Model Checking Tap Withdrawal in C. Elegans

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Abstract. We present what we believe to be the first formal verification of a biologically realistic (nonlinear ODE) model of a neural circuit in a multicellular organism: Tap Withdrawal (TW) in C. Elegans, the common roundworm. TW is a reflexive behavior exhibited by C. Elegans in response to vibrating the surface on which it is moving; the neural circuit underlying this response is the subject of this investigation. Specially, we perform reach-tube-based reachability analysis on the TW circuit model of Wicks et al. (1996) to estimate key model parameters. Underlying our approach is the use of Fan and Mitra’s recently developed technique for automatically computing local discrepancy (convergence and divergence rates) of general nonlinear systems.

The results we obtain are a significant extension of those of Wicks et al. (1996), who equip their model with fixed parameter values that reproduce the predominant TW response they observed experimentally in a population of 590 worms. In contrast, our techniques allow us to much more fully explore the model’s parameter space, identifying in the process the parameter ranges responsible for the predominant behavior as well as the non-dominant ones. The verification framework we developed to conduct this analysis is model-agnostic, and can thus be re-used on other complex nonlinear systems.

1 Introduction

Although neurology and brain modeling/simulation is a popular field of biological study, formal verification has yet to take root. There has been cursory study into neurological model checking (see Sect. 2), but not with the nonlinear ODE models used by biologists. The application of verification technology to hardware circuits has played a key role in the Electronic Design Automation (EDA) industry; perhaps it will play a similar role with neural circuits.

For our initial neurological study, we have selected the round worm, \textit{Caenorhabditis Elegans}, due to the simplicity of its nervous system (302 neurons, \textasciitilde 5,000 synapses) and the breadth of research on the animal. The complete connectome of the worm is documented, and there have been a number of interesting experiments on its response to stimuli.
For model-checking purposes, we were particularly interested in the tap withdrawal (TW) neural circuit. The TW circuit governs the reactionary motion of the animal when the petri dish in which it swims is perturbed. (A related circuit, touch sensitivity, controls the reaction of the worm when a stimulus is applied to a single point on the body.) Studies of the TW circuit have traditionally involved using lasers to ablate the different neurons in the circuit of multiple animals and measuring the results when stimuli are applied.

A model of the TW circuit was presented by Wicks, Roehrig, and Rankin in [16]. Their model is in the form of a system of nonlinear ODEs with an indication of polarity (inhibitory or excitatory) of each neuron in the TW circuit. Additionally, Wicks and Rankin had a previous paper in which they measure the three possible reactions of the animals to TW with various neurons ablated [15]; see also Fig. 3. The three behaviors—acceleration, reversal of movement, and no response—are logged with the percentage of the experimental population to display that behavior.

The [16] model has a number of circuit parameters, such as gap-junction conductance, capacitance, and leakage current, that crucially affect the behavior of the organism. A single value for each parameter is given in [16]. With this single set of parameter values, the model produces predominant behavior in most ablation groups with a few exceptions.

While the experimental work in [15,16] and the model presented in [16] were by no means insubstantial, the exploration of the model is vastly incomplete. The fixed parameter values fit through experimentation cause the model to replicate the predominant behavior seen in said experiments, but little can be said about the model beyond that. The ranges that can produce the predominant behavior, as well as the two other behaviors, are completely missing. This is not to fault the authors of [16], however, as the technology needed to uncover these ranges simply did not exist at the time.

The missing technology was the ability to automatically generate local discrepancy functions [2], and has only recently been developed [5]. With this technique, we can theoretically compute reach tubes used in verification. In reality, this is not a simple plug-and-play situation. To make use of [5], we needed to create the verification framework in Fig. 1. Through careful model engineering Fig. 1 (1–3) and verification engineering Fig. 1 (4–6) we were able to explore and verify the full parameter ranges in the Wicks et al. model to produce all three behaviors in the TW circuit. Such an understanding of the model is critical to morphospace exploration [14] of the animal. A detailed description of our framework and its application to the [16] model Fig. 1(b) is given in Sect. 4.

This verification framework has the additional benefit of being model agnostic. It can be reused to verify other complex nonlinear ODE models.

