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An RNAi Screen Identifies Genes that Regulate GABA Synapses

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SUMMARY

GABA synapses play a critical role in many aspects of circuit development and function. For example, conditions that perturb GABA transmission have been implicated in epilepsy. To identify genes that regulate GABA transmission, we performed an RNAi screen for genes whose inactivation increases the activity of *C. elegans* body muscles, which receive direct input from GABAergic motor neurons. We identified 90 genes, 21 of which were previously implicated in seizure syndromes, suggesting that this screen has effectively identified candidate genes for epilepsy. Electrophysiological recordings and imaging of excitatory and inhibitory synapses indicate that several genes alter muscle activity by selectively regulating GABA transmission. In particular, we identify two humoral pathways and several protein kinases that modulate GABA transmission but have little effect on excitatory transmission at cholinergic neuromuscular junctions. Our data suggest these conserved genes are components of signaling pathways that regulate GABA transmission and consequently may play a role in epilepsy and other cognitive or psychiatric disorders.

INTRODUCTION

Inhibitory synapses play a critical role in the proper development and function of neuronal circuits. Fast synaptic inhibition in the brain is primarily mediated by GABA. During development, GABA transmission is required for ocular dominance plasticity (Fagiolini and Hensch, 2000) and for refinement of receptive fields in the visual system (Tao and Poo, 2005). Finally, conditions that perturb GABA transmission have been implicated in epilepsy and other cognitive disorders (Prosser et al., 2001; Schuler et al., 2001; Snodgrass, 1992). Consequently, identifying genes that specifically regulate GABA transmission may reveal important insights into function and development of circuits as well as the contribution of circuit dysfunction to diseases of the nervous system.

Systematic RNAi screens in cultured cell lines have allowed extensive progress in identifying molecular components for various cellular processes (Perrimon and Mathey-Prevot, 2007). In the model organisms *C. elegans* and *Drosophila*, it has become possible to do RNAi screens on intact animals (Dietzl et al., 2007; Fraser et al., 2000; Kamath et al., 2003). A prior systematic RNAi screen focused on identifying genes in *C. elegans* that are required for synaptic function (Sieburth et al., 2005). It has been proposed that the next logical step is to apply similar systematic in vivo RNAi screens to the study of systems level problems such as the regulation of behavioral circuits (Bargmann, 2005).

To identify genes that regulate GABA transmission, we designed an RNAi screen using *C. elegans* body muscles as a model. Body muscles receive both excitatory (acetylcholine, ACh) and inhibitory (GABA) inputs. The activity of body muscles can be indirectly measured using the acetylcholinesterase inhibitor aldicarb. Aldicarb treatment causes accumulation of ACh at neuromuscular junctions (NMJs), leading to acute paralysis. Hypersensitivity to aldicarb-induced paralysis is caused by mutations that increase ACh secretion (Gracheva et al., 2006; McEwen et al., 2006) or that decrease inhibitory GABA input (Loria et al., 2004). As described below, our screen identified several conserved signaling pathways, including several that specifically regulate GABA transmission.

RESULTS

An RNAi Screen for Increased Muscle Activity

Initial experiments using the acetylcholinesterase inhibitor aldicarb showed it could be used to identify genes that regulate GABA transmission. For example, mutants lacking genes required for GABA transmission (e.g., *unc-25* glutamic acid decarboxylase [GAD], *unc-47* vesicular GABA transporter [VGAT], and

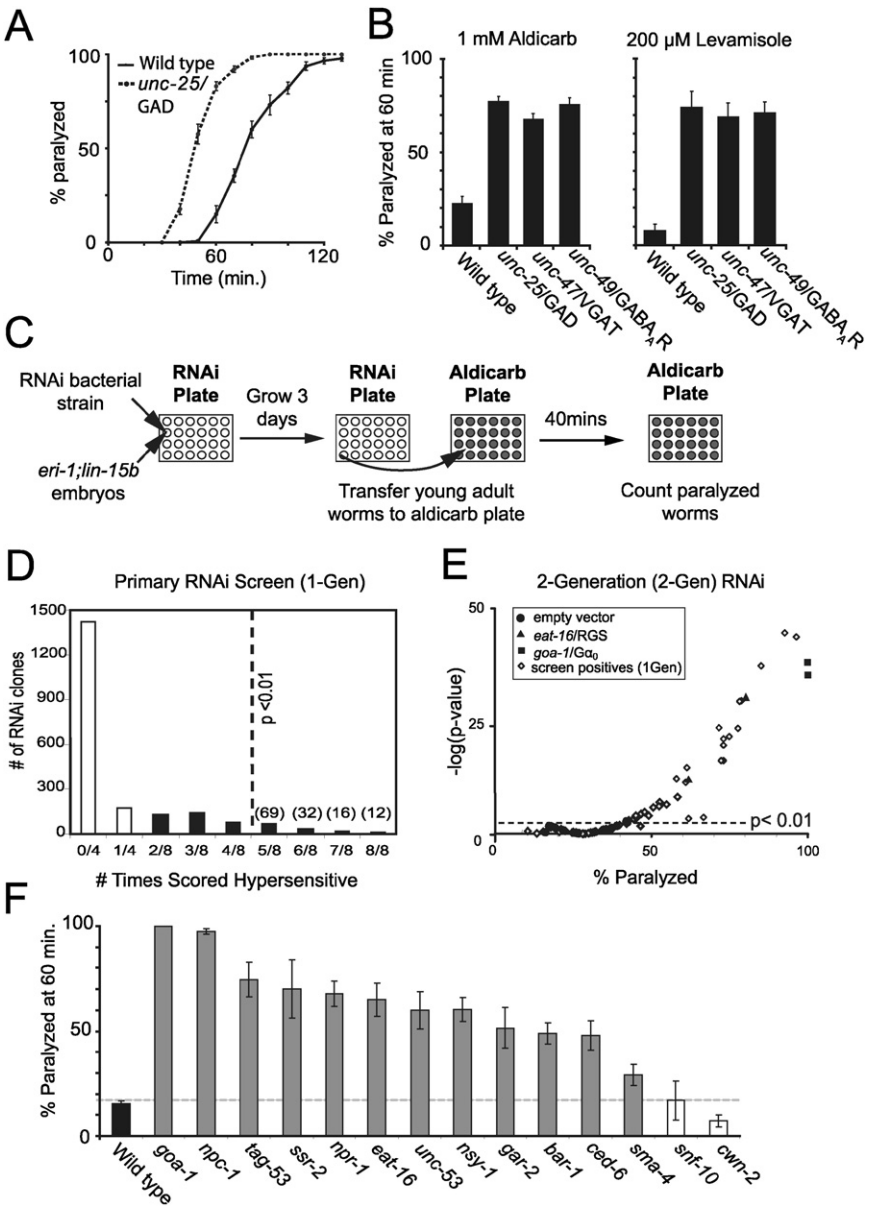

Figure 1. Screen for Aldicarb Hypersensitivity

(A) Time course of paralysis for *unc-25* GAD versus wild-type on 1 mM aldicarb (n = 9 and 6 trials, respectively, 20–25 worms per trial). Future assays and RNAi screen were scored at the 40 or 60 min time point to determine hypersensitivity. Error bars indicate SEM.

(B) Responsiveness of GABA mutants to aldicarb and levamisole. Percent paralyzed *unc-25* GAD, *unc-47* VGAT, and *unc-49* $GABA_A$ animals at 60 min on 1 mM aldicarb (n = 12, 16, 6, and 6 trials for wild-type, *unc-25*, *unc-47*, and *unc-49*, respectively; 20–25 animals per trial) and 200 µM levamisole (n = 5 trials for each genotype). GABA transmission mutants are hypersensitive to both drugs compared to wild-type ($p < 0.0001$). Error bars indicate SEM.

(C) Schematic of screen protocol. Embryos from an enhanced RNAi strain (*eri-1;lin-15b*) were placed in wells containing bacteria expressing dsRNA, and adults were scored for hypersensitivity to aldicarb 3 days later.

(D) Histogram of results from primary RNAi screen. All clones were tested four times, and clones that were scored as hypersensitive at least two out of four times were retested four additional times (black), while clones that scored hypersensitive less than two times were not rescreened (white). Clones that were scored as hypersensitive five out of eight times or more were significantly different from empty vector controls ($p < 0.01$ Fisher's exact test, 129 clones).

(E) Secondary screening by 2-Gen RNAi. The F1 generation of worms exposed to the 129 RNAi clones from the primary screen were tested (in quadruplicate) for hypersensitivity to aldicarb (second generation, 2-Gen, RNAi). Negative log p value is plotted against percent paralyzed. Forty-nine clones were hypersensitive to aldicarb following 2-Gen RNAi ($p < 0.01$ χ^2 test versus empty vector controls, threshold indicated). Positive controls *eat-16* RGS (filled triangle) and *goa-1* $G\alpha_0$ (filled square) are indicated.

(F) Aldicarb sensitivity of available loss-of-function mutants for genes identified as hypersensitive to aldicarb by 2-Gen RNAi. Twelve out of fourteen (86%) of the mutants tested confirmed the aldicarb hypersensitivity phenotype. Grey bars = hypersensitivity ($p < 0.01$, t test); open bars indicate wild-type sensitivity; error bars indicate SEM.

unc-49 $GABA_AR$) were hypersensitive to paralysis induced by aldicarb and by a cholinergic agonist (levamisole) (Jiang et al., 2005; Loria et al., 2004; Figures 1A and 1B). These results are consistent with the idea that GABA mutants have increased muscle activity and suggest that genes regulating GABA transmission should be identified in a screen for hypersensitivity to aldicarb-induced paralysis.

