Synaptic polarity and sign-balance prediction using gene expression data in the *Caenorhabditis elegans* chemical synapse neuronal connectome network

Short title: Synaptic polarity balance in a neuronal network

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Abstract

Graph theoretical analyses of nervous systems usually omit the aspect of connection polarity, due to data insufficiency. The chemical synapse network of *Caenorhabditis elegans* is a well-reconstructed directed network, but the signs of its connections are yet to be elucidated. Here, we present the gene expression-based sign prediction of the *C. elegans* connectome, incorporating presynaptic neurotransmitter and postsynaptic receptor gene expression data (3,638 connections and 20,589 synapses total). We made successful predictions for more than two-thirds of all chemical synapses and determined a ratio of excitatory-inhibitory (E:I) interneuronal ionotropic chemical connections close to 4:1 which was found similar to that observed in many real-world networks. Our open source tool (http://EleganSign.linkgroup.hu) is simple but efficient in predicting polarities by integrating neuronal connectome and gene expression data.

Author Summary

The fundamental way neurons communicate is by activating or inhibiting each other via synapses. The balance between the two is crucial for the optimal functioning of a nervous system. However, whole-brain synaptic polarity information is unavailable for any species and experimental validation is challenging. The roundworm Caenorhabditis elegans possesses a fully mapped connectome with a comprehensive gene expression profile of its 302 neurons. Based on the consideration that the polarity of a synapse must be determined by the neurotransmitter(s) expressed in the presynaptic neuron and the receptors expressed in the postsynaptic neuron, we conceptualized and created a tool that predicts synaptic polarities based on connectivity and gene expression information. We were able to show for the first time that the ratio of excitatory and inhibitory synapses in *C. elegans* is around 4 to 1 which is

in line with the balance observed in many natural systems. Our method opens a way to include spatial and temporal dynamics of synaptic polarity that would add a new dimension of plasticity in the excitatory:inhibitory balance. Our tool is freely available to be used on any network accompanied by any expression atlas.

Introduction

Chemical synapses of a neuronal network are both directed and signed, since a neuron is able to excite or inhibit another neuron. The nervous system of the nematode *Caenorhabditis elegans* has been fully mapped and reconstructed [1–3]. However, except for a few connections there is no comprehensive chemical synapse polarity data available [1]. While the direction of a synaptic connection can be inferred from its structure, the experimental determination of its polarity requires delicate electrophysiological methods (e.g. patch-clamping) which have a limited system-level use. Instead, *in silico* approaches using reverse engineering have efficiently predicted synaptic signs for subnetworks of the *C. elegans* connectome [4–6].

Many synaptic sign prediction models have relied on the widely accepted assumption that the polarity of a chemical synapse is solely determined by the type of neurotransmitter released by the presynaptic neuron [4,5]. Therefore, in *C. elegans* excitatory glutamatergic and cholinergic, as well as inhibitory ɣ-aminobutyric acid (GABA)-ergic ionotropic chemical connections have been modeled. However, with this approach, approximately 95% of the connections turned to be excitatory [7,8]. This would possibly result in an unbalanced, over-excited network, as has been shown by previous publications [9–11]. Moreover, there is evidence of unconventional postsynaptic effects of neurotransmitters, such as cholinergic inhibition [12,13] or glutamatergic inhibition [14–16], meaning that a neuron can

simultaneously excite and inhibit its postsynaptic partners with the same neurotransmitter due to variable neurotransmitter receptor expression on the postsynaptic neuron membrane. For example, the cholinergic AIY interneuron can activate RIB neurons and inhibit AIZ neurons in an acetylcholine-mediated fashion [13].

We aimed to predict synaptic polarities of ionotropic chemical connections of the *C. elegans* connectome relying on presynaptic and postsynaptic gene expression data. In this study of the *C. elegans* nervous system, we successfully predicted the polarity of more than 70% of ionotropic chemical synapses and predicted a sign-balance of excitatory:inhibitory connections close to 4:1 that was observed and functionally stable in many real-world circumstances. Presenting the first connectome-scale work, we show that the concept of gene expression-based polarity prediction can efficiently be applied to demonstrate a natural balance in a nervous system.

Results

Creating a prediction tool of the synaptic polarities of the *C. elegans* connectome

Our primary goal was to infer synaptic polarity from combining connectivity and gene expression data. We created a simple, yet powerful algorithmic database (S1 Data) that takes as input connectome and gene expression data to predict synaptic polarity of ionotropic chemical connections. We used the *C. elegans* WormWiring connectome data primarily in the form of a weighted edge list representing 20,589 chemical synapses in 3,638 connections. Our prediction tool is available here: http://EleganSign.linkgroup.hu.

Update of the previous neurotransmitter expression tables

We updated the *C. elegans* neuronal neurotransmitter tables previously published [17–20] with recent evidence [8,21] (Methods). After this update, 256 neurons had a single neurotransmitter expressed, while 12 neurons had double neurotransmitter expression (Fig 1A). There were 34 neurons which did not express any of the three neurotransmitters investigated.