The rest of the paper develops as follows. Section 2 reviews related work. Section 3 provides requisite background material on the TW neural circuit, its reactionary behavior, and the ODE model of [16]. Section 4 describes our reach-tube reachability analysis and associated property checking. Section 5 presents our extensive collection of model-checking/parameter-estimation results. Section 6 offers our concluding remarks and directions for future work.
Fig. 1. Verification framework of nonlinear ODE model based on automatic computation of discrepancy function. (a) The general framework, (b) Application to [16] model.

2 Related Work

Iyengar et al. [10] present a Pathway Logic (PL) model of neural circuits in the marine mollusk *Aplysia*. Specifically, the circuits they focus on are those involved in neural plasticity and memory formation. PL systems do not use differential equations, favoring qualitative symbolic models. They do not argue that they can replace traditional ODE systems, but rather that their qualitative insights can support the quantitative analysis of such systems. Neurons are expressed in terms of rewrite rules and data types. Their simulations, unlike our reachability analysis, do not provide exhaustive exploration of the state space. Additionally, PL models are abstractions usually made in collaboration between computer scientists and biologists. Our work meets the biologists on their own terms, using the pre-existing ODE systems developed from physiological experiments.

Tiwari and Talcott [13] build a discrete symbolic model of the neural circuit Central Pattern Generator (CPG) in *Aplysia*. The CPG governs rhythmic foregut motion as the mollusk feeds. Working from a physiological (non-linear ODE) model, they abstract to a discrete system and use the Symbolic Analysis Laboratory (SAL) model checker to verify various properties of this system. They cite the complexity of the original model and the difficulty of parameter estimation as motivation for their abstraction. Neuronal inputs can be positive, negative, or zero and outputs are boolean: a pulse is generated or not. Our approach uses the original biological model of the TW circuit of *C. Elegans* [16], and through reachability analysis, we obtain the parameter ranges of interest.
We have extensive experience with model checking and reachability analysis in the cardiac domain, e.g. [6,8,9,12]. In fact, much of our previous work has focused on the cardiac myocyte, a computationally similar cell to the neuron. This is not surprising as both belong to the class of excitable cells. The similarities are so numerous that we have used a variation of the Hodgkin-Huxley model of the squid giant axon [7] to model ion channel flow in cardiac tissue.

3 Background

In C. Elegans, there are three classes of neurons: sensory, inter, and motor. For the TW circuit, the sensory neurons are PLM, PVD, ALM, and AVM, and the inter-neurons are AVD, DVA, PVC, AVA, and AVB. The model we are using abstracts away the motor neurons as simply forward and reverse movement.

Neurons are connected in two ways: electrically via bi-directional gap junctions, and chemically via uni-directional chemical synapses. Each connection has varying degrees of throughput, and each neuron can be excitatory or inhibitory, governing the polarity of transmitted signals. These polarities were experimentally determined in [16], and used to produce the circuit shown in Fig. 2.

![Fig. 2. Tap Withdrawal Circuit of C. Elegans. Rectangle: Sensory Neurons; Circle: Inter-neurons; Dashed Undirected Edge: Gap Junction; Solid Directed Edge: Chemical Synapse; Edge Label: Number of Connections; Dark Gray: Excitatory Neuron; Light Gray: Inhibitory Neuron; White: Unknown Polarity. FWD: Forward Motor system; REV: Reverse Motor System.](image)

The TW circuit produces three distinct locomotive behaviors: acceleration, reversal of movement, and a lack of response. In [15], Wicks et al. performed a series of laser ablation experiments in which they knocked out a neuron in a group of animals (worms), subjected them to a tapped surface, and recorded the magnitude and direction of the resulting behavior. Figure 3 shows the response types for each of their experiments.
The dynamics of a neuron’s membrane potential, $V$, is determined by the sum of all input currents, written as:

$$C \dot{V} = \frac{1}{R} (V^{\text{leak}} - V) + \sum I_{\text{gap}} + \sum I_{\text{syn}} + I_{\text{stim}}$$

where $C$ is the membrane capacitance, $R$ is the membrane resistance, $V^{\text{leak}}$ is the leakage potential, $I_{\text{gap}}$ and $I_{\text{syn}}$ are gap-junction and the chemical synapse currents, respectively, and $I_{\text{stim}}$ is the applied external stimulus current. The summations are over all neurons with which this neuron has a (gap-junction or synaptic) connection.

