Invert Neurosci (2006) 6:189–205
DOI 10.1007/s10158-006-0033-z

ORIGINAL PAPER

Characterization of the *Caenorhabditis elegans* G protein-coupled serotonin receptors

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Received: 31 August 2006 / Accepted: 4 October 2006 / Published online: 3 November 2006

Abstract Serotonin (5-HT) regulates a wide range of 'behaviors in *Caenorhabditis elegans*, including egg laying, male mating, locomotion and pharyngeal pumping. So far, four serotonin receptors have been described in the nematode *C. elegans*, three of which are G protein-coupled receptors (GPCR), (SER-1, SER-4 and SER-7), and one is an ion channel (MOD-1). By searching the *C. elegans* genome for additional 5-HT GPCR genes, we identified five further genes which encode putative 5-HT receptors, based on sequence similarities to 5-HT receptors from other species. Using loss-of-function mutants and RNAi, we performed a systematic study of the role of the eight GPCR genes in serotonin-modulated behaviors of *C. elegans* (F59C12.2, Y22D7AR.13, K02F2.6, C09B7.1, M03F4.3, F16D3.7, T02E9.3, C24A8.1). We also examined their expression patterns. Finally, we tested whether the most likely candidate receptors were able to modulate adenylate cyclase activity in transfected cells in a 5-HT-dependent manner. This paper is the first comprehensive study of G protein-coupled serotonin receptors of *C. elegans*. It provides a direct comparison of the expression patterns and functional roles for 5-HT receptors in *C. elegans*.

Electronic supplementary material Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s10158-006-0033-z and is accessible for authorized users.

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Introduction

Serotonin (5-HT) is a neurotransmitter present in most of the animal kingdom. In mammals, serotonin controls a wide range of responses and behaviors, including temperature regulation, pain perception, sleep, aggression and feeding (for review, see Weiger 1997). Serotonin signaling is also involved in diseases such as depression, anorexia, and autism (Schloss and Williams 1998). In mammals, 5-HT acts either as a neurotransmitter or as a neurohormone, through seven classes of serotonin receptors (5-HT_{1-7}). Six of them are seven transmembrane domains, G protein-coupled receptors (GPCR) (for review, see Hartig 1997). The 5-HT_1 receptor class is subdivided into five subtypes: A, B, C, D and E, all of which can attenuate adenylate cyclase activity through $G_{i/o}$ protein signaling. Another 5-HT receptor type, 5-HT_5, attenuates adenylate cyclase activitythrough G_s signaling (Amlaiky et al. 1992; Maroteaux et al. 1992; Plassat et al. 1992). In contrast, adenylate cyclase activity is activated via G_s protein signaling in 5-HT_4, 5-HT_6, and 5-HT_7 receptor types (Gerald et al. 1995; Meyerhof et al. 1993; Monsma et al. 1993). The 5-HT_2 receptor class consists of 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} which couple to $G_{q/11}$ to increase phospholipase C activity, thus increasing

inositol phosphate hydrolysis and elevating cytosolic Ca^{2+} (Foguet et al. 1992a, b; Pritchett et al. 1988). The 5-HT_3 receptor is a ligand-gated non selective cation channel (Dubin et al. 1999; Hanna et al. 2000).

The first 5-HT invertebrate receptor to be molecularly identified was the *Drosophila* 5-HT-dro1 receptor (Witz et al. 1990). It shares most similarity wiith the 5-HT_7 human receptor (Bard et al. 1993). Like its human counterpart, it is able to activate adenylate cyclase activity in transfected cells when stimulated by 5-HT (Bard et al. 1993; Saudou et al. 1992). Saudou et al. (1992) identified two additional 5-HT_{1A}-like receptors in *Drosophila*: 5-HT_{1ADro} and 5-HT_{1BDro}. When expressed in mammalian cells, they inhibit adenylate cyclase activity and activate phospholipase C, similar to the 5-HT_1 mammalian receptor (Gerhardt et al. 1996). These two receptors are expressed in the central nervous system of late embryos and adult flies where they are believed to play roles in motor control. A fourth *Drosophila* receptor, 5-HT_{2Dro}, is related in structure and pharmacology to mammalian 5-HT_2 receptors (Colas et al. 1995). It is expressed during early embryogenesis where it is thought to function first as a patterning gene, and later in the central nervous system of adult flies (Colas et al. 1995). Lastly, a 5-HT_1-like receptor from the Southern cattle tick, *Boophilus microplus*, was cloned and found to inhibit adenylate cyclase activity when expressed in mammalian cells (Chen et al. 2004).

Several molluscan 5-HT receptors are known, two from the pond snail *Lymnea stagnalis* (Gerhardt et al. 1996; Sugamori et al. 1993), and three from *Aplysia californica* (Angers et al. 1998; Li et al. 1995). The two receptors from *L. stagnalis*, 5-HT_{1Lym} and 5-HT_{2Lym}, are related to 5-HT_1 and 5-HT_2 mammalian receptors respectively. The *A. californica* Ap5-HT_{B1} and Ap5-HT_{B2} receptors activate phospholipase C (Li et al. 1995), linking them to 5-HT_2 mammalian receptors, but their molecular structure do not resemble this class of receptors. A third *A. californica* receptor, 5-HT_{1Ap}, shares molecular features with 5-HT_1 mammalian receptors (Angers et al. 1998). Moreover, when expressed in mammalian cells, 5-HT_{1Ap} was able to inhibit adenylate cyclase activity. 5-HT receptors have also been described in the parasitic nematode *Ascaris suum* (Huang et al. 1999).

Thus, numerous receptors have been identified in invertebrates. Their type classification with respect to mammalian classes is often uneasy because numerous mammalian receptor agonists have a different spectrum in invertebrates. To our knowledge, no study has characterized the full list of 5-HT receptors in a single organism.

5-HT is also present in the nematode *Caenorhabditis elegans* (Horvitz et al. 1982) where it regulates basic behaviors including locomotion, pharyngeal pumping, egg laying and male mating (Horvitz et al. 1982; Loer and Kenyon 1993; Sze et al. 2000). More complex behaviors of the worm are also modulated by serotonin (Chao et al. 2004; Sawin et al. 2000; Zhang et al. 2005). In the past few years, four 5-HT receptors have been identified in *C. elegans*: Three are G protein-coupled receptors: SER-4, which resemble the 5-HT_1 receptors of other species (Olde and McCombie 1997), SER-1, a 5-HT_2-like receptor (Hamdan et al. 1999), and SER-7, which presents some homology to the 5-HT_7 mammalian receptor (Hobson et al. 2003). The fourth serotonin receptor is a 5-HT gated chloride channel called MOD-1 (Ranganathan et al. 2000).

Since the *C. elegans* genome is fully sequenced, since *C. elegans* is amenable to genetic studies and has several simple 5-HT-modulated behaviors, it was therefore possible to perform a comprehensive study of all of the 5-HT receptors of *C. elegans* and of their involvement in the animal behaviors.

In this study, we first tried to establish the complete list of the *C. elegans* 5-HT GPCRs. Then, we systematically described their expression patterns and their involvement in 5-HT-modulated behaviors. We also tried to determine whether the most likely candidates could modulate adenylate cyclase activity in transfected mammalian cells.

This is the first comprehensive study of proven and putative 5-HT receptors in *C. elegans*.

Materials and methods

C. elegans strains

The wild-type strain used was the Bristol N2 strain. Mutant strains were provided by the Caenorhabditis Genetics Center (Minnesota) and are RB745: Y22D7AR.13/*ser-4*(*ok512*) which is a deletion of 1,336 bp, DA1774: K02F2.6/*ser-3*(*ad1774*) which is a deletion of 1,560 bp, and DA1814: F59C12.2/*ser-1*(*ok345*) which is a putative null allele (Carnell et al. 2005). *C. elegans* strains were grown on NGM plates seeded with *E. coli* (OP50). *C. elegans* strains were grown at 15°C except for RNAi assays.

RNA interference

RNA interference was performed by feeding N2 worms with HT115 *E. coli* transformed with the

appropriate plasmid (Timmons et al. 2001). The N2 strain was preferred to the RNAi-hypersensisitive *rrf-3* strain to avoid the appearance of synthetic phenotypes. The bacteria were seeded on NGM plates containing carbenicillin (25 μg/ml) and IPTG (1 mM) to induce expression of double-stranded (ds) RNA targeting the gene of interest. Control worms were grown on plates seeded with HT115 transformed with the empty vector, pL4440. Plates were used 5 days after seeding to allow bacterial growth. Animals were grown at 23°C and were exposed from hatching to adulthood to the dsRNA-producing bacteria.

