Supplementary Table S1. Sequences of primers used for cloning of neuropeptide-coding genes. Fw: Forward primer; Rv: Reverse primer.

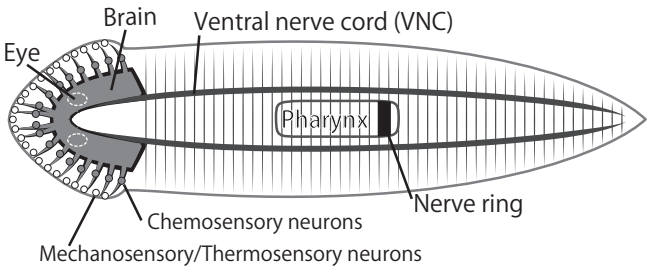
Gene name	Primer sequences
DjNp3	Fw: 5'-ATAAATGAATATGAAAACAATAATTGC-3' Rv: 5'-TAATTCAATTAGTATCTTATTCCTCCA-3
DjNp4	Fw: 5'-CAATTCCAAATCTTTTATATCGAATAC-3' Rv: 5'-TTATTTTCTTTGCAATTCTGTTAATAA-3'
DjNp9	Fw: 5'-ATAAATCCCATTCAAAAACTGACTACT-3' Rv: 5'-TCTTTCACAATAAGAAATTTTTAACCA-3'
DjNp12	Fw: 5'-CATGATATATTTCGATTGAAGAAAGGT-3' Rv: 5'-AAGAAAATTATTCGCAATTATCACAGA-3'
DjNp17	Fw: 5'-AATCATTTTCCTTGTATTTAATATCGG-3' Rv: 5'-AATACAAGCTGTCTGGAATGATTATTT-3'
DjNp19	Fw: 5'-AATCGTCATTGCTGTTAAGGTAATATC-3' Rv: 5'-AATCATTCTTTTCTGATAAATCATTCG-3'
DjNp23	Fw: 5'-GTAATCTTCTTGAGTAATTCGATCGGA-3' Rv: 5'-GAAAAACGATTTGACTACCCATTTCAA-3'
DjNp25	Fw: 5'-GCCAAATTCATTTTTTTTTTCAATATT-3' Rv: 5'-GAGAAAAGACCAAATTGGAAAGATATG-3'
DjNp28	Fw: 5'-ATGAACGGTTATAAAACGCATTTATTG-3' Rv: 5'-AAAAATTCGTTTTCCATATCTTGGTCT-3'
DjNp34	Fw: 5'-CAGATGATCCATTAGTTTTAGGTCTCA-3' Rv: 5'-AAACAGAGAAATCAGTGGTTTTACAAA-3'
DjNp35	Fw: 5'-AAAGAATATTCACTGAAAATCCTTTGG-3' Rv: 5'-TTCTTGTTTTCTCTTCAGTAGCATTTG-3'
DjNp40	Fw: 5'-CATTTTCTTAGTTCTATTTGTGGTAGT-3' Rv: 5'-TATCTTGATAATTTCTCCACATTTTAA-3'
DjNp41	Fw: 5'-AGCCCAATCTCATTAATCGTAAACCTC-3' Rv: 5'-AATTGTGTCTTATGCCGATGGTGATTA-3'
DjNp42	Fw: 5'-ATTATTGCTTTATTGTTTATCGTAGTG-3' Rv: 5'-ATTCATTTTTATCGTAGATTAGCGAAG-3'
DjNp47	Fw: 5'-TGGAAATTATTATCAAAGAACACGAAA-3' Rv: 5'-GTAATTTTCCTCAGACGTGTAGTTGGA-3'
DjNp49	Fw: 5'-TAGCCAAGGTAGATAAATGAAAGAATA-3' Rv: 5'-AAATAAACATGGCAGAAATAAACATAT-3'
DjNp51	Fw: 5'-TTTTAAAATATAACAGGTCCAACGGAA-3' Rv: 5'-TCATTATGATAAAAGATTCGACCCAAT-3'
DjNp52	Fw: 5'-GTGAACTTGGGTCACATTTAAAAATCT-3' Rv: 5'-TCGATTAAACGATATACCCGATTATTT-3'
DjNp56	Fw: 5'-TTAATGAATTTTCAGACCTTGATGATC-3' Rv: 5'-TCATTAATCGTAAACCTCTTTTTTTCA-3'

Supplementary Table S2. Primer sequences for qPCR. Fw: Forward primer; Rv: Reverse primer.