**Fig. 3.** Effect of ablation on Tap Withdrawal reflex (experimental results). The length of the bars indicate the fraction of the population demonstrating the particular behavior [15].

The current flow between neuron $i$ and $j$ via a gap-junction is given by:

$$I_{ij}^{\text{gap}} = n_{ij}^{\text{gap}} g_m^{\text{gap}} (V_j - V_i)$$

where the constant $g_m^{\text{gap}}$ is the maximum conductance of the gap junction, and $n_{ij}^{\text{gap}}$ is the number of gap-junction connections between neurons $i$ and $j$. The conductance $g_m^{\text{gap}}$ is one of the key circuit parameters of this model that dramatically affects the behavior of the animal.
The synaptic current flowing from pre-synaptic neuron $j$ to post-synaptic neuron $i$ is described as follows:

$$I_{ij}^{\text{syn}} = n_{ij}^{\text{syn}} g_{ij}^{\text{syn}}(t)(E_j - V_i)$$

where $g_{ij}^{\text{syn}}(t)$ is the time-varying synaptic conductance of neuron $i$, $n_{ij}^{\text{syn}}$ is the number of synaptic connections from neuron $j$ to neuron $i$, and $E_j$ is the reversal potential of neuron $j$ for the synaptic conductance.

The chemical synapse is characterized by a synaptic sign, or polarity, specifying if said synapse is excitatory or inhibitory. The value of $E_j$ is assumed to be constant for the same synaptic sign; its value is higher if the synapse is excitatory rather than inhibitory.

Synaptic conductance is dependent only upon the membrane potential of presynaptic neuron $V_j$, given by:

$$g_{ij}^{\text{syn}}(t) = g_{\infty}^{\text{syn}}(V_j)$$

where $g_{\infty}^{\text{syn}}$ is the steady-state post-synaptic conductance in response to a presynaptic membrane potential.

The steady-state post-synaptic membrane conductance is modeled as:

$$g_{\infty}^{\text{syn}}(V_j) = \frac{g_{m}^{\text{syn}}}{1 + \exp \left( k \frac{V_j - V_{eq}^j}{V_{Range}} \right)}$$

where $g_{m}^{\text{syn}}$ is the maximum post-synaptic membrane conductance for the synapse, $V_{eq}^j$ is the pre-synaptic equilibrium potential, and $V_{Range}$ is the pre-synaptic voltage range over which the synapse is activated. $k$ is an experimentally derived constant, valued at $-4.3944$.

Combining all of the above pieces, the mathematical model of the TW circuit is a system of nonlinear ODEs, with each state variable defined as the membrane potential of a neuron in the circuit. Consider a circuit with $N$ neurons. The dynamics of the $i^{th}$ neuron of the circuit is given by:

$$C_i \dot{V}_i = \frac{V_i - V_i}{R_i} + \sum_{j=1}^{N} I_{ij}^{\text{gap}} + \sum_{j=1}^{N} I_{ij}^{\text{syn}} + I_{i}^{\text{stim}}$$

$$I_{ij}^{\text{gap}} = n_{ij}^{\text{gap}} g_{m}^{\text{gap}} (V_j - V_i)$$

$$I_{ij}^{\text{syn}} = n_{ij}^{\text{syn}} g_{ij}^{\text{syn}} (E_j - V_i)$$

$$g_{ij}^{\text{syn}} = \frac{g_{m}^{\text{syn}}}{1 + \exp \left( k \frac{V_j - V_{eq}^j}{V_{Range}} \right)}$$

