Computational Inferences on Alteration of Neurotransmission in Chemotaxis Learning in *Caenorhabditis elegans*

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Abstract. *Caenorhabditis elegans* changes its NaCl-associated behavior from attraction to avoidance following exposure to NaCl in the absence of food (salt chemotaxis learning). To understand the changes induced by chemotaxis learning at the neuronal network level, we modeled a neuronal network of chemotaxis and estimated the changes that occurred in the nervous system by comparing the neuronal connection weights prior to and after chemotaxis learning. Our results revealed that neurotransmission involving ASE and AIA neurons differed prior to and after chemotaxis learning. This partially corresponded to the experimental findings of previous studies. In addition, our computational inference results suggest the involvement of novel synapse connections in chemotaxis learning. Our approach to estimate changes of neurotransmission corresponding to learning may help in planning experiments in order of importance.

Keywords: Computational neuroscience, neuronal network model, salt chemotaxis learning, neurotransmission, *C. elegans*.

1 Introduction

The nematode *C. elegans* is one of the major model organisms for the nervous system. Its neuronal networks consisting of 302 neurons [1] enable it to respond adequately to various stimuli such as attractant/repellent chemicals, variations in temperature, and mechanical stimulation [2]. *C. elegans* typically approaches NaCl, as a soluble chemoattractant. Behavioral plasticity is observed in this organism after it experiences a particular combination of multiple stimuli [3]; for example, *C. elegans* modifies its movement response to NaCl from attraction to avoidance following exposure to NaCl in the absence of food for several hours (see Fig. 1). Since the anatomical structure of the nervous system in *C. elegans* is well-characterized [1] and does not change at the adult stage, by using this organism it may be possible to understand the specific changes in neuronal states (the

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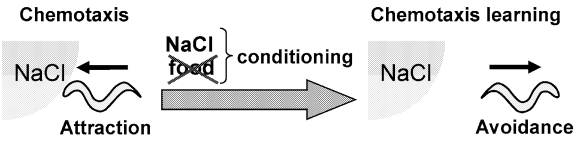

Fig. 1. Salt chemotaxis learning in *C. elegans*

response-characteristic of a neuron and the synaptic transmission efficiency at a certain time) corresponding to behavioral changes. Because of this advantage, in recent years many studies on the mechanisms of learning and memory have been carried out using *C. elegans* [3]–[6].

The behavioral plasticity in response to NaCl, termed 'salt chemotaxis learning', can be explained, at the neuronal network level, as the changes over time of both the response-characteristics of each neuron and the degree of synapse transmission (neurotransmission). In the previous studies, the involvement of some neurons in learning was determined from molecular experiments. However, even using physiologic and/or advanced imaging techniques [7]–[8], it is not possible to measure signal transduction in whole synapse connections and gap junctions in *C. elegans* at the same time. For this reason, whether the change of neuronal states corresponding to the behavioral changes extends to the whole nervous system or only to a limited part of the nervous system is not known.

Therefore, to understand the behavioral changes induced by learning at the neuronal network level, we here propose a novel approach in which the neuronal network is modeled based on the actual neuronal connections, and the neuronal changes corresponding to learning are estimated. The purpose of our computational inference study is to provide novel information that cannot be obtained using conventional experimental techniques. These results will help us to plan experiments in order of importance. This paper covers estimation of the neuronal changes relating particularly to salt chemotaxis learning.

