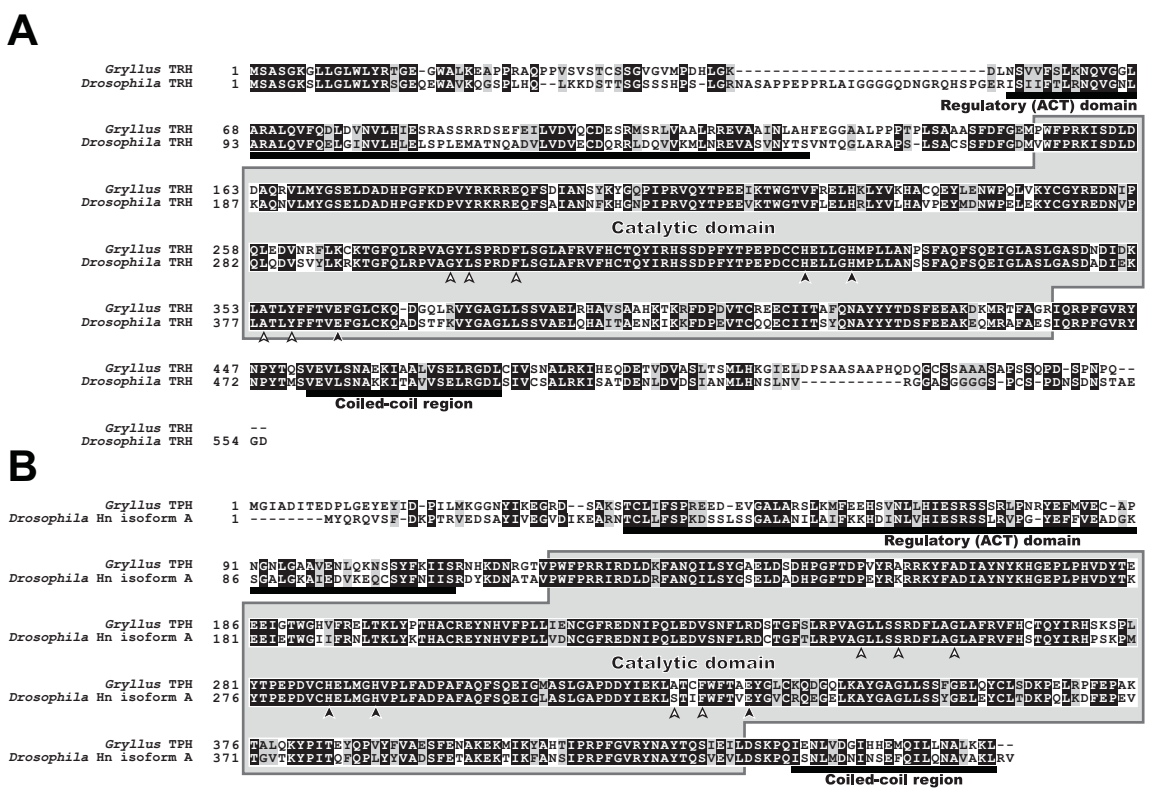


