Computational Modeling of Neural Circuit-Like
Coupled Belousov-Zhabotinsky Reactions

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Paul Miller, PhD

By: Alexander Mitchell

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Abstract

Computational Modeling of Neural Circuit-Like Coupled Belousov-Zhabotinsky Reactions

A thesis presented to the Interdepartmental Program in Neuroscience.

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Brandeis University
Waltham, Massachusetts

By Alexander Mitchell

We seek to adapt simplified circuit models of neural central pattern generators (CPGs) for use in a system of chemical oscillators using the Belousov-Zhabotinsky reaction (BZ). The BZ reaction lends itself well to the analysis of these complex oscillators, as it shares a number of properties with model neurons. BZ produces periodic all-or-nothing spike-like behavior, this spike behavior can be induced or prevented by perturbations to the reaction mixture, and critically reactors can be chemically coupled to one another. Microreactors of BZ mixture can be coupled with either hollow channels for the diffusion of aqueous solution (activator coupling) or with PDMS-filled channels for bromine (inhibitor coupling). Using existing computational models for single BZ reactors, we constructed and tested various simple arrangements of wells and chemical couplings that resembled different CPGs from the crustacean stomatogastric ganglion, lamprey spinal cord movement, and others. Using MatLab, we simulated the dynamics of oscillations in these arrangements under different conditions that could be used to disrupt the circuit, such as the presence chemical gradients across channels, light exposure, and the physical dimensions and arrangement of the circuits themselves. We then used these conditions to control the frequency and phase relationships of BZ oscillators. We successfully constructed models for a number of potential circuits and characterized their behavior. We hope that this work will encourage future exploration into applying neuroscience models to further the growing field of BZ research.
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Chapter 1

Introduction

For decades, neuroscientists have developed extremely powerful and precise mathematical models to describe rhythmic neural activity. These models provide insights into decision-making [1], respiration [2], visual perception [3], and motor activity [4]. These models, which have been constructed for a wide range of organisms, teach us the principles by which biological systems organize complicated actions from repeating autonomous units.

We seek to create novel synthetic circuits inspired by the same principles that govern neural circuits, but built in a totally different medium. Our particular system is not a biological organism, but a chemical reaction, the Belousov-Zhabotinsky oscillator (BZ). BZ is a mixture of chemicals, including a metal catalyst whose oxidation state oscillates, causing distinctive changes in coloration of the reaction mixture [5]. These changes occur rapidly, resulting in sharply visible spikes in oxidation state more akin to the nonlinear voltage spikes that characterize neuroscience than physical harmonic oscillators. Our lab has previously shown that reactors of BZ can influence each other through the diffusion of different reagents through coupling channels [6]. The diffusion of these chemicals allows BZ reactors to influence one another, exchanging information that changes the phase and frequency of individual units’ oscillations to create complex syncopated networks [7].

1.1 Neurons and BZ

Neuroscientists have discovered many simple circuits used outside of the brain for controlling various biological functions. Rhythmic movements such as swimming, mastication, walking, and others require predictable behavior from stable neural circuits, and over the years neuroscientists have done an excellent job characterizing many of these systems and forming powerful explanatory models [8]. These models describe the behavior of neural circuits, but the strongest models incorporate phenomena such as channel turnover and neuromodulation that are hard to account for in our BZ system [9].

An important caveat: we are not hoping to inform the study of neural systems with
our models, but rather take the ideas from neuroscience and apply them to our physical system. A neural circuit is much more complicated than a BZ circuit. We can never hope to fully replicate the elegant complexity of a biological system in such a constrained chemical environment. Our goal is not to outperform biological systems, but to engineer reaction-diffusion networks that will provide fundamental insight to the design constraints all oscillatory networks face.

We also see a bright future in the potential engineering applications of BZ. A small but growing literature describes mechanically oscillating polymer gels controlled by the BZ reaction [10–12]. The expansion and contraction of these gels somewhat resembles the activity of muscle cells, which change shape similarly to cause locomotion. We hope to one day combine mechanically oscillating gels with stable neuro-inspired circuits of BZ oscillators in order to create a new class of chemically controlled soft robots.

1.2 The Stomatogastric Ganglion

The crustacean stomatogastric ganglion (STG) provides some of the most robust and well-studied circuit models in all of computational neuroscience. The STG drives mastication, a process in which it churns the contents of its stomach to aid in digestion [8]. The circuit involves dozens of neurons [14], and despite great physiological variability between animals, certain cell types are highly conserved [15]. The STG provides us with two interesting circuits. The first, a four-cell-type model, relies on almost all-to-all inhibitory synapses [16]. The other model, a more recent addition to the literature, uses five cell types to create an oscillator that can switch between rhythms. Gutierrez, et al. proposed a model in which a hub neuron, the inferior cardiac (IC), is electrically coupled to the lateral pyloric neuron, which oscillates antiphase to the pyloric dilator, a cell that itself inhibits IC [17]. The IC is also electrically coupled to the lateral gastric (LG) neuron, which oscillates antiphase to the interneuron Int1, another inhibitor of IC. Together, Int1 and LG oscillate much faster than the pyloric circuit. These five cell-types come together in a model proposed by (Gutierrez et al. 2013) [17], in which the IC receives competing inputs from the pyloric circuit and the Int1-LG circuit. This model has a number of advantages for our BZ system. It is computationally viable and demonstrates robustness, and is therefore worth investigating. Many other strong candidates exist, and they are worth mentioning.