2 Computational Inference of Neuronal States Using a Neuronal Network Model

2.1 Stimulation Response in *C. elegans* and Its Neuronal Structure

C. elegans has a simple cylindrical body approximately 1 mm in length and the body is composed of 959 cells. Neuronal networks consisting of 302 neurons include approximately 5000 chemical synapse connections, approximately 600 gap junctions and approximately 2000 connections between neurons and muscles [1]. The neuronal network processes information from various kinds of stimuli inside and outside the body, and produces differing types of movement appropriate for each stimulus; for example, avoiding obstacles or repellent chemicals. As mentioned previously, in addition to transient responses, *C. elegans* has the capacity to learn some amount of environmental information [3]. Although *C. elegans* usually prefers NaCl and approaches the high-concentration area of a NaCl gradient,

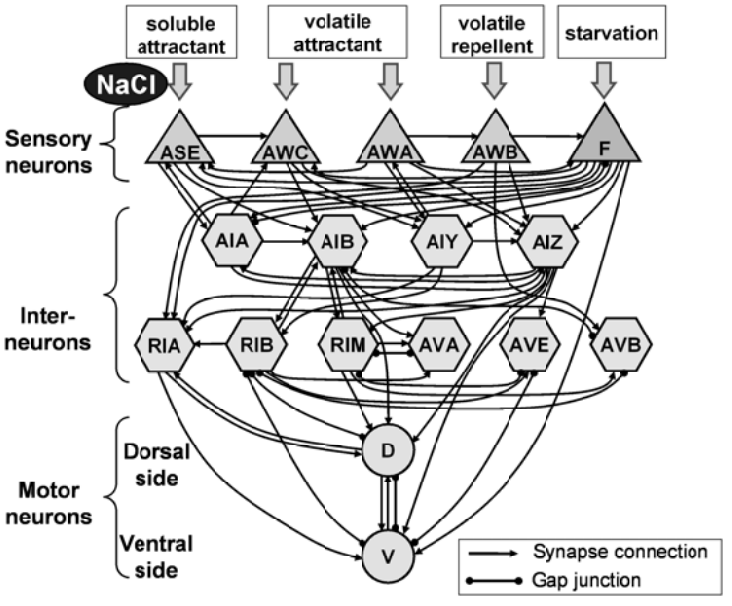

Fig. 2. A model of the chemotactic neuronal network in *C. elegans*

after having experienced starvation and NaCl at the same time, the response changes to avoidance of NaCl. Neurons of *C. elegans* are classified into three main groups by function: sensory neurons, interneurons and motor neurons. The sensory neurons detect external stimuli first, and then the interneurons process information from the stimuli. Finally, the motor neurons control the muscles on the basis of signals from the interneurons. These neuronal networks enable *C. elegans* to respond adequately to various stimuli.

2.2 A Model of the Chemotactic Neuronal Network in *C. elegans*

Estimation of the neurotransmission prior to and after chemotaxis learning is meaningful towards an understanding of the changes in the nervous system induced by learning. Therefore, we here propose a neuronal network model of *C. elegans*, and use this model to estimate the changes in neurotransmission relating particularly to salt chemotaxis learning. Figure 2 shows a model of the neuronal network in relation to chemotaxis, in which the neuronal connections were connected based on the anatomical structure [1] of *C. elegans*. There are 66 connections in this model.

'ASE' represents a pair of sensory neurons as one neuron, in which the sensory neurons ASEL and ASER, which sense soluble chemicals such as NaCl, were simplified. We expressed the other sensory neurons relating to chemotaxis in our model, in which AWA(L/R) and AWC(L/R) sense volatile chemoattractants and AWB(L/R) senses volatile repellents. Neurons that have the same

function were presented as one neuron in the same way as ASE neuron. On the other hand, the details of the neurons that sense starvation are not known. We here focused on sensory neurons, ADF(L/R), ASG(L/R) and ASI(L/R), which relate to the formation of dauer larvae under conditions without food at the larval stage [9] and/or sense stress at the adult stage [10]. We represented these as a sensory neuron 'F' that sense starvation. Subsequently, 10 types of interneurons, AIA(L/R), AIB(L/R), AIY(L/R), AIZ(L/R), RIA(L/R), RIB(L/R), RIM(L/R), AVA(L/R), AVE(L/R) and AVB(L/R), connecting with the 5 sensory neurons were included in this model. Finally, we modeled motor neurons. For various types of movement such as turn and locomotion in *C. elegans*, muscles are controlled by motor neurons existing in the whole body. In fact, turns in response to stimuli are considered to be dependent on neuromuscular controls in the head. Therefore, we considered only the outputs of 14 motor neurons for head control, and these neurons were simplified as only 2 neurons, i.e., a dorsal (D) motor neuron and a ventral (V) motor neuron, where the former controls the dorsal side of the head and the latter controls the ventral side.

