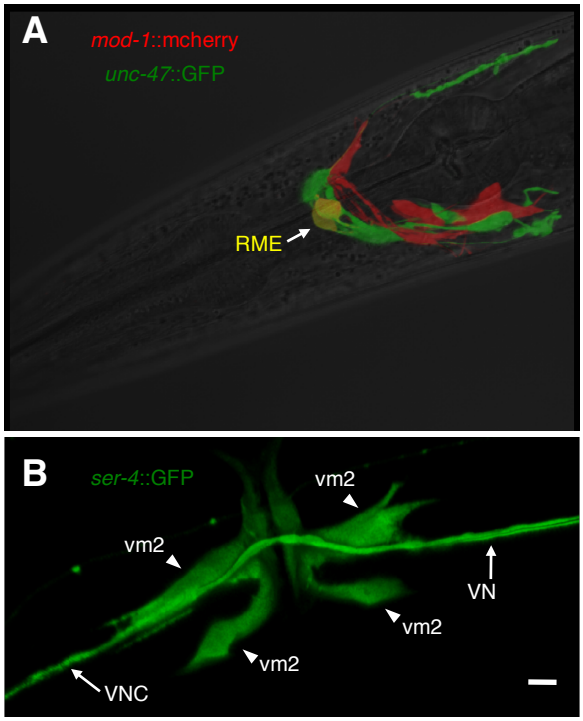

Figure S1 Further details of *mod-1* and *ser-4* reporter expression patterns.

(A) Adult head region showing *mod-1*::mCherry and *unc-47*::GFP fluorescence superimposed on a brightfield image of the animal. The *unc-47* reporter labels GABAergic neurons. This image demonstrates that not all GABAergic neurons expressed *mod-1*::mCherry. The two doubled-labeled GABAergic cells (arrow) that did express *mod-1*::mCherry were RME interneurons.

(B) Ventral view of the vulval region of a young adult expressing *ser-4::*GFP. Arrows, the fluorescent ventral nerve cord (VNC) running the length of the animal. Arrowheads, the four vm2 muscle cells also labeled by *ser-4*::GFP. Bar=40 µm.

File S1

Wild-type animals moving in water

A 30-second video clip showing wild-type animals in a microtiter well containing 50 µl of water and no serotonin. These animals were all scored as "moving," in that they showed continuous full-body thrashing movements.

File S1 is available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.112.142125/-/DC1 as a Quicktime movie.

File S2

Wild-type animals paralyzed by exogenous serotonin

A 30-second video showing wild-type animals in a microtiter well containing 50 µl water with a serotonin concentration (5 mM) sufficient to fully paralyze the animals, prepared as for the assays shown in Figure 2B. These animals were all scored as not "moving," in that they did not show the continuous full-body thrashing movements. One animal briefly made body bends halfway through the video.

File S2 is available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.112.142125/-/DC1 as a Quicktime movie.

File S3

Head neurons expressing *mod-1* and *ser-4* reporter transgenes

Three-dimensional rotation of the head region of a double-transgenic animal carrying the *mod-1*::mCherry and *ser-4*::GFP transgenes showing a merge of the red and green fluorescence. The bilaterally symmetric double-labeled neurons visualized in yellow are the left and right AIB neurons.

File S3 is available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.112.142125/-/DC1 as a Quicktime movie.

File S4

Optogenetic stimulation of endogenous serotonin release

Two 15-second videos showing slowing of locomotion during optogenetic stimulation of serotonin release. Animals shown carried the *ljIs102* transgene, which expresses ChR2::YFP in the NSM and ADF serotonergic neurons, as well as a mutation in *lite-1*, to block the endogenous blue light response of *C. elegans*. Animals are shown prior to, during, and after a several second illumination with blue light.

File S4 is available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.112.142125/-/DC1 as a Quicktime movie.