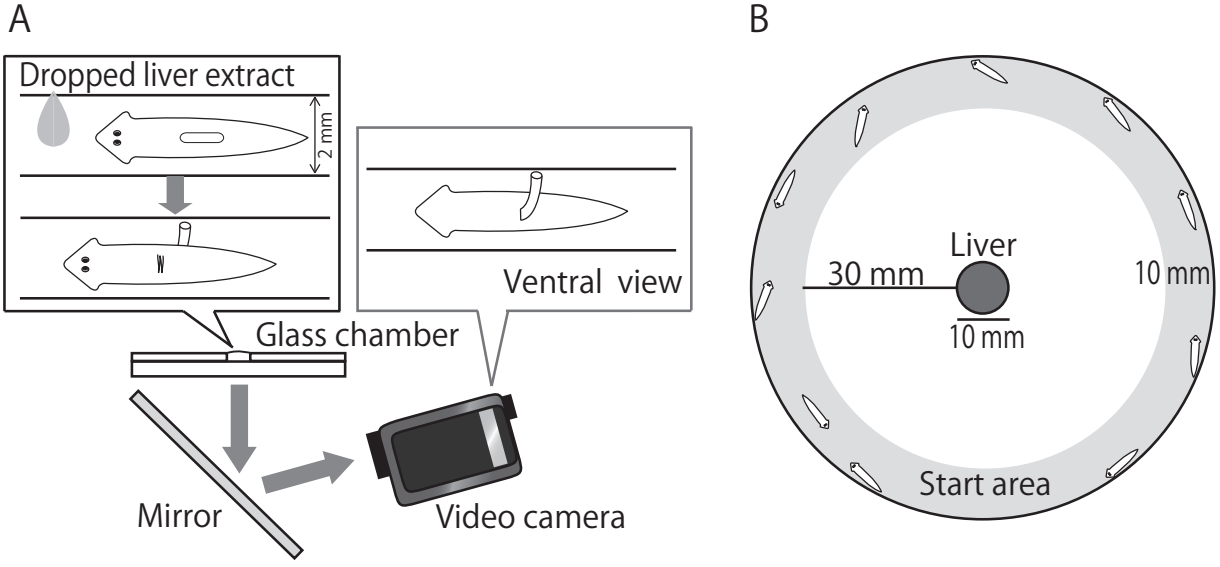
Gene name	Sequences of forward and reverse primers	Reference
DjEF-1	Fw: 5'-TGGTTACTCTCCAGTCTTAGA-3' Rv: 5'-CAGCTTTCTTAGTTACCTCCTT-3'	Mineta et al., 2003
Tyrosine hydroxylase; DjTH	Fw: 5'-CAATGTACACAATACACCAGGCATCAT-3' Rv: 5'-ACATCGGAACATGGCCAAGTAAT-3'	Nishimura et al., 2007a
Tyramineβ-hydroxylase; DjTBH	Fw: 5ACCACGATTGGAACAAAAGCATCACAT-3' Rv: 5'-CCCATTGGTTTGCTTTCTGCATTACAC-3'	
Tryptophan hydroxylase; DjTPH	Fw: 5'-AATTGGCAACGATAACAAATGCAT-3' Rv: 5'-TTTTCCTATATTGATTGATCGGCC-3'	Nishimura et al., 2007b
Choline acetyltransferase; DjChAT	Fw: 5'-AGCAGCATCGATTCCAGCTTTGA-3' Rv: 5'-TGCCCCATAGCACATAAATGCGT-3'	
Glutamic acid decarboxylase; DjGAD	Fw: 5'-AAGAGGAAAAATGGATACTGAAAACC-3' Rv: 5'-ATTTTCAATTCCATTCAGTAAATAGCG-3'	Nishimura et al., 2008b
Djglutaminase	Fw: 5'-AATTGGTTTATGGTCTCCACCTCTT-3' Rv: 5'-ATTTCTGCACAAGTTCCATGCA-3'	Higuchi et al., 2008
Synaptotagmin; Djsyt	Fw: 5'-GTGTTCGTACATCCTATGGCGATATAACAT-3' Rv: 5'-TAGACTAATCACAATGGCTATGACGAATGT-3'	Tazaki et al.,1999
Prohormone convertase2; DjPC2	Fw: 5'-CTCATGTGAGTTCAATTCCAAGTTGGA-3' Rv: 5'-TGGAAGTGGAATACGATCTTGCTTCTT-3'	
Dj1020HH	Fw: 5'-CTTGTGCCAATGGCTATTTTTCTG-3' Rv: 5'-CAAATAGTTTCTTCGCTTCCATCTG-3'	Inoue et al., 2004
Djeye53	Fw: 5'-GAAATGGATCCGAACAAAAGAAGT-3' Rv: 5'-ATACACTCCAACGAAATATCAATTATCG-3'	Inoue et al., 2004