In this model, multiple connections existing between a pair of neurons were simplified as a single connection and the efficiency (information content) of neurotransmission of each connection was expressed by the connection weight, w_i $(i = 1, 2, \cdots, 66)$. Signal transductions on chemical synapse connections are one way transductions, while gap junctions are interactive. A positive value of w_i indicates an excitatory signal and a negative value signifies an inhibitory signal.

2.3 Description of the Characteristics of Neurons

Output of the sensory neurons O_j ($j \in$ {ASE, AWC, AWA, AWB, F} was expressed by the following nonlinear equation based on the general neuronal characteristics:

$$O_j = c_j / [1 + \exp\{-a_j(I_j - b_j)\}] \tag{1}$$

where a_j is an inclination with output function, b_j is the value of the stimulation input at which the output of the neuron takes a central value, and c_j is a gain $(0 < c_j \leq 1)$ to the output and is equivalent to the stimulation reception sensitivity. The input I_j to each neuron is the sum of a value that multiplies the connection weight w_i by the stimulation input S_j and/or the output of the connected neuron. Stimulation inputs S_j to sensory neurons are the step-less inputs of the range of [0, 1], which quantifies the strength of the stimulation. Therefore, O_j outputs the continuation value of [0, 1] which is normalized by the maximum output from the actual neuron. The output characteristics O_k ($k \in$ {AIA, AIB, AIY, AIZ, RIA, RIB, RIM, AVA, AVE, AVB, D, V}) of interneurons and motor neurons were also represented by Eq. (1).

2.4 Settings for Output of Motor Neurons Based on Behavior

In the proposed model of the neuronal network, if the output of motor neurons D and V corresponding to stimulation input sensed by sensory neurons is known,

Table 1. Settings for output of motor neurons based on behavior

Stimulation	Behavior	Motor neuron	State	Output
Non-stimuli	Forward (Nomal)	D	Inhibition	$T_{\mathrm{D}} = 0$
		V	Inhibition	$T_{\mathrm{V}} = 0$
Attractants	Forward (Attraction)	D	Excitation	$T_{\mathrm{D}} = 1$
		V	Excitation	$T_{\mathrm{V}} = 1$
Repellents (Case 1)	Dorsal-side turn (Avoidance)	D	Excitation	$T_{\mathrm{D}} = 1$
		V	Inhibition	$T_{\mathrm{V}} = 0$
Repellents (Case 2)	Ventral-side turn (Avoidance)	D	Inhibition	$T_{\mathrm{D}} = 0$
		V	Excitation	$T_{\mathrm{V}} = 1$

we can estimate the neurotransmission for each synapse connection and each gap junction on the basis of the input-output relationship. However, it is impossible to measure the output of each motor neuron even using advanced techniques.

Therefore, we provided the output of the motor neurons from the corresponding behavioral responses, such as forward movement or turn. Here we assumed that *C. elegans* moves forward when the internal states of 2 motor neurons balance, and it turns when the states do not balance. Turns are classified into 2 cases, i.e., the dorsal-side turn (Case 1) and ventral-side turn (Case 2). Motor neuron D is in an excited state when the dorsal-side muscles contract and *C. elegans* turns to its dorsal side, and motor neuron V is in an excited state when the ventral-side muscles contract and the worm turns to its ventral side. Based on this, the settings for outputs of the motor neurons corresponding to each stimulation input were provided as shown in Table 1. Outputs for forward movement (attraction) were given as $T_{\mathrm{D}} = T_{\mathrm{V}} = 1$. In the same way, for turn (avoidance), the desired outputs were given as $T_{\mathrm{D}} = 1$ and $T_{\mathrm{V}} = 0$ or $T_{\mathrm{D}} = 0$ and $T_{\mathrm{V}} = 1$. Therefore, in the case of normal chemotaxis (prior to learning), response to stimulation sensed by the ASE, AWC or AWA neurons is forward movement, and the desired outputs of the motor neurons were $T_{\mathrm{D}} = T_{\mathrm{V}} = 1$. Also, the response to stimulation sensed by the AWB neuron is turn, and the desired outputs of the motor neurons were $T_{\mathrm{D}} = 1$ and $T_{\mathrm{V}} = 0$ or $T_{\mathrm{D}} = 0$ and $T_{\mathrm{V}} = 1$.