We screened an enhanced RNAi strain, which allows detection of neuronal defects (Wang et al., 2005), for hypersensitivity to aldicarb (Figure 1C). Two thousand seventy-two genes were selected for this screen, based on the presence of domains that predict involvement in signal transduction, synaptic localization, cytoskeletal regulation, or membrane trafficking (Sieburth et al., 2005) and on their availability as clones in the *C. elegans* RNAi feeding library (Kamath et al., 2003). In this primary screen, RNAi treatment was initiated after embryonic and early larval development was completed. One hundred twenty-nine genes were identified whose inactivation caused aldicarb hypersensitivity in significantly more replicates than did the empty vector control ($p < 0.01$, Fisher's exact test; Figure 1D).

To validate the results of the primary screen, we subjected the positive genes to several secondary assays. First, all positive genes were rescreened using two-generation RNAi treatments (2-Gen RNAi), which typically result in stronger and more consistent phenotypes. By 2-Gen RNAi, we confirmed that inactivation of 49 genes caused significant ($p < 0.01$, χ^2 test) aldicarb

hypersensitivity compared to empty vector controls (Figure 1E; see Table S1 available online). For some positive genes, 2-Gen RNAi treatments were not possible due to lethality, sterility, or growth defects. For these genes, we repeated 1-Gen RNAi, confirming that inactivation of 30 genes caused significant ($p < 0.01$, χ^2 test) aldicarb hypersensitivity (Table S1).

To further confirm the positive gene list, we tested whether inactivation of these genes caused aldicarb hypersensitivity across a range of experimental conditions. We used a panel of mutants and drug treatments that cause aldicarb resistance and tested whether 2-Gen RNAi of each gene could still produce significant aldicarb hypersensitivity (Figure 2). We also tested whether inactivation of each gene increased muscle responsiveness to the nicotinic agonist levamisole as an indication of increased muscle sensitivity or excitability. These results demonstrate that many of these genes regulate aldicarb responses across a wide range of experimental conditions (Figure 2).

Finally, we confirmed RNAi results by analyzing the effect of loss-of-function mutations in positive genes. For the 2-Gen RNAi positive genes, 12/14 (86%) available loss-of-function alleles caused aldicarb hypersensitivity ($p < 0.01$, χ^2 test; Figure 1F; Table S1). Based on their annotated functions, a small number of genes not screened by RNAi were also tested for aldicarb hypersentivity using available loss-of-function alleles. In this manner, eleven additional positive genes were identified (Table S1). Taken together, these secondary assays defined a set of 90 positive genes (Table S1). Fifty-six percent (51) of the positive genes had mouse or human orthologs (Table S1). Twenty-one positive genes had been previously implicated in GABA transmission or seizure syndromes (Table 1). These results provide confidence that our screen has effectively identified conserved genes whose inactivation increases the activity of neuronal circuits, including several that regulate GABA transmission.

Assaying ACh and GABA NMJs

Increased muscle activity could result from changes in either excitatory or inhibitory synaptic transmission. We performed a variety of experiments to distinguish between these possibilities. To maximize the severity of the observed defects, this analysis was restricted to the subset of positive genes for which viable loss-of-function mutants were available. To determine if genes altered motor neuron development, we examined the morphology of the GABA and cholinergic motor neurons. To assay synaptic transmission at NMJs, we recorded endogenous excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs, respectively) in body muscles.

We expect that some synaptic defects may not be detected in these recordings, since the dissected preparation may not precisely mirror synaptic transmission in the intact animal. To assay synaptic function in intact animals, we utilized a GFP-tagged synaptic vesicle protein (SNB-1/Synaptobrevin) as a probe to detect changes in presynaptic morphology and function. Expression of GFP-tagged SNB-1 in motor neurons creates a continuous pattern of fluorescent puncta in the nerve cords, where each punctum corresponds to a single NMJ (Jin et al., 1999).

Changes in the distribution of GFP::SNB-1 have been used to infer changes in the development or functional status of nerve terminals. The density of SNB-1 puncta is a measure of synaptic density. The fluorescence intensity of SNB-1 puncta correlates with the number of synaptic vesicles (SVs) at a synapse (as determined by ultrastructural studies; Richmond et al., 1999; Weimer et al., 2003). By contrast, the intensity of the diffuse SNB-1 axon fluorescence (i.e., between puncta) correlates with the abundance of Synaptobrevin in the plasma membrane (Dittman and Kaplan, 2006), which is a steady-state measure of the ratio of the rates of SV exocytosis and endocytosis (Fernandez-Alfonso and Ryan, 2004). For example, the exocytosis deficient mutants *unc-13* Munc13 and *unc-18* Munc18 have an increased number of SVs at synapses (Richmond et al., 1999; Weimer et al., 2003), increased SNB-1 punctal fluorescence (Sieburth et al., 2005) and decreased SNB-1 axon fluorescence (Dittman and Kaplan, 2006). The converse changes in SV numbers and SNB-1 fluorescence are found in the endocytosis deficient mutants *unc-57* Endophilin and *unc-11* AP180 (Alfonso et al., 1993; Dittman and Kaplan, 2006; Schuske et al., 2003; Sieburth et al., 2005). By combining the electrophysiological and imaging approaches, we should maximize our ability to detect synaptic defects.

Since we analyze both cholinergic and GABAergic synapses, our experiments are internally controlled for several potential outcomes. For example, gene inactivations that perturb the health or development of the muscle cells should produce equivalent defects in EPSCs and IPSCs and would likely produce morphological changes detected by imaging synaptic markers. Furthermore, the excitatory input to GABAergic motor neurons is provided exclusively by ACh released at cholinergic NMJs (White et al., 1986); consequently, gene inactivations that decrease excitatory drive should produce approximately equivalent changes in the EPSC and IPSC rates. To increase our chances of finding regulatory pathways, we focused our analysis on genes that differentially regulate excitatory and inhibitory transmission. Using these assays, we identified several genes that alter different aspects of ACh and GABA synapses (Table 1).

Categorizing Genes Identified in the Screen

The objective of the screen was to identify genes that regulate GABA synaptic transmission. Nevertheless, genes identified in our screen could alter muscle activity by several mechanisms. In particular, we expected to find three general categories of genes. First, general presynaptic factors would be expected to be recovered in our screen if their inactivation produces disproportionately stronger defects in GABA transmission, thereby resulting in a net increase in excitation. An example of this is provided by the *rpm-1* gene, which encodes a large perisynaptic E3 ubiquitin ligase (Schaefer et al., 2000; Zhen et al., 2000). Mutants lacking RPM-1 have defects in both cholinergic and GABAergic synapses; however, the GABA defects are more severe (Nakata et al., 2005). Thus, *rpm-1* is predicted to increase muscle activity, and was recovered in our screen (Table S1).

Second, genes required for the proper development of GABAergic neurons should be isolated in our screen. Prior to our screen, only the *unc-30* gene (which encodes a goosecoid homeodomain protein) had been shown to specifically regulate differentiation of GABA motor neurons (Jin et al., 1994). In our