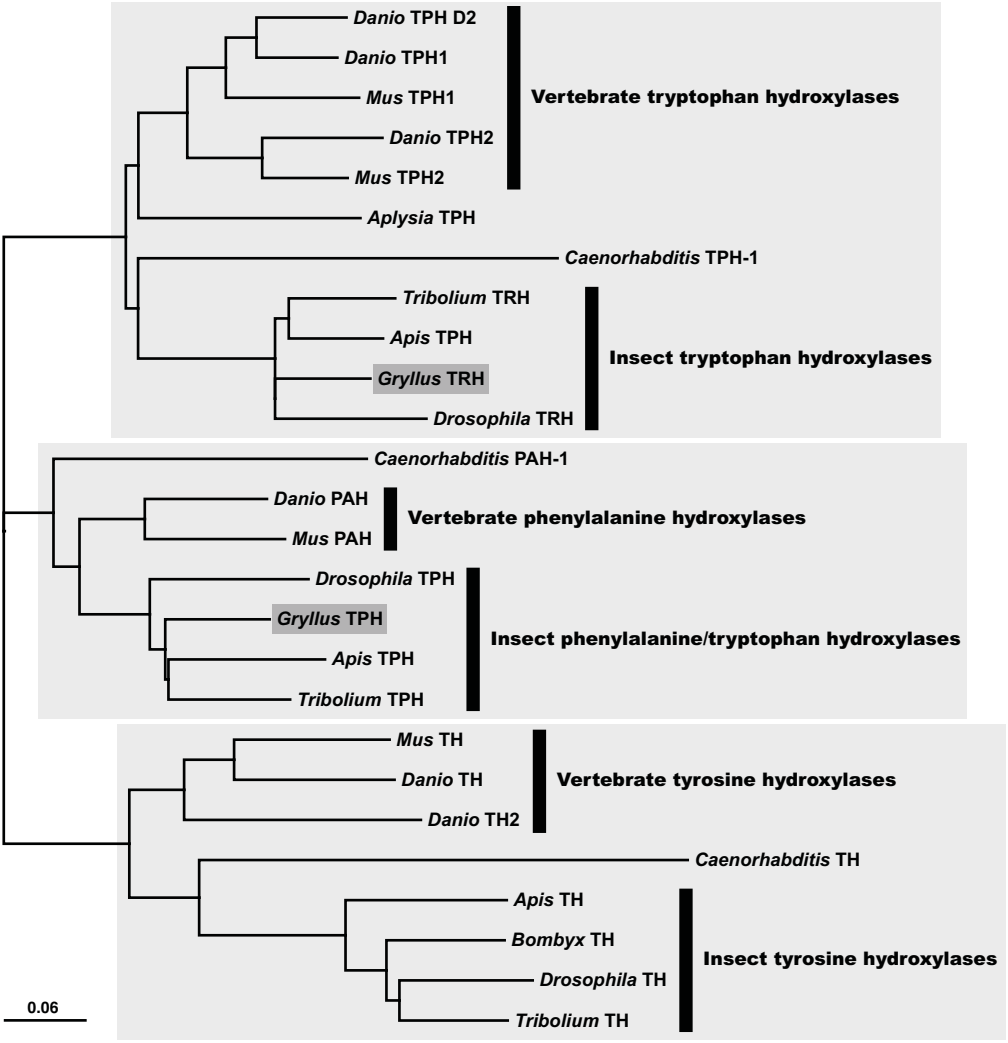
5-Hydroxy-L-tryptophan
(5-HTP)