2.5 Optimization of the Neuronal Network Model by a Real-Coded Genetic Algorithm (GA)

In this study, the desired outputs of the motor neurons, T_{D} and T_{V}, were provided so as to correspond to each of u ($u = 1, 2, \cdots, U = 10$) patterns of stimulation inputs to sensory neurons. Note that T_{D} and T_{V} for responses after learning were set to different values from those for prior to learning only in the response to NaCl which was sensed by the ASE neuron. To search for an adequate set of neuronal connection weights that fulfills the input-output relationship provided in Table 2, we employed a real-coded genetic algorithm (GA) that we previously used for parameter tuning of some neuronal network models of *C. elegans* and confirmed its effectiveness [11]. All the connection weights, w_i ($i = 1, 2, \cdots, 66$), included

Table 2. Desired outputs of motor neurons corresponding to stimulation inputs (Case 1)

	Input					Output prior to learn.			Output after learn.		
u	S_{ASE}	S_{AWC}	S_{AWA}	S_{AWA}	S_{F}	T_{D}	T_{V}	Behav.	T_{D}	T_{V}	Behav.
1	1	0	0	0	0	1	1	FW	1	0	DT
2	0	1	0	0	0	1	1	FW	1	1	FW
3	0	0	1	0	0	1	1	FW	1	1	FW
4	0	0	0	1	0	1	0	DT	1	0	DT
5	0	0	0	0	1	0	0	FW	0	0	FW
6	1	0	0	0	1	1	1	FW	1	0	DT
7	0	1	0	0	1	1	1	FW	1	1	FW
8	0	0	1	0	1	1	1	FW	1	1	FW
9	0	0	0	1	1	1	0	DT	1	0	DT
10	0	0	0	0	0	0	0	FW	0	0	FW

FW denotes forward movements and DT denotes dorsal-side turns.

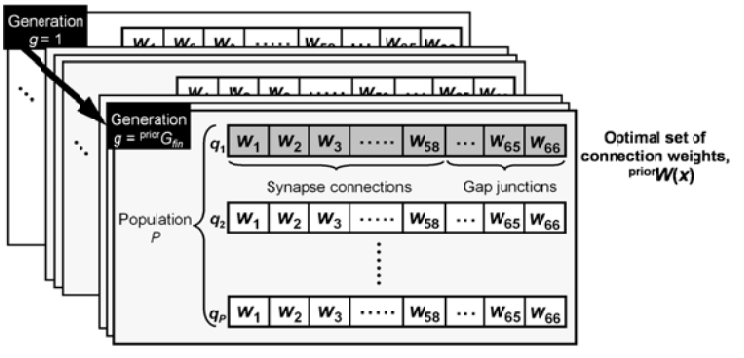

Fig. 3. The outline of the GA method for searching for an adequate set of connection weights prior to chemotaxis learning. The method for connection weights after learning is similar to this.

in the model shown in Fig. 2 were represented as individual genes (see Fig. 3). A string including all the connection weights (genes) of the model was treated as an individual in the GA, and the procedures, (1) selection, (2) crossover, and (3) mutation, were repeated at each generation g ($g = 1, 2, \cdots, G_{\mathrm{fin}}$). An individual of a GA consisted of a string arraying a set of connection weights included in the neuronal network model.