The equilibrium potentials ($V_{eq}^j$) of the neurons are computed by setting the left-hand side of Eq. (1) to zero. This leads to a system of linear equations, that can be solved as follows:

$$V_{eq}^i = A^{-1} b$$
where matrix $A$ is given by:

$$A_{ij} = \begin{cases} -R_i n_{ij}^{gap} g_m^{gap} & \text{if } i \neq j \\ 1 + R_i \sum_{j=1}^{N} n_{ij}^{gap} g_{ij}^{syn} g_m^{syn} / 2 & \text{if } i = j \end{cases}$$

and vector $b$ is written as:

$$b_i = V_{li} + R_m \sum_{j=1}^{N} E_j n_{ij}^{syn} g_m^{syn} / 2.$$  

The potential of the motor neurons $AVB$ and $AVA$ determine the observable behavior of the animal. If the integral of the difference between $V_{AVA} - V_{AVB}$ is large, the animal will reverse movement. By extension, if the difference is a large negative value, the animal will accelerate, and if the difference is close to zero there will be no response. The equation that converts the membrane potential of $AVB$ and $AVA$ to a behavioral property, (e.g. reversal), is given by:

$$\text{Propensity to Reverse} \propto \int (V_{AVA} - V_{AVB}) dt \quad (6)$$

where the integration is computed from the beginning of tap stimulation until either the simulation ends or the integrand changes sign. To allow initial transients after the tap, the test for a change of integrand sign occurs only after a grace period of 100 ms.

For the purpose of reachability analysis (Sect. 4), we normalize the system of equations with respect to the capacitance. This correlates to step (1) in Fig. 1. Combining Eqs. (1) and (4) and taking $C_{mi}$ to the right-hand side, we have:

$$\dot{V}_i = \frac{V_{li} - V_i}{R_i C_i} + g_{gap}^{\text{gap}} \frac{N}{C_i} \sum_{j=1}^{N} n_{ij}^{gap} (V_j - V_i) + g_{syn}^{\text{syn}} \frac{N}{C_i} \sum_{j=1}^{N} n_{ij}^{syn} (E_j - V_i) + \frac{1}{C_i} I_{stim}$$

Now letting $g_i^{\text{leak}} = \frac{1}{R_i C_i}$, $g_i^{\text{gap}} = \frac{g_m^{gap} n_{ij}^{gap} (V_j - V_i)}{C_{mi}}$, $g_i^{\text{syn}} = \frac{g_m^{syn} n_{ij}^{syn} (E_j - V_i)}{C_{mi}}$ and $I_{ext}^{i} = \frac{1}{C_{mi}}$ the system dynamics can be written as:

$$\dot{V}_i = g_i^{\text{leak}} (V_i - V_i) + g_i^{\text{gap}} \sum_{j=1}^{N} n_{ij}^{gap} (V_j - V_i) + g_i^{\text{syn}} \sum_{j=1}^{N} n_{ij}^{syn} (E_j - V_i) + I_{ext}^{i}$$

This is the 9 dimensional ODE model of the TW circuit. The key circuit parameters are the gap conductances, $g_i^{\text{gap}}$, and we aim to characterize the ranges of these conductances that produce acceleration, reversal, and no response.

### 4 Reachability Analysis of Nonlinear TW Circuit

Reachability analysis for verifying properties for general nonlinear dynamical systems is a well-known hard problem. The verification framework introduced in Fig. 1 combines model and verification engineering to perform reachability analysis on the Wicks et al. [16] model, discovering crucial parameter ranges to produce all three behaviors of the TW circuit. Our framework can be applied to any nonlinear ODE model.
3. For each \( t, t' \) of the system is a function \( \xi : \mathbb{R}^n \times [0, \infty) \rightarrow \mathbb{R}^n \) such that for any initial point \( x_0 \in \mathbb{R}^n \) and at any time \( t > 0 \), \( \xi(x_0, t) \) satisfies the differential Eq. (8). A state \( x \) in \( \mathbb{R}^n \) is reachable from the initial set \( \Theta \subseteq \mathbb{R}^n \) within a time interval \([t_1, t_2]\) if there exists an initial state \( x_0 \in \Theta \) and a time \( t \in [t_1, t_2] \) such that \( x = \xi(x_0, t) \). The set of all reachable states in the interval \([t_1, t_2]\) is denoted by \( \text{Reach}(\Theta, [t_1, t_2]) \). If \( t_1 = 0 \), we write \( \text{Reach}(t_2) \) when set \( \Theta \) is clear from the context. If we can compute or approximate the reach set of such a model, then we can check for invariant or temporal properties of the model. Specifically, \textit{C. Elegans} TW properties such as accelerated forward movement or reversal of movement fall into these categories. Our core reachability algorithm [2,3,8] uses a simulation engine that gives sampled numerical simulations of (8).