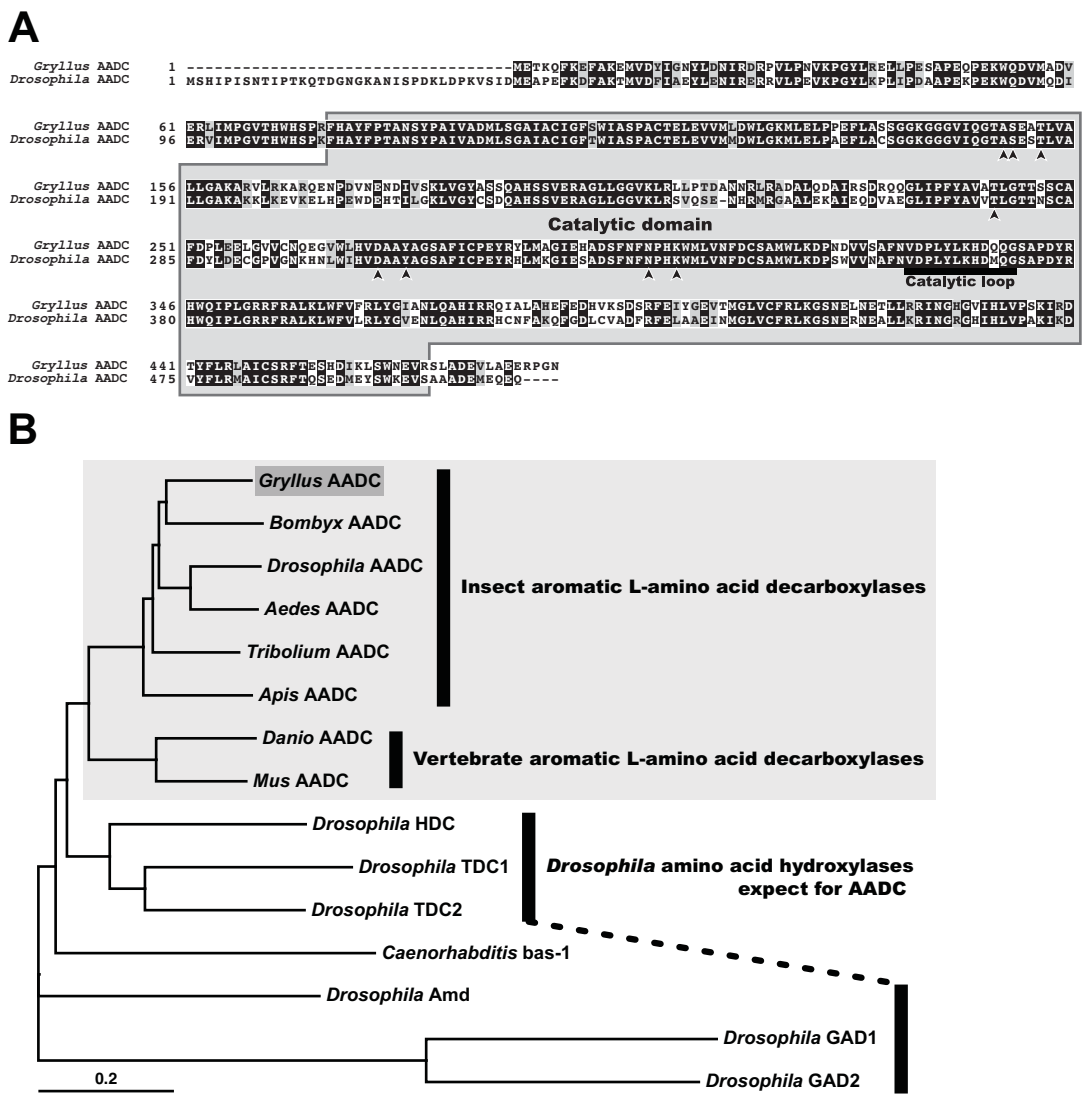
AADC

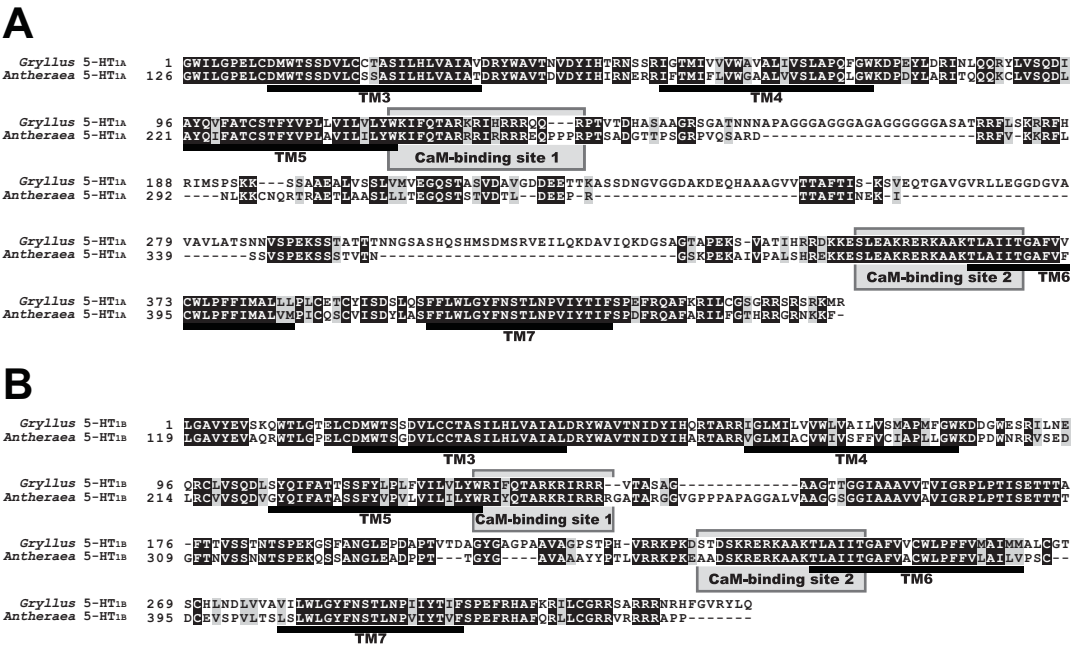