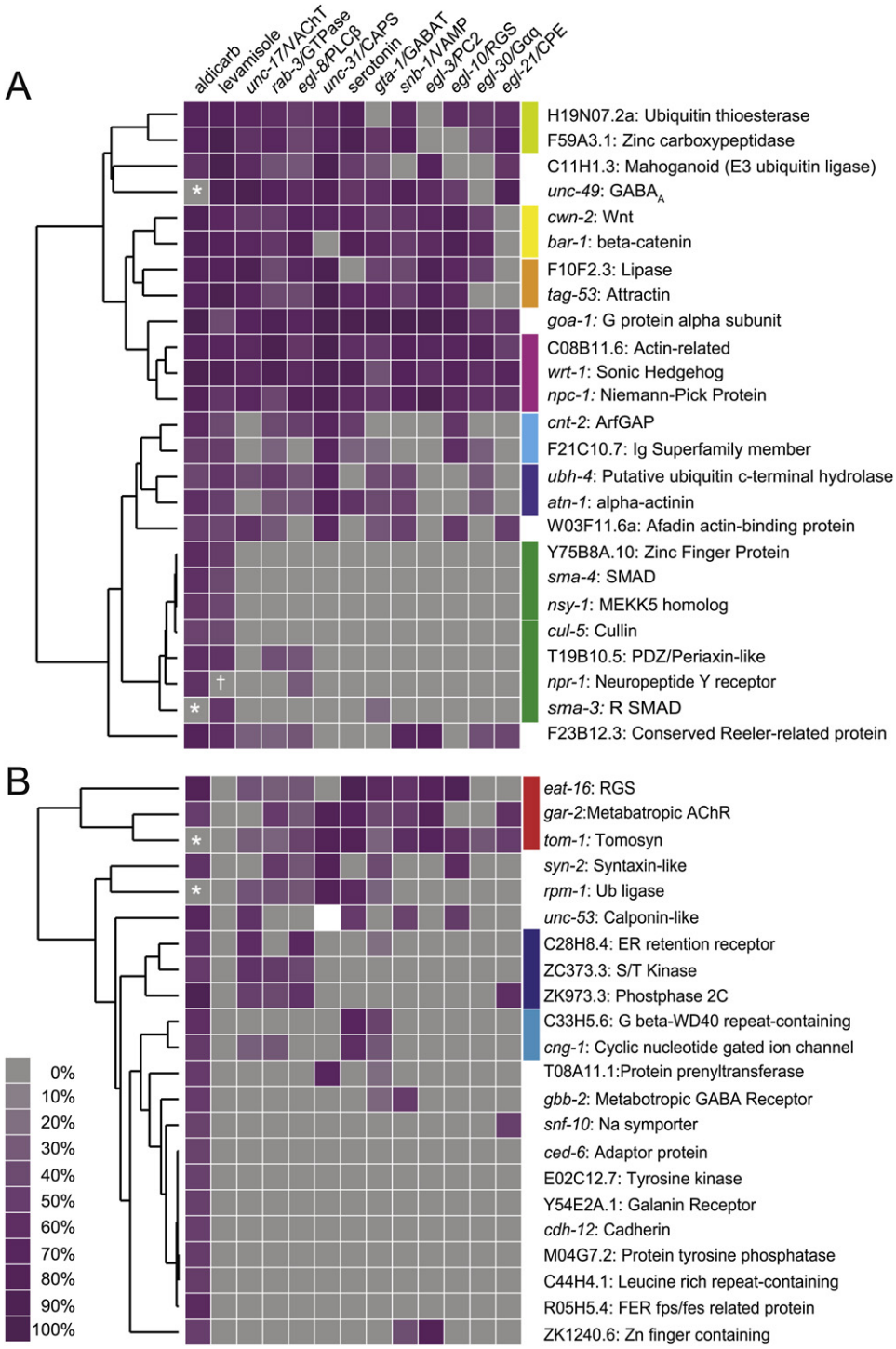

Figure 2. Patterns of Aldicarb Responses of Following Inactivation of Positive Genes

Columns represent different genetic backgrounds or drug treatments. Here, we illustrate patterns for 25 clones whose inactivations cause hypersensitivity to the cholinergic agonist levamisole (A) and 22 clones whose inactivations did not alter levamisole responses (B). The average percentage of animals paralyzed in each condition is indicated by the shading. Scores not meeting the threshold for significance ($p < 0.01$, $\chi 2$ test) were set to zero. These patterns of drug sensitivity were clustered using eight different algorithms. Robust clusters, which remain together under at least six algorithms, are indicated by colored bars. Four RNAi clones were included in analysis based on their mutant aldicarb phenotype (*) and *npr-1* was clustered with levamisole hypersensitive genes based on mutant data (†).

Table 1. Pathways Implicated in GABA Transmission or Seizure Syndromes

Gene	Locus	Description	Human Hom/ Orth	Expression: Motor Neuron	Expression: Body Muscle	GABA Imaging Defect	Ephys. Defect*	Seizure-Related Phenotypes
GABA-Related								
Y37D8A.23	*unc-25*	GAD	O	Yes (GABA)[1]	No			Inc. seizure susceptibility[17]
T21C12.1	*unc-49*	GABAAR	H	No	Yes[2]			Inc. seizure susceptibility[17]
T20G5.6	*unc-47*	VGAT	O	Yes (GABA)[3]	No			Inc. seizure susceptibility[17]
Presynaptic GPCR Pathways								
C26C6.2	*goa-1*	$G\alpha_o$ subunit	O	Yes (ACh, GABA)[4]	No	Yes	E	
C16C2.2	*eat-16*	RGS	H	Yes (ACh, GABA)[5]	No			Regulated by seizure activity[18,19]
ZK180.1	*gbb-2*	mGABAR	H					Inc. seizure susceptibility[20,21]
F47D12.1	*gar-2*	mAChR	H	Yes (ACh, GABA)[6]	No			Seizure resistance[22]
Wnt Signaling								
C54D1.6	*bar-1*	β-catenin	H	Yes[7]	No	Yes		Inc. seizure susceptibility[23]
W10C8.2	*pop-1*	TCF	O	Yes[8]	No	Yes		
W01B6.1	*cwn-2*	Wnt	O					
W06F12.1	*lit-1*	Nemo-like S/T Kinase	O					
Endocrine Regulators								
C39E6.6	*npr-1*	NPY-like R	H	Yes (GABA)[9]	No	No	I	Inc. seizure susceptibility[24–26]
F33C8.1	*tag-53*	Attractin/Mahogany	O					Hypomyelination, tremors[27–29]
C11H1.3		Mahoganoid	O	Yes[10]	Yes[10]			Hypomyelination[30]
Y54E2A.1		GPCR/Galanin R	H					Inc. seizure susceptibility[31,32]
TGFβ Pathway								
T25F10.2	*dbl-1*	TGFβ	O	Yes (ACh)[11]	No	Yes	I	Inc. Kainate-induced excitoxicity[33–35]
R12B2.1	*sma-4*	Co-SMAD	O	No[8]	No[8]			TGFβ signaling induced by Kainate[36]
R13F6.9	*sma-3*	R-SMAD	O	No[12]	No[12]			TGFβ signaling induced by Kainate[36]
Kinase Pathways								
K11E8.1	*unc-43*	CAMKII	O	Yes[13]	No	Yes	I	Inc. seizure susceptibility[37]
R03G5.2	*sek-1*	MAPKK	O	#[14]	No	Yes	I	Induced by Kainate[38]
F59A6.1	*nsy-1*	ASK1/MAPKKK	O	#[15]	No			Induced by Kainate[39]
B0478.1	*jnk-1*	JNK/MAPK	O	Yes (ACh, GABA)[16]	No			Resistant to Kainate-induced excitotoxicity and seizure[40]
Other Seizure-Implicated								
T26A5.9	*dlc-1*	dynein	O	Yes[10]	No			Worm seizure model[41]
C17H12.1	*dli-1*	dynein intermed. chain	O					Worm seizure model[41]

Human orthology (O) was determined based on reciprocal best BLAST hits; homologs (H) have e-value <1e−6.
Published expression patterns in motor neurons indicated as GABA and/or ACh where known. # neuronal expression described but ventral cord expression not analyzed.
GABA imaging defects: yes for a change in GABAergic SNB-1 localization where $p < 0.01$ and/or a change in soluble GFP.
Ephys. defects*: newly described electrophysiology in this paper, I or E for changes in IPSC or EPSC recordings where $p < 0.05$.
Seizure-related phenotypes: in response to loss of function. Induction or regulation indicates gene responses in wild-type.
[1]McIntire et al. (1993); [2]Bamber et al. (1999); [3]Eastman et al. (1999); [4]Ségalat et al. (1995); [5]Hajdu-Cronin et al. (1999); [6]Lee et al. (2000); [7]Natarajan et al. (2004); [8]Reece-Hoyes et al. (2007); [9]de Bono and Bargmann (1998); [10]Hunt-Newbury et al. (2007); [11]Morita et al. (1999); [12]Wang et al. (2002); [13]Reiner et al. (1999); [14]Tanaka-Hino et al. (2002); [15]Sagasti et al. (2001); [16]Kawasaki et al. (1999); [17]Snodgrass (1992); [18]Gold et al. (1997); [19]Ingi et al. (1998); [20]Prosser et al. (2001); [21]Schuler et al. (2001); [22]Hamilton et al. (1997); [23]Campos et al. (2004); [24]El Bahh et al. (2005); [25]Noe et al. (2007); [26]Vezzani et al. (1999); [27]Bronson et al. (2001); [28]Gunn et al. (2001); [29]Kuramoto et al. (2001); [30]He et al. (2003); [31]Mazarati et al. (2000); [32]Mazarati et al. (1998); [33]Brionne et al. (2003); [34]Mesples et al. (2005); [35]Tesseur et al. (2006); [36]Luo et al. (2006); [37]Butler et al. (1995); [38]Jeon et al. (2000); [39]Shinoda et al. (2003); [40]Yang et al. (1997); [41]Williams et al. (2004).

screen, we isolated several genes encoding components of the canonical Wnt signaling pathway (Figure 3A; Table 1). We subsequently showed that some of these Wnt signaling genes are required for outgrowth of GABA axons. In wild-type animals, the GABAergic D type motor neurons extend axons that form a contiguous pattern filling the dorsal and ventral nerve cords. In *bar-1* mutants (which lack a β-catenin) and *pop-1* mutants (which lack the TCF transcription factor), there were prominent

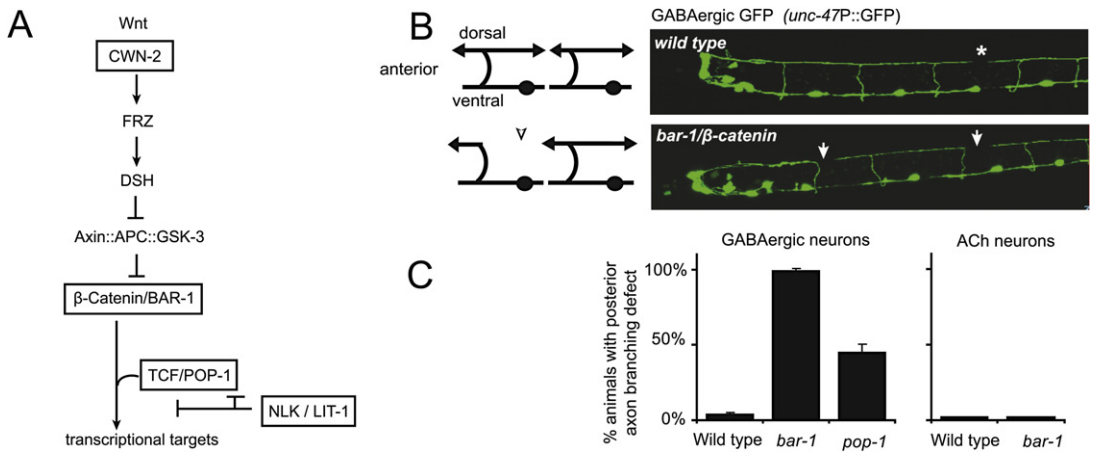

Figure 3. Wnt Signaling Genes Regulate GABA Neuron Development

(A) The schematic shows the canonical Wnt signaling pathway. Boxes indicate genes identified in our screen or known by mutant phenotype to be aldicarb hypersensitive.