1.3 Vertebrate and Invertebrate Locomotion

We have already had success in duplicating one neural circuit: the rhythms that govern the swimming motions of the lamprey. Lampreys, dogfish, and other aquatic mammals
Figure 1.1: Sample image from two experiments with the lamprey-like CPG geometry. The wells on the right are contralaterally activator-coupled, while the one on the left has contralateral inhibitory coupling. Lampreys have two large columns of neurons in the spinal cord, whose interactions allow for this undulating S-like behavior. Within each column, excitatory inputs descend from the brain to control centers made up of diverse neuron types, including motor neurons, ipsilaterally excitatory coupled interneurons, contralaterally coupled inhibitory neurons, and more [22]. Each segment along the spinal cord connects to multiple motor units, and has...
intrinsic bursting and rhythm generation capability. Each segment serves as a local control center that receives descending inputs from the brain and other segments, ensuring that the passage of the signal from the head of the animal to its tail occurs without interruption [23]. Critically, each of these local control centers fires with a constant phase difference, allowing for a uniform contortion in the body [24]. In addition, the neurons provide cross-inhibition to the motor unit opposite them in the spinal cord. Neurons on one side of the column fire in series, beginning at the top and working their way down, before the other column begins to fire in a similar pattern [20,25]. At this point, we must emphasize again that these models do not fully capture the true complexity of lamprey locomotion. These fish can change direction and speed quickly, adjust for collisions, initiate reflex responses, and perform other complex behaviors our simple models do not account for. Nevertheless, in terms of understanding the simple, rhythmic motion of the lamprey, this model will suffice.

Vertebrates do not have a monopoly on interesting locomotive circuits. Invertebrates like shrimp and crayfish move through the water using a series of fine-tuned swimmerets [8,26], whose neural control does not rely on the same schematic as the lamprey, as shown in Figure 1.2. Each swimmeret pushes the crayfish forwards with a power stroke, and then returns to resting position once the shrimp is moving slower via a return stroke, much like oars on a rowboat [13]. This motion is more efficient when the strokes are done in sequence, specifically when they are phase locked at $\frac{\pi}{4}$, as this minimizes drag. Nearly all long-tailed crustaceans use this mechanism, and hydrodynamicists have shown this pattern of locomotion is the most energy-efficient possible [13].

Of course, many more viable circuit designs exist in the animal kingdom. Not all of them are as well-documented as these three, but we hope to continue investigations into
others at a later date. Potential candidates include embryonic xenopus [27, 28] and zebrafish locomotion [29].

1.4 The Belousov-Zhabotinsky Reaction

Non-biological chemical oscillators have emerged as a growing field in physical chemistry. Beginning with work by Belousov in the 1950s [30], study of the reaction really took off in the west beginning in the 1970s and continuing to the present day. Researchers have not only fully characterized the mechanism of BZ but also fully described the reaction’s kinetics and provided many powerful mathematical models to predict its behavior [5, 31]. The hallmark of the BZ reaction is the oscillating oxidative state of its metal catalysts, which corresponds to distinctive changes in the color of the reaction Figure 1.3. Through a series of complicated competing reactions, the reaction avoids equilibrium and the catalyst’s oxidation state changes back and forth between oxidized and reduced states.

![Image of the Belousov-Zhabotinsky Reaction](image)

Figure 1.3: The Belousov-Zhabotinsky Reaction. The ferroin catalyst is periodically oxidized, turning the mixture blue.

Rigorous study of the reaction began with work done by Field, Kraft, and Noyes [5].
They viewed the reaction as a cycle of three steps, shown in Figure 1.4. As the catalyst is reduced, production of hypobromous acid from the inhibitor bromine increases. The catalyst then becomes oxidized in a rapid autocatalytic reaction. While in its oxidized state, the catalyst accelerates production of the inhibitor bromine, which in turn begins to produce hypobromous acid, and the cycle begins anew.

Figure 1.4: An overview of the BZ reaction. The oxidation of the catalyst produces the recognizable changes in color.

Our particular recipe of the reaction uses two metal catalysts: ferroin and Tris(bipyridine) Ruthenium (II) Chloride (Rubipy). Because of the change in the coloration of the oxidized catalyst, we can easily determine experimentally when a BZ reactor spikes. Rubipy, unlike the ferroin or the classical cerium catalyst, is photosensitive. When the catalyst is exposed to direct green light (λ = 452 nm), the catalyst oxidizes and promotes the formation of the inhibitor bromine [32]. Our lab uses a setup containing a Programmable Illuminating Microscope (PIM) to project light onto precise points in the apparatus. The PIM is able to focus light onto individual wells only a few microns in diameter and change intensity over time, giving experimentalists the ability to control the oscillations of wells [33, 34]. The strength of this photoinhibition is directly proportional to the intensity of light used, so a stronger light exposure will do a better job of slowing or suppressing the reaction. Through these advances, we have developed a system of well-characterized chemical oscillators that can be directly controlled by experimentalists.
1.5 Coupled BZ Reactors

Microfabricated wells provide a well-characterized physical system for us to use to create BZ circuits. Although our project seeks to merely provide a computational model of a BZ network, we ought to stay mindful of design constraints for when our model is eventually realized. Tompkins et al. described the manufacturing process of BZ coupled well systems [7]. The process produces thin silicon chips with wells and channels etched into them. Chips can also be made partially from Polydimethyl Siloxane (PDMS). Etched silicon channels allow aqueous BZ mixture to flow between wells. PDMS channels allow only the diffusion of nonpolar species between wells, most notably bromine. The coupling strength of the network is dependent on the physical dimensions of the channels and wells. All coupling depends on a reaction-diffusion system, so the depth, height, and width of the channel determine the strength of coupling.