Tissue expression

The expression patterns were determined by using *gfp* reporter gene fused with the promoter region of each of the gene of interest. The strains are BC10137 for M03F4.3, BC10143 for F16D3.7, BC10186 for C09B7.1/*ser-7b*, BC10208 for T02E9.3, BC10206 for F59C12.2/*ser-1* and BC13771 for C24A8.1. GFP-constructs were created by a modification of the Hobert technique (Hobert 2002; McKay et al. 2003). Promoter::GFP fusions were stitched together and then co-injected with wild-type *dpy-5* plasmid (pCeh-361) into *dpy-5*(*e907*) animals (Thacker et al. 2006). To isolate promoter containing regions for PCR-stitching we selected the most probable ATG start codon and used the Primer 3 program (http://www.genome.wi.mit.edu/genome software/other/primer3.html) to generate appropriate primer sequences. We normally designed a 'B' primer close to the start codon (within 100 bp) or to cross the start site (in which case we mutated the start codon's ATG sequence). An 'A' primer was designed to abut against the next upstream gene, unless that gene was more than 3,000 bp away in which case we designed the 'A' primer to be approximately 3,000 bp upstream. In order to eliminate false positive PCR products we designed a nested primer (A*) immediately downstream from the 'A' primer. List of primers used (5′ to 3′): M03F4.3-A GGGTCTTGAGAAAATGTGCAG, M03F4.3-A* ATTCACAAGGGACCGTTTTT, M03F4.3-B AGTC GACCTGCAGGCATGCAAGCTTTCAACCCACG ATAAGAAAATTG; F16D3.7-A CCCCTACTTTTT GCAGGACA, F16D3.7-A* CAGGACATCTTCGGA CCTCA, F16D3.7-B AGTCGACCTGCAGGCATGC AAGCTGAAAGATGACGGCGATGAC, C09B7.1-A ATGTTTCGGCGAAGTCGATA, C09B7.1-A* TCG GCGAAGTCGATAAAATC, C09B7.1-B AGTCGA CCTGCAGGCATGCAAGCTTCAGGCTGTGTCT GACTGCT, T02E9.3-A TTTCCTTTGATTGGGAA AACA, T02E9.3-A* CTTTGATTGGGAAAACAT TG, T02E9.3-B AGTCGACCTGCAGGCATGCA AGCTAGATGTTTTGTCGTCGTGGT, F59C12.-A CGCAGAAATATTTTTCAACTGAC, F59C12.-A* CAACTGACTGTTCTATTT, F59C12.-B AGTCGA CCTGCAGGCATGCAAGCTAGGAGTGCACCT CTGAAACAA, C24A8.1-A GGCACAATTCGGTT GAAGTT, C24A8.1-A* AACATGGTTTTTGAACT GGAAAGT, C24A8.1-B AGTCGACCTGCAGGC ATGCAAGCTATTTCCGATTCGTTGATCAGT.

We used pPD95.67 variant S65C (kindly provided by A. Fire) for our marker. It contains a GFP-cassette and a region that has sequence overlap with the B primer thus allowing for PCR stitching of a promoter containing region to the GFP-cassette to make a promoter::GFP fusion. For observations, adult worms were mounted on 2% agarose pads containing 0.1% sodium azide, and were observed under a Zeiss Axiovert fluorescence microscope.

Brood size

For each gene, 12 young adult hermaphrodites were picked on a single plate. Each worm was transferred to a new NGM plate with OP50 bacteria every day for 4 days. Two days later, the number of progeny was counted on each plate and summed up for each worm. The mean and SEM values of each experiment were calculated. The means were compared by a Student's *t* test.

Eggs in utero

Synchronized gravid adults were put on 0.1% sodium azide pads and the number of eggs in utero was observed under a Zeiss microscope. Twenty worms were counted for each experiment. The mean and SEM values of each experiment were calculated. The means were compared by a Student's *t* test.

Behavioral assays

For behavioral assays, 20–30 well fed adults were washed thoroughly in M9 buffer. They were put on NGM plates without food, with or without 10 mM 5-HT (creatinine sulfate salt, 4 mg/ml, Sigma). Animals were assayed for the serotonin-dependent behaviors after 2 h at room temperature (22°C). Controls were the N2 strain for mutants and empty RNAi vector for RNAi experiments. Since not all the experiments were performed simultaneously, results were normalized to the control value of each experiment.

Egg laying The number of eggs laid in two hours was observed on plates and was divided by the number of animals on the plate.

Pharyngeal pumping The pharyngeal pumping of at least 20 animals per experiment was observed during 30-s periods. The mean and SEM values were calculated. The means were compared by a Student's *t* test.

Locomotion The number of body bends/minute was observed for at least 20 animals per experiment. The mean and SEM values were calculated. The means were compared by a Student's *t* test.

Male mating At least 20 *him-8* adult males were picked onto fresh plates without food, with or without 5-HT (4 mg/ml). After 2 h, the proportion of males displaying a mating behavior, i.e. curling ventrally their tail, was counted at four different time points separated by 2 min.

All assays were scored by an observer blind as to treatment and genotype.

cAMP assays

To express the receptors in COS-7 cells, we cloned their coding sequence into the pEGFP-N1 expression vector. The N-terminal gfp tag was used to monitor receptor expression and localization after transfection. The *gfp* construction for C09B7.1/*ser-7* was made from the yk1036g04 cDNA provided by Yuji Kohara (Mishima, Japan) inserted in pEGFP-N1 in order to couple *gfp* expression to the expression of C09B7.1/*ser-7*. The M03F4.3-*gfp* construct was made in the same way from the yk1339e08 cDNA provided by Yuji Kohara. Since no cDNA was available for F16D3.7-*gfp*, an artificial cDNA was constructed by PCR-amplifying the DNA corresponding to the exons predicted by Wormbase, and inserted in pEGFP-N1 vector. COS-7 cells were transiently transfected with 1 μg of these constructs using the FuGENE6™ (Roche, Indianapolis, IN, USA), in 12-well plates. Twenty-four hours after transfection, 1 ml of Dulbecco's modified Eagle-medium (DMEM) with 10% serum was added. 48 h after transfection, *gfp* expression was controlled under microscope. Then, the medium was aspirated the reaction mix containing 300 μM 3-isobutyl-L-methylxanthine (IBMX), 10 μM pargyline HCl with or without the tested ligand in DMEM with 10% serum was added to the cells and incubated for 15 min at 37°C. The biogenic amine tested were: 5-HT, 1 μM; dopamine, 1 μM; tyramine, 1 μM and octopamine, 1 μM, obtained from Sigma-Aldrich (L'Isle d'Abeau, France). Reactions were terminated by aspiration of media and addition of 300 μl 0.1 M HCl. Then, intracellular cAMP levels were determined by spectrophotometry using the Amersham cAMP Biotrak EnzymeImmunoAssay System (Indianapolis, IN, USA), in 12-well plates. Protein concentrations were determined by the method of Bradford (1976).

Results and discussion

Established and putative 5-HT G protein-coupled receptors of *C. elegans*

Defining the list of known and putative C. elegans receptors

Three bona fide *C. elegans* 5-HT GPCR receptors are already known: F59C12.2/*ser-1* (Hamdan et al. 1999), Y22D7AR.13/*ser-4* (Olde and McCombie 1997), and C09B7.1/*ser-7* (Hobson et al. 2003).

To identify additional putative 5-HT receptors of *C. elegans*, we used a multistep procedure. First, we screened the *C. elegans* genome for predicted GPCR showing homologies to human 5-HT1A and 5-HT2 receptors. The genes giving the best 50 Blast scores were selected. Then, a phylogenetic tree comprising the 50 candidates and human 5-HT and non-5-HT receptors was built by the parsimony method (not shown). Since their position in the tree was closed to human 5-HT receptors, 16 *C. elegans* GPCRs were considered as putative candidate 5-HT receptors after this first round of analysis (Y22D7AR.13, C09B7.1, M03F4.3, T02E9.3, F59C12.2, F16D3.7, K02F2.6, C52B11.3, C24A8.1, T14E8.3, F14D12.6, K09G1.4, C02D4.2, F01E11.5, F15A8.5 and Y40H4A.1). This list coincides with the *C. elegans* list of biogenic amine receptors established by Tsalik et al. (2003). A second round of analysis led to the exclusion of the last six genes from this list because their sequence revealed strong similarities with octopamine, dopamine, tyramine, or muscarinic receptors. In addition, C52B11.3 and T14E8.3 were excluded when they were functionally demonstrated to be dopamine receptors (Sugiura et al. 2005). Our final list of putative 5-HT receptors based on sequence analysis and functional data is therefore composed of eight genes, which are shown in Table 1. Our search method is validated by the fact that the three receptors already shown to be bona fide 5-HT receptors, F59C12.2/*ser-1*, Y22D7AR.13/*ser-4*, and C09B7.1/*ser-7* are included in this list. A partial alignment of the sequences is shown in Fig. 1. The full peptide alignment of the *C. elegans* 5-HT receptors is given in Fig. S1 (supplementary materials).