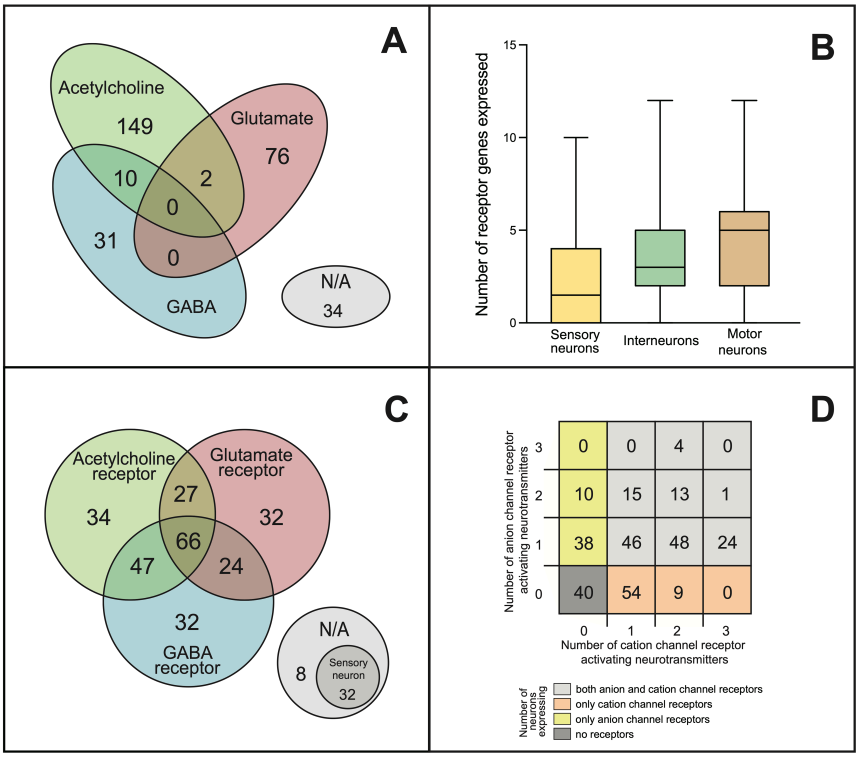

Fig 1. Neurotransmitter and receptor expression patterns of *C. elegans* neurons. Expression data of the three major synaptic neurotransmitters and their receptors of *C. elegans* were collected from multiple datasets and were manually curated (see Methods). **(A)** Distribution of neurons according to their neurotransmitter expression: glutamate (red), acetylcholine (green), GABA (blue) or none (grey). **(B)** Distribution of neurons based on their neurotransmitter receptor gene expression (colors are the same as in panel **a**). **(C)** Number of receptor genes expressed by neurons, grouped by neuron modality. **(D)** Distribution of neurons according to the number of neurotransmitters for which anion and/or cation channel receptor genes are expressed.

Extraction of gene expression data

In parallel, we extracted gene expression data from Wormatlas [22], Wormbase (www.wormbase.org), and a recent RNA-sequencing dataset [21] which we curated manually to assign ionotropic receptor expression pattern to each neuron (see Methods). To do this we first sorted the previously identified 62 ionotropic receptor genes into six functional classes based on their suggested neurotransmitter ligand (glutamate, acetylcholine or GABA) and putative ion channel type (cationic or anionic, i.e. excitatory or inhibitory), as shown in Table 1. We found evidence for expression of 42 out of the 62 receptor genes in the *C. elegans* nervous system (genes marked bold in Table 1; also S1 Data). We also found an increasing average number of receptor genes in sensory, inter- and motor neurons, respectively (Fig 1B).

Table 1. Neurotransmitter receptor genes

	Glutamate	Acetylcholine		GABA
cation channel receptor gene	***glr-1***	*acr-1*	***acr-16***	***exp-1***
	glr-2	***acr-2***	*acr-17*	***lgc-35***
	glr-3	***acr-3***	***acr-18***	
	glr-4	*acr-4*	*acr-19*	
	glr-5	***acr-5***	***acr-20***	
	glr-6	***acr-6***	*acr-21*	
	glr-7	*acr-7*	***acr-23***	
	glr-8	*acr-8*	*acr-25*	
	nmr-1	*acr-9*	***deg-3***	
	nmr-2	*acr-10*	***des-2***	
		acr-11	***lev-8***	
		acr-12	***unc-29***	
		acr-13	*unc-38*	
		acr-14	***unc-63***	
		acr-15		

anion channel receptor gene	*glc-1* *glc-2* ***glc-3*** *glc-4* ***avr-14*** ***avr-15***	***acc-1*** ***acc-2*** *acc-3* ***lgc-46*** ***lgc-47*** *lgc-48* *lgc-49*	***gab-1*** ***ggr-1*** ***ggr-2*** *ggr-3* ***lgc-36*** ***lgc-37*** ***lgc-38*** *unc-49*

The *Caenorhabditis elegans* genome contains 62 ionotropic postsynaptic receptor genes for glutamate, acetylcholine, and GABA. In this table genes are grouped according to their neurotransmitter ligand and whether forming cationic (+) or anionic (-) ion channels ([22] and Table 1 in Supporting Information). In *C. elegans* "unconventional signaling", namely, glutamate-mediated inhibition, cholinergic inhibition and GABA-ergic excitation, is facilitated by 6, 7, and 2 receptor genes, respectively. In the gene expression database used in this work, expression in at least one neuron was found in the case of 42 genes (marked **bold**), while for 20 genes no neuronal expression was found.

Next, for all the 302 neurons of the *C. elegans* connectome we determined which receptor classes were expressed. 164 neurons had an overlapping expression of receptors for two or three different neurotransmitters (Fig 1C). The distribution of neurons according to their expression of cationic and/or anionic glutamate, acetylcholine and/or GABA receptors suggested functional diversity due to the high number of neurons expressing both excitatory and inhibitory receptors (Fig 1D). Surprisingly, 85 neurons expressed both excitatory and inhibitory receptor genes for the same neurotransmitter (S1 Data). Forty out of 302 neurons showed no receptor expression, out of which 32 neurons were primarily sensory neurons (S1 Data). The average number of receptor genes expressed was 3.6 per neuron (Table 2 in S1 Supporting Information).

Neurotransmitter and receptor gene expression-based polarity prediction

After assigning neurotransmitter and receptor expression patterns to each neuron, we predicted synaptic polarities by looking for matches between the neurotransmitter expression of the presynaptic neuron and the receptor gene expression of the postsynaptic neuron (Fig 2A). This way, we labeled synapses as one of the following: excitatory, inhibitory, complex, or unpredicted (see Methods). “Excitatory” or “inhibitory” label were given when the neurotransmitter-matched postsynaptic receptor genes were only cation or anion channel related, respectively. A synapse was labeled as “complex” if data suggested both excitatory and inhibitory function. With this approach, we successfully predicted synaptic polarity for 73% of chemical synapses of the *C. elegans* connectome (Fig 2B). We couldn’t predict polarity for the remaining synapses due to missing neurotransmitter data or mismatch in neurotransmitter/receptor expression (Fig 2B). We predicted that 9,034 of the synapses are excitatory and 2,580 are inhibitory, while 3,431 synapses have complex function (Fig 3A; S1 Data). These findings suggest that the overall ratio of excitatory and inhibitory synapses (E:I ratio) in the *C. elegans* ionotropic chemical synapse network is close to 4:1 (Fig 3A, *NT+R method*).