For each GA generation, the adequacy of each individual was evaluated to determine which individuals will be included in the next generation. The function for evaluating error values during GA-searching was defined by the following equation.

$$F(p) = \sum_{u=1}^{U} (|T_{\mathrm{D}}(u) - O_{\mathrm{D}}(p,u)| + |T_{\mathrm{V}}(u) - O_{\mathrm{V}}(p,u)|) \tag{2}$$

where p ($p = 1, 2, \cdots, P$) is the serial number of the GA individual. Searching for an adequate set of connection weights for prior to and after learning was conducted using a GA in each case, and a set of connection weights that provides a minimal value of $F(p)$ in the final generation, G_{fin} ($\in \{{}^{\text{prior}}G_{\text{fin}}$ (for weights prior to learning), ${}^{\text{after}}G_{\text{fin}}$ (for weights after learning)$\}$) was employed in the model.

3 Estimated Changes in Neurotransmission After Chemotaxis Learning

In searching for an adequate set of connection weights by a GA, we set the desired outputs of motor neurons corresponding to sensory inputs prior to and after chemotaxis learning as shown in Table 2. We repeated the search ${}^{\text{prior}}N = 50$ times under the same calculation conditions to ensure statistical power, and 50 distinct sets of neuronal connection weights were thus obtained. On the other hand, searching for connection weights for after learning was conducted where each of the previous 50 sets of connection weights were used as initial values, and the calculations were repeated ${}^{\text{after}}N = 50$ times for each set of initial values. Finally, the average variation in the sets of connection weights after learning was derived from the results of 2500 (50 50) sets of connection weights. Because turns to another direction occurred, we partially changed the desired outputs (Case 2), in which the outputs of motor neurons for turn were inverted from those of Case 1 shown in Table 2. Under these settings, we conducted the same searching as described above.

We evaluated quantitatively the change in neurotransmission on each neuronal connection after salt chemotaxis learning, based on a variation, $v_i(x, y)$. The variation of neurotransmission of each neuronal connection, prior to and after learning, was calculated by the following equation:

$$v_i(x, y) = |{}^{\text{prior}}w_i(x) - {}^{\text{after}}w_i(x, y)| \tag{3}$$

where ${}^{\text{prior}}w_i(x)$ is the i ($i = 1, 2, \cdots, 66$)-th connection weight prior to learning that is included in the x ($x = 1, 2, \cdots, {}^{\text{prior}}N$)-th adequate set of weights, and ${}^{\text{after}}w_i(x, y)$ is the i-th connection weight after learning that was obtained from an initial value of ${}^{\text{prior}}w_i(x)$ and is included in the y ($y = 1, 2, \cdots, {}^{\text{after}}N$)-th adequate set of weights. Subsequently, the mean variation of $\bar{v_i}(x)$ was calculated by:

$$\bar{v_i}(x) = \frac{1}{{}^{\text{after}}N} \sum_{y=1}^{{}^{\text{after}}N} v_i(x, y) \tag{4}$$

For each of the 50 sets of initial connection weights prior to learning, this calculation was performed and the integrated value of mean variation V_i of each neuronal connection was calculated by:

$$V_i = \sum_{x=1}^{{}^{\text{prior}}N} \bar{v_i}(x) \tag{5}$$

Note that V_i in Case 1 and Case 2 were individually calculated.

