Supplementary Materials and Methods

Strains

Culturing and genetic manipulation of *C. elegans* were performed as described (Brenner, 1974). The alleles used in this study are as follows: *goa-1(sa734) I* (Robatzek and Thomas, 2000), *cat-2(e1112) II* (Sulston et al, 1975), *tph-1(mg280) II* (Sze et al, 2000), *daf-7(e1372) III* (Riddle et al, 1981), *ser-4(ok512) III* (Dempsey et al, 2005), *daf-1(m40) IV* (Riddle et al, 1981), *dop-2(vs105) V* (Chase et al, 2004), *daf-3(e1376) X* (Riddle et al, 1981), *dop-1(ev748, vs100) X* (Chase et al, 2004; Sanyal et al, 2004), *dop-3(vs106) X* (Chase et al, 2004), *dop-4(ok1321) X* (isolated by *C. elegans* Gene Knockout Project, Oklahoma Medical Research Foundation), *tbh-1(ok1196) X* (Suo et al, 2006), and *tzIs3[cre::gfp;lin-15(+)]* (Kimura et al, 2002). The strains carrying *cre::gfp* reporter were constructed by mating *tzIs3* males with other mutants. The genotypes of the crossed animals were determined by PCR for deletion mutants, PCR-RFLP for the *cat-2* mutant, and Daf phenotype for daf mutants. *goa-1(sa734);tzIs3* and *tbh-1(ok1196);tzIs3* were constructed previously (Suo et al, 2006).

Construction of fusion genes

The following primers were used to make the fusion gene constructs.

A: CCGGAATCTTCTTCTTATGTATCTC

B: AGTCGACCTGCAGGCA TGCAAGCTAAGAGCAACGGCACTTCTC

C: GCTATTCTTACCGGAAGCTTCTTATGTATCTC
D: GAAGAGAAGAGGATCCGCACTTCTCATTCTTCTG
E: GCTATGACCATGATTACGCC
F: GTATTCATACCGGCTCGCTGGGAACAGATGG
G: CCGCACTTACCGGTTCCTGAAAAATCGTATAAGTAATGG
H: CGCTCGAGACCGGTAAAAATGGAGGCGCGAGAG
I: GAGTTTTTTGTGCACCGGCCTTAGACATGCACGCTGC
J: GACATTGAACCGGTAAAAATGGTTGTGGTTATGGCG
K: GTTTTTTGTGCACCGGCCTCATCTTTTTTTGAATATCCCG

dop-3::gfp was constructed by the fusion PCR method (Hobert, 2002). The region corresponding to 2.9 kb upstream and a part of exon 1 of dop-3 gene was amplified with primers A and B and were fused to 2-1876 of pPD95.75.

The promoter region of the tbh-1 gene was amplified with primers C and D using genomic DNA as the template. The PCR product was digested with BamH I and Hind III and cloned into BamH I- and Hind III-digested pPD95.75 to obtain tbh-1::gfp.

ceh-17::dsred (Suo et al, 2006) was digested with Kpn I and Apa I and the 1.5 kb fragment that contains DsRed cDNA was cloned into Kpn I- and Apa I-digested tbh-1::gfp to obtain tbh-1::dsred.

ceh-17::dsred and tbh-1::dsred contains small portions of exon 1 of ceh-17 and tbh-1 gene, respectively. To remove these region, the promoter region of ceh-17 gene was amplified with primers E and F using ceh-17::dsred as the template. The PCR product was digested with BamH I and Age I and re-cloned into ceh-17::dsred to obtain ceh-17(2)::dsred. The promoter region of tbh-1 gene was amplified with primers E and G.
using \textit{tbh-1::dsred} as the template. The PCR product was digested with Bgl II and Age I and re-cloned into \textit{tbh-1::dsred} to obtain \textit{tbh-1(2)::dsred}.

The coding sequences of \textit{dop-2S} and \textit{dop-2L} were amplified with primers H and I using the corresponding cDNAs (Suo et al, 2003) as template. The coding sequences of \textit{dop-3fl} were amplified with primers J and K using the \textit{dop-3fl} cDNA (Sugiura et al, 2005) as template. The PCR products were digested with Age I and Not I and cloned into \textit{ceh-17(2)::dsred} or \textit{tbh-1(2)::dsred} to obtain \textit{ceh-17::dop-2S, ceh-17::dop-2L, ceh-17::dop-3fl, tbh-1::dop-2S, tbh-1::dop-2L,} and \textit{tbh-1::dop-3fl}. 
Supplementary Figure Legends

Supplementary Figure 1 CRE-mediated GFP expression in the SIA neurons of dopamine receptor double and triple mutants. The number of GFP-expressing SIA neurons per animal was determined after animals were incubated on plates containing no amine (NA), octopamine (OA), dopamine (DA), and octopamine + dopamine (O+D) for 6 hr. Error bars indicate the standard errors of the means. At least 80 animals were tested. *1P<0.001 (Tukey-Kramer multiple comparison test), compared to NA of dop-2;dop-3 double mutants. *2P<0.001, compared to NA of dop-1;dop-2;dop-3 triple mutants. *3P<0.001, compared to NA of dop-2;dop-3 double mutants.

Supplementary Figure 2 Effect of serotonin on CRE-mediated GFP expression in the SIA neurons. (A, B) The number of GFP-expressing SIA neurons per animal was determined after animals were incubated on plates containing no amine (first column), or octopamine (3 mg/ml) plus increasing concentrations of serotonin. (C-E) The number of GFP-expressing SIA neurons per animal was determined after animals were incubated in well-fed (WF), starvation (ST), starvation + sephadex (SX), and soaking (SO) conditions for 6 hr. Results for (A, C) wild type (wt) animals, (B, H) ser-4 mutants, and (D) tph-1 mutants carrying cre::gfp are shown. Error bars indicate the standard errors of the means. At least 80 animals were tested.
References


Supplementary Figure S1
Supplementary Figure S2