(B) Confocal images of soluble GFP expression in GABAergic neurons of wild-type and *bar-1* early larval stage 2 animals. White arrowheads indicate prominent gaps in the dorsal cord where the main axonal processes posterior to the GABA neuron commissures normally occur (diagram). Asterisk indicates gaps present in some wild-type animals between DD4 and DD5 at this developmental stage. These gaps are morphologically different from those seen in *bar-1* animals in that posterior axonal projections are developing normally from commissures.

(C) Quantification of posterior axon branching defects for wild-type, *bar-1*, and *pop-1* in the GABAergic motor neurons (n = 58, 68, and 50) and wild-type and *bar-1* in the cholinergic neurons (n = 42 and 44).

gaps in D neuron dorsal cord axons, visualized by expressing either soluble GFP or SNB-1::GFP (Figures 3B and 3C and data not shown). These gaps were apparently caused by incomplete extension of posteriorly-directed D neuron axons in the dorsal cord and were most frequently observed in DD2 and DD4 axons. By contrast, the morphology of cholinergic DA neuron axons in the dorsal nerve cord was normal in *bar-1* mutants (Figure 3C). Thus, Wnt signaling mutants selectively disrupt GABA axon outgrowth, decreasing GABA transmission. These results are consistent with recent studies showing that Wnt signaling regulates other cell and axon migrations in *C. elegans* (Hilliard and Bargmann, 2006; Pan et al., 2006).

Third, we expected to identify genes encoding components of a previously described G protein signaling network (Figure 4A; Table 1). The heterotrimeric GTP-binding protein α subunits GOA-1 $G\alpha_o$ and EGL-30 $G\alpha_q$ have antagonistic effects on aldicarb responses, and these effects are thought to be mediated by their antagonistic regulation of ACh secretion (Lackner et al., 1999; Miller et al., 1999; Nurrish et al., 1999). Consistent with this prediction, electrophysiological recordings indicated that *goa-1* $G\alpha_o$ mutants had an increased rate of endogenous EPSCs (53%, $p = 0.02$), whereas the EPSC amplitudes were not significantly different from wild-type controls (Figures 4B and 4C and Figure S1A). The density of GFP::SNB-1 puncta in cholinergic axons was not increased in *goa-1* $G\alpha_o$ mutants (Figures 4D and 4E). Therefore, the increased EPSC rate observed in *goa-1* $G\alpha_o$ mutants was unlikely to be caused by increased synapse numbers. These results indicate that *goa-1* $G\alpha_o$ mutants have an increased rate of ACh secretion, as previously proposed based on genetic analysis of aldicarb responses.

The excitatory input to GABAergic motor neurons is provided exclusively by ACh released at cholinergic NMJs (White et al., 1986). Consequently, we expected that the increased rate of EPSCs observed in *goa-1* mutants would be accompanied by a corresponding increase in the IPSC rate. However, we found that the rate and amplitude of endogenous IPSCs recorded from *goa-1* mutants were not significantly different from wild-type controls (Figure 4C). This discrepancy may be explained by a decrease in the density of GABAergic NMJs, since the density of SNB-1::GFP puncta in GABAergic axons was slightly decreased in *goa-1* mutants (19%, $p < 0.001$) (Figure 4F).

While prior studies have identified many components of this G protein signaling network, much less is known about the upstream G protein-coupled receptors (GPCRs) that impinge on this network. Genes encoding several classes of GPCRs were isolated in our screen (Figure 4A; Table 1), identifying additional pathways that could modulate ACh release through this G protein signaling network.

Thus, the genes identified in our screen include 21 previously implicated in seizure disorders, and include examples of each of these three predicted classes of genes (Table 1). These results suggest that our list is enriched for genes that influence muscle activity by altering circuit properties, such as the relative balance of excitatory and inhibitory inputs to the muscle. Beyond these predicted classes of genes, we show that several genes identified in our screen modulate GABA transmission, thereby altering muscle activity, as detailed below.

Endocrine Regulation of GABA Transmission

Several genes that encode components of endocrine regulatory pathways were identified in our screen including TAG-53 Attractin, NPR-1 (related to the NPY receptor) (de Bono and Bargmann, 1998), and Y54E2A.1 (a predicted Galanin receptor) (Figure 5A; Table 1). Attractin is an auxiliary subunit of the Agouti

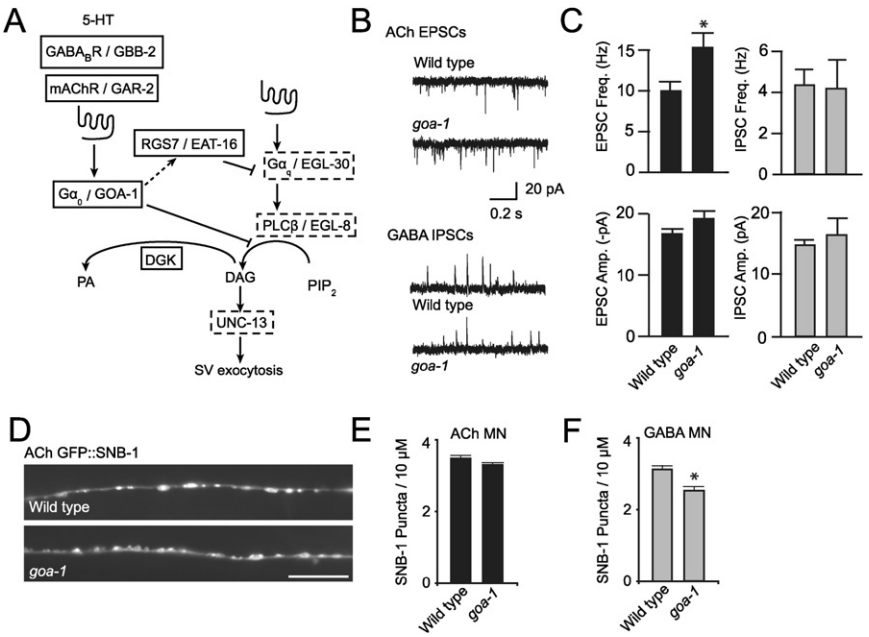

Figure 4. G Protein Signaling Pathways Regulate ACh Secretion

(A) The schematic shows the G protein signaling networks that regulate synaptic vesicle exocytosis at NMJs. Boxes indicate genes identified in our screen or known to cause hypersensitivity to aldicarb when inactivated (Miller et al., 1999; Nurrish et al., 1999). Mutants of components previously shown to be resistant to aldicarb are shown in dashed boxes (Lackner et al., 1999; Miller et al., 1996).

(B) Representative traces of endogenous EPSCs (top panel) and IPSCs (bottom panel) recorded from adult wild-type (n = 29) and *goa-1* (n = 6) animals in 1 mM $CaCl_2$ and 4 mM $MgCl_2$.

(C) Mean frequency (top panel) and amplitude (bottom panel) of endogenous EPSCs (black bars) and IPSCs (gray bars) for wild-type and *goa-1* animals. An asterisk indicates a significant difference between wild-type and *goa-1* animals for EPSC frequency (53% increase, $p = 0.02$). Error bars indicate SEM.

(D) Representative images of SNB-1::GFP expressed in the cholinergic motor neurons of wild-type and *goa-1* animals.

(E) Quantification of the number of SNB-1 puncta per unit length in cholinergic axons. n = 55 and 54 animals for *goa-1* and wild-type, respectively.

(F) Quantification of SNB-1 puncta per unit length in GABAergic axons (n = 31 and 35 animals for *goa-1* and wild-type.) A double asterisk indicates a significant difference between wild-type and *goa-1* for SNB-1 puncta density (19% decrease, $p < 0.001$.) Error bars indicate SEM.

receptor (Haqq et al., 2003; He et al., 2001). A second component of the Attractin signaling pathway, the E3 ubiquitin ligase C11H1.3/Mahoganoid (He et al., 2003) was also identified in our screen. We previously showed that inactivation of two genes encoding insulin-like growth factors (*ins-22* IGF and *ins-31* IGF) caused aldicarb-resistance (Sieburth et al., 2005). Thus, these results identify endocrine pathways that antagonistically regulate aldicarb responsiveness, and presumably muscle activity.