Therefore, in creating a circuit with BZ, we must be mindful of a few design constraints. Firstly, we must minimize the number of one-way connections. Because all coupling in BZ wells occurs through diffusion, creating a system where chemical species can only flow in one direction would be extremely difficult. One-way connections exist in the literature, but they make use of pulse coupling [35] or photochemical coupling [36]. Strictly one-way diffusive coupling should be impossible. While our models assume inhibitory diffusion through inhibitory channels, activator coupling is mediated by reaction-diffusion waves [37]. For models that require one-way connections, such as some of the crustacean locomotion circuits, we hope to exploit this fact to create a one-way connection by changing the widths of the mouths of the channels, but that will be left for future projects.

In designing these models, we rely heavily on the existing theory of chemical oscillators in literature. The complete Field-Körös-Noyes mechanism (FKN) is overly complicated for our purposes [5], and the conventional simple Oregonator model does not allow for an inhibitory variable [38], so we instead turn to the more recent Vanag-Epstein model [31]. This model simplifies the FKN into a four-dimensional system, with bromine as a communicator of inhibition [6, 37]. We also drew heavily from previous work by the Fraden Lab. Building off work by Tompkins et al., Wang et al. performed experiments on Euclidean geometries of inhibitor-coupled BZ microdroplets [7, 39]. Li et al. began work on activator-coupled systems with small one-dimensional arrays of BZ droplets coupled with fluorinated oil [6], but this system limited the diffusion of many excitatory reagents between droplets and could not be easily translated to a fixed-well geometry. We hope to expand beyond simple geometric patterns into neural-circuit inspired geometries, complete with the integration of both inhibitor and activator channels.

In conclusion, we have outlined a number of different neural circuits worth investiga-
tion as potential frameworks for new BZ-driven oscillator networks. These different networks all represent evolutionarily refined schematics that have been shown to be highly effective in biological systems. We will also test our models to ensure they are stable across a wide range of temperatures and small perturbations, in homage to the homeostatic mechanisms of neural systems. We will now design theoretical circuits informed by these models that can be used in a BZ system.

1.6 Homeostasis in a BZ System

One of our greatest ongoing challenges is attempting to emulate the robustness of biological systems. Neurons and neural networks display homeostatic behavior, such that networks can remain stable despite changes in temperature [16], pH [40], and synaptic modulation [41, 42]. Several mechanisms govern neural circuit homeostasis [43]. One such mechanism is synaptic scaling [44], the process by which relative synaptic weights are changed over time in order to strengthen connections between neurons that fire onto one another frequently. Modulatory molecules like proctolin and dopamine also help to maintain consistent firing rhythms [24, 45]. Neurons also make use of activity-dependent conductances [46, 47], in which the microscopic properties of ion channels change in response to activity. Active neurons can even phosphorylate their ion channels, which change synaptic charge and membrane conductance [48]. Neural circuits have no shortage of tools to ensure homeostatic output.

Unfortunately, many of these mechanisms of homeostasis depend on complicated biological processes such as protein expression levels or channel phosphorylation and offer few options for our simplified system. No work has yet been published on developing a form of long-term memory in BZ systems. Cellular-level learning models necessitate changes at the level of the individual neuron or synapse via Hebbian plasticity, but we do not yet have the experimental means for such a system, so long-term learning experiments will need to wait for further advances in chemistry.

1.7 Potential Concerns

Neural and chemical systems have many differences. Neurons are not simple chemical wells; they possess diverse cellular properties we cannot hope to replicate. Neuromodulators can control intrinsic excitability, connection strengths, and other properties. Neurons can possess complex firing properties like bursting. Channel properties can provide post-inhibitory rebound and other activity-dependent processes [46]. Transport through neurons follows active cytosolic mixing, a much more active process than Fick’s diffusion. Perhaps
most critically, synapses do not function like diffusive channels. Synapses are unidirectional, and their effects on the postsynaptic cell are not linearly proportional to the membrane voltage in the presynaptic cell. We have no expectation that the chemistry of the BZ system could ever match the level of complexity and diversity displayed in animal nervous systems.

The above are all legitimate concerns, but they do not impact the motivation for our model. We will not even attempt to recapitulate every faculty of neuron behavior, but rather we strive to provide the computational framework to build physical models that emulate some aspects of these circuits. Regarding the dissimilarities between a neuron and a BZ well, we do not see an obvious immediate effect of particular firing properties on circuit dynamics. Computational neuroscientists use point models in their own work, and they still provide an accurate approximation of circuit behavior. Moreover, many models assume timescales that do not allow for changes in expression level, channel phosphorylation, and the other beautiful but mathematically intractable mechanisms of cell biology. Aggressive simplifying assumptions of neuron properties are nothing new, and any reasonable model would need to make at least a few.

