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.