**Definition 1.** A \((x_0, \tau, \epsilon, T)\)-simulation of (8) is a sequence of time-stamped sets \((R_0, t_0), (R_1, t_1), \ldots, (R_n, t_n)\) satisfying:

1. Each \( R_i \) is a compact set in \( \mathbb{R}^n \) with \( \text{dia}(R_i) \leq \epsilon \).
2. The last time \( t_n = T \) and for each \( i, 0 < t_i - t_{i-1} \leq \tau \), where the parameter \( \tau \) is called the sampling period.
3. For each \( t_i \), the trajectory from \( x_0 \) at \( t_i \) is in \( R_i \), i.e., \( \xi(x_0, t_i) \in R_i \), and for any \( t \in [t_{i-1}, t_i] \), the solution \( \xi(x_0, t) \in \text{hull}(R_{i-1}, R_i) \).

The algorithm for reachability analysis uses a key property of the model called a discrepancy function.

**Definition 2.** A uniformly continuous function \( \beta : \mathbb{R}^n \times \mathbb{R}^n \times [0, \infty) \rightarrow [0, \infty) \) is a discrepancy function of (8) if

1. for any pair of states \( x, x' \in \mathbb{R}^n \), and any time \( t > 0 \),
   \[ \| \xi(x, t) - \xi(x', t) \| \leq \beta(x, x', t), \] (9)
2. for any \( t \), as \( x \rightarrow x' \), \( \beta(\ldots, t) \rightarrow 0 \).

If a function \( \beta \) meets the two conditions for any pair of states \( x, x' \) in a compact set \( K \) then it is called a \textit{K-local discrepancy function}. Uniform continuity means that \( \forall \epsilon > 0, \forall x, x' \in K, \exists \delta \) such that for any time \( t \), \( \| x - x' \| < \delta \Rightarrow \beta(x, x', t) < \epsilon \). The verification results in [2–4,8] required the user to provide the discrepancy function \( \beta \) as an additional input for the model. A Lipschitz constant of the dynamic function \( f \) gives an exponentially growing \( \beta \), contraction metrics [11] can give tighter bounds for incrementally stable models, and sensitivity analysis gives tight bounds for linear systems [1], but none of these give an algorithm for computing \( \beta \) for general nonlinear models. Therefore, finding the discrepancy can be a barrier in the verification of large models like the TW circuit.
Here, we use Fan and Mitra’s recently developed approach that automatically computes local discrepancy along individual trajectories [5]. Using the simulations and discrepancy, the reachability algorithm for checking properties proceeds as follows: Let the \( U \) be the set of states that violate the invariant in question. First, a \( \delta \)-cover \( C \) of the initial set \( \Theta \) is computed; that is, the union of all the \( \delta \)-balls around the points in \( C \) contain \( \Theta \). This \( \delta \) is chosen to be large enough so that the cardinality of \( C \) is small. Then the algorithm iteratively and selectively refines \( C \) and computes more and more precise over-approximations of \( \text{Reach}(\Theta, T) \) as a union \( \cup_{x_0 \in C} \text{Reach}(B_\delta(x_0), T) \). Here, \( \text{Reach}(B_\delta(x_0), T) \) is computed by first generating a \( (x_0, \tau, \epsilon, T) \)-simulation and then bloating it by a factor that maximizes \( \beta(x, x', t) \) over \( x, x' \in B_\delta(s_0) \) and \( t \in [t_{i-1}, t_i] \). If \( \text{Reach}(B_\delta(x_0), T) \) is disjoint from \( U \) or is (partly) contained in \( U \), then the algorithm decides that \( B_\delta(x_0) \) satisfies and violates \( U \), respectively. Otherwise, a finer cover of \( B_\delta(x_0) \) is added to \( C \) and the iterative selective refinement continues. We refer to this in this paper as \( \delta \)-refinement. In [2], it is shown that this algorithm is sound and relatively complete for proving bounded time invariants.