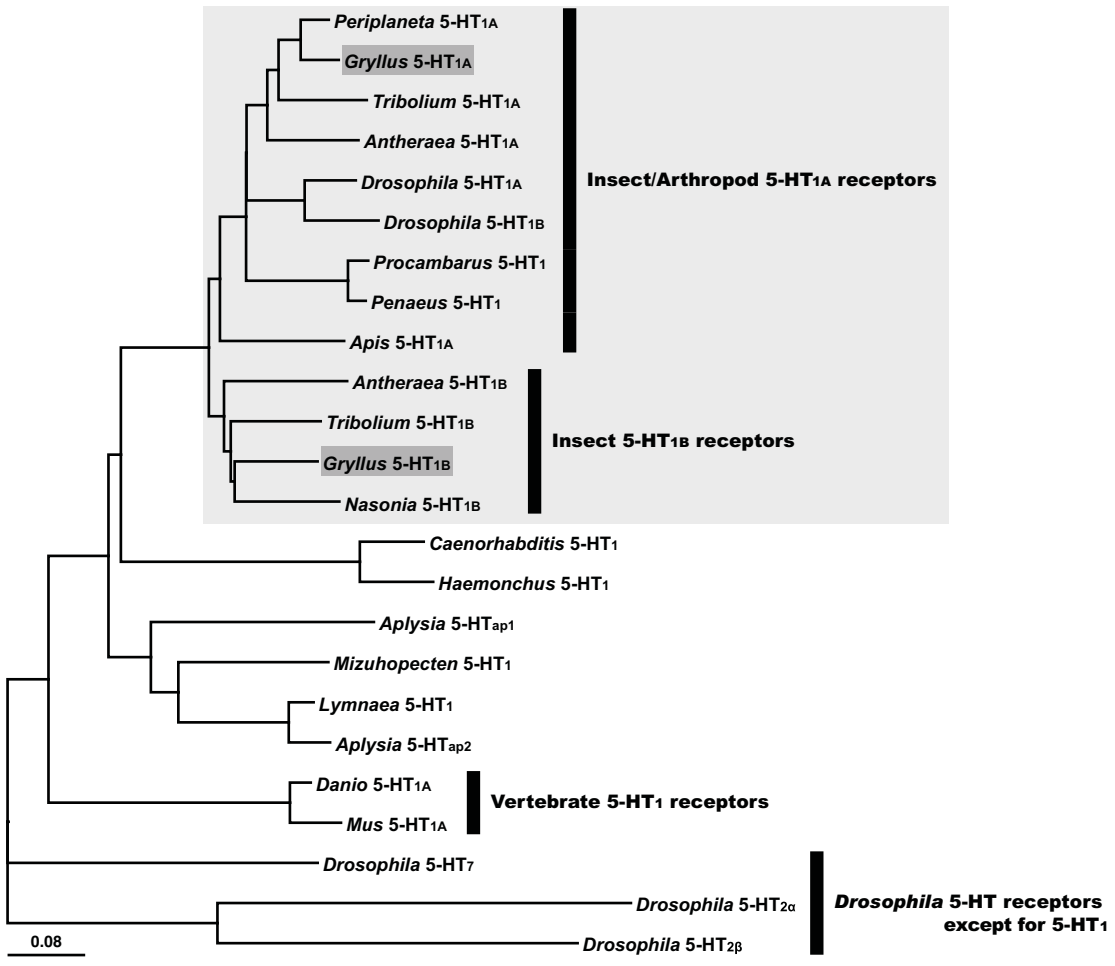
Serotonin (5-HT)