Table 1 Nomenclature

Gene name (cosmid)	Gene name (3 letters)	Receptor type	Functional characterisation in
F59C12.2	*ser-1*	5-HT2	Hamdan et al. (1999)
Y22D7AR.13	*ser-4*	5-HT1	Olde et al. (1997)
K02F2.6	*ser-3*	–	
C09B7.1	*ser-7*	5-HT7	Hobson et al. (2003)
M03F4.3	–	–	
F16D3.7	–	–	
T02E9.3	–	–	
C24A8.1	–	–	

Classification of C. elegans receptors with respect to mammalian receptor types

Functional data obtained on the three established receptors (F59C12.2/*ser-1*, Y22D7AR.13/*ser-4*, C09B7.1/*ser-7)* have led to the following classification: F59C12.2/*ser-1* is of 5-HT_2 type (Hamdan et al. 1999), Y22D7AR.13/*ser-4* is of 5-HT_1 type (Olde and McCombie 1997), and C09B7.1/*ser-7* is of 5-HT_7 type (Hobson et al. 2003).

In order to gain insight into the 5-HT type of the remaining five receptors, the eight receptors have been tentatively put on a phylogenetic tree (parsimony method) with human 5-HT GPCRs of various types (Fig. 2). The first observation arising from this tree is that the bootstrap values are small; this can be explained by the high divergence between human and *C. elegans* receptors. In this tree, the three established receptors have positions which are in accordance with their known functional properties. This validates the classification appearing on the tree despite of the small bootstrap values. Positioning the remaining five receptors with respect to human 5-HT is more difficult and the results given here are only speculative at this point.

K02F2.6/*ser-3* may be of the 5-HT_4 type. This is supported by the fact that (1) it is closer to this type than to any other type on the tree, (2) some key residues are consistent with this: in the second loop, K02F2.6/*ser-3* has an aspartic acid which is common to

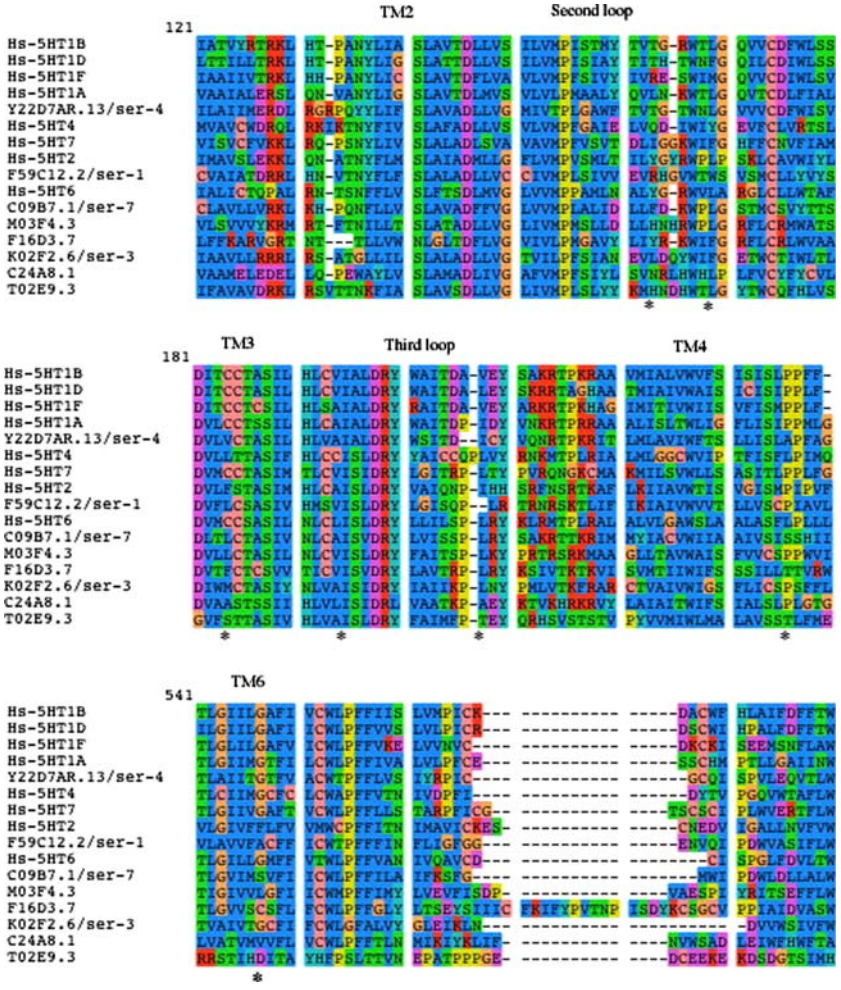

Fig. 1 Partial alignment of the peptide sequence of the *C. elegans* 5-HT GPCR and of the major types of human 5-HT GPCR showing type-specific residues. Only the regions of the sequences used to infer the receptor types are shown in this figure. The full alignment can be found in Fig. S1. The color code corresponds to the different amino acids classes. *Hs* Homo sapiens. The human receptor accession number is as follows: 5HT1A:NP_000515, 5HT1B:NP_000854, 5HT1D:NP_000855, 5HT1F:NP_000857, 5HT2:NP_000859, 5HT4:NP_000861, 5HT6:NP_000862, 5HT7:NP_000863. The sequence of the *C. elegans* receptors can be found on wormbase (http://www.wormbase.org). The numbering is with respect to the M03F4.3 sequence. *Stars* indicate residues positions discussed in the text. *TM* transmembrane segment

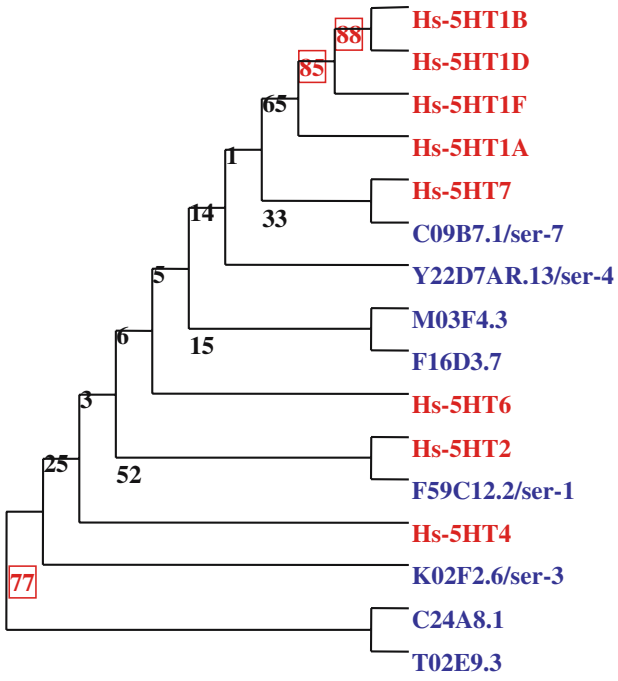

Fig. 2 Phylogenetic tree showing the position of the *C. elegans* 5-HT GPCR with respect to the human receptors. The tree was built by the maximum parsimony method. Bootstrap value are indicated at *branchpoints*

only 5-HT_4 among human receptors. Similarly, in the sixth loop, K02F2.6/*ser-3* has a serine which is close to the threonine specific of 5-HT_4 at this position. In the sixth transmembrane segment, K02F2.6/*ser-3* and 5-HT_4 both have a cysteine, which is also rare amongst 5-HT GPCRs (Fig. 1).

C24A8.1 may be of 5-HT_2 type because it shares a number of specific residues with human 5-HT_2, which are not found in the other types: a proline in the second loop instead of a conserved glycine, a serine in the third transmembrane segment instead of a conserved cysteine, a 5-HT_2 specific leucine/isoleucine in the fourth transmembrane segment and a 5-HT_2 specific leucine in the fifth transmembrane segment (Fig. 1).

T02E9.3, although closer to C24A8.1 than to any other *C. elegans* GPCR, only shares the last two of the four residues mentioned above. It is therefore more difficult to predict its type.

M03F4.3 and F16D3.7 are even more difficult to assign to a receptor type. Their most distinctive feature is a positively charged residue in the third loop, which is unique to 5-HT_6 among human receptors, the other ones carrying a negatively charged or neutral residue at this position.

These observations are summarized in Table 2.