Dj_aH_308_M24	Fw: 5'-CCTATTCCTTGTGACAACAGTTTTAGC-3' Rv: 5'-ATCCTCGTCTTTTACCAATTAAACCTC-3'	
Dj_aH_019_P02	Fw: 5'-AGCGTTGTTTAATCCCAAGAGATGTAC-3' Rv: 5'-AGAAGAGCAGAAAGTTCCGATAGAAAA-3'	
Dj_aH_401_P19	Fw: 5'-TTCAGGTGTCGCCATTCTATTGACATC-3'; Rv: 5'-ATTCTTGTGCCCAGTATGCCTTTTTC-3'	
DjNp4	Fw: 5'-GAATACCACCGGTTAAATAGTTAAGTC-3' Rv: 5'-ATAACTAAAGCACTGTCAATGTCTGTC-3'	
DjNp9	Fw: 5'-ATCCAATTCAAAGCGAGCTCTTGTTCC-3' Rv: 5'-TTTTTATGTCCCAATCATCCCAAGCGT-3'	
DjNp17	Fw: 5'-TCGGAAAATACAAATGAAAAAAGAGCC-3' Rv: 5'-AGATGAATCATAGCTTTTCTTGCCCAG-3'	
DjNp19	Fw: 5'-CTTGCACCACGTTTTCCAAATCTAGTA-3' Rv: 5'-TGATAACCCATTTGAATTTGTTGATCC-3'	
DjNp23	Fw: 5'-AATCTTTTACCGAACATTATTGGATCG-3' Rv: 5'-TTGAAAAACGATTTGACTACCCATTTC-3'	
DjNp25	Fw: 5'-TCTTGTTTAGAGGCATGTATTGATCAC-3' Rv: 5'-GTAAACGTAAGTGCATGGAGAGATATG-3'	
DjNp40	Fw: 5'-TCTGCTCATGACGATACAGAGGATTCA-3' Rv: 5'-CGTTTCCCCATTCTCAGTATTCTCAAA-3'	
DjNp41	Fw: 5'-ATAACATTGTCATCTTCCAAATTCCTC-3' Rv: 5'-ATGGTGATTATTCGTCCTTTAATGAAT-3'	
DjNp42	Fw: 5'-CGATGGGTGCACTTTGTGAATTGGAGA-3' Rv: 5'-GAAGCCTGATCCGAATGTCATTGGGTC-3'	
DjNp47	Fw: 5'-AAGAACACGAAAATTGTCTATTTGCAC-3' Rv: 5'-TCAGACGTGTAGTTGGAAAAAGAAATC-3'	
DjNp49	Fw: 5'-CTACTCATTCTACTCCATTCTCGATAC-3' Rv: 5'-TGATTTCTATGAAATTGTTGTTTTTTA-3'	
DjNp51	Fw: 5'-ATGGGATCAAATCGTTTTTCAAATCGA-3' Rv: 5'-AAAGATTCGACCCAATAATGTTTGGTA-3'	
DjNp52	Fw: 5'-TATGATGGATTATGCTGCCTTTTATAC-3' Rv: 5'-CGATTAAACGATATACCCGATTATTTA-3'	