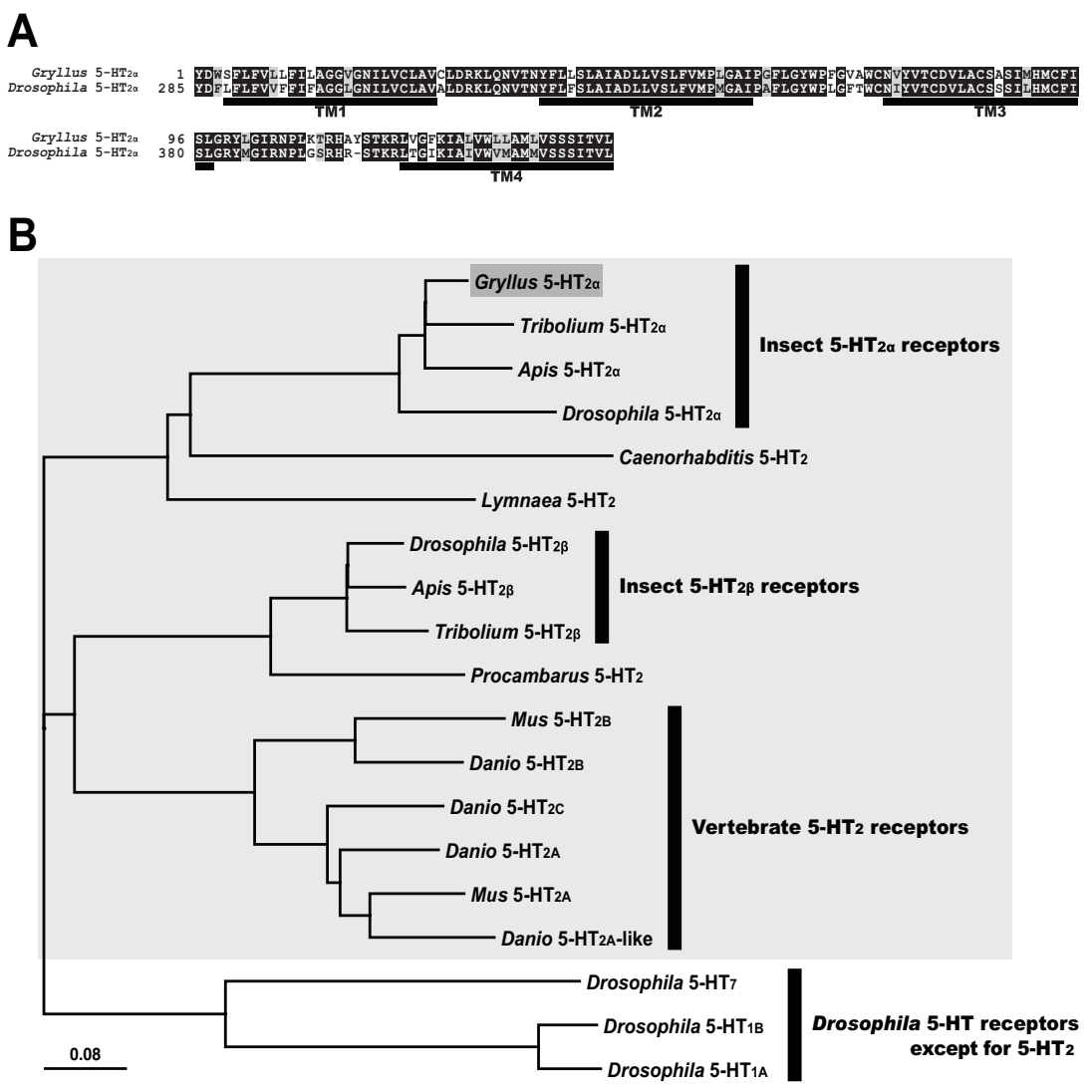

A
Gryllus TRH
Drosophila TRH
Regulatory (ACT) domain
Catalytic domain
Coiled-coil region
B
Gryllus TPH
Drosophila Hn isoform A
Regulatory (ACT) domain
Catalytic domain
Coiled-coil region