Tissue and stage expression

Overview

We investigated the stage and tissue expression of the eight receptors. For that purpose, we first constructed

Table 2 Receptor classification and signalling pathways

Gene	5-HT receptor type	Specificity	Pathway
F59C12.2/*ser-1*	5-HT_2[b]	Mixed 5-HT_1 and 5-HT_2 properties[b], low affinity for 5-HT[e], α-methyl-5HT as an agonist[f], mianserin as an agonist[g]	Coupled to Ca^{2+} signaling[b]
Y22D7AR.13/*ser-4*	5-HT_1[c]	Low affinity for 5-HT, overall 5-HT_1 properties[c]	Attenuate adenylate cyclase activity[c]
K02F2.6/*ser-3*	5-HT_4?	ND	ND
C09B7.1/*ser-7*	5-HT_7[d]	High affinity for 5-HT and tryptamine, no high affinity for 5-CT[d]	Activate adenylate cyclase activity[a,d]
M03F4.3	?	ND	Independent of adenylate cyclase activity[a]
F16D3.7	?	ND	Independent of adenylate cyclase activity[a]
T02E9.3	?	ND	ND
C24A8.1	5-HT_2?	ND	ND

[a] Our data
[b] Hamdan et al. 1999
[c] Olde et al. 1997
[d] Hobson et al. 2003
[e] Xie et al. 2005
[f] Carnell et al. 2005
[g] Dempsey et al. 2005

gfp lines carrying promoter regions of the genes. The results are given in Table 3 and are illustrated in Fig. 3.

We also matched our results with expression data obtained by different means. The first one is based on transcriptome data analysis performed on DNA microarrays (Kim et al. 2001). Clustering programs group together genes which have resembling transcriptome profiles across a large set of experimental conditions. Genes are associated together by such programs in clusters (or mountains). These clusters often contain genes which are expressed and functionally relevant to the same tissue. The 19,000 *C. elegans* genes can be grouped in 43 mountains (Kim et al. 2001). We looked on http://www.wormbase.org at the mountain associated with each of the eight genes of interest. This analysis showed that five of the eight genes (K02F2.6/*ser-3*, C24A8.1, M03F4.3, F16D3.7, T02E9.3) belong either to mountain 1 (which is enriched in neuronal and muscle genes) or to mountain 13 (neuronal genes). Two other genes (F59C12.2/*ser-1* and C09B7.1/*ser-7*) belong to mountains 0 and 10, which are less specific. The mountain data for Y22D7AR.13/*ser-4* were not available (Table 3).

Another useful dataset to follow gene expression comes from serial analysis of gene expression (SAGE), which is a direct way of measuring the abundance of a gene transcript in a tissue. SAGE *C. elegans* libraries are made either from total animals at a given developmental stage, or from cell-sorted dissociated embryonic cells of a given lineage (McKay et al. 2003). Data relative to the eight genes of this study were collected on http://www.elegans.bcgsc.bc.ca/home/ and are shown in Table 3. (A more detailed version of this Table can be found in supplementary materials, Table S1).

Table 3 Time and tissue expression

Gene	Microarray data[a]		SAGE data[b]	Gfp expression
	Mountain#	Functional group		
F59C12.2/*ser-1*	10	ND	Young adults	Head neurons[c], pharyngeal muscles, pharyngeal nerve ring[d], vulval muscles, many neurons[g] , neurons of the head and the tail, motoneurons of the ventral cord, vulva muscles, uterine cells, plus in male-specific diagonal muscles[h]
Y22D7AR.13/*ser-4*	ND		Pharynx	Head neurons[c], RIB and RIS head interneuron classes, pharyngeal neuron, a pair of sublateral inter- or motorneurons, pair of neurons in the retrovesicular ganglion, the PVT tail neuron and either the DVA or DVC tail interneuron[d]
K02F2.6/*ser-3*	1	Muscle and neuronal genes	Muscle cells pan-neuronal cells pharynx	Head and tail neurons[d]
C09B7.1/*ser-7*	0	ND	Larvae neuronal and pharynx cells	M4 pharyngeal neuron[e], MCs, M4, I2s, I3, M5, M3s, I4, I6 and M2s pharyngeal neurons, vulval muscles[f], head and tail neurons[c,d]
C24A8.1	1	Muscle and neuronal genes	Neuronal cells	No expression detected[c]
M03F4.3	13	Neuronal genes	Embryos, larvae muscle and neuronal cells	Head neurons (anterior deirid, cephalic sensilla), vulva[c], head and tail neurons[d]
F16D3.7	1	Muscle and neuronal genes	L1 larvae and young adults neuronal and pharynx cells	Body-wall muscle cells, vulva muscle cells, neurons in the head[c]
T02E9.3	13	Neuronal genes	L3 larvae	Head and tail neurons[d] no expression detected[c]

[a] Data extracted from http://www.wormbase.org

[b] Data extracted from http://www.elegans.bcgsc.bc.ca/home/

[c] Our data

[d] Tsalik et al. (2003)

[e] Hobson et al. (2003)

[f] Hobson et al. (2006)

[g] Dempsey et al. (2005)

[h] Carnell et al. (2005)

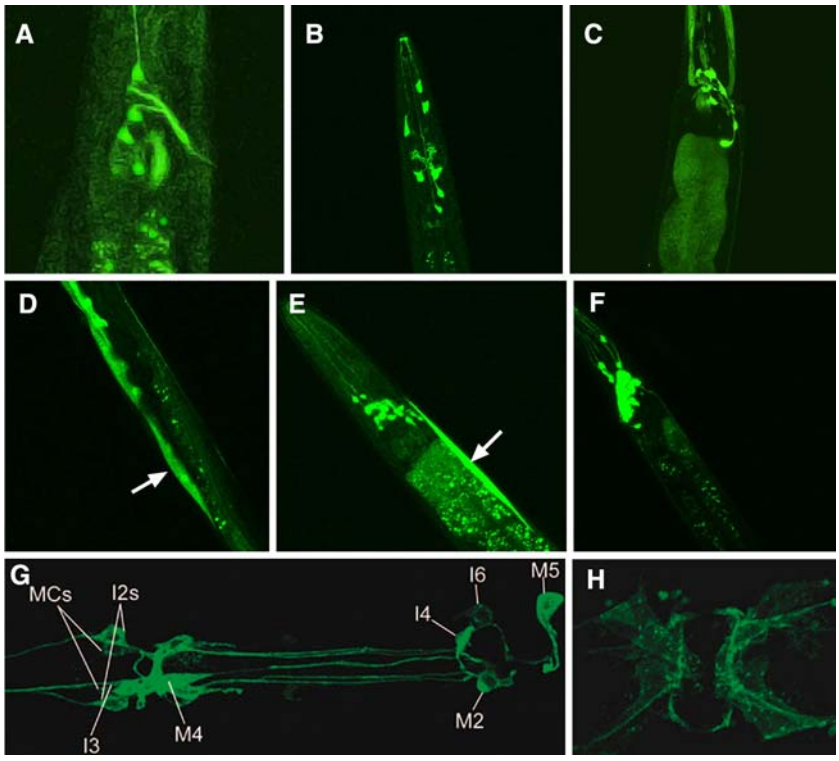

Fig. 3 Tissue expression of *C. elegans* 5-HT GPCRs. Expression was determined by gfp reporter gene in *C. elegans* transgenic animals. **a** F59C12.2/*ser-1*, gfp expression is seen in head and pharynx neurons, as well as in pharyngeal muscles. **b** Y22D7AR.13/*ser-4*, gfp expression is seen in a few head and pharynx neurons. **c** M03F4.3, gfp is seen in head neurons. **d**–**f** F16D3.7, gfp is seen in head and tail neurons, as well as in body wall muscles (*arrows*). **g**, **h** C09B7.1/*ser-7*, expression is seen pharynx and head neurons, pharyngeal muscles and vulva muscles (reproduced from Hobson et al. 2006 with permission). For a complete description of gfp patterns, see main text and Table 3. No gfp staining was detected for the other genes

Two major observations arise from the SAGE data about the eight 5-HT GPCR genes studied here. First, the level of expression is weak. Four of eight genes have only 0 or 1 occurrences across the nine SAGE libraries considered. The remaining four genes have at most three occurrences in a given library, and are present in only a few of the stage-specific libraries. Second, SAGE patterns vary from gene to gene, confirming the gfp observations that the genes have overlapping but distinct spatial expressions.

Tissue expression of individual genes

F59C12.2/ser-1 was reported to be expressed in most pharyngeal muscles and possibly in pharyngeal neurons by a gfp construct by Tsalik et al. (2003). Using a larger piece of the upstream sequences (4.9 kb instead of 1.7 kb), Dempsey et al. (2005) observed the expression of *F59C12.2/ser-1* in vulva muscles, in the ventral neural cord, in several neurons and interneurons in the head and in the PVT neuron in the tail in addition to pharyngeal muscles. By using a gfp transgene including a 8 kb promoter region, Carnell et al. (2005) showed additional expression of F59C12.2/*ser-1* in motoneurons of the ventral cord from L1 to adulthood, in adults vulval muscles, in adults uterine cells and a faint expression in posterior intestine cells of adult worms (Carnell et al. 2005; Dempsey et al. 2005; Tsalik et al. 2003). Our gfp strains also showed head neuron expression. The discrepancies in expression patterns may be due to the fact that F59C12.2/*ser-1* encodes 2 isoforms with different 5′ ends. It is to be noted that F59C12.2/*ser-1* was never detected in either the HSNs neurons or the VC neuron, which are known to regulate egg-laying (Trent et al. 1983; Waggoner et al. 1998).