File S5

Extended Materials and Methods

***C. elegans* strains**

The wild-type strain was Bristol N2. Additional *C. elegans* strains used in this work are listed below, followed by a table listing the transgenes shown within the genotypes and a description of the plasmids used to generate the transgenes.

Figure 2: N2, AQ866 *ser-4(ok512)* III, MT9668 *mod-1(ok103)* V, LX1834 *ser-4(ok512)* III; *mod-1(ok103)* V, LX1166 *lin-15(n765ts)* X; *vsIs123*, LX1835 *ser-4(ok512)* III; *lin-15(n765ts)* X; *vsIs123*

Figure 3: LX1851 *lin-15(n765ts) vsIs163* X, LX1858 *lin-15(n765ts)* X; *vsIs154*; *ljIs570*, LX1857 *oxIs12 lin-15(n765ts)* X; *vsIs163*

Figure 4: AQ2050 *lite-1(ce314)* X; *ljIs102*, LX1841 *bas-1(ad446)* III; *lite-1(ce314)* X; *ljIs102*, LX1838 *mod-1(ok103)* V; *lite-1(ce314)* X; *ljIs102*, LX1839 *ser-4(ok512)* III; *lite-1(ce314)* X; *ljIs102*, LX1842 *ser-4(ok512)* III; *mod-1(ok103)*; *lite-1(ce314)* X; *ljIs102*

Figure 5: N2, MT9668 *mod-1(ok103)* V, MT9667 *mod-1(nr2043)* V, MT9772 *mod-5(n3314)* I, MT14121 *mod-5(n3314)* I; *ser-4(ok512)* III, MT9849 *mod-5(n3314)* I; *mod-1(ok103)* V, MT10143 *mod-5(n3314)* I; *mod-1(nr2043)* V, MT14126 *mod-5(n3314)* I; *ser-4(ok512)* III*; mod-1(ok103)* V, MT17972 *mod-5(n3314)* I; *ser-4(ok512)* III*; mod-1(ok103)* V; *nEx1403*, MT17973 *mod-5(n3314)* I; *ser-4(ok512)* III*; mod-1(ok103)* V; *nEx1404,* MT14984 *tph-1(n4622)* II

Figure S1: LX1857 *oxIs12 lin-15(n765ts)* X; *vsIs163,* LX1858 *lin-15(n765ts)* X; *vsIs154*; *ljIs570*

File S1: N2

File S2: N2

File S3: LX1858 *lin-15(n765ts)* X; *vsIs154*; *ljIs570*

File S4: AQ2050 *lite-1(ce314)* X; *ljIs102*

Construction of transgenes

The *ser-4*::GFP reporter transgene *adEx1616* developed by Tsalik *et al*. (2003) was used to produce the chromosomally-integrated transgene *ljIs570* by S. Shyn and W. Schafer and kindly provided to us for these studies. The *mod-1*::mCherry reporter plasmid pGG17 was constructed by inserting a 1645 bp *mod-1* promoter fragment upstream and the 1172 bp 3' untranslated region (UTR) of *mod-1* downstream of the mCherry coding sequences to generate plasmid pGG17. The primers used to amplify the promoter were GACTCTGCAGGCGTTCGTCACATTCTGCCG and CTGAGGTACCAATTTTCTTTCACCGCATTGGC. The primers used to amplify the 3' UTR were GACTGAGCTCTTGAAGTTTATCCCTT and GACTGGGCCCTAATCACAGGTGTCATCGG. Injection of pGG17 into *C. elegans* gave transgenes showing very weak mCherry expression, but following the method of Etchberger and Hobert (2008) we found that PCR amplification of the promoter::mCherry::3' UTR cassette from the plasmid and injection of the linear amplified DNA gave much stronger expression. An extrachromosomal transgene generated in this manner was chromosomally integrated using psoralen/UV mutagenesis to produce two independent integrated transgenes, *vsIs154* and *vsIs163*. For double labeling, animals carrying these mCherry transgenes were crossed with animals carrying the *unc-47*::GFP transgene *oslx12* (McIntire *et al*. 1997), which labels GABAergic neurons or the *unc-17*::GFP transgene *vsIs48* (Chase *et al*. 2004), which labels cholinergic neurons.