Regarding concerns over bidirectional channels, we make two considerations. First, most of the models we utilized contained mutual inhibition between cells. That is, if A has an inhibitory projection onto B, then B has an inhibitory projection onto A. Second, the impact of inhibition on the dynamics of BZ oscillations is essentially unidirectional. When one cell inhibits the other, the chemicals flow one way. This inhibits the receiving cell, but has little effect on the first cell, whose oscillatory period is great enough that the diffusion of inhibitor may not diminish its own frequency. In other words, while we have bidirectional coupling between two wells, a setup with two independent one-way connections would likely produce similar behavior in the wells. We do not model any explicit excitatory synapses in this paper, but instead treat electrical synapses as a form of activator coupling. Electrical synapses rely on diffusion across concentration gradients and are bidirectional, so in this respect our channels match the existing models quite well.

Lastly, BZ differs significantly from neural networks in its timescale. Neurons frequently produce action potentials at frequencies of 10-100 Hz, while coupled BZ wells have a slower frequency [14,49]. Traditionally, BZ oscillators have periods measured in seconds or even minutes [50], although changes in chemistry can result in frequencies as high as 10 Hz, largely by controlling temperature and reactant concentrations [51]. In reaction-diffusion coupled networks like ours, frequencies of that magnitude could jeopardize stability, particularly given the sensitivity of the lamprey system to changes in phase lag. However, based on our own experiments, in which diffusion across a well takes place on the order of hundreds of milliseconds, increasing the firing rate of these wells by a couple orders of magnitude
should not harm the stability of the system. Indeed, our preliminary data suggest that for much higher concentrations of sulfuric acid, frequencies reach up to ten times faster than the traditional mixture, with no appreciable loss in network stability. While we understand that our findings are not directly relevant to the neural circuits we investigated, our work has been inspired by their behavior. This work takes the study of neural circuits in a new direction, and applies the many remarkable advances of the field to a brand new system.
Chapter 2

Methods and Theory

2.1 Chemistry

The original mechanism for the Belousov-Zhabotinsky reaction was worked out by Field, Körös, and Noyes in 1972 [5]. The full mechanism is reproduced below. Note that our form of the reaction uses a $[\text{Ru(bpy)}_3]^2^+$ catalyst (Rubipy), which can be either reduced or oxidized. MA is an abbreviation for Malonic acid, which has the formula $\text{CH}_2(\text{COOH})_2$

\[
\text{HOBr} + \text{Br}^- + \text{H}^+ \iff \text{Br}_2 + \text{H}_2\text{O}
\]

\[
\text{HBrO}_2 + \text{Br}^- + \text{H}^+ \longrightarrow 2\text{HOBr}
\]

\[
\text{BrO}_2^- + \text{Br}^- + 2\text{H}^+ \longrightarrow \text{HBrO}_2 + \text{HOBr}
\]

\[
2\text{HBrO}_2 \longrightarrow \text{BrO}_2^- + \text{HOBr} + \text{H}^+
\]

\[
\text{BrO}_2^- + \text{HBrO}_2 + \text{H}^+ \iff 2\text{BrO}_2^\bullet + \text{H}_2\text{O}
\]

\[
[\text{Ru(bpy)}_3]^2^+ + \text{BrO}_2^\bullet + \text{H}^+ \longrightarrow [\text{Ru(bpy)}_3]^4^+ + \text{HBrO}_2
\]

\[
[\text{Ru(bpy)}_3]^2^+ + \text{BrO}_2^\bullet + \text{H}_2\text{O} \longrightarrow [\text{Ru(bpy)}_3]^4^+ + \text{BrO}_2^- + 2\text{H}^+
\]

\[
\text{Br}_2 + \text{MA} \longrightarrow \text{BrMA} + \text{Br}^- + \text{H}^+
\]

\[
[6\text{Ru(bpy)}_3]^4^+ + \text{MA} + 2\text{H}_2\text{O} \longrightarrow 6[\text{Ru(bpy)}_3]^2^+ + \text{HCOOH} + \text{Br}^- + \text{H}^+
\]

\[
4[\text{Ru(bpy)}_3]^2^+ + \text{BrMA} + 2\text{H}_2\text{O} \longrightarrow \text{Br}^- + 4[\text{Ru(bpy)}_3]^4^+ + \text{HCOOH} + 2\text{CO}_2 + 5\text{H}^+
\]

In addition, the Rubipy catalyst is photosensitive, and has its own associated reactions [31, 32, 52].

\[
\text{Ru(bpy)}_3^{2^+} + h\nu \longrightarrow \text{Ru(bpy)}_3^{2^+\bullet}
\]

\[
\text{BrMA} + \text{Ru(bpy)}_3^{2^+\bullet} \longrightarrow \text{Ru(bpy)}_3^{3^+} + \text{Br}^-
\]
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<th>Chemical Constant</th>
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<td>$C_h$</td>
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<td>$c_0$</td>
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<td>$b_C$</td>
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<td>$b$</td>
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<td>$c_{max}$</td>
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<td>$k(I)$</td>
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Table 2.1: Reaction Parameters