Y22D7AR.13/*ser-4* expression is restricted to neurons. Tsalik et al. (2003) used a reporter gene with a promoter region including 4.1 kb upstream of the predicted ATG of Y22D7AR.13/*ser-4*. They saw gfp expression in the RIB and RIS head interneuron classes and a fainter expression in a pharyngeal neuron, a pair of sublateral inter- or motorneurons. They also reported rare observations of expression in a pair of neurons in the retrovesicular ganglion, the PVT tail neuron and either the DVA or DVC tail interneuron. We also observed Y22D7AR.13/*ser-4* expression in head neurons which have not been identified.

Tsalik et al. (2003) used a reporter gene comprising 4.1 kb upstream of the predicted start codon of K02F2.6/*ser-3* (middle of exon 2) fused to gfp. They observed an expression pattern restricted to a subset of head and tail neurons but did not described it further.

C09B7.1/*ser-7* was first reported to be expressed in a restricted subset of head and tail neurons (Tsalik et al. 2003). With a slightly different gfp construct, Hobson

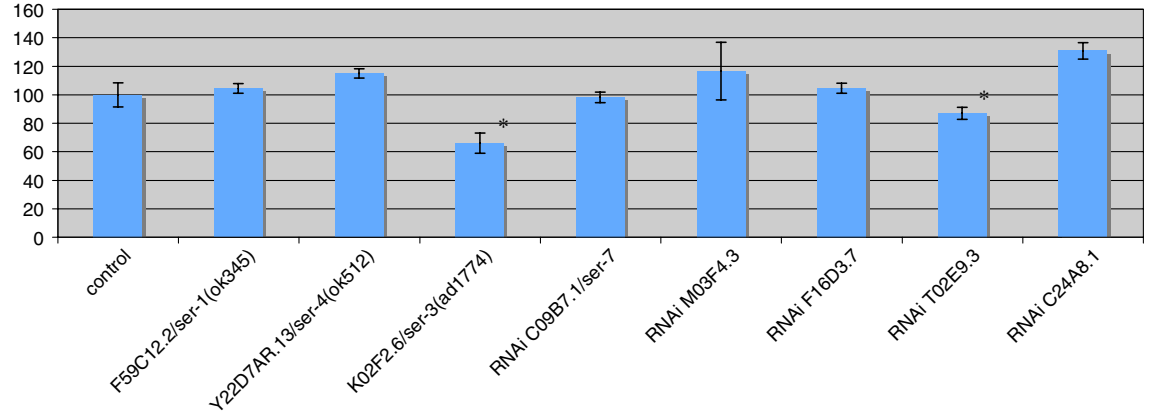

Fig. 4 Brood size. *Bars* indicate the number of live offspring obtained from mutant lines of genotype F59C12.2/*ser-1*(*ok345*), Y22D7AR.13/*ser-4*(*ok512*), K02F2.6/*ser-3*(*ad1774*), or from N2 animals in which the receptor genes have been inactivated by RNAi. Since not all the experiments were performed simultaneously, the results are normalized to the control value of each experiment. *Significantly lower than control value ($P < 0.05$)

et al. (2003) reported the expression of C09B7.1/*ser-7* exclusively in the M4 pharyngeal neuron from L1 to adulthood. The same group later described a more precise expression pattern with a full length functional C09B7.1/*ser-7* construct, in which the gfp coding sequence was included in the third exon (predicted third intra-cellular loop of the receptor) (Hobson et al. 2006). Expression was observed in the MCs, M4, I2s, I3, M5 pharyngeal neurons and occasionally in the M3s, I4, I6 and M2s pharyngeal neurons. They also observed a strong fluorescence in the vulval muscles. However the transgenic strains did not carry very well, possibly because of an adverse effect. Injected at lower concentrations, their construct produced a fainter fluorescence pattern but it was sufficient to rescue the pumping phenotype (Hobson et al. 2006). We also detected gfp expression in unidentified head and tail neurons.

We did not detect gfp fluorescence in C24A8.1:gfp transgenic animals.

Tsalik et al. (2003) observed the expression in unidentified head and tail neurons. We observed M03F4.3 expression in some head neurons and in the vulva.

We saw F16D3.7 reporter gene expression in body-wall muscle cells, in vulva muscles and in some neurons in the head.

Tsalik et al. (2003) observed the expression of the gene in unidentified head and tail neurons. We did not detect any fluorescence with our gfp construct.

In summary, with the exception of C24A8.1, gene expression could be detected by gfp for each gene. The most striking feature of the eight genes studied here is their low expression level, as was already seen with the SAGE data. Expression appears to be mostly in neurons, and less often in muscles. Neuron expression is a common feature of the genes, although the neurons expressing the receptors differ from gene to gene. The three well characterized 5-HT receptors of *C. elegans* (F59C12.2/*ser-1*, Y22D7AR.13/*ser-4*, and C09B7.1/*ser-7*) are present in the pharynx (muscles and neurons), and 4/8 receptors are expressed in the vulva muscles. The expression profile which can be inferred from the SAGE data is in line with the role played by some of the receptors in the 5-HT modulation of pharyngeal pumping and egg-laying (see below).

Role of 5-HT GPCR in *C. elegans* growth and behaviors

To determine the involvement of the eight receptors in the physiology and behaviors of *C. elegans*, we quantitated the brood size, as well as 5-HT modulated behaviors, in animals where the target genes had been inactivated by mutations or RNAi.

Brood size

The brood size was significantly lower for two out of the eight receptors tested (K02F2.6/*ser-3*, and T02E9.3) when compared to the wild-type N2 strain. A reduction of brood size upon inactivation had not been reported before for these genes. We did not investigate further the cause of the brood size reduction. Although these mutants have been backcrossed, one cannot rule out the possibility that the reduction of brood size observed here results from another mutation genetically linked to the receptor. Inactivation of

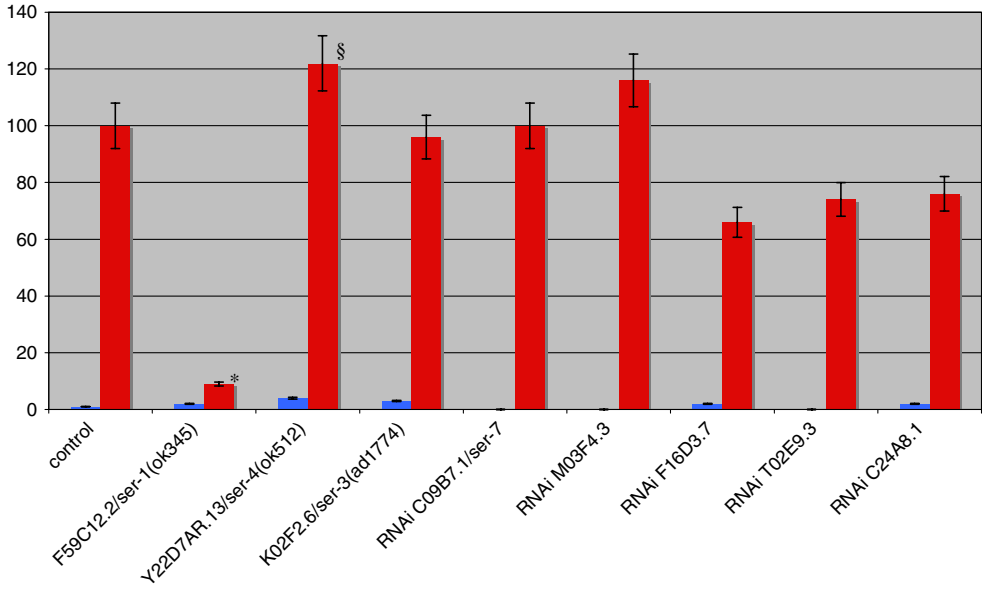

Fig. 5 Egg-laying rate. *Bars* indicate the number of eggs laid in a 2-h period by animals of genotype F59C12.2/*ser-1*(*ok345*), Y22D7AR.13/*ser-4*(*ok512*), K02F2.6/*ser-3*(*ad1774*), or by N2 animals in which the receptor genes have been inactivated by RNAi. *Blue bars* without 5-HT, *red bars* with 4 mg/ml 5-HT. Since not all the experiments were performed simultaneously, the results are normalized to the control value of each experiment. *Significantly lower than control value ($P < 0.05$); §Significantly higher than control value ($P < 0.05$)

the other genes did not significantly affect the brood size (Fig. 4).