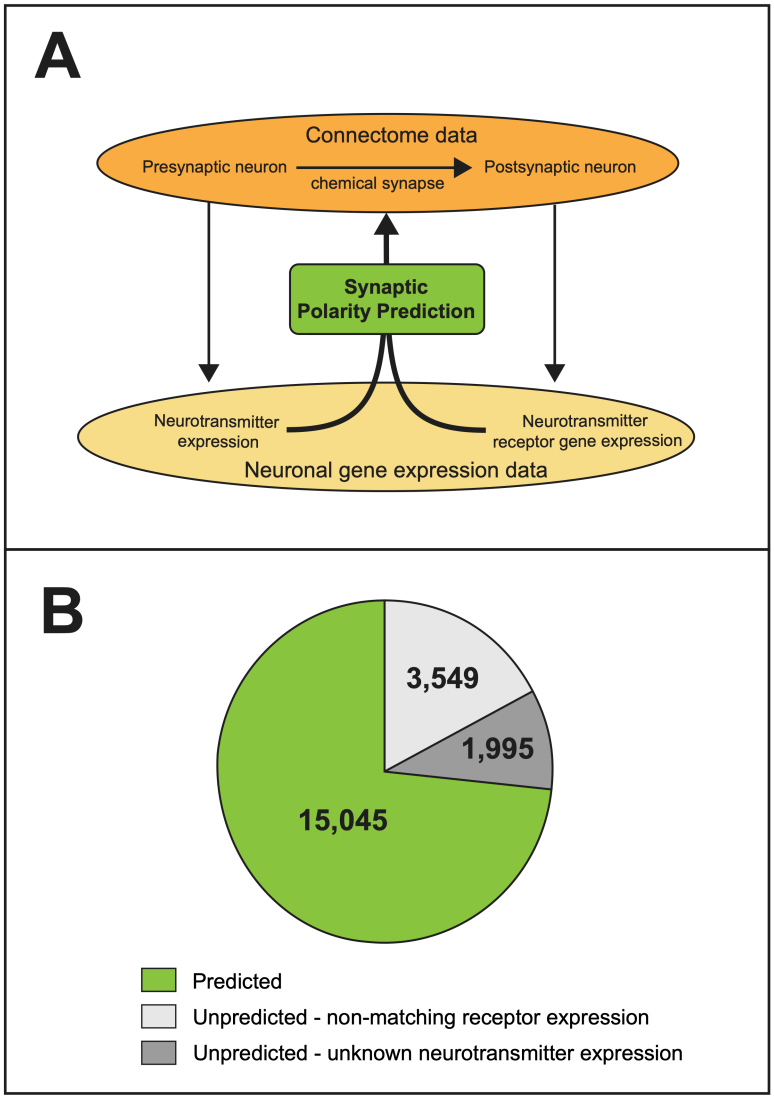

Fig 2. Prediction of synaptic polarities of the *C. elegans* connectome. (A) Prediction method. Connectome and gene expression data were manually curated (see Methods). Polarities of chemical synapses were predicted based on the neurotransmitter expression of presynaptic neurons and the matching receptor gene expression of the postsynaptic neurons. **(B)** Distribution of predicted and unpredicted synapses. We were able to predict the polarity of 73% of chemical synapses (green). The polarities of the rest of synapses were unpredicted due to unknown neurotransmitter expression of the presynaptic neurons (dark grey) or non-matching receptor gene expression of the postsynaptic neurons (light grey).

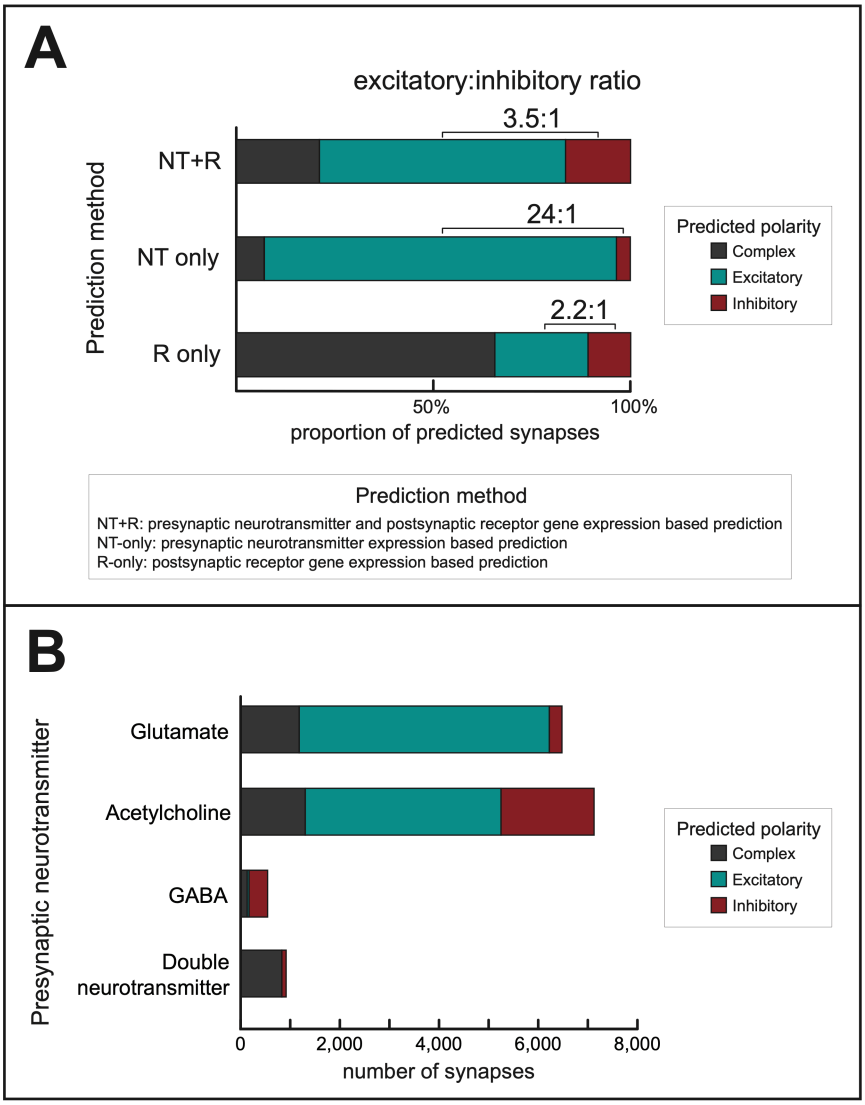

Fig 3. Predicted synaptic polarities. **(A)** Distributions of predicted polarities, using the method developed in this paper (*NT+R*) and two alternative methods as comparison (*NT-only* and *R-only*). Polarities were predicted by considering the neurotransmitter expression of the presynaptic neuron and/or the receptor gene expression of the postsynaptic neuron (see Methods). **(B)** Distributions of predicted synaptic polarities (using the *NT+R method*) according to the presynaptic neurotransmitter. Colors are the same as in panel **A**. Unpredicted synapses are not shown.