Supplementary Table S3. Pharynx extension rate of planarians that were offered different dilutions of liver extract. $^{**}P < 0.005$; $t = 120$ sec; $n = 35$ or 36.

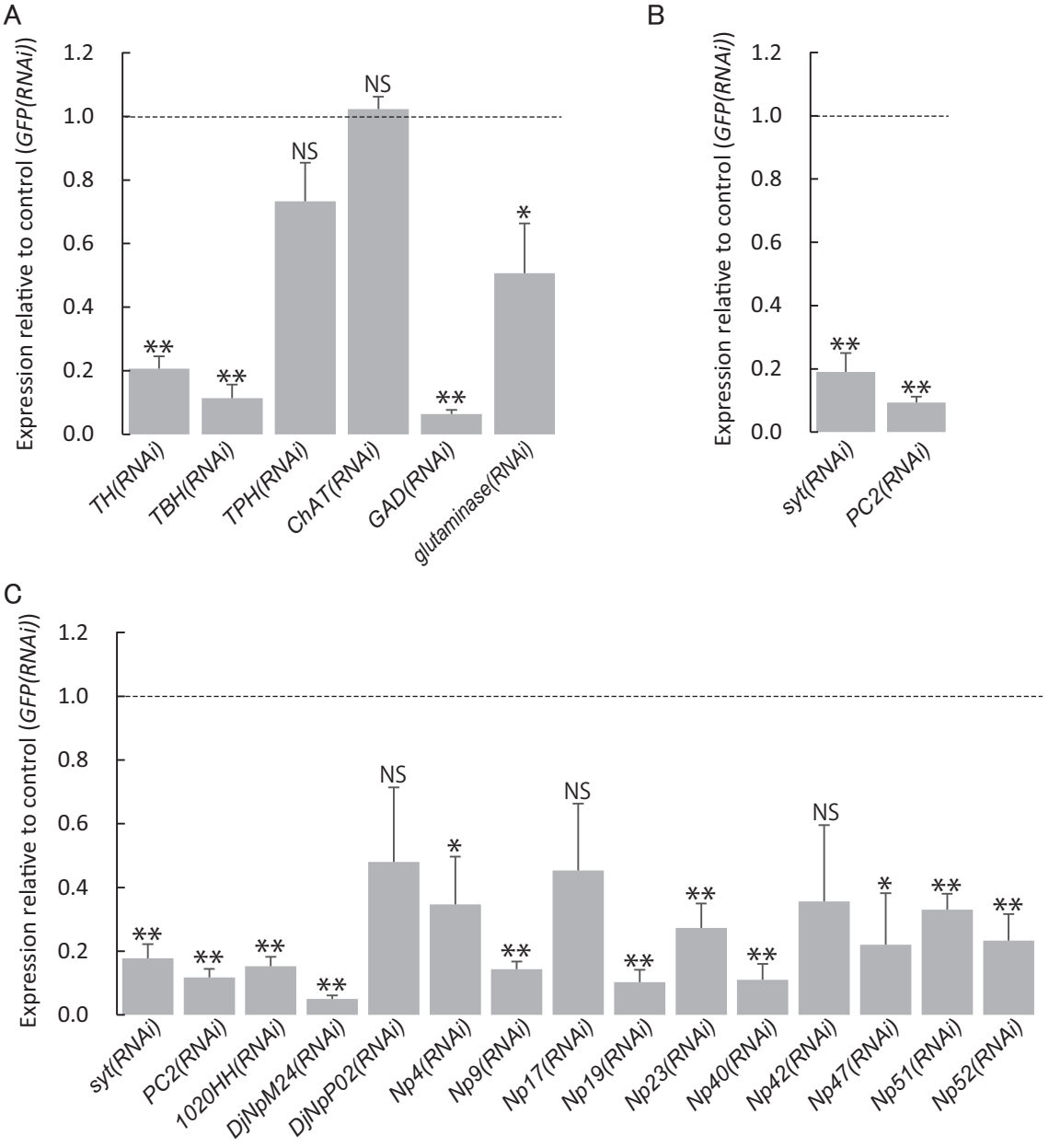
Dropped sample	No. of planarians that extended pharynx/Number of planarians				Avg. pharynx extension rate ± s.e.m.
	Ex.1	Ex.2	Ex.3	Total	
Control(Water)	0/12	0/12	0/12	0/36	0.0 ± 0.0%
1/1000 Liver extract	0/12	0/12	0/12	0/36	0.0 ± 0.0%
1/100 Liver extract	0/12	1/12	3/12	4/36	11.1 ± 7.4%
1/10 Liver extract	10/11	10/12	10/12	30/35**	85.9 ± 2.5%
Undiuted Liver extract	11/12	10/12	11/12	32/36**	88.9 ± 2.8%