Serotonin-modulated behaviors characterized in this study

5-HT modulates numerous behaviors of *C. elegans*. Notably, it depresses locomotion, stimulates pharyngeal pumping, stimulates egg-laying (Horvitz et al. 1982), and stimulates the male mating behavior (Loer and Kenyon 1993). We characterized the response to 5-HT of animals in which the receptors had been inactivated.

Egg laying and eggs in utero

Results obtained from this study The involvement of 5-HT receptors in the egg laying behavior was quantitated in two ways. First, the egg laying rate was determined in presence and in absence of 5-HT. Secondly, the number of eggs in the uterus of non-stimulated animals was quantitated.

For the egg-laying behavior, the basic rate (in absence of food and 5-HT) of all mutants and RNAi treated animals was not different from controls. In stimulated animals (4 mg/ml 5-HT) F59C12.2/*ser-1* and Y22D7AR.13/*ser-4* responded differently from controls. F59C12.2/*ser-1* mutants were almost insensitive to 5-HT, whereas Y22D7AR.13/*ser-4* mutants appeared hypersensitive (Fig. 5).

The number of egg in the uterus of well-fed animals on NGM plates is also a good indicator of 5-HT signalling in the vulva (Trent et al. 1983). F59C12.2/*ser-1* mutants had more eggs in utero than wild-type (Fig. 6), which correlated with its poor sensitivity to exogenous 5-HT and reveals a defect in 5-HT signalling. Conversely, Y22D7AR.13/*ser-4* animals had fewer eggs in utero, indicating that they might be slightly egg-laying constitutive (egl-c phenotype). K02F2.6/*ser-3* mutants had even fewer eggs in utero. However, since these animals have a reduced brood size, it is unclear whether the reduced number of eggs in utero is caused by an egl-c phenotype, or by a reduced fertility.

Comparison to results obtained by others The resistance to 5-HT of F59C12.2/*ser-1* mutants has already been noted by others (Carnell et al. 2005; Dempsey et al. 2005; Komuniecki et al. 2004). Dempsey et al. (2005) showed a total rescue of the 5-HT dependent egg-laying response when F59C12.2/*ser-1* is re-expressed in muscle cells whereas Carnell et al. (2005) showed only partial rescue of that response. However, Dempsey et al. (2005) also showed that re-expression of F59C12.2/*ser-1* in neurons could partially restore the egg-laying response to 5-HT. Thus, it is still unclear whether F59C12.2/*ser-1* is required in neurons or muscles or both.

Regarding Y22D7AR.13/*ser-4*, our results are similar to those of others who also observed an hypersensitivity of mutants to 5-HT (Hobson et al. 2006; Komuniecki et al. 2004). Dempsey et al. (2005) did not observe this effect. Hobson et al. (2006) found that C09B7.1/*ser-7*(*tm1325*) and C09B7.1/*ser-7*(*tm1728*) mutants were no longer sensitive to 5-HT-induced egg-laying. We did not observe such an effect. The discrepancy might come from the fact that we used RNAi inactivation.

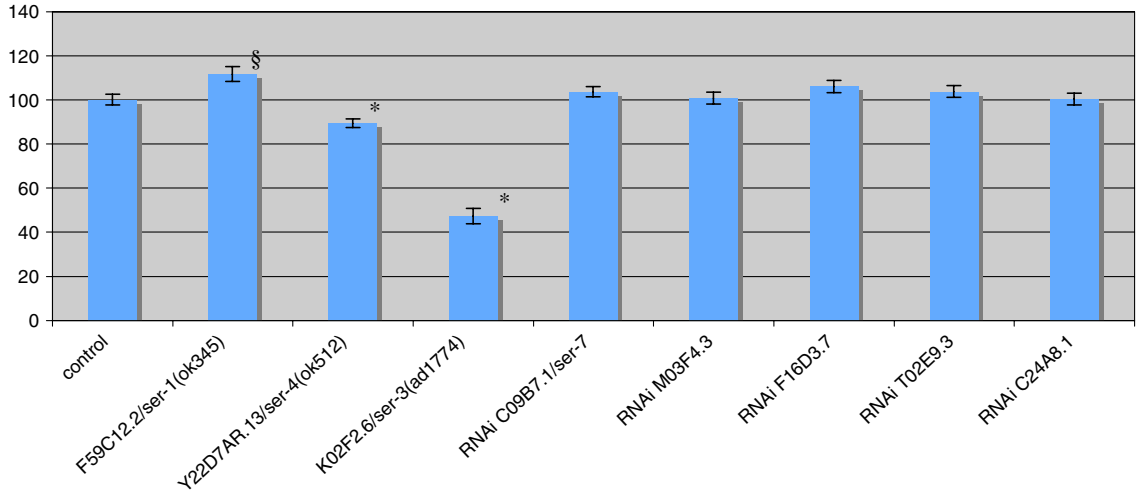

Fig. 6 Eggs in utero. *Bars* indicate the number of eggs present in the uterus of synchronized gravid adults of genotype F59C12.2/*ser-1*(*ok345*), Y22D7AR.13/*ser-4*(*ok512*), K02F2.6/*ser-3*(*ad1774*), or of N2 animals in which the receptor genes have been inactivated by RNAi. Since not all the experiments were performed simultaneously, the results are normalized to the control value of each experiment. *Significantly lower than control value ($P < 0.05$); §Significantly higher than control value ($P < 0.05$)

Pharyngeal pumping

Results obtained from this study Like egg-laying, pharyngeal pumping was assayed in absence and in presence of exogenous 5-HT. On NGM plates, three genes (K02F2.6/*ser-3*, C09B7.1, and T02E9.3) led to a reduced pumping rate when inactivated (Fig. 7, Table 4). On 5-HT, two genes (F59C12.2/*ser-1* and K02F2.6/*ser-3*) showed a slightly reduced pumping rate (Fig. 7). These four genes are expressed in pharyngeal neurons, or in head neurons which have not been identified precisely but which are likely to be pharyngeal neurons by their position. It is to be noted however, that the reduction of the pumping rate is small. This suggests that the regulation of pumping in response to 5-HT may be modulated by a redundant mechanism.

Comparison to results obtained by others Komuniecki et al. (2004) did not observe a significant difference of the pumping rate between the F59C12.2/*ser-1*(*ok345*) worms and the N2 worms on 5-HT. However, although not statistically significant, they observed a 10% reduction of the pumping rate compared to N2 (Komuniecki et al. 2004). The amplitude of the variation is similar to our observations.

Our results regarding Y22D7AR.13/*ser-4* are in line with those of others (Hobson et al. 2006; Komuniecki et al. 2004). Using mutants of C09B7.1/*ser-7*, Hobson et al. (2006) observed a reduced rate of pumping on 5-HT. In our hands, it was the basal rate which was slightly reduced, and not the response to 5-HT.

Locomotion

Results obtained from this study Next, locomotion was assayed in absence and in presence of exogenous 5-HT. 5-HT is a strong modulator of the locomotion rate. Animals deficient in 5-HT signalling (like *goa-1*) (Segalat et al. 1995), are spontaneously hyperactive. Three genes (Y22D7AR.13/*ser-4*, K02F2.6/*ser-3*, and M03F4.3) led to hyperactive locomotion when inactivated (Fig. 8, Table 4). They are therefore supposed to be involved in the 5-HT modulation of locomotion. Surprisingly, on 5-HT, none of the genes tested led to an hyperactive locomotion. On the contrary, RNAi inactivated K02F2.6/*ser-3* animals were more sluggish than controls, as if these animals were hypersensitive to 5-HT. It is to be noted that F16D3.7, the only genes on the list to be expressed in body wall muscles, was not different from control, indicating again that locomotion may be modulated by 5-HT at the level of the neurons, not the muscles.