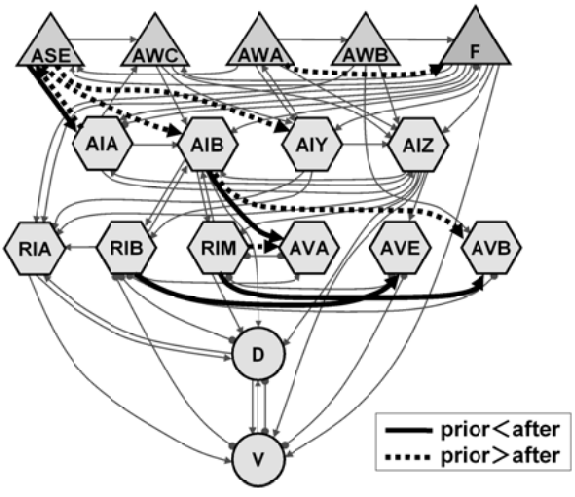

Fig. 4. Estimated changes in neuronal networks induced by salt chemotaxis learning

We focused on the neural connections whose connection weight changed in the same way in the 2 types of input-output settings (Case 1 and Case 2). Ten connections where substantial changes ($V_i > 20$) occurred are shown as heavy lines in Fig. 4. The solid lines denote connections that resulted in excitatory neurotransmission, and the dotted lines denote those that resulted in inhibitory neurotransmission. Among the 10 connections, 4 connections connected with ASE neuron. It is known that ASE sensory neuron and AIY interneuron play a significant role in chemotaxis learning [4]. In addition, the activity of ASE neuron is inhibited by the function of AIA neuron, which results in the inhibition of neurotransmission to AIB or AIY neuron from an ASE neuron [5]. Our results partially corresponded to this experimental finding.

Furthermore, the connections at which neurotransmission was barely altered ($V_i < 10$) were concentrated in those connections from the F sensory neuron (Figure not shown). This indicates that neurotransmission relating to starvation maintains a constant level regardless of learning. The weights of neuronal connections from the F neuron were values in the range of -0.3 to 0.3 on average, which were lower than that of other connections. These results suggest the possibility that salt chemotaxis learning can be realized by inhibiting the activity of neurotransmission involving ASE neuron. Nevertheless, the substantial changes corresponding to chemotaxis learning were newly estimated in this study on synapse connections to AVA and AVB interneurons. Although biological experiments using advanced imaging techniques could measure the changes of neurotransmission at a few neuronal-levels, our method could estimate the changes in neurotransmission concerned with learning at a neuronal network level.

4 Discussion and Conclusions

The purpose of our study was to establish a novel computational approach, which provides information that cannot be obtained using well-known physiological and/or advanced imaging techniques, and that provides information for selecting experiments in order of importance. To understand the changes induced by chemotaxis learning in *C. elegans* at the neuronal network level, we modeled the chemotactic neuronal network based on the actual neuronal connections. In this model, we simplified the neuronal connections and properties of neurons on the basis of their function and estimated the changes that occurred in the nervous system by comparing the neuronal connection weights prior to and after salt chemotaxis learning.

The results revealed that signal transduction in several connections, such as that from AIA interneuron to ASE sensory neuron, differed prior to and after salt chemotaxis learning. This corresponded partially to the experimental findings of previous studies which suggested the involvement of some synapse connections in salt chemotaxis learning. The significant point is that we used the simplified model and obtained results similar to the actual experimental results. These results are meaningful with respect to discussions concerning the adequacy of simplification and assumptions on modeling of living organisms. Another important point is that we could estimate involvement of some novel neuronal connections in chemotaxis learning by computational approach. The involvement in chemotaxis learning will need to be examined in greater detail through biological experiments at the neuronal-level.

On the other hand, comparative studies on learning dynamics between the neuronal network model and the actual *C. elegans* are also important, particularly focusing on the effects of external noisy input and the time needed for learning. These results will provide suggestive knowledge on learning. We will investigate these relationships through more-detailed analyses of the data presented in this paper. Furthermore, updating the model to correspond to the novel knowledge is important to obtain more accurate results. Since it is much important to establish a framework for estimation of neurotransmission that does not change even if the targeted model is changed, we developed such the method for computational inferences in this study. The method we proposed is not dependent on a model and can also be applied to the distinct neuronal-network model for estimating changes in neurotransmission prior to and after learning. We will use the proposed method to estimate the neurotransmission underlying various types of phenomena.

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