The *mod-1* overexpressing transgene *vsIs123* was generated by directly microinjecting a long-range PCR product containing the entire *mod-1* gene into a *lin-15(n765ts)* strain of *C. elegans* at 20 ng/µl with the *lin-15* rescuing plasmid pL15EK at 50 ng/µl, selecting non-Lin progeny, and subsequently using psoralen/UV mutagenesis to chromosomally integrate the transgene. The *mod-1* PCR product was amplified from *C. elegans* genomic DNA using the primers CTAATCACAGGTGTCATCGG and GCGTTCGTCACATTCTGCCG.

The *ser-4* rescuing plasmid pMG12 contained a 5 kb fragment of the *ser-4* promoter region followed by a *ser-4* cDNA and the 3' untranslated region from the *unc-54* gene. The *ser-4* promoter fragment was PCR amplified using the primers GCGCGCATGCCAGAGGAGTTCGCCACACAACACGTCAC and GCGCGCATGCGTGGAGTTGCACACAACACCGGAAGC containing the restriction sites *SphI* and *BamHI,* respectively. We amplified the *ser-4* cDNA yk1731h09 (kindly provided by Y. Kohara) using the primers GCGCGGTACCATGATCGACGAGACGCTTCJTCAATC and GCGCGATATCACTAGTCTAGCGGCCGCGACCTGCAGC containing the restriction sites *KpnI* and *EcoRV,* respectively. These restriction sites were used to ligate the two fragments into the vector pPD49.25 (kindly provided by A. Fire), which supplied the *unc-54* 3' untranslated region. A negative control plasmid, pMG13, was identical to pMG12 but carried a frameshift mutation in the *ser-4* cDNA: we inserted two G residues after nucleotide 91 of *ser-4* exon 1. The transgenes *nEx1403* and *nEx1404* were generated by microinjecting pMG12 or pMG13, respectively, at 10 ng/µl, along with the *lin-15* rescuing plasmid pL15EK at 20 ng/µl, into a *ser-4(ok512); lin-15(n765ts)* strain and selecting non-Lin progeny.

Additional References for Extended Materials and Methods

Chase, D. L., J. S. Pepper, and M. R. Koelle, 2004 Mechanism of extrasynaptic dopamine signaling in *Caenorhabditis elegans*. Nat. Neurosci. 7: 1096-103.

Etchberger, J. F. and O. Hobert, 2008 Vector-free DNA constructs improve transgene expression in *C. elegans*. Nat. Methods 5: 3.