Alternative polarity prediction methods.

To put our results in context, we applied two alternative prediction methods for comparison (*NT-only* and *R-only*; see Methods). The *NT-only* method yielded a much higher E:I ratio (Fig 3A, *NT-only method*; S2 Data), which is in line with the dominance of purely glutamatergic or cholinergic (traditionally excitatory) neurons over GABA-ergic (traditionally inhibitory) neurons (Fig 1A). To explain the difference further, the *NT+R* method predicted that 30% of cholinergic and 5% of glutamatergic synapses were *inhibitory* (Fig 3B) which is a significant fraction of synapses otherwise predicted excitatory with the *NT-only* method. The other, *R-only* method yielded a markedly smaller E:I ratio, however predicted an excessive number of complex synapses (Fig 3A, *R-only method*, S3 Data). This is due to the fact that many neurons express both cation and anion channel receptor genes (Fig 1D).

Feedback inhibition between neuron groups

Notably, in subsets of connections which connect neurons of different modalities of sensory neurons, motor neurons, interneurons and polymodal neurons, the E:I ratios varied between 1:10 (motor –> sensory) and 14:1 (inter –> motor). Importantly, we observed dominant inhibition in the motor –> sensory, motor –> inter, and inter –> sensory directions (Fig 4A), exhibiting inhibitory backwards signaling as discussed in the literature previously [23,24]. In addition, significant presence of inhibitory and complex connections was found in the locomotion circuit as well (Fig 4B).

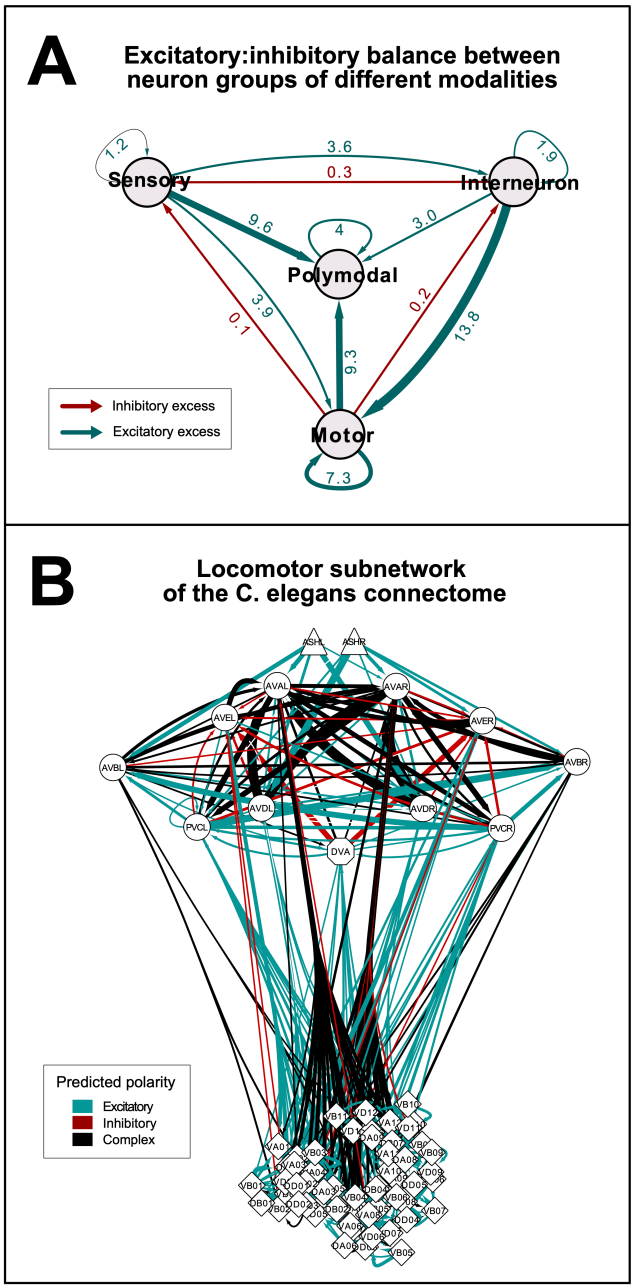

Fig 4. Excitatory:inhibitory balance of different neuron groups. (A) Excitatory:inhibitory balance between neuron groups of different modalities. Nodes represent groups of neurons by modality. Edges are weighted according to the excitatory:inhibitory (E:I) ratios (see numbers). Green and red colors represent excitatory (E:I>1) and inhibitory (E:I<1) excess in sign-balances, respectively. **(B)** Network representation of the locomotion subnetwork. Edges represent excitatory (blue), inhibitory (red), or complex (black) chemical connections. Edges are weighted according to synapse number. Shape of vertices (△,○,◇) represent the modality (sensory, inter, motor, respectively) of neurons.

Network representations of the signed *C. elegans* connectome

Network representations of synaptic polarities in the *C. elegans* chemical synapse connectome using the EntOptLayout plugin of Cytoscape [25] are on Fig 5. Fig 5A shows that the modular structure of the *C. elegans* connectome visualized by the EntOptLayout method nicely captures the anatomical locations of the anterior, ventral and lateral ganglions, as well as the premotor interneurons of the worm. While the anterior and lateral ganglions show a large glutamate expression, this is much less characteristic to the ventral ganglion (Fig 5A). Fig 5B shows that the ventral ganglion has predominantly inhibitory connections, while connections in the other locations are predominantly excitatory if predicted by our *NT + R method*. Fig 5C demonstrates that the prediction of using neurotransmitters only (*NT-only method*) results in a large excitatory excess, which makes the system unstable. The anatomical locations of neurons expressing various neurotransmitters (Fig 5D) correspond well to the network representation shown on Fig 5A. Links between inhibitory function and anatomical structures have been shown in the human brain [26,27], but have not been demonstrated previously in the nematode.