Several results suggest that NPR-1 receptor effects on muscle activity are caused by changes in GABA transmission. Mutants lacking NPR-1 receptors had a significantly reduced rate of endogenous IPSCs (53%, $p = 0.01$), while IPSC amplitudes were normal (Figures 5B and 5C and Figure S1B). The density of SNB-1 puncta in the GABAergic axons was unaltered in *npr-1* mutants ($p = 0.88$) (Figures 5D and 5E), suggesting that the decreased IPSC rate was not caused by a change in the number of GABA synapses. SNB-1 axon fluorescence in GABAergic neurons of *npr-1* mutants was indistinguishable from wild-type controls ($p = 0.62$; Figure 5F). Thus, although endogenous IPSC rates were decreased, we did not detect a corresponding change in the distribution of SNB-1 (Figures 5D–5F). Several factors could account for this discrepancy. For example, if the rates of SV exocytosis and endocytosis were decreased to the same extent, one would expect that IPSC rates would decrease but the distribution of SNB-1 would be unaltered.

The decreased inhibition observed in *npr-1* mutants cannot be explained by a corresponding decrease in the excitatory drive on the GABA motor neurons since the rate of endogenous EPSCs was actually increased in *npr-1* mutants (32%, $p = 0.1$), although this effect was not significant (Figures 5B and 5C). Similarly, there was no change in amplitude of endogenous EPSCs nor in the density of SNB-1 puncta in the cholinergic axons (Figure 5G). Taken together, these results suggest that *npr-1* mutants have a selective reduction in the rate of GABA transmission.

A TGFβ Pathway Regulates GABA Transmission

Three genes encoding components of a TGFβ signaling pathway were identified in our screen (Figure 6A; Table 1). Inactivation of *dbl-1* TGFβ, *sma-4* co-SMAD, and *sma-3* R-SMAD resulted in hypersensitivity to aldicarb and levamisole, consistent with increased muscle activity.

Mutants lacking DBL-1 had several defects in GABAergic transmission. These mutants had a significantly decreased IPSC rate (91%, $p < 0.0001$) compared to wild-type controls, consistent with a decreased rate of GABA secretion in these mutants (Figures 6B and S1C). The rate and amplitude of endogenous body muscle EPSCs were normal in *dbl-1* mutants lacking (Figures 6B and 6C), as was the density of SNB-1 puncta in cholinergic axons (Figure 6D). These results suggest that the decreased inhibition observed in *dbl-1* mutants were not caused by a corresponding decrease in the excitatory drive on the GABA motor neurons. The density of GABAergic NMJs, as measured by both presynaptic (SNB-1) and postsynaptic (UNC-49 $GABA_A$) puncta densities was actually slightly increased in *dbl-1* mutants (12%, $p = 0.005$ and 13%, $p < 0.0001$), suggesting that the decreased IPSC rate was not caused by a decrease in the density of the GABAergic NMJs (Figures 6E, 6F, and 6I). SNB-1 fluorescence in GABA axons was unaltered in *dbl-1* mutants, suggesting that the decreased GABA secretion was not accompanied by a change in the relative rates of SV exocytosis and endocytosis (Figures 6E and 6G). These results indicate that *dbl-1* mutants have presynaptic defects leading to decreased GABA secretion.

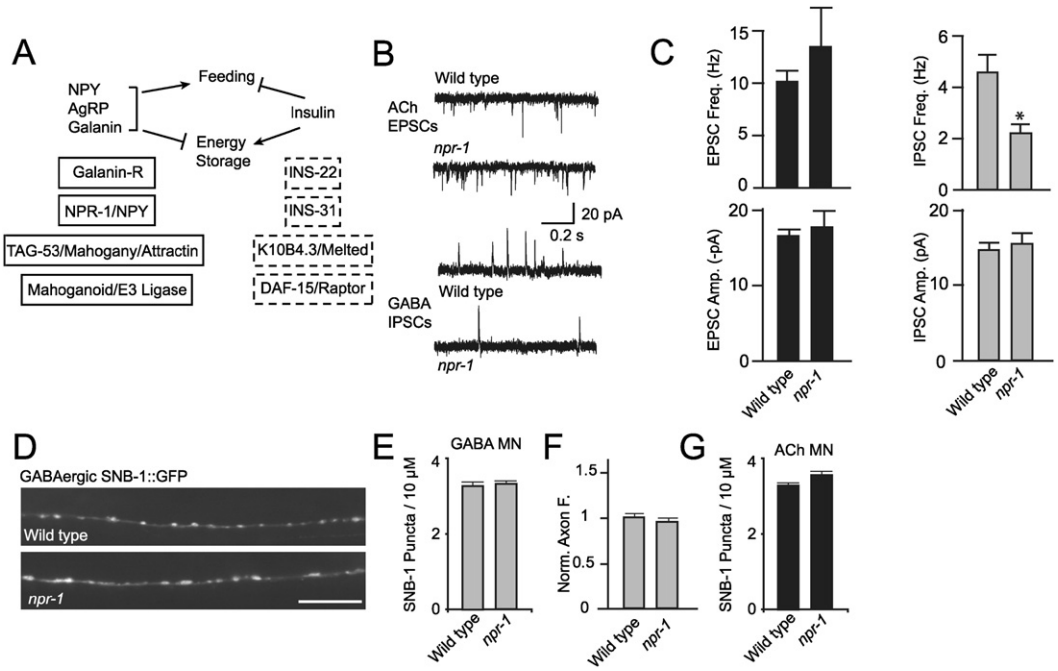

Figure 5. Regulation GABA Transmission by *npr-1*/ Neuropeptide Y-like Receptor

(A) The schematic above summarizes neuropeptides that regulate energy homeostasis in mammals. Below, we summarize the effects of orthologous genes on *C. elegans* aldicarb responses. Genes whose inactivation cause aldicarb hypersensitivity are indicated by the solid boxes while those causing resistance are indicated by dashed boxes (Sieburth et al., 2005).

(B) Representative traces of endogenous EPSCs (top panel) and IPSCs (bottom panel) recorded from adult wild-type (n = 29) and *npr-1* (n = 6) animals in 1 mM $CaCl_2$ and 4 mM $MgCl_2$.

(C) Mean frequency (top panel) and amplitude (bottom panel) of endogenous EPSCs (black bars) and IPSCs (gray bars) for wild-type and *npr-1* animals. An asterisk indicates a significant difference between wild-type and *npr-1* animals for IPSC frequency (53% decrease, p = 0.01). Error bars indicate SEM.

(D) Representative images of SNB-1::GFP expressed in the GABAergic motor neurons of wild-type and *npr-1* animals.

(E and F) Quantification of the number of SNB-1 puncta per unit length (E) and the nonsynaptic, axonal SNB-1 fluorescence (F) in the GABAergic neurons. n = 31 and 28 animals for *npr-1* and wild-type.

(G) Quantification of the number of SNB-1 puncta per unit length in the cholinergic axons. n = 31 and 25 animals for *npr-1* and wild-type. Error bars indicate SEM.

In addition to the decreased GABA secretion, several results suggest that *dbl-1* mutants also had postsynaptic defects at GABAergic NMJs. IPSC amplitudes were significantly reduced in *dbl-1* mutants (40%, $p < 0.01$; Figure 6C). Changes in the amplitude of postsynaptic currents can occur when muscle responsiveness to neurotransmitter is altered, or when the size of presynaptic quanta is altered. To distinguish between these possibilities, we recorded the muscle currents evoked by application of the GABA agonist muscimol. The amplitude of muscimol-evoked currents were also significantly reduced in *dbl-1* mutants (33%, $p < 0.01$), consistent with decreased muscle sensitivity to GABA (Figure 6H).

We did several experiments to further characterize the mechanism for this change in muscle sensitivity. The abundance of GFP-tagged UNC-49 $GABA_A$ subunits in the nerve cord was not altered in *dbl-1* mutants, suggesting that decreased GABA sensitivity was not caused by decreased expression or targeting of $GABA_A$ receptors (Figure 6I). Decreased muscle sensitivity might be caused by expression of functionally impaired UNC-49 $GABA_A$ receptors or by a structural change in GABAergic NMJs leading to inefficient activation of $GABA_A$ receptors. To test these possibilities, we analyzed the kinetics of endogenous IPSCs, which should provide a sensitive assay for both UNC-49 receptor function and for changes in the geometry of GABAergic synapses. The shapes of the average endogenous IPSCs in *dbl-1* mutants and wild-type controls were indistinguishable, suggesting that the kinetics of synaptic GABA responses were not significantly altered in *dbl-1* mutants (Fig. S2A). To more directly assess the structure of of GABAergic NMJs, we analyzed the morphology of these synapses. We found that the alignment of RAB-3 (RFP-tagged) and UNC-49 (GFP-tagged) at these synapses was unaltered in *dbl-1* mutants (Fig. S3). These results suggest that DBL-1 regulates the function of UNC-49 receptors, e.g., by regulating their insertion into the plasma membrane, or their activation by GABA. Our results indicate that the increased muscle activity observed in *dbl-1* mutants was caused by both pre and postsynaptic defects at GABAergic synapses.