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<th>Symbol</th>
<th>Chemical</th>
<th>Initial Concentration (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>Bromous Acid</td>
<td>1.6</td>
</tr>
<tr>
<td>Y</td>
<td>Bromide</td>
<td>0.2</td>
</tr>
<tr>
<td>Z</td>
<td>Oxidized Catalyst</td>
<td>0.01</td>
</tr>
<tr>
<td>U</td>
<td>Bromine</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Table 2.2: Dynamic Variable Definitions

For most reactions, the initial conditions and reaction constants were set to those above. During some simulations, we modified $k(I)$, which stayed on the order of $10^{-4}$ and the initial concentrations of $y$ and $u$, which varied from their current values to as much as ten times higher. We discovered that when simulations with identical concentrations of all reagents in both wells, the wells would perfectly synchronize, reaching what we assumed to be an unstable fixed point. To correct for this, in reactions without asymmetric initial conditions we would give one well a slight advantage by increasing the initial concentrations of all reagents by 20%. This did not noticeably affect the frequency of any of the wells.

2.2 Diffusion and Coupling

The chemical composition at a given time point of each well depends on two factors: the chemical reaction occurring within the well at that time and the diffusion of chemicals to and from connected wells. Wang et al. 2016 used similar models, and their intuition informs this section [39]. The first part of the problem can be handled simply enough. Although diffusion through the BZ medium is not instantaneous, we can safely assume that diffusion throughout the well occurs much faster than through a channel and treat wells as homogenous solutions. These assumptions let us treat these wells as points in a graph, as
Wang et al. did. Thus for each well $i$ we have

$$\frac{d\vec{C}_i}{dt} = R(\vec{C}_i) + D_{ihb} \nabla \vec{C} + D_{act} \nabla \vec{C}$$

Where $R$ represents the intrawell chemical reactions from $S_{ihb}$ the set of all wells with inhibitory coupling to well $i$, $S_{act}$ the set of all wells with excitatory coupling to well $i$. We treat the concentrations of a given well as a vector that $R$ can act on:

$$\vec{C}_i = \begin{bmatrix} x_i \\ y_i \\ z_i \\ u_i \end{bmatrix}$$

We model $R$ using the equations from Vanag and Epstein [31]:

$$\begin{align*}
\frac{\partial x}{\partial t} &= -k_1 xy + k_2 y - 2k_3 x^2 + k_4 x (c_0 - z)/(c_0 - z + c_{min}) \\
\frac{\partial y}{\partial t} &= -3k_1 xy - 2k_2 y - k_3 x^2 + k_4 u + k_5 z + k(I)(c_0 - z)/(b_C/b + 1) \\
\frac{\partial z}{\partial t} &= 2 \ast k_4 x (x_0 - z)/(c_0 - z + c_{min}) - k_5 z - k_4 0z + k(I)(c_0 - z)/(b_C/b + 1) \\
\frac{\partial u}{\partial t} &= 2k_1 xy + k_2 y + k_3 x^2 - k_4 u
\end{align*}$$

$D$ is diffusion through the channel according to Fink’s Law. The value for $D$ actually depends on the physical dimensions of the channel, which is equal to the product of the diffusion constant $D$, times the area, $A$, times its density, $\phi$ (which we take as a constant) divided by the product of the volume, $V$, of the channel times its length, $d$. Recall that activator coupling allows all four reagents to pass between wells, while inhibitor coupling allows only bromine to pass. Unless stated otherwise, we set the total diffusion constants to be $D_{act} = 5.0 \ast 10^{-2}$ and $D_{ihb} = 4.0 \ast 10^{-3}$. When each well spikes, on the order of 1-10% of its concentration flows into the next well.

$$D = \frac{D_i A \phi}{V d}$$

To calculate the effect of diffusion, we multiplied a linear vector containing all concentrations, $\vec{C}$ with the Kronecker product of the laplacian matrix and a vector representing the reagents diffusing through that medium, $v$. $v_{act}$ is the identity matrix, as all four dynamic reagents can flow through activator-coupling channels. $v_{ihb}$ has only one nonzero entry, as only the
inhibitor $u$ can flow through inhibitor channels.

$$v_{\text{act}} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \quad v_{\text{ihb}} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}$$

We first create two graphs of all connections between wells representing either activator or inhibitor channels. This graph can be visualized as a laplacian matrix $L$ representing the flow of chemicals at each time step. The graph below represents the linear activator-coupled three-well geometry.

$$< (C_{1,\text{act}}, C_{2,\text{act}}), (C_{2,\text{act}}, C_{3,\text{act}}) > \iff \begin{bmatrix} -1 & 1 & 0 \\ 1 & -2 & 1 \\ 0 & 1 & -1 \end{bmatrix}$$

Thus we have $\tilde{D}_{\text{ihb}} = v_{\text{ihb}} \otimes L_{\text{ihb}}$ and $\tilde{D}_{\text{act}} = v_{\text{act}} \otimes L_{\text{act}}$