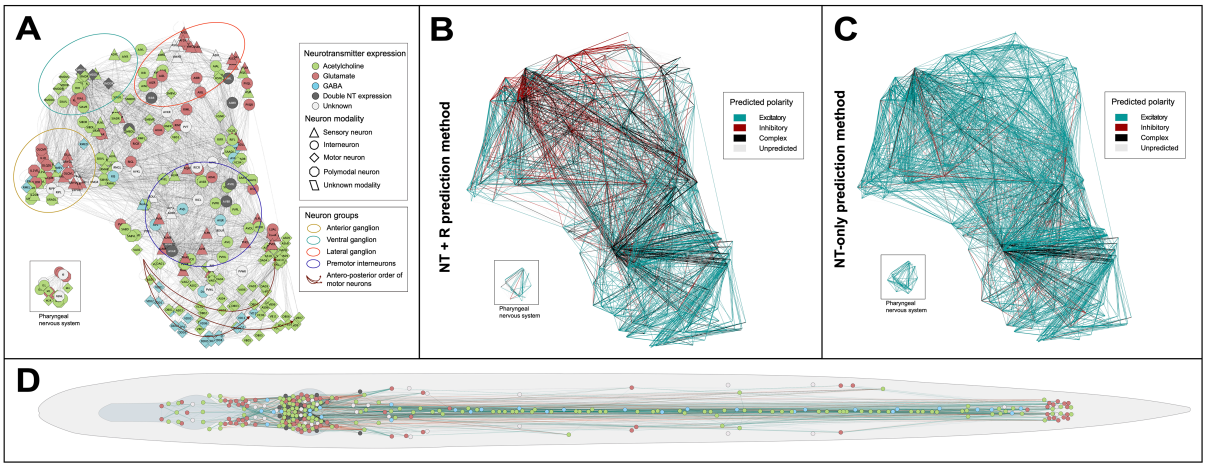

Fig 5. Network representations of the *C. elegans* chemical synapse network. (A-C) Network representations using the EntOpt layout plugin in Cytoscape[25]. **(A)** Color and shape of vertices represent neurotransmitter expression and modality of neurons, respectively (see inset for definitions). **(B)** Edges represent excitatory (blue), inhibitory (red), or complex (black) chemical connections predicted by the NT+R method (see Methods), weighted according to synapse numbers. **(C)** Colors of edges (see panel **B**) represent the polarities of chemical synapses predicted by the NT-only method. **(D)** Layout of vertices is representing the anatomic position of neurons. Node and edge colors are as in panels **A** and **B,** respectively**.**

Validation of our predictions

To validate our results, we compared them with an *in silico* prediction work of the locomotion circuit of *C. elegans* [28] and confirmed that 70% of the synapses were predicted the same (Table 3 in S1 Supporting Information), albeit using a completely different concept. As a further validation step, we tested our predictions against experimental evidence and found that the majority of predicted polarities using our method were consistent with earlier findings in

C. elegans: only a single interneuronal connection out of 12 showed opposing polarity (Table 4 in S1 Supporting Information).

To test the robustness of our *NT+R* prediction method, we applied the same rules to predict polarities of connections regardless of synapse number data, and after perturbations in the network like deletion of the 20 pharyngeal nervous system neurons or deletion of potentially variable (i.e. single-synapse) connections (S4 Data). Furthermore, we repeated our analyses in other published connectomes [2,3] of different sizes as well (Table 5 in S1 Supporting Information and S5 Data). In all five cases, the excitatory:inhibitory ratios were in the range of 3.1 to 4.1 (S1 Fig). When predicting not using the yet preprint-published RNA-seq expression dataset [21], this range was 4.1-4.6 (data not shown). All together, these findings suggest that the observed sign-balance is a remarkably robust property of the *C. elegans* neuronal network.

Discussion

Nervous systems are not only directed but signed networks as well, since neurons either activate or inhibit other neurons [10]. The balance of excitatory and inhibitory connections (i.e. the sign-balance) is a fundamental feature of brain networks, clearly marked by the variety of disorders associated with its impairment [10,29,30]. However, direct evidence of single synapse polarity is rather sporadic even in simple species. In this work we predicted that the sign-balance in the *C. elegans* chemical synapse network is approximately 4:1 (excitatory-inhibitory, E:I). This is consistent with previous *in vitro* and *in vivo* studies of nervous systems [31–34], and also with observations of different social networks [35,36], as shown in Table 2. However, this ratio can only be predicted if not only the neurotransmitter expression of the presynaptic neuron but also the receptor gene expression of the postsynaptic neuron is taken into consideration. Its significance is due to fifteen receptor genes which are presumed to encode inhibitory glutamatergic/cholinergic or excitatory GABA-ergic ion

channel receptors. This concept of unconventional signaling is not new, but has already been described in *C. elegans* [12,37,38] and other primitive species [39–41], and also in mammals in the postnatal period [42,43]. This concept has already motivated the prediction of connection polarity instead of neuron polarity, yet on a subcircuit level [28]. By developing a gene expression-based methodology, our work is the first attempt to predict polarities of all ionotropic chemical synapses of the *C. elegans* connectome.

Table 2. Proportions of negative edges in signed networks

Network	Proportion of negative edges	Reference
***C. elegans* chemical synapse network**	**20%**	**this work**
Rat hippocampus (*in vivo*)	**5-30%**	[33]
Rat excitatory neocortical neurons	**20%**	[34]
Cerebral cortex (*in vivo*; GAD expression)	**10-20%**	[15]
Cerebral cortex (*in vivo;* GABA neurons)	**20-25%**	[15]
Optimal network for synchronized bursting activity (*in silico*)	**10-20%**	[6]
Primary visual cortex (V1; *in silico)*	**25%**	[44]
Neuronal network (e*x vivo*) and neuronal network model *(in silico)*	**20%**	[16]
Wikipedia (social network)	21%	[35]
Epinions (social network)	15%	[35]
Slashdot (social network)	23%	[35]
University freshman network (social)	12-14%	[36]

A surprisingly high proportion of synapses were predicted to have a complex, i.e. both excitatory and inhibitory polarity. This is due to parallel expression of cationic and anionic receptor genes – often for the same neurotransmitter – in half of the neurons. This suggests a highly complex functioning of neuronal connections that extends beyond the permanently exclusive concept of excitation-inhibition dichotomy. Since our work is based on gene expression rather than protein expression data, predictions made are derived from genetic permissibility rather than direct receptor presence. Obviously, a single synapse cannot simultaneously be excitatory and inhibitory, therefore the predicted "complexity" can be resolved mainly in two physiologically well-established ways. One is that postsynaptic receptors are not homogenously distributed across the plasma membrane but their subcellular localization is regulated. This allows the receptors to act independently [45–50] and allows the same neurotransmitter to excite and inhibit at distinct postsynaptic sites. For example, such mechanisms have been identified in the AIA [51–53] and AIB neuron groups [54]. Another explanation of "complexity" is the dynamic change of gene expression in time which is observed all through the life-span of a worm e.g. during development, learning (synaptic plasticity), and aging [55–63]. Ultimately, changes in gene expression can lead to neurotransmitter-switching and consequential up- and downregulation of receptors of opposing polarity [64,65].