Kinase Pathways Regulate GABA Transmission

Several genes encoding components of MAP kinase signaling cascades were identified in our screen, including NSY-1 ASK1 (MAPKKK), SEK-1 (MAPKK), and JNK-1 (MAPK) (Figure 7A; Table 1). In addition to the MAPK components, we found that inactivation of another serine/threonine protein kinase, the type II

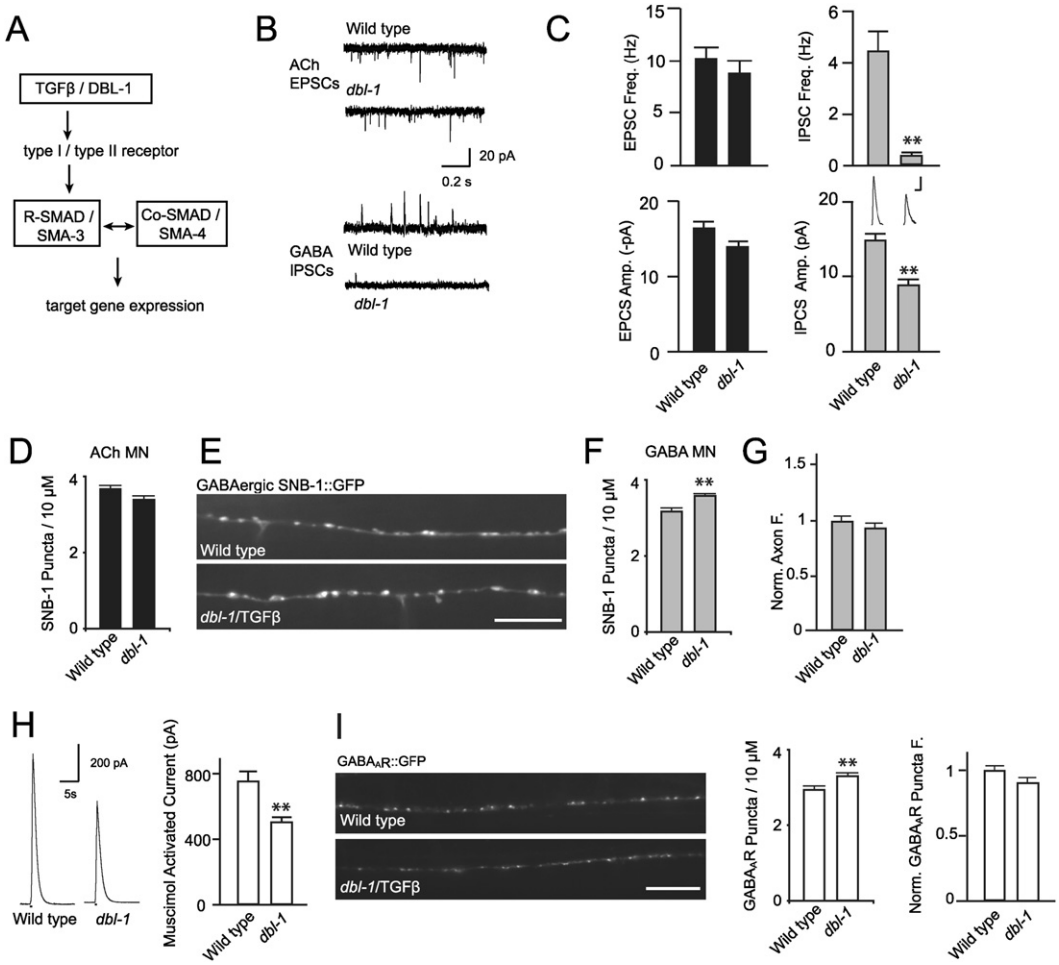

Figure 6. Regulation of GABA Transmission by a TGFβ Pathway

(A) Schematic of a TGFβ signaling pathway. Boxes indicate genes identified in our screen.

(B) Representative traces of endogenous EPSCs (top panel) and IPSCs (bottom panel) recorded from adult wild-type (n = 29) and *dbl-1* (n = 6) animals in 1 mM $CaCl_2$ and 4 mM $MgCl_2$.

(C) Mean frequency (top panel) and amplitude (bottom panel) of endogenous EPSCs (black bars) and IPSCs (gray bars) for wild-type and *dbl-1* animals. A double asterisk indicates a significant difference between wild-type and *dbl-1* animals for IPSC frequency (91% decrease, $p < 0.0001$) and amplitude (40% decrease, $p < 0.01$). Averaged endogenous IPSCs are shown above the respective bars for wild-type and *dbl-1*, scale bars are 5 pA and 5ms. Error bars indicate SEM.

(D) Quantification of the number of SNB-1 puncta per unit length in the cholinergic axons. n = 42 and 34 animals for *dbl-1* and wild-type.

(E) Representative images of SNB-1::GFP expressed in the GABAergic motor neurons of wild-type and *dbl-1* animals.

(F) Quantification of SNB-1 puncta per unit length in GABA axons (n = 51 and 42 animals for *dbl-1* and wild-type). A double asterisk indicates a significant difference between wild-type and *dbl-1* for SNB-1 puncta density (12% increase, $p = 0.005$).

(G) Quantification of the nonsynaptic, axonal SNB-1 fluorescence in the GABAergic neurons. n = 51 and 42 animals for *dbl-1* and wild-type.

(H) Muscimol-activated currents in wild-type and *dbl-1* body muscles. The GABA agonist Muscimol (100 µM) was pressure ejected onto body muscles for 0.5 s for wild-type (n = 8) and *dbl-1* (n = 6). Averaged responses for Muscimol-activated currents from wild-type and *dbl-1* are shown (left) and average peak responses are quantified (right). An asterisk indicates a significant difference between wild-type and *dbl-1* for Muscimol-activated current, (33% decrease, $p < 0.01$). Error bars indicate SEM.

(I) Postsynaptic $GABA_AR$::GFP imaging. Representative images of $GABA_AR$::GFP in wild-type and *dbl-1* animals (left panel) are accompanied by quantification of the number of $GABA_AR$ post synaptic sites per unit length (left) and the abundance of $GABA_AR$ at synapses (right). A double asterisk indicates a significant difference between wild-type and *dbl-1* for $GABA_AR$ puncta density (13% increase, $p < 0.0001$). n = 30 for both *dbl-1* and wild-type. Scale bar = 10µm. Error bars indicate SEM.

calcium- and calmodulin-dependent protein kinase (CaMKII) UNC-43, also causes aldicarb hypersensitivity (Robatzek and Thomas, 2000; Table S1). Thus, our results suggest that muscle activity may be regulated by one or more protein kinase cascades.

Several results suggest that UNC-43 and SEK-1 regulate GABA transmission. The rate of endogenous IPSCs was significantly reduced in both *unc-43* CaMKII (77%, $p = 0.01$) and *sek-1* (47%, $p = 0.04$) mutants (Figures 7B and 7C and Figures S1F and S1I). The rate and amplitude of endogenous EPSCs, and the

density of SNB-1 puncta in cholinergic axons were normal in both *unc-43* and *sek-1* mutants (Figures 7B–7D, S1F, and S1I), suggesting that the decreased inhibition was not caused by a decrease in the excitatory input to the GABA motor neurons. The density of SNB-1 puncta in GABA axons was unaltered in these mutants, suggesting that the decreased GABA secretion was not caused by a decrease in the number of GABA NMJs (Figures 7E and 7F). SNB-1 fluorescence in GABA axons was significantly decreased in both *unc-43* CaMKII and *sek-1* mutants (18%, $p < 0.001$ and 26% $p < 0.0001$) (Figures 7E and 7G). Thus, GABA secretion was reduced in these mutants, as assayed by both imaging and electrophysiological recordings.

Transgenes expressing UNC-43 in all neurons completely rescued the aldicarb hypersensitivity defect of *unc-43* mutants, while partial rescue was produced by expression in the GABA motor neurons (Figure 7J). By contrast, UNC-43 expression in cholinergic neurons or body muscles failed to rescue aldicarb hypersensitivity (Figure 7J). Similarly, transgenes expressing SEK-1 in GABA motor neurons partially rescued the aldicarb hypersensitivity defect of *sek-1* mutant Figure 7K). These data suggest that UNC-43 and SEK-1 act in the D neurons to regulate GABA secretion, although both are likely to act in additional neurons as well.

We also observed evidence for a postsynaptic GABA defect in *unc-43* CaMKII mutants. The amplitude of endogenous IPSCs and the amplitude of muscimol-evoked currents were both significantly reduced in *unc-43* CaMKII mutants (36%, $p < 0.01$, and 44%, $p < 0.05$; Figures 7B, 7C, 7I, and S1G). Both were normal in *sek-1* mutants. The shapes of the average IPSCs in *unc-43* and wild-type controls were identical, suggesting that UNC-43 CaMKII did not alter the kinetics of the synaptic $GABA_A$ receptors nor the kinetics of synaptic GABA responses (Figure S2B). These results demonstrate that *unc-43* CaMKII mutant muscles are less responsive to GABA. The abundance of GFP-tagged UNC-49 $GABA_A$ was significantly reduced in *unc-43* CaMKII mutants (14%, $p < 0.01$), which could contribute to the decreased GABA sensitivity (Figure 7J). These results suggest that UNC-43 CaMKII and SEK-1 MAPKK regulate inhibitory input to the body muscles. These data are consistent with the finding that CaMKII potentiates GABA whole-cell currents and IPSCs in mammalian neurons (Kano et al., 1996; Wang et al., 1995). Thus, in addition to its presynaptic role, CaMKII also plays a conserved role in regulating the postsynaptic response to GABA.