Comparison to results obtained by others Similar to our findings, Komuniecki et al. (2004) did not observe any difference in the locomotion rate of F59C12.2/*ser-1*(*ok345*) mutants worms compared to N2, with or without 5-HT. Contrarily to our findings, Keating et al. (2003) did not observe any difference between locomotion rates of RNAi induced M03F4.3 worms and N2. Komuniecki et al. (2004) reported a partial resistance of Y22D7AR.13/*ser-4* mutants in the presence of 5-HT whereas we found a more pronounced effect on

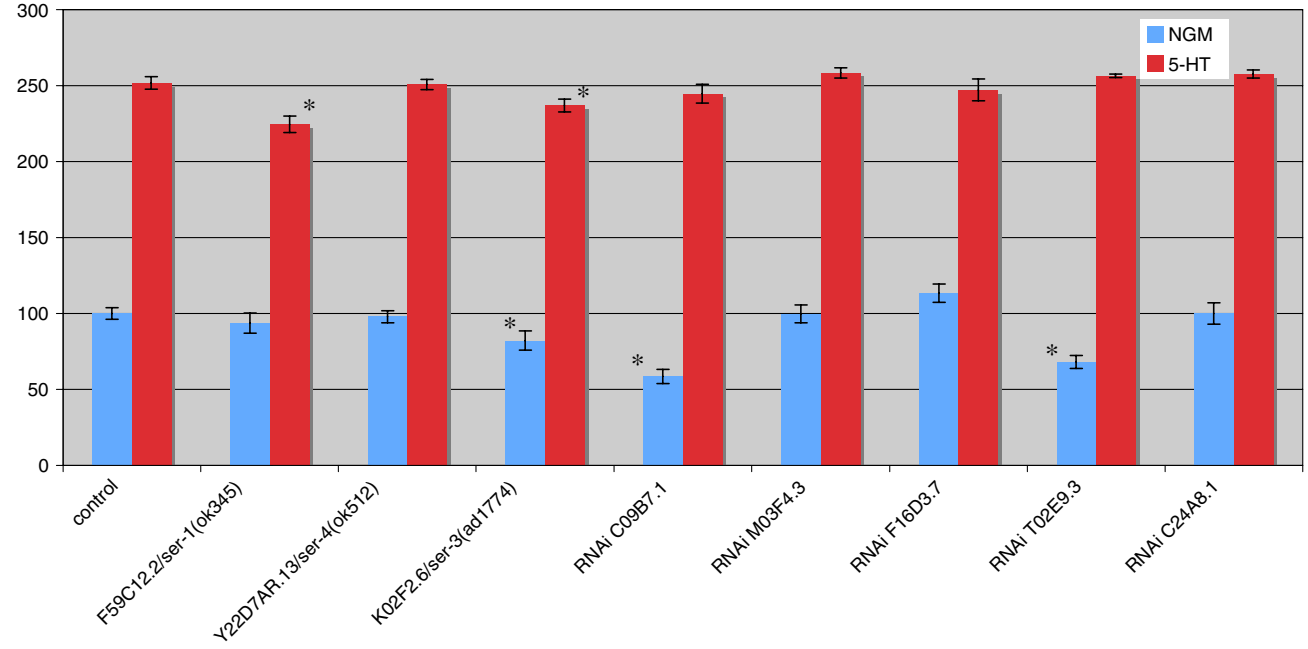

Fig. 7 Pharyngeal pumping. *Bars* indicate the pumping rate adults of genotype F59C12.2/*ser-1*(*ok345*), Y22D7AR.13/*ser-4*(*ok512*), K02F2.6/*ser-3*(*ad1774*), or of N2 animals in which the receptor genes have been inactivated by RNAi. *Blue bars* without 5-HT, *red bars* with 4 mg/ml 5-HT. Since not all the experiments were performed simultaneously, the results are normalized to the control value of each experiment. *Significantly lower than control value ($P < 0.05$)

the basal level. This difference might be explained by the way body bends are counted, or by the time animals are left on the 5-HT plates before they are scored.

Male mating

Results obtained from this study Finally we looked at male tail curling, a male-specific mating behavior also modulated by 5-HT (Loer and Kenyon 1993). Like previous 5-HT-dependent behaviors, male tail curling was assayed in absence and in presence of exogenous 5-HT. On regular NGM plates, no statistically significant difference could be detected between mutants and controls (Fig. 9). On 5-HT, two genes (Y22D7AR.13/*ser-4* and T02E9.3) showed a clearly reduced response to 5-HT. These two genes are expressed in tail neurons. K02F2.6/*ser-3* animals were significantly more active on 5-HT than controls, suggesting that these animals were hypersensitive to 5-HT.

Comparison to results obtained by others Carnell et al. (2005) found that F59C12.2*/ser-1(ok345)* mutants are partly resistant to 5-HT. Although our results were not statistically significant, we observed a similar tendency. This effect on male mating is consistent with the expression of the gene in the male-specific diagonal muscles (Carnell et al. 2005).

Activation of adenylate cyclase by *C. elegans* 5-HT GPCRs

Several classes of vertebrate and invertebrate 5-HT receptors activate adenylate cyclase, thereby increasing intracellular cAMP levels (Hartig 1997). Demonstration of such an activity upon 5-HT binding is a definitive proof that a candidate 5-HT receptor is a 5-HT receptor. In a attempt to demonstrate that F16D3.7, M03F4.3, and T02E9.3 were bona fide 5-HT receptors, we set out to measure their cAMP levels in response to 5-HT.

Results obtained from this study

We tested whether the putative receptors were able to stimulate adenylate cyclase activity in response to 5-HT in transfected mammalian cells. This work was performed only on C09B7.1*/ser-7* which served as a positive control, F16D3.7, and M03F4.3. Attempts to clone a T02E9.3 cDNA in the expression vector were unsuccessful. cAMP level were measured in transfected cells after stimulation by 5-HT. In order to assess the ligand specificity of the receptors, the response to dopamine, tyramine, and octopamine was measured in parallel. After 5-HT stimulation, cells transfected with C09B7.1*/ser-7* showed a 34 fold increase of cAMP concentration over cells transfected with the empty

Table 4 Behaviors of mutant and RNAi-treated animals

Gene	Brood size	Eggs in utero	Egg laying	Pharyngeal pumping	Locomotion	Male mating
F59C12.2/*ser-1*	No difference[a]	More than N2[a,c,d], no difference[b]	Resistant to 5-HT[a,c,d,e]	Lower than N2 on 5-HT[a], no difference[e]	No difference[a,e]	No difference[a], partially resistant to 5-HT[d]
Y22D7AR.13/*ser-4*	No difference[a]	Fewer than N2[a,b], no difference[c]	Hypersensitive to 5-HT[a,b,e], no difference[c]	No difference[a,b,e]	Higher than N2 on control medium[a], partially resistant to 5-HT[e]	Resistant to 5-HT[a]
K02F2.6/*ser-3*	Smaller than N2[a]	Fewer than N2[a]	No difference[a]	Lower than N2 on control medium and on 5-HT[a]	Higher than N2 on control medium and partially resistant to 5-HT[a]	Hypersensitive to 5-HT[a]
C09B7.1/*ser-7*	No difference[a]	No difference[a,b]	No difference[a] resistant to 5-HT[b]	Lower than N2 on control medium[a], lower than N2 on 5-HT[b]	No difference[a]	No difference[a]
M03F4.3	No difference[a]	No difference[a]	No difference[a]	No difference[a]	Higher than N2 on control medium[a], no difference[f]	No difference[a]
F16D3.7	No difference[a]	No difference[a]	No difference[a]	No difference[a]	No difference[a]	No difference[a]
T02E9.3	Smaller than N2[a]	No difference[a]	No difference[a]	Lower than N2 on control medium[a]	No difference[a]	Partially resistant to 5-HT[a]
C24A8.1	No difference[a]	No difference[a]	No difference[a]	No difference[a]	No difference[a]	No difference[a]

[a] Our data

[b] Hobson et al. (2006)

[c] Dempsey et al. (2005)

[d] Carnell et al. (2005)

[e] Komuniecki et al. (2004)

[f] Keating et al. (2003)

vector (Table 5). In addition to 5-HT, C09B7.1/*ser-7* was also responsive to dopamine, albeit to a lesser extent. 5-HT-stimulated cells transfected with F16D3.7 and M03F4.3 did not show any significant increase in cAMP levels (Table 5). These two receptors were also insensitive to the other ligands (Table 5).

These results indicate that (1) C09B7.1/*ser-7* is a *bona fide* 5-HT receptor activating adenylate cyclase, and (2) F16D3.7 and M03F4.3, if 5-HT receptors, are coupled to a signalling pathway independent of adenylate cyclase.

Comparison to results obtained by others

F59C12.2/*ser-1* was shown in COS-7 cells to have mixed 5-HT_1 and 5-HT_2 ligand binding properties. It did not respond to dopamine or octopamine (Hamdan et al. 1999). Xie et al. (2005) showed that SER-1 display a low affinity for its ligand, 5-HT (K_i = 9.7 μM). Antagonist of mammalian 5-HT_2 receptors, mianserin, was shown to be effective to block SER-1 dependent egg-laying response to 5-HT (Dempsey et al. 2005). SER-1 appears to be coupled to a Ca^{2+} mediated signalling pathway and is likely to be coupled to a $G_{\alpha q}$ protein (Bastiani et al. 2003; Hamdan et al. 1999).