4.2 Applying Local Discrepancy to TW Circuit

Fan and Mitra’s algorithm (see details in [5]) for automatically computing local discrepancy relies on the Lipschitz constant and the Jacobian of the dynamic function, along with simulations. The Lipschitz constant is used to construct a coarse, one-step over-approximation \( S \) of the reach set of the system along a simulation. Then the algorithm computes an upper bound on the maximum eigenvalue of the symmetric part of the Jacobian over \( S \), using a theorem from matrix perturbation theory. This gives a piecewise exponential \( \beta \), but the exponents are tight as they are obtained from the maximum eigenvalue of the linear approximation of the system in \( S \). This means that for models with convergent trajectories, the exponent of \( \beta \) over \( S \) will be negative, and the \( \text{Reach}(T) \) approximation will quickly become very accurate. In the rest of this section, we describe key steps involved in making this approach work with the TW circuit.

The model of the TW circuit from Sect. 3 can be written as \( \dot{V} = f(V) \), where \( V \in \mathbb{R}^9 \) has components \( V_i \) giving the membrane potential of neuron \( i \). The Jacobian of the system is the matrix of partial derivatives with the \( ij^{th} \) term given by:

\[
\frac{\partial f_i}{\partial V_j} = -g_i^{lak} - g_i^{gap} \sum_{j=1, j \neq i}^N n_i^{gap} - g_i^{syn} \sum_{j=1, j \neq i}^N n_i^{syn} \frac{n_j^{syn}}{v_{Range}} \\
= g_i^{gap} n_i^{gap} - g_i^{syn} n_i^{syn} \frac{v_{Range}}{k} \exp\left(\frac{k}{v_{Range}}(E_j - V_j - V_{Range})\right) / \left(1 + \exp\left(\frac{k}{v_{Range}}(E_j - V_j - V_{Range})\right)^2\right).
\]
Fig. 4. Model Checking Reversal Property of Control Group, with $\delta = 5 \times 10^{-5}$, varying $g_{AVM}^{gap}$.

Fig. 5. Model Checking Reversal Property of Control Group by refining $\delta$.

of models, instead of analyzing just a single member of that family. Here the parameters of interest are the quantities $p_{i}^{\text{leak}} = 1/g_{i}^{\text{leak}}, p_{i}^{\text{gap}} = 10/g_{i}^{\text{gap}}, p_{i}^{\text{syn}} = 1/g_{i}^{\text{syn}}$.

Consider, for example, $1/g_{i}^{\text{leak}}$ as a parameter:

$$
\begin{bmatrix}
\dot{V} \\
1/g_{i}^{\text{leak}}
\end{bmatrix} = 
\begin{bmatrix}
f(V)
\end{bmatrix}.
$$

In this case the Jacobian matrices for the system with parameters will be singular because of the all-zero rows that come from the parameter dynamics. The zero eigenvalues of these singular matrices are taken into account automatically by the algorithm for computing local discrepancy. In this paper we focus on $p_{i}^{\text{gap}}$, leaving the others for future work.
4.3 Checking Properties

Once the reach sets are computed, checking the acceleration, reversal, and no-response properties are conceptually straightforward. For instance, Eq. (6) gives a method to check reversal movement. Instead of computing the integral of \((V_{AVA} - V_{AVB})\), we use the following sufficient condition to check it:

\[
\phi_{rev} : \forall t \in T_{int}, \forall x \in \text{Reach}(\Theta, [t, t]), V_{AVA}(x) > V_{AVB}(x).
\]

Here, \(T_{int}\) is a specific time interval after the stimulation time, \(\Theta\) is the initial set with parameter ranges, and recall that \(\text{Reach}(\Theta, [t, t])\) is the set of states reached at time \(t\) from \(\Theta\). We implement this check by scanning the entire reach-tube and checking that its projection on \(V_{AVB}(x)\) is above that of \(V_{AVA}(x)\) over all intervals. If this check succeeds (as in Fig. 4(a)), we conclude that the range of parameter values produce the reversal movement. If the check fails, then the reversal movement is not provably satisfied (Fig. 5(a)) and in that case we \(\delta\)-refine the initial partition (Fig. 5(b)). In some cases, such as Fig. 4(b), \(\delta\)-refinement can not prove the property satisfied or unsatisfied. This often occurs when two tubes intersect within the interval of interest. In this case, the property is considered to be unknown.