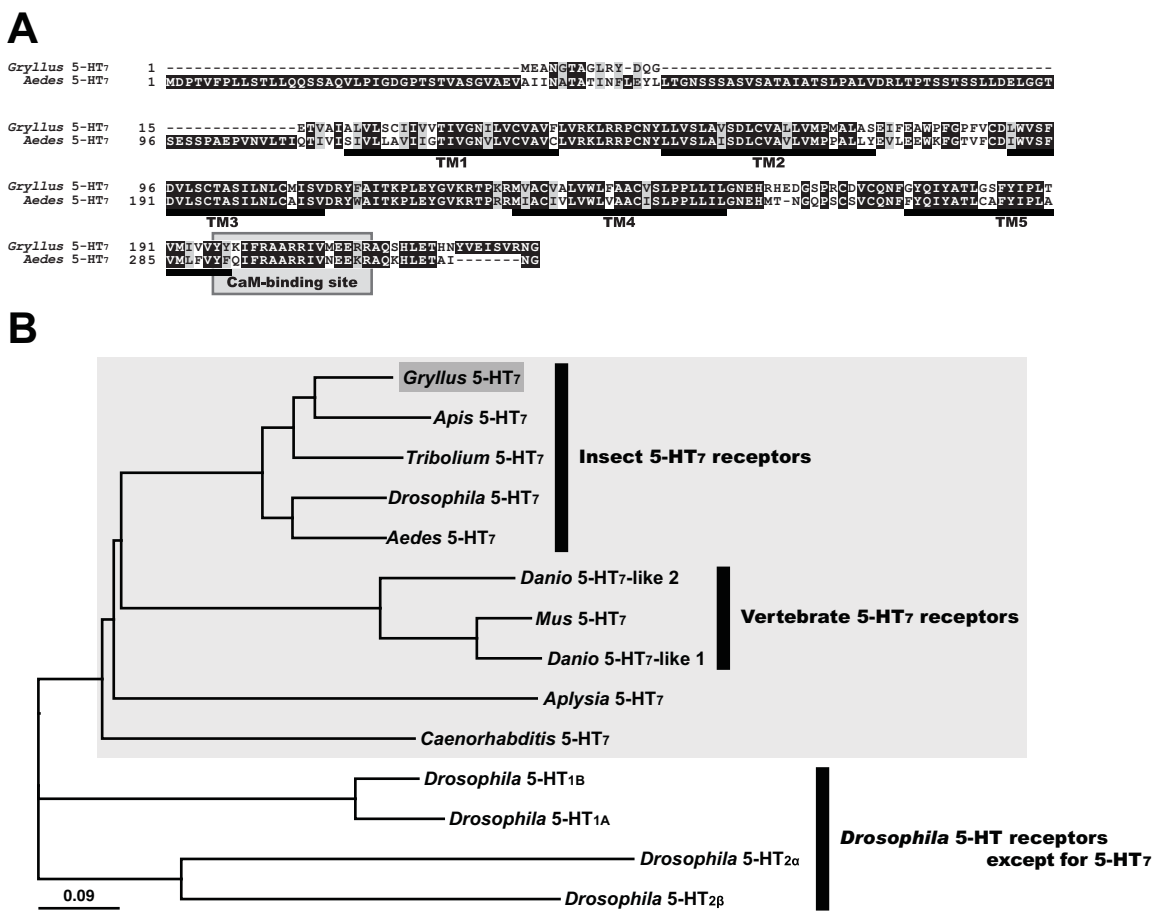

Danio TPH D2
Danio TPH1
Mus TPH1
Danio TPH2
Mus TPH2
Aplysia TPH
Vertebrate tryptophan hydroxylases
Caenorhabditis TPH-1
Tribolium TRH
Apis TPH
Gryllus TRH
Drosophila TRH
Insect tryptophan hydroxylases
Caenorhabditis PAH-1
Danio PAH
Mus PAH
Vertebrate phenylalanine hydroxylases
Drosophila TPH
Gryllus TPH
Apis TPH
Tribolium TPH
Insect phenylalanine/tryptophan hydroxylases
Mus TH
Danio TH
Danio TH2
Vertebrate tyrosine hydroxylases
Caenorhabditis TH
Apis TH
Bombyx TH
Drosophila TH
Tribolium TH
Insect tyrosine hydroxylases
0.06