DISCUSSION

An emerging area of interest in systems neuroscience is the importance of balancing excitatory and inhibitory synaptic inputs (E/I balance) in a circuit. Our results indicate that aldicarb responsiveness can be used as a systems-level assay for the balance of excitatory and inhibitory input to *C. elegans* body muscles. Using this assay, we identified 90 genes whose inactivation increased muscle activity, including components of several phylogenetically conserved pathways. Although these genes were identified as regulators of muscle activity, several results suggest that the genes identified here may perform similar functions in the brain. First, in mammals, GABA is utilized in the CNS and not at NMJs; consequently, conserved genes regulating GABA synapses are likely to act in the brain. Second, 21 of the identified genes were previously implicated in GABA transmission or seizure disorders in mammals, providing further evidence that these genes have conserved functions regulating activity in the brain.

We identify four genes as modulators of GABA transmission. These genes are components of conserved signaling pathways, (TGFβ, MAP kinase, and NPY signaling pathways), suggesting these pathways may differentially regulate GABA transmission. Mutations in these genes decreased GABA transmission but did not reduce cholinergic transmission. While there has been extensive analysis of pathways that modulate excitatory synaptic transmission, much less is known about regulation of inhibitory transmission. Recent studies suggest that the adhesion molecules Neuroligin-2 and Sema4D promote formation of GABA synapses but not glutamatergic synapses in cultured rodent neurons (Chih et al., 2005; Graf et al., 2006; Paradis et al., 2007). Beyond these precedents, relatively little is known about molecules that differentially impact the development or function of inhibitory synapses. Below we briefly discuss the potential implications our results have for the mechanisms regulating inhibitory transmission and circuit activity.

Regulation of GABA Transmission by Conserved Kinase Pathways

Several genes encoding components of MAP kinase signaling cascades were identified in our screen, including NSY-1 ASK1 (MAPKKK), SEK-1 (MAPKK), and JNK-1 (MAPK) and the kinase UNC-43 CAMKII. These kinases were previously implicated in asymmetric expression of an odorant receptor (STR-2) and in innate immune responses to bacterial pathogens (Sagasti et al., 2001; Tanaka-Hino et al., 2002). Our results suggest that a similar MAPK pathway regulates GABA transmission. Previous studies showed that UNC-43/CAMKII is required for the normal activity of enteric, egg-laying, defecation, and male copulatory muscles (LeBoeuf et al., 2007; Reiner et al., 1999); however, the mechanism underlying these effects has not been determined. A recent study showed that mutants lacking UNC-43 have decreased body muscle IPSC rates and amplitudes, which are consistent with the results we report here (Liu et al., 2007). The function of these kinases in regulating circuit activity may be conserved, since mouse knockouts lacking CaMKII are seizure prone (Butler et al., 1995) and orthologous mouse MAPK components (ASK1/MKK6/JNK) have been implicated in seizure induced neuronal cell death (Brecht et al., 2005; Shinoda et al., 2003). We propose that the corresponding pathways may also modulate inhibitory neurotransmission in mammals.

Regulation of GABA Transmission by Conserved Endocrine Regulators

We identify several endocrine pathway components (e.g., NPR-1/NPY-R, Y54E2A.1/Galanin-R, Insulin-like growth factors, and agouti pathway components) as potential regulators of muscle activity. In particular, NPR-1 receptors regulate the rate of GABA secretion by ventral cord motor neurons. Previously, insulin signaling pathways were implicated in diapause and longevity (Kimura et al., 1997), while NPR-1/NPY-R signaling was implicated in foraging behavior (de Bono and Bargmann, 1998). In mammals, the orthologous genes regulate energy homeostasis

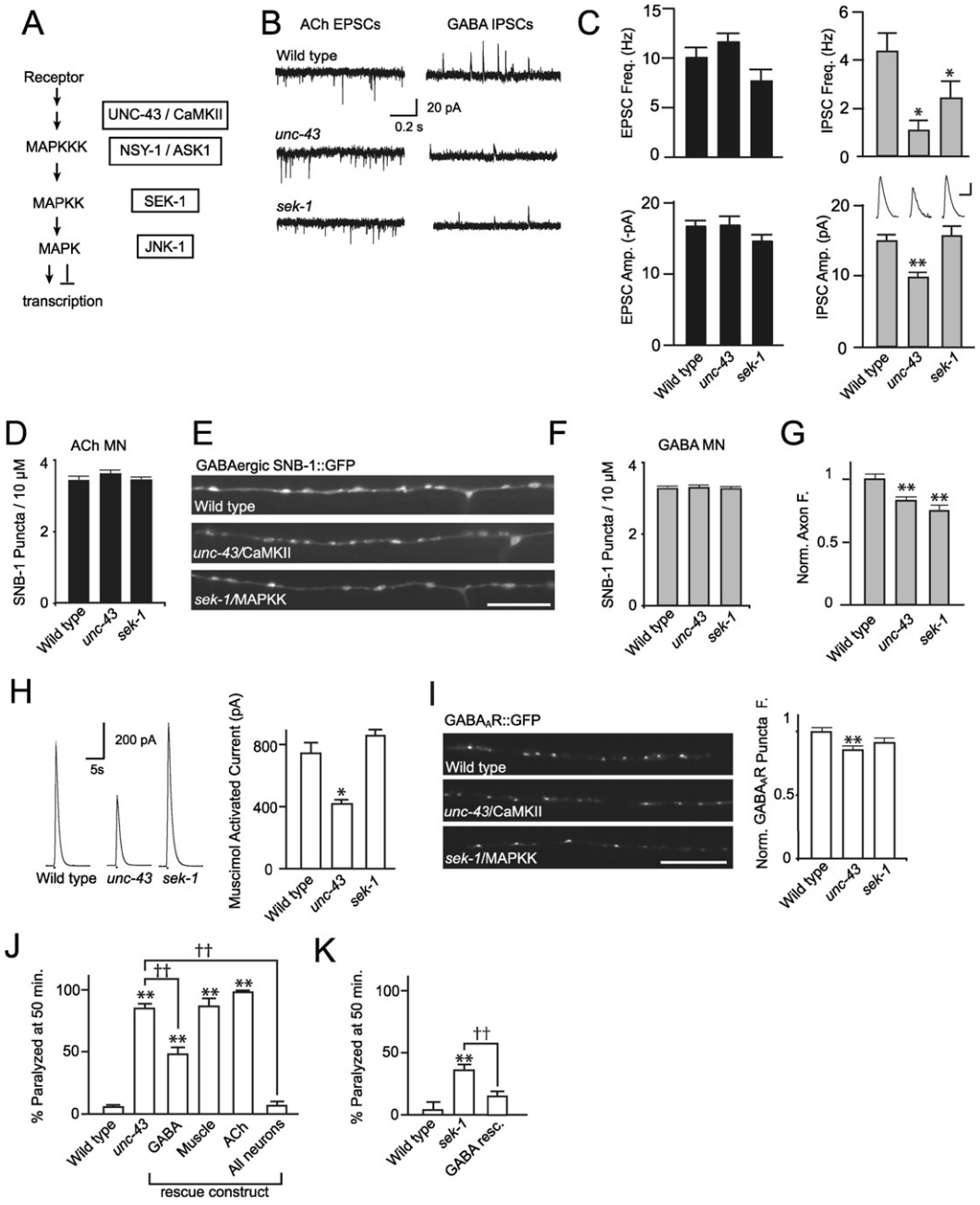

Figure 7. Regulation of GABA Transmission by Kinase Pathways

(A) Schematic of a MAP kinase signaling pathway. On the right, we list several genes encoding components of MAP kinase signaling cascades that were identified in our screen (boxed).

(B) Representative traces of endogenous EPSCs (left panel) and IPSCs (right panel) recorded from adult wild-type (n = 29), *unc*-43 (n = 6), and *sek-1* (n = 6) animals in 1 mM $CaCl_2$ and 4 mM $MgCl_2$.

(C) Mean frequency (top panel) and amplitude (bottom panel) of endogenous EPSCs (black bars) and IPSCs (gray bars) for wild-type, *unc-43*, and *sek-1* animals. Averaged endogenous IPSCs are shown for wild-type, *unc-43*, and *sek-1* above the respective IPSC amplitude bars. Scale bars are 5 pA and 5 ms. An asterisk indicates a significant difference between wild-type and *unc-43* (77% decrease, p = 0.01) or *sek-1* (47% decrease, p = 0.04) animals for IPSC frequency, and a double asterisk indicates a significant difference between wild-type and *unc-43* for IPSC amplitude (36% decrease, p < 0.01).

(D) Quantification of the number of SNB-1 puncta per unit length in the cholinergic axons. n = 31, 28, and 34 animals for *unc-43, sek-1,* and wild-type.

(E) Representative images of SNB-1::GFP expressed in the GABAergic neurons of wild-type, *unc-43*, and *sek-1* animals.

(F) Quantification of the number of SNB-1 puncta per unit length in these GABAergic neurons.