Table S1 Molecular Lesions of Mutations Identified in This Work

Serotonin resistance mutation	Nucleotide sequence of the wild type[a]	Nucleotide sequence of the mutant[a]	Affected amino acid in the wild-type protein	Amino acid in the mutant protein
goa-1(vs115)	tgcgtatatt**C**aagcacaatt	tgcgtatatt**T**aagcacaatt	Q305	Stop
goa-1(vs134)	agacggcatg**C**aagcggcaaa	agacggcatg**T**aagcggcaaa	Q29	Stop
goa-1(n4093)	tgcaccacatacagtgagtca	tgcaccacat[Mos1]acagtgagtca		
goa-1(n4402)	cttcgtggat**G**cggcttgtat	cttcgtggat**A**cggcttgtat	C351	Y
goa-1(n4405)	ctagcgccat**G**ggttgtacca	ctagcgccat**A**ggttgtacca	M1	N/A
goa-1(n4439)	catattttca**G**aaccgaatgc	catattttca**A**aaccgaatgc	intron 5 splice acceptor	N/A
goa-1(n4492)	ttttcagaac**C**gaatgcacga	ttttcagaac**T**gaatgcacga	R243	Stop
goa-1(n4493)	aacggttgtg**G**ggagacgcag	aacggttgtg**A**ggagacgcag	W132	Stop
goa-1(n4494)	agcgaataag**G**taagaaaaaa	agcgaataag**A**taagaaaaaa	intron 7 splice donor	N/A
eat-16(n4403)	aaccctcgat**C**agacattgga	aaccctcgat**T**agacattgga		
abts-1(n4094)	actttcatcgattatacagct	actttcatcga**[Mos1]**ttatacagct	N/A	N/A
ser-4(vs122)	atttttgcag**G**ttacctaaac	atttttgcag**A**ttacctaaac	G410	D
emb-9(vs114)	ggtcagccag**G**ttatccagga	ggtcagccag**A**ttatccagga	G1197	D
flp-1(n4491)	ttattattca**G**gtgtcggcag	ttattattca**A**gtgtcggcag	intron 1 splice acceptor	N/A
flp-1(n4495)	atcattttca**G**gtcgaagtga	atcattttca**A**gtcgaagtga	intron 4 splice acceptor	N/A
mod-1(vs107)	atggatgtgt**G**gatgcttgga	atggatgtgt**A**gatgcttgga	W305	Stop
mod-1(n3791)	ttgtatcttg**G**gtttcattct	ttgtatcttg**A**gtttcattct	W258	Stop
mod-1(n4054)	ctacgtcttactttccagttt	ctacgtcttac**[Mos1]**tttccagttt	N/A	N/A
elpc-3(vs119)	catgtatacg**G**atccgtcgtt	catgtatacg**A**atccgtcgtt	G475	E

[a] The affected nucleotide is capitalized and 10 nucleotides on either side are shown.

Table S2 ***C. elegans*** **Transgenes Used in This Work**

Transgene	Purpose	Used in	Source
vsIs123	Overexpression of MOD-1. Carries multiple copies of wild-type *mod-1* genomic DNA.	Figure 2	This work. Microinjection of a *mod-1* long-range PCR product at 20 ng/µl and pL15EK (a *lin-15* rescuing plasmid used as a coinjection marker) at 50 ng/µl. Chromosomal integration via UV/psoralen mutagenesis
vsIs154 *vsIs163*	Expression of mCherry from the *mod-1* promoter. These two alleles are independent chromosomal integrants.	Figures 3, S1, File S3	This work. Microinjection of the *mod-1*::mCherry plasmid pGG17 at 65 ng/µl and pL15EK at 50 ng/µl. Chromosomal integration via UV/psoralen mutagenesis
oxIs12	Expression of GFP in GABAergic neurons from the *unc-47* promoter.	Figures 3, S1	McIntire *et al*. 1997[a]
ljIs102	Expression of Channelrhodopsin2::YFP in the serotonergic NSM and ADF neurons.	Figure 4 File S4	Ezcurra *et al*. 2011
nEx1403	Rescue of the *ser-4* mutant phenotype by expression of a *ser-4* cDNA from the *ser-4* promoter.	Figure 5	This work. Microinjection of the plasmid pMG12 at 10 ng/µl and pL15EK at 20 ng/µl
nEx1404	Negative control for *ser-4* rescue. Similar to *nEx1403* except that the *ser-4* cDNA used carries a frame shift mutation that prevents expression of SER-4 protein.	Figure 5	This work. Microinjection of the plasmid pMG13 at 10 ng/µl and pL15EK at 20 ng/µl
ljIs570	Expression of GFP from the *ser-4* promoter.	Figure 3 File S3	A gift of S. Shyn and W. Schafer. Chromosomal integrant of the extrachromosomal transgene *adEx1616* from Tsalik *et al*. (2003)
vsIs48	Expression of GFP in cholinergic neurons from the *unc-17* promoter.	Data not shown	Chase *et al*. 2004

[a] McIntire, S. L., R. J. Reimer, K. Schuske, R. H. Edwards, and E. M. Jorgensen, 1997 Identification and characterization of the vesicular GABA transporter. Nature 389: 870-876.