Currently, there is not enough data to address either the spatial or the temporal aspects of receptor expression regulation on the worm-scale. As expression-profiling methods will provide whole-brain and dynamic proteomics data of subcellular resolution, complex synapses might be further resolved.

This paper covers neurotransmitter-mediated ionotropic connections, which account for the fast-acting system of the nervous system. Other, generally slower-acting components of the signaling system, such as G-protein coupled metabotropic or extrasynaptic signal

transmission were not studied but can be targets of future research. Although the strength of prediction of our work is generally acceptable (>70%), as new data of connectivity and gene expression emerge, our method can be used to provide more accurate predictions of synaptic polarity.

Within the scope of our aims and subject to the limitations of the available connectome and gene expression data [2,21,55,66], we predicted synaptic polarities of ionotropic chemical connections in the complete *C. elegans* neuronal network, for the first time. We developed and applied a novel method that combines connectivity with presynaptic and postsynaptic gene expression data and made its tool available for users at the website http://EleganSign.linkgroup.hu/. Importantly, the natural balance of excitatory and inhibitory connections can be well approximated only if one considers both pre- and postsynaptic gene expression, a concept that was lacking from previous work. Our method opens a way to include spatial and temporal dynamics of synaptic polarity that would add a new dimension of plasticity in the excitatory:inhibitory balance. When sufficient data is available, our polarity prediction method can be applied to any neuronal (and as a concept non-neuronal) network.

Methods

Description of *C. elegans* connectome data

Connectome reconstruction of the adult hermaphrodite worm published by WormWiring (http://wormwiring.org) consists of 3,638 chemical connections and 2,167 gap junctions, connecting 300 neurons (the two canal-associated neurons, CANL and CANR remained isolated in this reconstruction, and therefore were omitted from the connectome-related analyses). Each of the connections have 4 attributes: the presynaptic neuron, the postsynaptic neuron, the type of the connection (chemical or electrical), and the number of synapses. The chemical connections subset consisting of 20,589 synapses connecting 297 neurons was used in our work (the sensory neuron pair PLML and PLMR, and the pharyngeal neuron M5 is isolated in the chemical synapse network). Additionally, two other connectome reconstructions – both covering a smaller number of neurons and synapses – were used for validation (Table 5 in S1 Supporting Information).

Description of gene expression data and processing

Initially, binary gene expression data was obtained from a previous publication based on Wormbase [19]. This was extended with data of neuronal neurotransmitter [17,18] and receptor [22] expression, and with expression data from the recently published CenGen database [21] after transformation to binary information (Text 1 and Table 6 in S1 Supporting Information). For receptor expression scoring, only the genes coding ionotropic receptor subunits were evaluated according to the six functional classes based on their suggested neurotransmitter ligand (glutamate, acetylcholine or GABA) and putative ion channel type

(cationic or anionic, i.e. excitatory or inhibitory). Expression of one or more genes in a functional class was considered positive.

Prediction of synaptic polarities

Polarities were predicted for connections based on presynaptic neurotransmitter and postsynaptic receptor expression data, using nested logical and conditional formulas. In case of the method referenced as the *NT+R method* throughout the paper, synapses were predicted as *excitatory* or *inhibitory* if only cation channel or only anion channel receptor genes matched the presynaptic neurotransmitter, respectively; *complex* if both types of receptor genes matched; and *unpredicted* if no receptor gene matched. Alternative prediction methods were used according to the following rules. *NT-only method*: synapses were predicted *excitatory* or *inhibitory* if the presynaptic neurotransmitter was acetylcholine and/or glutamate or GABA, respectively; *complex* if acetylcholine and/or glutamate and GABA; and *unpredicted* if the neurotransmitter was none of these. *R-only method:* synapses were predicted *excitatory* or *inhibitory* if the postsynaptic receptor genes expressed were only cation channel or anion channel coding, respectively; *complex* if both types of ion channel receptor genes were expressed; and *unpredicted* if no receptor gene was expressed.
Exact formulas are available in Supplementary Data.

Software and data

Data were processed and predictions were made using Microsoft Excel (ver. 16.32) and R (RStudio 1.1.456) using standard packages.

Data availability

Original data are available as Supplementary Information and Supplementary Data. The sign prediction tool is available at http://EleganSign.linkgroup.hu. Scripts are available on GitHub [https://github.com/bank-fenyves/CeConn-SignPrediction].

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Author contribution

B.G.F contributed to conception, design, acquisition, analysis and wrote the manuscript; G.S.S contributed to acquisition, data analysis, and to the manuscript; Z.V. contributed to the interpretation of data and provided the web-surface of the tool; C.S. contributed to interpretation of data; P.C. contributed to conception, interpretation of data, and to the manuscript.

Competing interests

The authors declare no competing interests.

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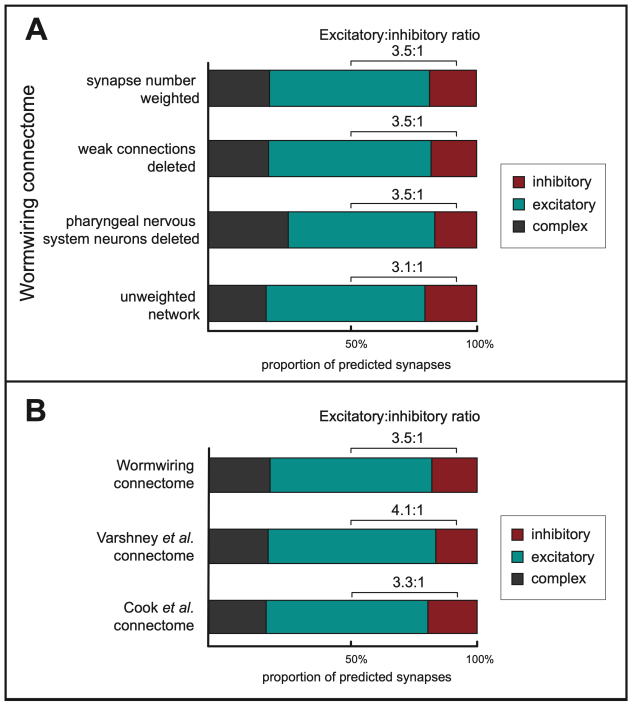
Supporting Information

S1 Supporting Information. Supplemental text and tables.