(G) Quantification of the nonsynaptic axonal SNB-1 fluorescence in these GABAergic neurons (gray bars). A double asterisk indicates a significant difference between wild-type and *unc-43* (18% decrease, p < 0.001) or *sek-1* (26% decrease, p < 0.0001) for GABA neuron SNB-1 axonal fluorescence. n = 46, 44, and

and feeding behaviors. For example, feeding behavior and consumption of energy stores is promoted by neuropeptide Y (NPY) and the agouti related peptide, while insulin has the converse effects (Saper et al., 2002). Our results suggest the intriguing possibility that energy homeostasis pathways play a conserved role in regulating inhibitory transmission, providing a mechanism to couple metabolic status to changes in circuit activity.

Several results from human and rodent studies demonstrate that circuit activity and behavior are regulated by metabolic status. First, a low-carbohydrate ketogenic diet effectively reduces the incidence of seizures in patients with drug resistant forms of epilepsy (Freeman et al., 1998; Thiele, 2003). Second, hypoglycemic shock, e.g., following insulin overdose, induces seizures in humans and rodents (Kaplan and Fisher, 2005). Third, NPY and Galanin knockout mice are seizure prone (Baraban et al., 1997; Jacoby et al., 2002). Thus, we speculate that these pathways may be enriched for genes that regulate susceptibility to epilepsy and perhaps other psychiatric disorders.

Regulation of GABA Transmission by a TGFβ Pathway

We show that another secreted ligand, DBL-1 TGFβ, also promotes GABA transmission, being required for both a normal IPSC rate and for normal muscle responsiveness to GABA. DBL-1 TGFβ is endogenously produced by the cholinergic motor neurons (Suzuki et al., 1999). Thus, the activity of the cholinergic neurons may enhance inhibitory input via changes in DBL-1 TGFβ secretion. DBL-1 TGFβ secretion also regulates body size (Suzuki et al., 1999); therefore, this pathway coordinately regulates the size and activity of muscle cells. Interestingly, TGFβ signaling was previously implicated in controlling developmental growth of *Drosophila* NMJs, which are glutamatergic (Aberle et al., 2002; Sweeney and Davis, 2002).

Implications for Regulating Circuit Activity

Our results suggest that muscle activity is tightly regulated in *C. elegans* and that much of this regulation occurs at the circuit level. The extensive control of activity could reflect several aspects of *C. elegans* development and anatomy. First, it is possible that changes in the balance of excitation and inhibition are required to compensate for the large increase in body size or for changes in the motor circuit that occur during larval and adult development, as has been reported for other invertebrate model systems (Li et al., 2002; Lnenicka and Mellon, 1983; Zito et al., 1999). In particular, it is noteworthy that at all stages of development, there are roughly 3-fold more cholinergic motor neurons than GABAergic motor neurons (embryo 16 cholinergic and 6 GABAergic motor neurons; adult 56 cholinergic and 19 GABAergic). Given this disparity in cell numbers, it seems likely that modulatory pathways are required to maintain appropriate muscle activity. Excitatory input to the GABA motor neurons is exclusively provided at cholinergic NMJs (White et al., 1986); consequently, changes in synaptic drive would produce proportionate changes in excitation and inhibition. Thus, to modulate muscle activity by altering the balance of excitation and inhibition, it is necessary to invoke nonsynaptic signaling pathways (e.g., the NPY and DBL-1 TGFβ pathways identified here).

The intricate regulation of excitation and inhibition observed in *C. elegans* is strikingly similar to that seen in mammals. In many brain regions, excitatory and inhibitory conductances are held in a constant ratio (Haider et al., 2006; Liu, 2004; Marder and Goaillard, 2006; Turrigiano, 2007). In cultured neurons, this balance results from a constant ratio of excitatory and inhibitory synapses along individual dendrites (Liu, 2004). Relatively little is known about how this ratio is maintained. We speculate that some of the genes identified here may play a role in this process.

Candidate Genes for Epilepsy and Other Psychiatric Disorders

Many cognitive and affective disorders are thought to arise from perturbations of E/I balance. Increased activity, resulting from increased excitatory inputs or decreased inhibitory inputs, leads to seizure activity, such as in epilepsy (McNamara et al., 2006). Decreased inhibition has been implicated in schizophrenia (Akbarian and Huang, 2006) and autism (Rubenstein and Merzenich, 2003). Conversely, excess inhibition has been proposed to occur in mental retardation syndromes, such as Down's and Rett Syndromes (Dani et al., 2005; Kleschevnikov et al., 2004). Pharmacological blockade of inhibition enhances long term potentiation (LTP) and ameliorates learning defects in a mouse model for Down's Syndrome (Fernandez et al., 2007; Kleschevnikov et al., 2004). Thus, identifying genes that regulate GABA synapses will provide candidate genes for inherited forms of these disorders, may provide new insights into the mechanisms underlying cognitive and affective disorders, and may suggest potential treatments for these disorders. For example, altered secretion of

52 animals for *unc-43*, *sek-1*, and wild-type for GABA neuron quantification.

(H) Muscimol-activated currents in wild-type, *unc-43*, and *sek-1* body muscles. The GABA agonist Muscimol (100 μM) was pressure ejected onto body muscles for 0.5 s for wild-type (n = 8), *unc-43* (n = 6), and *sek-1* (n = 6). Averaged responses for Muscimol-activated currents from wild-type, *unc-43*, and *sek-1* are shown (left) and average peak responses are quantified (right). An asterisk indicates a significant difference between wild-type and *unc-43* for Muscimol-activated current, (44% decrease, $p < 0.05$). Error bars indicate SEM.

(I) Postsynaptic $GABA_AR$::GFP imaging. Representative images of $GABA_AR$::GFP in wild-type, *unc-43*, and *sek-1* animals (left) are accompanied by quantification of the abundance of $GABA_AR$ at synapses (right). A double asterisk indicates a significant difference between wild-type and *unc-43* for $GABA_AR$ puncta fluorescence (14% decrease, $p < 0.01$). n = 30 for each genotype. Scale bar = 10μm. Error bars indicate SEM throughout figure.

(J) Aldicarb hypersensitivity of wild-type (n = 12 trials), *unc-43*(n = 15 trials), and *unc-43* mutants carrying transgenes driving *unc-43* expression in GABA motor neurons (P*unc-25*::*unc-43*, n = 10 trials, 3 independent lines), muscle (P*myo-3*::*unc-43* n = 6 trials, 3 independent lines), cholinergic neurons (P*unc-17*::*unc-43* n = 4 trials, 2 independent lines), and all neurons (P*snb-1*::*unc-43* n = 6 trials, 3 independent lines). A double asterisk indicates a significant difference compared to wild-type ($p < 0.0001$). A double dagger indicates a significant difference between *unc-43* mutants and transgenic rescue ($p < 0.0001$).

(K) Aldicarb hypersensitivity of wild-type (n = 8 trials), *sek-1* (n = 7 trials), and *sek-1* mutants carrying a transgene (P*unc-30*::*sek-1*, n = 13 trials, 4 independent lines) driving expression in GABA motor neurons (GABA resc.). A double asterisk indicates a significant difference compared to wild-type ($p < 0.01$). A double dagger indicates a significant difference betweens *sek-1* and transgenic rescue ($p < 0.01$). Error bars indicate SEM throughout figure.

the ligand for NPR-1 receptors or of DBL-1 would be expected to alter the balance of excitation and inhibition, thereby altering circuit activity. Thus, manipulation of these pathways may provide a useful therapeutic strategy for treating cognitive or psychiatric disorders in which E/I balance has been disrupted.

EXPERIMENTAL PROCEDURES

A detailed description of all methods is included in the Supplemental Data.

C. elegans Strains

Strains were maintained at 20°C. OP50 *E. coli* was used for feeding. The wild-type reference strain was N2 Bristol.

RNAi Feeding Experiments and Screen

The 2072 genes selected for this screen were previously described (Sieburth et al., 2005). RNAi feeding screens were performed as described (Kamath and Ahringer, 2003; Kamath et al., 2003). Aldicarb assays were performed on young adults as described (Lackner et al., 1999).

We used a panel of mutants that cause aldicarb resistance (each in an *eri-1;lin-15b* background) and tested whether RNAi of each gene could still produce significant aldicarb hypersensitivity. Hierarchical clustering was performed using Cluster 3.0 (Eisen et al., 1998). Clusters were initially identified using an average variance method with a Euclidian measure of distance and then robustness was assessed as detailed in Supplemental Experimental Procedures.

Fluorescence Microscopy and Quantitative Analysis

All imaging experiments were done using a Zeiss Aviovert 100 microscope equipped with an ORCA CCD camera (Hamamatsu). Animals were immobilized with 30 mg/ml BDM (Sigma). Line scans of dorsal cord fluorescence (for GFP::SNB-1) or punctal fluorescence intensity (to estimate the abundance of synaptic $GABA_AR$ for UNC-49::GFP) were analyzed in Igor Pro (WaveMetrics) using custom-written software (Burbea et al., 2002), and all statistics reported from imaging experiments are from Kolmogorov-Smirnov tests (KS tests).

Electrophysiology

Electrophysiology was done on dissected *C. elegans* as previously described (Richmond and Jorgensen, 1999). All recording conditions were as described (Sieburth et al., 2007). Statistical significance was determined on a worm-by-worm basis using the Mann-Whitney test for Muscimol puff experiments, Student's t test for comparison of mean frequency and amplitude for EPSCs and IPSCs. The Kolmogorov-Smirnov test was used to determine statistical significance for all cumulative probabilities.

SUPPLEMENTAL DATA

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/58/3/346/DC1/.

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