SER-4 exhibits low affinity for 5-HT (16 μM) (Olde and McCombie 1997). When expressed in Ltk mammalian cells and stimulated by serotonin, SER-4 was able to lower cAMP levels, showing that SER-4 can attenuate adenylate cyclase activity (Olde and McCombie 1997). The authors suggested that SER-4 might be coupled to a protein G similar to a mammalian protein $G_{\alpha i}$.

When expressed in COS-7 cells, SER-7 exhibited high affinity for 5-HT and tryptamine but not the other biogenic amines (Hobson et al. 2003). SER-7 pharmacology differ from that of the 5-HT_7 mammalian receptor, to which it is closely related since it presents low affinity for many of the specific agonists of the 5-HT_7 mammalian receptor (Hobson et al. 2006).

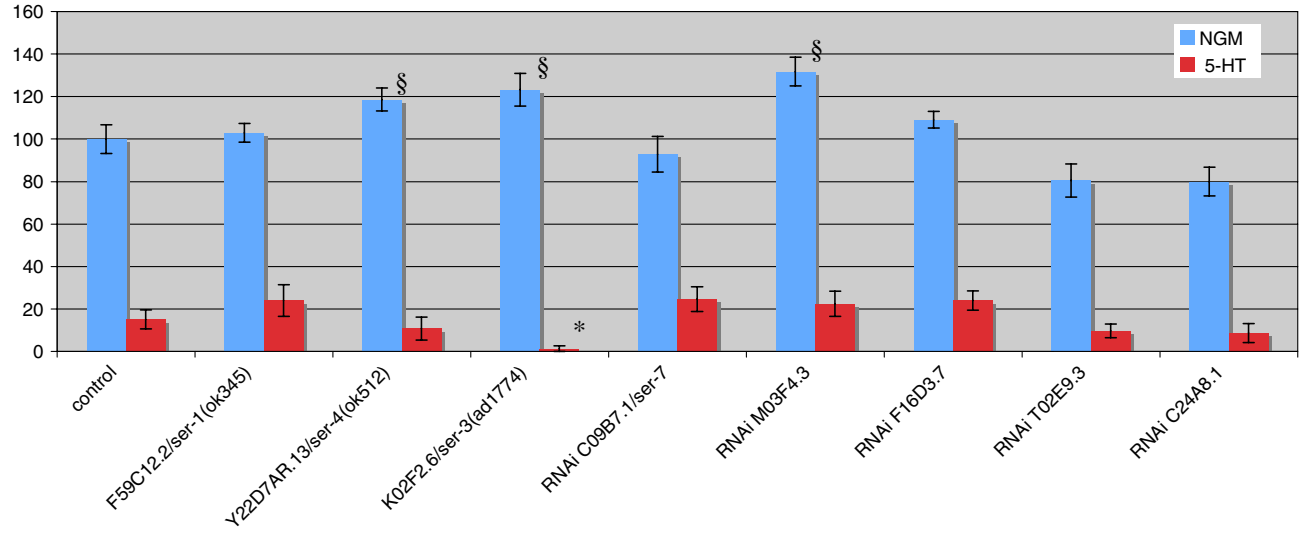

Fig. 8 Locomotion. *Bars* indicate the locomotion rate adults of genotype F59C12.2/*ser-1*(*ok345*), Y22D7AR.13/*ser-4*(*ok512*), K02F2.6/*ser-3*(*ad1774*), or of N2 animals in which the receptor genes have been inactivated by RNAi. *Blue bars* without 5-HT, *red bars*, with 4 mg/ml 5-HT. Since not all the experiments were performed simultaneously, the results are normalized to the control value of each experiment. *Significantly lower than control value ($P < 0.05$), §Significantly higher than control value ($P < 0.05$)

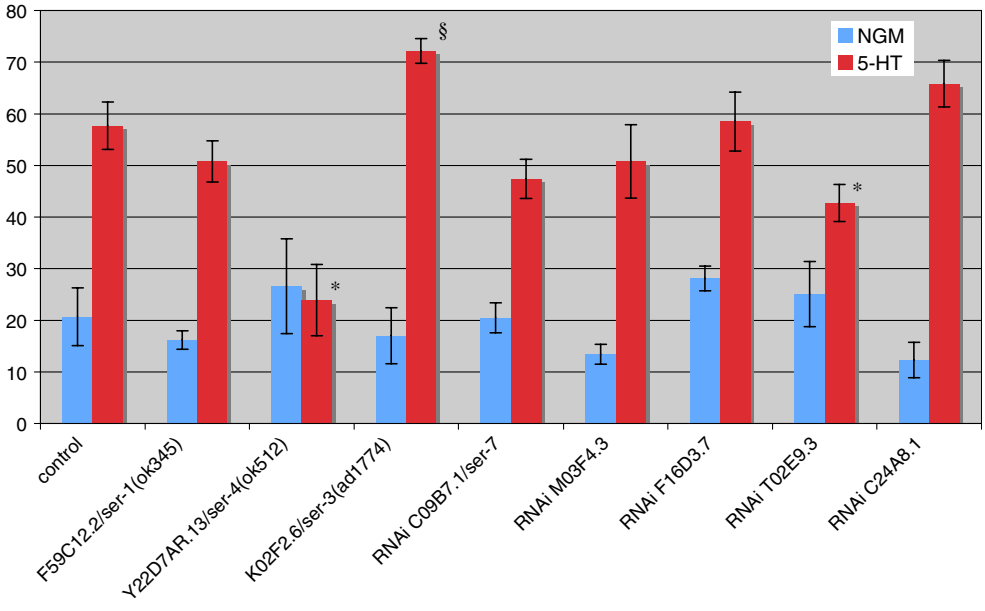

Fig. 9 Male mating. *Bars* indicate the percentage of adult males displaying a male mating behavior. *Blue bars* without 5-HT, *red bars* with 4 mg/ml 5-HT. *Significantly lower than control value ($P < 0.05$), §Significantly higher than control value ($P < 0.05$)

However, such differences in the pharmacological profile of invertebrate 5-HT GPCRs are frequent and are explained by the protein sequence divergence between mammalian and worm counterparts (Tierney 2001).

Conclusion

The purpose of this study was to set the list of existing and putative *C. elegans* 5-HT GPCRs; and to characterize (1) their expression, (2) their involvement in 5-HT-modulated behaviors, (3) their signalling pathways.

The basal information regarding *C. elegans* 5-HT GPCRs has been up to now fragmented in numerous papers, most of them focused on a single gene or a single behaviors, thus making comparisons uneasy. In this paper, despite the limitation of the RNAi technique which produces only partial gene inactivation for some genes tested, we have attempted to provide a comprehensive study of the receptors, reassembling

Table 5 cAMP level determination in COS-7 cells expressing known and putative 5-HT GPCR genes

Gene	cAMP levels compared to control				
	Basal	5-HT	Dopamine	Tyramine	Octopamine
Control	1.00 ± 0.05	0.99 ± 0.02	1.33 ± 0.08	1.45 ± 0.05	1.45 ± 0.05
C09B7.1/*ser-7*	8.97 ± 3.60*	34.00 ± 0.60*	6.35 ± 0.20*	ND	ND
F16D3.7	0.78 ± 0.19	0.58 ± 0.12	0.81 ± 0.08	1.20 ± 0.05	0.92 ± 0.10
M03F4.3	0.65 ± 0.02	0.89 ± 0.10	1.17 ± 0.30	ND	ND

Values are given in arbitrary units ± SEM

ND not determined

*Significantly different from control ($P < 0.05$). The cAMP levels are normalized to the control ($n = 4$)

both new data obtained in standardized experiments and preexisting published information.

The main conclusions of this study are:

- There are between three and eight 5-HT GPCRs in *C. elegans*.
- The *C. elegans* 5-HT GPCRs have a predominantly neuronal expression.
- Inactivaction of the 5-HT GPCRs leads to behavioral profiles which differ from gene to gene, indicating a level of specificity among the receptors functions.
- Inactivation of a given 5-HT GPCRs did not totally impair any of the major 5-HT-modulated behaviors, indicating a level of redundancy among 5-HT GPCRs.
- Inactivation of at least one demonstrated and one putative 5-HT receptors lead to hypersensitivity of 5-HT, suggesting that some 5-HT receptors might act antagonistically to others.
- F16D3.7 and M03F4.3, if 5-HT receptors, are coupled to a signalling pathway independent of adenylate cyclase.

Acknowledgments The authors thank Theresa Stiernagle and the *Caenorhabditis* Genetic Center (CGC) for providing various strains used in this study, and J. Starck, G. Mouchiroud and F. Morlé for technical advice. The authors thank P.O. Barome, P. Vernier, D. Rognan, and F. Bonneton for phylogenetic analysis. This work was supported by grants from the Association Française contre les Myopathies (AFM) and the European Community (MYORES) to L.S., and grants from Genome Canada and Genome BC, NSERC, and a CRC Chair to D.L.B.

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