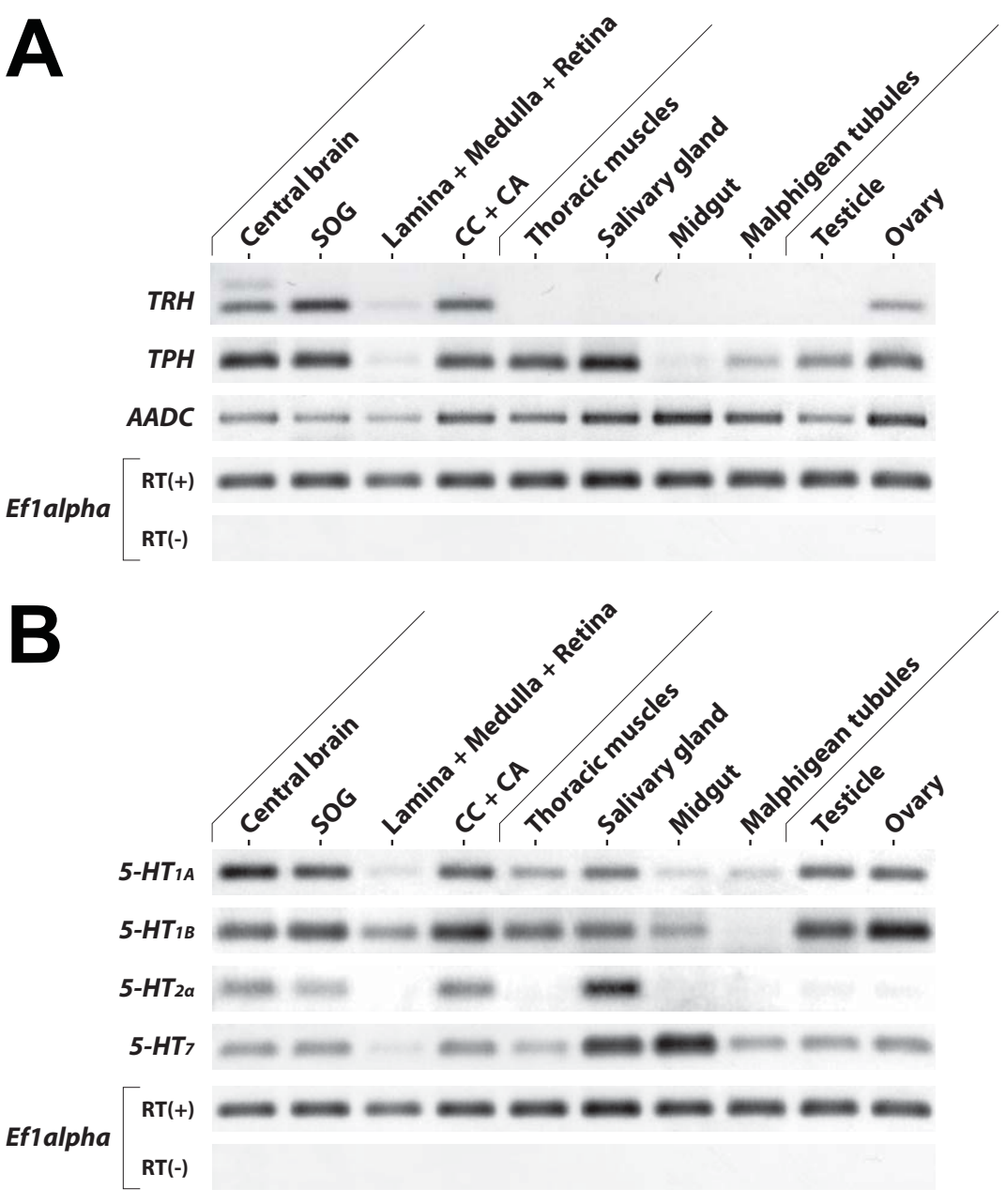

A
Gryllus AADC
Drosophila AADC
Catalytic domain
Catalytic loop
B
Gryllus AADC
Bombyx AADC
Drosophila AADC
Aedes AADC
Tribolium AADC
Apis AADC
Insect aromatic L-amino acid decarboxylases
Danio AADC
Mus AADC
Vertebrate aromatic L-amino acid decarboxylases
Drosophila HDC
Drosophila TDC1
Drosophila TDC2
Drosophila amino acid hydroxylases expect for AADC
Caenorhabditis bas-1
Drosophila Amd
Drosophila GAD1
Drosophila GAD2
0.2