S1 Fig. Proportions of predicted synaptic polarities in alternative *C. elegans* neuronal networks. Predictions were made based on the neurotransmitter and receptor gene expression patterns of the presynaptic and postsynaptic neurons, respectively (*NT+R method*, see Methods). Red, blue, and grey colors mark inhibitory, excitatory, and complex polarities, respectively. **(A)** Excitatory-inhibitory balances in alternative networks of the WormWiring connectome reconstruction. Bars from top to bottom: 1. synapse weighted network for comparative purpose (same as on Figure 3A); 2. weak links (defined by synapse number of 1) deleted [3]; 3. links connecting any of the pharyngeal nervous system neurons deleted. The rationale is that many previous work analyzed the connectome without the pharyngeal

nervous system [2,67]; 4. unweighted network. **(B)** Predicted synaptic polarities for two connectome reconstructions other than Wormwiring, covering a variable number of neurons and synapses [2,3] (Table 5 in S1 Supporting Information). In summary, excitatory:inhibitory sign-balance ratios were similar in all cases, ranging between 3.1 – 4.1. Source data are provided in S1, S4 and S5 Data.

S1 Data. Prediction of synaptic signs based on neurotransmitter and receptor expression data

S2 Data. Prediction of synaptic signs based on neurotransmitter expression data

S3 Data. Prediction of synaptic signs based on neurotransmitter receptor expression data

S4 Data. Prediction of synaptic and edge signs based on neurotransmitter and receptor expression data in different subnetworks

S5 Data. Prediction of synaptic signs based on neurotransmitter and receptor expression data (alternative connectome reconstructions)

Supporting Information to

Synaptic polarity and sign-balance prediction using gene expression data in the *Caenorhabditis elegans* chemical synapse neuronal connectome network

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Supporting Text

Text 1. Gene expression

The primary sources of gene expression data used in this work [1,2] contained binary (i.e. 'expressed' or 'not expressed') information. Therefore, any extension of gene expression data needed to be carried out in a binary fashion as well. In case of the CenGEN database [3] where relative expression levels were published, data was transformed into binary information using the following rules: criteria for positive expression was avg_logFC > 0 and p_val_adj < 0.05. This way, genes that were found significantly "over-expressed" in a cell cluster compared to the rest of the cells were considered as "expressed" in that cell cluster, while the other genes were not.

Our approach of gene expression data extension was intentionally more sensitive than specific. Thus, in case of conflicting information (i.e. positive expression in one dataset and no expression data in another) evidence of positive expression was considered. Although, this method might have resulted in some false positive predictions of synaptic polarities, the balanced number of unpredicted and complex synapses observed suggested that our method was equally sensitive and specific.

Supporting Tables

Table 1. Channel type (cation or anion) of ionotropic neurotransmitter receptor genes of *C. elegans*

Gene name	Neurotransmitter	Type of channel	Validated?	Reference
acc-1	Ach	-	Yes	[4]
acc-2	Ach	-	Yes	
acc-3	Ach	-	Yes	
acr-1	Ach	+	Yes	[5]
acr-10	Ach	+	Yes	
acr-11	Ach	+	Yes	
acr-12	Ach	+	Yes	
acr-13	Ach	+	No	
acr-14	Ach	+	Yes	[5]
acr-15	Ach	+	Yes	
acr-16	Ach	+	Yes	
acr-17	Ach	+	Yes	
acr-18	Ach	+	Yes	
acr-19	Ach	+	Yes	
acr-2	Ach	+	Yes	
acr-20	Ach	+	Yes	
acr-21	Ach	+	Yes	
acr-23	Ach	+	Yes	
acr-25	Ach	+	Yes	
acr-3	Ach	+	Yes	
acr-4	Ach	+	No	
acr-5	Ach	+	Yes	[5]
acr-6	Ach	+	Yes	
acr-7	Ach	+	Yes	
acr-8	Ach	+	No	
acr-9	Ach	+	Yes	[5]
avr-14	Glu	-	Yes	[6]
avr-15	Glu	-	Yes	
deg-3	Ach	+	Yes	[5]
des-2	Ach	+	Yes	
exp-1	GABA	+	Yes	[7]
gab-1	GABA	-	Yes	[8]
ggr-1	GABA	-	Yes	[5]
ggr-2	GABA	-	Yes	
ggr-3	GABA	-	Yes	
glc-1	Glu	-	Yes	[9]
glc-2	Glu	-	Yes	
glc-3	Glu	-	Yes	[10]
glc-4	Glu	-	No	
glr-1	Glu	+	Yes	[11]
glr-2	Glu	+	Yes	
glr-3	Glu	+	Yes	

glr-4	Glu	+	Yes	
glr-5	Glu	+	Yes	
glr-6	Glu	+	Yes	
glr-7	Glu	+	Yes	
glr-8	Glu	+	Yes	
lev-8	Ach	+	Yes	[12]
lgc-35	GABA	+	Yes	[13]
lgc-36	GABA	-	Yes	[14]
lgc-37	GABA	-	Yes	
lgc-38	GABA	-	Yes	
lgc-46	Ach	-	Yes	[15]
lgc-47	Ach	-	Yes	
lgc-48	Ach	-	No	
lgc-49	Ach	-	No	
nmr-1	Glu	+	Yes	[11]
nmr-2	Glu	+	Yes	
unc-29	Ach	+	Yes	[5]
unc-38	Ach	+	Yes	
unc-49	GABA	-	Yes	[14]
unc-63	Ach	+	Yes	[5,16]

The *Caenorhabditis elegans* genome contains 62 ionotropic postsynaptic receptor genes for glutamate, acetylcholine, and GABA. In this table genes are listed in alphabetic order, and the type of channel (**+** for cation channel, **–** for anion channel) is presented with relevant reference. All 42 genes that were expressed in at least one neuron in our database (marked **bold**) have been validated previously for being cationic or anionic.