Simulations were performed using the ode15s solver on MatLab [53]. We used a time step of 10µs, which is about 1/10,000 of the period of a BZ oscillator. We elected to exclude noise from our simulations, as it could make comparing across conditions more difficult. However, starting simulations with all wells at identical concentrations can lead to unstable fixed points. For example, two inhibitor coupled wells that start at exactly identical concentrations with no noise will produce perfect synchronization due to an unstable fixed point at a phase difference of 0. To correct for these effects while still maintaining reproducibility, we introduced a small bias in the initial concentrations of the wells. To do this, we increased the starting concentrations of one or more of the wells by 5 or 10%. These correspond to a small change in the phase, $\phi$, of the oscillator. As a result of this small change in phase, oscillators would not become perfectly entrained, and we avoided unstable fixed points.
Chapter 3

Results

We set out to build models of diffusion-coupled Vanag-Epstein BZ oscillators in geometries that resembled those of neural circuits. At each step of the way, we tried to characterize concrete steps that could be taken in the design process to control the properties of each circuit. We began with a single well, as shown in Figure 3.1. Eventually, we built up to more complicated models, which will be shown later in the paper. These models are presented in simplified geometric schemes throughout the paper, such as in 3.4. For these figures, black arrows indicate excitatory coupling, and red arrows indicate inhibitory coupling.

Figure 3.1: Oscillations of a single well. The yellow line follows the oxidized catalyst, z. We focus on this variable for determining properties of "spikes", as it corresponds to the most significant and distinct state the oscillator enters. The spikes in z represent the blue color shown in Figure ??.

From an experimental perspective, we have control over only a small number of pa-
rameters in the system. Firstly, because BZ is photosensitive, by shining a small, focused light on a particular cell, we can slow the reaction, or stop it altogether. This results from the photoinhibition of the Rubipy catalyst, as discussed earlier. Figure 3.2 shows the termination of oscillations in one well due to light inhibition. Secondly, we can change the initial concentrations of species in the reaction. Thirdly and finally, we can change the dimensions of the channels connecting wells. As stated previously, coupled BZ wells can affect each other’s chemical behavior, and controlling the width and height of channels connecting them will affect how significant changes in one cell will affect the others.

![Figure 3.2: Photoinactivation of a BZ oscillator. k(I) increases from 0 to 10^{-3} at the time indicated by the red arrow. The light oxidizes Rubipy, which quickly causes the inhibitor species bromine (u) to build up quickly.](image)

### 3.1 Single Well Simulations

We began by constructing a simple one-cell model of the particular BZ mechanism of interest. In this one-cell model, we are concerned first and foremost with the frequency of the well, and how this value responds to changes in experimentally pliable parameters. The frequency or firing rate of a well is defined as the number of oscillations completed per second, where we define an oscillation as the concentration of z reaching a peak. We ran simulations in which the strength of the light constant was adjusted, simulating changes in the intensity of the laser shown onto the wells. The effect of k(I) on the firing rate is shown in Figure 3.3. We also changed the initial conditions to reflect a wide range of physical possibilities. We found that the effect of the initial concentrations on the frequency of a single well was negligible at best. Firing rate changed by less than 1% across all tested initial conditions. Increasing the strength of the photoinhibition constant was found to have a more meaningful
impact on the frequency. When the photoinhibitory constant reached a value of $2.9 \times 10^{-4}$, we found that the oscillations disappeared entirely. Our findings on a single well show that initial conditions should have no noticeable impact on the frequency on the well and while changing the light can have a small impact on frequency, strong light easily switch off the oscillator.

Figure 3.3: Photoinactivation of a BZ oscillator. $k(l)$ increases from 0 to $10^{-3}$ at the time indicated by the red arrow. The light oxidizes Rubipy, which quickly causes the inhibitor species bromine ($y$) to build up quickly.

3.2 Two-Well Simulations

With the addition of a second well, we introduce a number of new variables into the equation. The two wells have a single inhibitory channel between them, as shown in Figure 3.4, whose dimensions we can manipulate to change the coupling strength. We can vary the strength of this coupling quite easily in simulation, but changing this value in an experiment would require making a whole new chip, which would be significantly more difficult and expensive than simply changing the chemicals or the light levels. We also introduce the idea of phase difference, $\phi$, of the two oscillators. The phase difference is the fraction of the first oscillator’s cycle the second oscillator activates. You can see an illustration of this in Figure 3.5.

Figure 3.4: Two Inhibitor-Coupled Wells
Figure 3.5: Two coupled BZ oscillators. We define the phase $\phi$ of the second oscillator relative to the first as shown in the figure.

### 3.2.1 Phase Relationships

Ideally, through inhibitory coupling, these two wells will be perfectly antiphase with the same frequency. In order to determine the phase relationship, we adjusted initial conditions and coupling strengths. Because two wells that begin with perfectly equal initial conditions will oscillate in phase regardless of initial conditions, we ran the experiment varying the initial conditions of one of the two wells by a very small amount.

In testing the initial conditions, we quickly discovered that the relative initial concentrations of $x$ and $z$ had virtually no impact on the phase relation between the two oscillators. Initial concentrations of $u$ and $y$, however, had a more meaningful impact. Curiously, in both the cases of varying the ratios of initial $y$ and initial $u$ between the wells, changes in phase seem to be very small up to a certain range of $y$ and $u$ concentrations, after which phase lag increased significantly up to and above $\frac{\pi}{2}$.