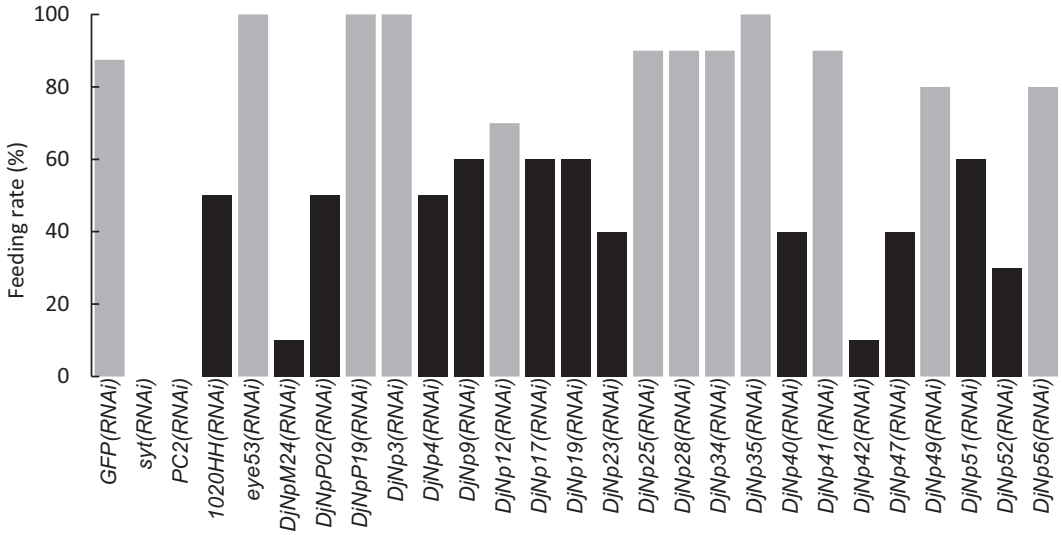
Supplementary Figure S1. Diagrammatic view of the planarian central nervous system from the ventral side. The planarian central nervous system (CNS) consists of an inverted U-shaped brain and ventral nerve cords (VNCs). There is a nerve ring in the tip of the pharynx. White circles represent mechanosensory and themosensory neurons, and gray circles represent chemosensory neurons concentrated in the head region. These sensory neurons project to the main lobes of the brain, where various inter-neurons such as serotonergic, dopaminergic and GABAergic neurons are distributed in particular patterns (Nishimura et al., 2007a, b, 2008b).



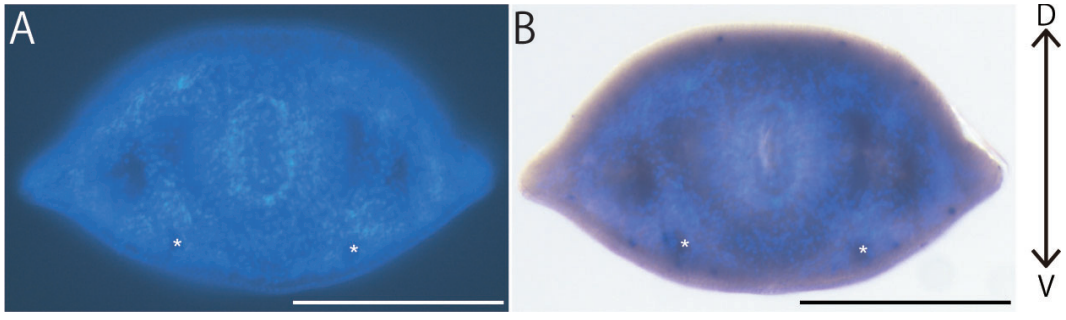
Supplementary Figure S2. Behavior assay systems. **(A)** Pharynx extension assay. One planarian moved straight in a narrow channel (2 mm width). Five microliters of liver extract were dropped in front of the planarian using a micropipette to induce pharynx extension, and the number of planarians with and without pharynx extension was counted over 2 min. Planarians were video-recorded from the ventral side using a mirror and a video camera. **(B)** Primary feeding behavior assay for feeding behaviors of several planarians. Several planarians were placed at the edge of a 90-mm-diameter Petri dish and a slice of chicken liver was placed as food in the center of the dish. The number of planarians feeding at 20 min after the liver was placed in the dish was counted.



Supplementary Figure S3. Relative expression values of the indicated genes determined by qPCR in RNAi-treated planarians. Expression level of each tested gene in *GFP*(*RNAi*) planarians was taken as = 1, indicated by a dashed line. Error bars indicate s.e.m. **(A)** Expression levels of the indicated genes in planarians with RNAi of genes for rate-limiting enzymes for synthesis of monoamine and amino acid neurotransmitters (See also Table 2). **(B)** Expression levels of the indicated genes in planarians with *syt*(*RNAi*) and *PC2*(*RNAi*) (See also Table 3). **(C)** Expression levels of the indicated genes in planarians with RNAi of neuropeptide coding genes (Also see Table 5). *$P < 0.05$; **$P < 0.005$; NS, no statistical difference by Student t-test.



Supplementary Figure S4. Feeding rate of RNAi planarians in primary feeding behavior assay. Black bars show the 10 lowest values of feeding rate of RNAi planarians (13 individuals were tested for RNAi of each gene). Gray columns indicate planarians with feeding rate higher than 60%. *t* = 20 min; *n* =10.



Supplementary Figure S5. In situ hybridization in transverse section for *DjNpP02* expression together with Hoechst staining (Also see Fig. 3D). **(A)** Samples were stained with Hoechst 33342 (for nuclei, shown in blue) to observe the VNCs, which were not stained. **(B)** Merge of Hoechst staining and bright field (in situ hybridization) images. *, VNC. Scale bar 250 μm.