A
Gryllus 5-HT1A
Antheraea 5-HT1A
TM3
TM4
TM5
CaM-binding site 1
CaM-binding site 2
TM6
TM7
B
Gryllus 5-HT1B
Antheraea 5-HT1B
TM3
TM4
TM5
CaM-binding site 1
CaM-binding site 2
TM6
TM7


Periplaneta 5-HT1A
Gryllus 5-HT1A
Tribolium 5-HT1A
Antheraea 5-HT1A
Drosophila 5-HT1A
Drosophila 5-HT1B
Procambarus 5-HT1
Penaeus 5-HT1
Apis 5-HT1A
Insect/Arthropod 5-HT1A receptors
Antheraea 5-HT1B
Tribolium 5-HT1B
Gryllus 5-HT1B
Nasonia 5-HT1B
Insect 5-HT1B receptors
Caenorhabditis 5-HT1
Haemonchus 5-HT1
Aplysia 5-HTap1
Mizuhopecten 5-HT1
Lymnaea 5-HT1
Aplysia 5-HTap2
Danio 5-HT1A
Mus 5-HT1A
Vertebrate 5-HT1 receptors
Drosophila 5-HT7
Drosophila 5-HT2α
Drosophila 5-HT2β
Drosophila 5-HT receptors except for 5-HT1
0.08


A
Gryllus 5-HT2α 1 YDWSFLFVLLFILAGGVGNILVCLAVCLDRKLQNVTNYFLLSLAIADLLVSLFVMPLGAIPGFLGYWPFGVAWCNVYVTCDVLACSASIMHMCFI
Drosophila 5-HT2α 285 YDFLFLFVVFFIFAGGLGNILVCLAVALDRKLQNVTNYFLFSLAIADLLVSLFVMPMGAIPAFLGYWPLGFTWCNIYVTCDVLACSSSILHMCFI
TM1
TM2
TM3
Gryllus 5-HT2α 96 SLGRYLGIRNPLKTRHAYSTKRLVGPKIALVWLLAMLVSSSITVL
Drosophila 5-HT2α 380 SLGRYMGIRNPLGSRHR-STKRLTGIKIALVWVMAMMVSSSITVL
TM4
B
Gryllus 5-HT2α
Tribolium 5-HT2α
Apis 5-HT2α
Drosophila 5-HT2α
Insect 5-HT2α receptors
Caenorhabditis 5-HT2
Lymnaea 5-HT2
Drosophila 5-HT2β
Apis 5-HT2β
Tribolium 5-HT2β
Insect 5-HT2β receptors
Procambarus 5-HT2
Mus 5-HT2B
Danio 5-HT2B
Danio 5-HT2C
Danio 5-HT2A
Mus 5-HT2A
Danio 5-HT2A-like
Vertebrate 5-HT2 receptors
Drosophila 5-HT7
Drosophila 5-HT1B
Drosophila 5-HT1A
Drosophila 5-HT receptors except for 5-HT2
0.08


A
Gryllus 5-HT7
Aedes 5-HT7
TM1
TM2
TM3
TM4
TM5
CaM-binding site
B
Gryllus 5-HT7
Apis 5-HT7
Tribolium 5-HT7
Drosophila 5-HT7
Aedes 5-HT7
Insect 5-HT7 receptors
Danio 5-HT7-like 2
Mus 5-HT7
Danio 5-HT7-like 1
Vertebrate 5-HT7 receptors
Aplysia 5-HT7
Caenorhabditis 5-HT7
Drosophila 5-HT1B
Drosophila 5-HT1A
Drosophila 5-HT2α
Drosophila 5-HT2β
Drosophila 5-HT receptors except for 5-HT7
0.09


A
Central brain
SOG
Lamina + Medulla + Retina
CC + CA
Thoracic muscles
Salivary gland
Midgut
Malphigean tubules
Testicle
Ovary
TRH
TPH
AADC
Ef1alpha
RT(+)
RT(-)
B
Central brain
SOG
Lamina + Medulla + Retina
CC + CA
Thoracic muscles
Salivary gland
Midgut
Malphigean tubules
Testicle
Ovary
5-HT1A
5-HT1B
5-HT2α
5-HT7
Ef1alpha
RT(+)
RT(-)