A number of robust patterns of interest from neuroscience feature phase differences of $\pi$ between oscillators [13, 54]. From our data, it looked possible that if we varied both $y$ and $u$ significantly enough, we could find a set of initial conditions that could give us $\pi$ phase-lag between the oscillators. When we increased the initial concentrations to a range all the way to the order of 10:1 at a number of different channel dimensions, although the latter had little effect. Changes in initial concentrations had very little effect on the firing rate (Figure 3.7).
Figure 3.6: Phase as a function of initial concentration in the two-well model. After crossing some threshold in starting concentrations, relative phase of the oscillators jumps up to the region of $\pi$ and increases slowly thereafter.

### 3.2.2 Effect of Light Inhibition

Phase is constant for lower-light conditions, but as light inhibition increased we noticed strange entrainment patterns. Because the two wells were coupled, accumulating bromine could diffuse out of the inhibited well. As a result, that well was able to fire even under light conditions that would have stopped oscillations in an uncoupled system. We began our simulation at a coupling constant of $\frac{mM}{s}$ found that wells approach a constant phase of $\frac{\pi}{10}$ and stay there at lower light levels. As light inhibition increases, eventually one of the wells slows and the phase relationship becomes less stable. As shown in Figure 3.8, increasing the strength of the light causes the inhibited oscillator to sometimes suffer a significant lag. This phase lag represents a 3:2 ratio of frequencies between the two oscillators. The two wells would fire together, then the uninhibited well, then the inhibited well, then both wells together. This eventually led to 2:1 entrainment, and then extinction of both oscillators. Other entrainment patterns, such as 5:4 were noticed at different values of the diffusion constant, as shown in Figure 3.9.

### 3.2.3 Limit Cycles

The limit cycles of the two wells show little change. Because of the mutual dependencies in the equations, the X/Z limit cycle is considered the canonical limit cycle to publish [5,31,55]. As seen in Figure 3.10, the limit cycles are the same for both oscillators,
Figure 3.7: Period as a function of initial concentrations. The period of the oscillator was almost completely unaffected by changes in Y and U, regardless of the entrainment properties.

3.3 Three-Well Simulations

We can construct a number of three-well models. One fascinating model is the all-to-all inhibitor coupled three-well model, shown in figure 3.12. The so called triangle model has been done in the Fraden lab before, both using droplets [39] and wells [7]. This model has already been investigated quite thoroughly, so we turned to its cousin, the linear three-well model. We arrange three wells linearly with only activator coupling, as shown in figure 3.13. The well at the right then received photoinhibition. This slow well competes with the fast well for entrainment with the center hub well. This system is similar to the five-cell model described by Gutierrez, in which the STG hub neuron receives competing inputs from two different rhythmic circuits. As the light level on the outer cell increases, phase relationships between the three oscillators become less coherent. However, at some threshold, the slow well captures the hub well and the two become almost perfectly entrained. The fast well, meanwhile, oscillates antiphase to these two wells. Similar to what we saw in the two-well model, the slow well continues to oscillate even when light levels exceed the threshold found in the one-well model (k(I) = 3 * 10^{-4}), thanks to the diffusion between the wells.

The obvious drawback of this model is that it is very easy for the three wells to
Figure 3.8: Relationship between light inhibition and phase difference in two-well simulations. Top Left: Concentration trace at $k(I) = 3 \times 10^{-4}$, when the phase difference is a constant and small value. In this and all subsequent plots, red shows $[z]$ for the uninhibited well, and blue shows $[z]$ for the inhibited well. Top Right: $k(I) = 4 \times 10^{-4}$, at which phase relation becomes aberrant. The oscillators fire almost in sync, then the inhibited one follows at some $\phi$, then they return to synchrony. Note that they are firing at a 4:3 ratio. Bottom Left: $k(I) = 5 \times 10^{-4}$, causing the oscillators to entrain at a 2:1 ratio with the same phase lag as when $k(I) = 3 \times 10^{-4}$. Further increases in $k(I)$ caused both oscillators to cease firing. Bottom Right: median phase as a function of the photoinhibition constant $k(I)$.

synchronize. If activator coupling outpaces the strength of the reaction, then the oscillators will quickly synchronize. As such, we need a system with a lower coupling constant and greater control over photoinhibition and initial conditions. We used a coupling constant of 0.06 mM/s, significantly less than the coupling required in the inhibitory models.

We found that photoinhibition alone accomplished very little. The reason is simple: unlike inhibitor-coupled wells, oxidized catalyst has the freedom to flow out of the slow well, which makes it harder to build up enough bromine to arrest the reaction’s progress. As a result, the effectiveness of photoinhibition relies strongly on the coupling strength. At higher coupling strengths, oscillators quickly entrain to one another 3.16. However, certain sets of initial conditions gave us substantial and consistent phase differences from the fast and slow cells. The key seemed to be increasing both the photoinhibition on the slow well and the $Y$ and $U$ of the hub well. Photoinhibiting the well slightly diminishes its ability to act as an activator on the hub well, as the slow well produces a more steady stream of bromine, making entrainment harder. Changing the starting concentrations of the hub well, on the other hand, prevents the wells from beginning the simulation entrained. When we increased light inhibition on the slow well by 150% to $2.5 \times 10^{-4}$ and increased initial $Y$ and $Z$ in the hub well to five times their usual concentration, we found the system switched from a fast/hub entrainment to a slow/hub entrainment. Changing the photoinhibitory constant had little effect on frequency, however.