Table 2. Distribution of neurons according to the number of ionotropic neurotransmitter receptor genes expressed

		Number of cation channel receptor genes										
		0	1	2	3	4	5	6	7	8	9	10
Number of anion channel receptor genes	0	**40**	**19**	**29**	**12**	**0**	**0**	**3**	**0**	**0**	**0**	**0**
	1	**29**	**11**	**7**	**9**	**4**	**4**	**0**	**0**	**0**	**0**	**0**
	2	**16**	**2**	**6**	**22**	**13**	**3**	**1**	**0**	**2**	**0**	**2**
	3	**1**	**3**	**8**	**6**	**2**	**0**	**1**	**9**	**0**	**0**	**0**
	4	**2**	**0**	**11**	**3**	**2**	**0**	**11**	**0**	**0**	**0**	**0**
	5	**0**	**4**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	6	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**
	7	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**

Neuronal gene expression database was compiled from available datasets and manually curated (Methods). Genes encoding ionotropic receptors for glutamate, acetylcholine or GABA were grouped according to the type of ion channel (cation or anion), i.e. whether being excitatory or inhibitory. Bold numbers represent number of neurons expressing certain numbers of excitatory and/or inhibitory receptor genes. 63 neurons express only cation-channel receptor genes (green), while 48 neurons express only anion-channel receptor genes (red). 151 neurons express a mixture of cation- and anion-channel receptor genes (grey). Source data is in S1 Data.

Table 3. Validation of results with a previous synaptic polarity prediction paper

		Target neuron						
		ASH	AVA	AVB	AVD	AVE	DVA	PVC
Source neuron	ASH		32	20	34			
	AVA				6	6		77
	AVB		47					
	AVD		167					
	AVE		67					
	DVA		8			36		5
	PVC		27	69	23	21	7	

Predicted polarities from our results (S1 Data) were compared to the polarities predicted by Rakowski and Karbowski [17] for the locomotion circuit of the *C. elegans* connectome. Each cell represents a connection between the named source and target neuron. Green colored cell means that the predicted polarity was the same in both cases. Orange colored cell means that the predicted polarity was different with the two methods. 456 of the 652 synapses (70%) were predicted the same.

Table 4. Validation of predictions with previously published experimental results

Source neuron	Target neuron	Number of synapses	Predicted polarity	Reference polarity	Validated?	Reference
AFD	AIY	54	-	-	Yes	[18]
AIA	AIB	35	-	-	Yes	[19,20]
AIA	AIZ	4	-	-	Yes	[19]
ASEL	AIB	29	+	-	No	[21]
ASER	AIB	36	+	+	Yes	[21]
ASH	AIA	52	complex	complex (?)	partially	[22] [20]
ASH	AVA	25	+	+	Yes	[23]
AWC	AIA	15	complex	-	partially	[24]
AWC	AIB	35	+	+	Yes	[25]
AWC	AIY	34	-	-	Yes	[25]
PVD	AVA	75	+	+	Yes	[26]
PVD	PVC	107	+	+	Yes	[26]

Predicted polarities from our results (S1 Data) were individually compared to previously published experimental data. Each row represents a single connection. If validated ("Yes" in column "Validated?"), the predicted polarity equals the reference polarity. Partial validation means that the predicted and/or reference polarity was bipolar or uncertain.

Table 5. Comparison of three chemical synapse connectome reconstructions

	WormWiring.org	Varshney *et al.*	Cook *et al.*
Number of chemical connections	3,638	2,575	3,242
Number of chemical synapses	20,589	6,394	10,203
Number of neurons (non-isolated; including the pharyngeal nervous system)	297	-	279
Number of neurons (non-isolated; without the pharyngeal nervous system)	278	279	259

The three most complete connectome reconstructions of *C. elegans ,* namely the WormWiring (http://wormwiring.org), as well as published by Varshney *et al.* [27] and Cook *et al*. [28], have fundamental differences in their coverage of chemical connections and synapse numbers.

Table 6. Manual curation and edits

Manual edit	Reference
RMED, RMEV, RMG, VA12, DA9: *avr-15*	[29]
ALM, PLM, PVD, PDE: *avr-14*	[6]
AS, DA, DB, VA, VB, VC: *unc-63*	[16]
RME, VNC, DA: *acr-14*	[30]
RIM neuron group: *avr-14*	[31]
all neurons: acc-4	[32]
RIB, SMDD/V, AVA, AVB, AVJ: GABA-expression	[33]
DA/VA: *lgc-46*	[34]
PVM: glutamate expression; AIA: *glc-3;* AIB: *acc-1, lgc-47, gar-2;* AIY: *gar-2;* ALM: *des-2;* BDU: *lgc-38;* CEPD/CEPV: *lgc-38, ggr-2;* DA01-DA07: *gar-2;* DB: *ggr-1, gar-2;* DD: *gar-3;* DVB: *acr-12;* DVC: *acr-20;* FLP: *deg-3;* I1: *avr-15;* IL1/IL1D/IL1V: *acr-2;* LUA: *lgc-38;* OLQD/OLQV: *lgc-38;* PDE: *lgc-47;* PLM: *des-2;* PVM: *des-2, deg-3;* PVN: *glr-2, des-2, deg-3, gar-2;* RIA: *glr-1, gar-3, avr-15;* RIB: *avr-15;* RIG: *acr-12;* RIH: *glr-2; RIM*: *lgc-47, acr-6, acr-3;* RIS: *ggr-2, avr-15;* RIV: *gar-2, lgc-47;* RMD/RMDD/RMDV: *lgc-47;* RMF: *glr-5, lgc-47, glr-2;* RMH: *glr-5;* SAAD/SAAV: *lgc-47, glr-5;* SDQL: *ggr-2;* SIAD/SIAV: *lgc-38, lgc-47;* SIBD/SIBV: *lgc-47, exp-1;* SMBV/SMBD/SMDD/SMDV: *lgc-47;* URAD/URAV: *ggr-2;* URB: *acr-11, ggr-2;* URYD/URYV: *lgc-38;* VC1-3/VC6: *ggr-2*	www.cengen.org (data accessed on 2019 September 1)
ADE: *acr-16*	www.wormatlas.org (2019)

Bulk gene expression data was updated manually according to literature data. Green and red text shows receptor gene additions and deletions, respectively, to (from) specified neuron groups. Blue text shows neurotransmitter expression additions. All neurons of a neuron group were updated unless specified otherwise.

Supplementary References

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