Figure 6 helps paint a picture of how the \(\delta\)-refinement process works with two parameters. We consider 4 refinement steps: \(\delta = 7 \times 10^{-5}, \delta = 6 \times 10^{-5}, \delta = 5.5 \times 10^{-5}\), and \(\delta = 5 \times 10^{-5}\). For \(\delta = 7 \times 10^{-5}\), the property of interest is unknown at all points. With \(\delta = 6 \times 10^{-5}\) the property is considered unknown for all red areas in the figure, including red and blue areas. Blue areas show where \(\delta = 5.5 \times 10^{-5}\) are satisfied, and in the blue and yellow area both \(\delta = 6 \times 10^{-5}\) and \(\delta = 5.5 \times 10^{-5}\) have a satisfied property. The property is satisfied for the

![Figure 6](Image)

**Fig. 6.** Example of 2-D Parameter Refinement. Red Regions are Unknown for both \(\delta = 6 \times 10^{-5}\) and \(\delta = 5.5 \times 10^{-5}\), Red/Blue Regions are Unknown for \(\delta = 6 \times 10^{-5}\), but Satisfied for \(\delta = 5.5 \times 10^{-5}\), and Yellow/Blue Regions are Satisfied for both (Color figure online).
5 Experimental Results

In this section, we apply our verification framework to the [16] model to estimate parameter ranges that produce three different behaviors (reversal, acceleration, no response) in the control and four ablation groups. We vary the gap-junction conductance of the sensory neurons \(g_{i}^{gap}, i \in \{AVM, ALM, PLM\}\) and keep all other parameters constant, as per [16]. Additionally, in the case of the no response behavior, we must lower the gap-junction conductance of the other neurons by a factor of \(10^3\).

In Sect. 4, we explain that we use \(p_{i}^{gap}\) as our parameter in the state vector instead of \(g_{i}^{gap}\), where \(p_{i}^{gap} = 10/g_{i}^{gap}\). The parameter space we explore can be considered a bounding box, where each \(p_{i}^{gap}\) ranges over \([0, 1]\). As exploring the entire parameter space is computationally intensive, we intelligently select a subspace to cover that lets us estimate contiguous ranges of parameters for each behavior. In Table 1, we present these ranges in terms of \(g_{i}^{gap}\).

In the following subsections, we will present our results for parameter range estimation for all three behaviors of the control and ablation groups. This process requires three experiments per group.

5.1 1-D Parameter Space

Here we vary \(p_{AVM}^{gap}\) in all groups, except the AVM,ALM- group. By varying this parameter, we are able to produce reversal behavior in all four groups. We are also able to produce acceleration in all groups but PLM-. The PLM neuron drives acceleration in the TW circuit [15]. Hence, its absence in the PLM- group prevents acceleration from being produced, justifying the result.

For the AVM,ALM- group, we vary \(p_{PLM}^{gap}\) and produce acceleration and no response behaviors. As both AVM and ALM, responsible for reversal of movement, are ablated, reversal cannot be produced by this group.

5.2 2-D Parameter Space

In this set of experiments, we vary two parameters simultaneously. First we vary \(p_{AVM}^{gap}\) and \(p_{ALM}^{gap}\) for the control and PLM- groups. In both cases we produce reversal behavior. For the same reasons given in the previous subsection, we are unable to produce acceleration in the PLM- group and no response behavior in both these groups.

Next, we vary \(p_{AVM}^{gap}\) and \(p_{PLM}^{gap}\) for the ALM- and ALM,DVA- groups. We are able to produce both all three behaviors in both groups.

