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, LX1857 oxIs12 lin-15(n765ts) X; vsIs163

Figure 4: AQ2050 lite-1(ce314) X; ljIs570, 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 *iljs570* 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 172 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 GACTCTGCAGCGGTACCATGGAGATTCGCCACACAACGTCAC and GCGGTACCATGCCATGCCAGAGGAGTTCGCCACACAACACGTCAC, respectively. We amplified the 3′ UTR cassette from the plasmid and injection of the linear amplified DNA gave much stronger expression. However, following the method of Etchberger and Hobert (2008) we found that PC 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, *vsls154* and *vsls163*. For double labeling, animals carrying these mCherry transgenes were crossed with animals carrying the *unc-47*:GFP transgene *ostx12* (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 GCTTTCTACATTTCTTCCCG which were used to amplify the 3′ UTR of the transgene.

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 GCGCGATACGAGGAGGTGTCCGCACACCAACGTCAC and GCGCGATGCAGGGAGTGTGCACACACACCGGAAGC containing the restriction sites SphI and BamHI, respectively. We amplified the *ser-4* cDNA yk1731h09 (kindly provided by Y. Kohara) using the primers GCGCGGATACGAGGAGGTGTCCGCACACCAACGTCAC and GCGCGATGCAGGGAGTGTGCACACACACCGGAAGC 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