5.3 3-D Parameter Space

Since the ablation groups we have used in this paper all feature at least one of the primary sensory neurons \((ALM, AVM, \text{and} PLM)\) ablated, we can only show the 3-D case for the original animal.
Table 1. Parameter ranges for all experiments, including δ and runtime information. REV=Reversal, ACC=Acceleration, NR=No Response.

| Group Name | Property | Parameters | Ranges            | δ               | Runtime (sec) |
|------------|----------|------------|-------------------|-----------------|---------------|
| Control    | REV      | g_{AVM}    | [46.2, 1000]      | 1 \times 10^{-6} | 6324.4        |
|            | NR       | g_{AVM}    | -                 | -               | -             |
| PLM-       | REV      | g_{AVM}    | [467.3, 1000]     | 1 \times 10^{-5} | 718.08        |
|            | NR       | g_{AVM}    | -                 | -               | -             |
| ALM-       | REV      | g_{AVM}    | [467.3, 1000]     | 1 \times 10^{-5} | 718.08        |
|            | NR       | g_{AVM}    | -                 | -               | -             |
| ALM,DVA-   | REV      | g_{AVM}    | [250, 500]        | 1 \times 10^{-5} | 1085.74       |
|            | NR       | g_{AVM}    | -                 | -               | -             |
| ALM,AVM-   | REV      | g_{PPLM}   | [33.33, 1000]     | 5 \times 10^{-5} | 3619.19       |
|            | NR       | g_{PPLM}   | -                 | -               | -             |

For the 3-D case, in addition to p_{AVM}^{gap} and p_{ALM}^{gap}, we have the p_{PPLM}^{gap} conductance. Finally, we get a non-zero value for no response in the control, but Table 1 shows that this value is an order of magnitude smaller than acceleration and several orders smaller than reversal.

5.4 Runtime and Memory Complexity Analysis

The time and memory needed for the procedure depends upon the value of δ used and the size of the parameter space. Assume L_d to be the interval length in
the $d^{th}$ dimension. The total number of $\delta$-balls required to cover the parameter space completely is:

$$T_N = \Pi_{d=1}^{D} N_d$$

where $D$ is the number of parameters added to the state vector and $N_d = 2L_d/\delta$. If $L_d$ is the same in all dimensions, $T_N = N_d^D$. We can analyze both runtime and memory complexity based on $T_N$. If we consider the time and memory required for verifying each $\delta$-ball to be $O(1)$, then the time and memory complexity will both be $O(T_N) = O(N_d^D)$. Note that the complexity also depends on the value of the $\delta$-refinement loop counter. Since we can safely assume that the loop will iterate only a constant number of times, this is not an issue.

Figure 7 illustrates how runtime relates to $T_N$ in one (a) and multiple (b) dimensions. The graph from (a) is the same as the 1D line in (b), but for a larger range of $T_N$. This increased range more clearly illustrates the linear relationship of runtime to $T_N$ when $D = 1$. Part (b) shows the rates for $D = 1$, $D = 2$ and $D = 3$ over a much smaller range of $T_N$ but helps to demonstrate the effect of dimensionality on time complexity. Since runtime grows at a trinomial rate when $d = 3$, we use the largest $\delta$ values (smallest $T_N$) that correctly cover the parameter space. This is what makes the $\delta$-refinement process imperative; it allows us to correctly verify a property while avoiding runtime blow-up.

6 Conclusions

In this paper, we performed reachability analysis with discrepancy to automatically determine parameter ranges for three fundamental reactions by *C. Ele-gans* to tap-withdrawal stimulation: reversal of movement, acceleration, and no response. We followed the lead of the *in vivo* experimental results of [15] to obtain parameter-estimation results for gap-junction conductances for a number of neural-ablation groups. The ranges we present are a significant expansion of the results in [16], where all of the parameters are constant and only the predominant behavior is produced. To the best of our knowledge, these results represent
the first formal verification of a biologically realistic (nonlinear ODE) model of a neural circuit in a multicellular organism.

The verification framework we develop is model-agnostic, and allows the techniques of [5] to be applied to general nonlinear ODE models. This is only possible through the careful model and verification engineering developed in this paper.

As alluded in Sect. 5, our results cannot necessarily cover the entire parameter space due to the $T_N$ required, but still enough to verify the properties in question. A potential solution to the incomplete coverage is parallelizing our approach. Luckily, calculating reach-tubes is a data-parallel computation and considered “trivially parallel” for the GPGPU (General-Purpose computing on a Graphics Processing Unit) architecture. This should allow us to run verification experiments in a fraction of the current required time, giving us a potential expansion of coverage.

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