Because light levels can be controlled dynamically, we can effectively switch synchrony of the network while an experiment is running. We utilized this fact to create a new simulation, in which the initial concentrations were set up such that the fast cell had de-
fault concentrations, the slow cell double default concentrations, and the hub cell quadruple default concentrations. At these levels, a $k(I)$ of 0 will lead to entrainment with the fast oscillator, while a $k(I)$ of $4.5 \times 10^{-4}$ will lead to entrainment with the slow oscillator. We began with $k(I) = 0$, but about halfway through the experiment at $t=40s$, we switched $k(I)$ to be $4.5 \times 10^{-4}$ instead. While the hub was initially entrained with the other slow oscillator, switching on the light targeting the slow well caused the hub to drift and synchronize with the fast well. The switch occurred within just a couple of cycles of the hub well, giving us further encouragement this technique may be experimentally robust.

3.4 Four-Well STG Simulations

The four-well circuit follows from the model used by a number of papers from the STG literature [16,56,57]. Our model simplifies the STG circuit, as shown in Figure 3.17 into something more experimentally tractable. We replaced all chemical synapses with two-way inhibitory connections and all electrical synapses with activator coupling. Our wells form the circuit, two of which have activator coupling. In addition two other wells provide inhibitory connections to the hub. These wells act similarly to the speed control mechanisms in the three-cell linear model. However, the linear model has wells that control the frequency of oscillation of the hub with activator coupling. This model uses inhibitor coupling, which has
Figure 3.10: Limit cycle for two-well model contrasting Y and Z. The figure on the left shows two synchronized wells, the figure on the right shows two wells that are synchronized 4:3. These limit cycles show no appreciable difference.

The model has three important connections. The hub well and auxiliary well, which both have the same light level, should be slightly out of phase. Because the two are coupled together by an activator channel, we can achieve the proper relationship by including an asymmetry in the initial conditions. The other two important connections are the inhibiting channels coming from the fast and slow wells. These control the rate of the hub well. Like in the three-cell model, the two outside wells compete for the activity of the hub well.

In order to get different rhythms between fast and slow, we set the $D_{\text{inhb}}$ to 2x as high as the $D_{\text{act}}$, for values of $5 \times 10^{-2} \frac{M}{V \cdot s}$ and $2.5 \times 10^{-2} \frac{M}{V \cdot s}$, respectively. We also applied a light with a photoinhibition constant of $5 \times 10^{-5}$ to the hub and auxiliary wells. From here, we tested the effect of different initial conditions and values of $k(1)$ exposure on the slow well.

Testing over a range of values of $k(1)$ on the slow well, we found that the hub well maintained a fairly consistent firing rate and essentially ignored input from the slow cell. We found that there is strong entrainment between the fast well, the hub, and auxiliary, and the three oscillate almost perfectly in phase, even when we apply greater photoinhibition to the aux and hub wells. Changing the light level on the slow well did lower its firing rate significantly. These findings suggest that activator coupling overpowers inhibitory coupling in circuits. When given competing influences, the entrainment between the auxiliary well...
Figure 3.11: Limit cycle for two-well model contrasting U and Z. The figure on the left shows two synchronized wells, the figure on the right shows two wells that are synchronized 4:3. U in each cell changes as a response to coupling, so a change in U indicates that one of the wells has fired. Note how the setup on the left (weak light inhibition) has these shifts occur at the same position in the limit cycle, while the setup on the right (stronger light inhibition) has them occur in multiple regions of the cycle.

One immediate finding was that this circuit allowed for a much greater control of the frequency of the slow well from light. As we increased the photoinhibitory constant, the slow well fell out of phase and its frequency decreased (Figure 3.18). Once $k(I) > 3 \times 10^{-4}$, the slow well stopped oscillating entirely. This was a much lower threshold than we found in the other models, and warrants further investigation.

Finally, we sought to create a "switch" similar to the one in the three-well model in which a tiny change in light intensity on one well could drastically alter the properties of the circuit. Ideally, by controlling the level of light inhibition on the slow cell, we could cause the hub to become entrained with it and substantially alter the frequency of the hub and even aux. The trick was to break the entrainment of the three wells. We changed initial conditions in all wells. At extremely high concentrations of Y or U, we did find a small decrease in the firing rate of the slow cell, going up to 4.5% in the most extreme cases. The firing rates of the other wells did not change. Their phase relations did not change much

and the hub well proves too strong for the outside wells to change. This could reflect the impact of the auxiliary providing stability. Indeed, the auxiliary and the hub entrained extremely quickly over every condition we proposed.
Figure 3.12: Schematic of a three-well array with all-to-all mutual inhibition

either, suggesting this is not the best approach for developing a new model. In addition, changing the initial concentration of any of the wells produced a similarly negligible effect on firing rate. We instead turn to our final model, the Gutierrez circuit, which was designed specifically with the idea of a switch in mind.

3.5 Five-Well STG Simulations

Having run a number of tests and produced a number of good models for smaller circuits, we turned finally to the Gutierrez circuit [58]. This five-well circuit has two simple rhythms that provide mixed connections to a hub, which should be able to alternate between two speeds based on the needs of relative photoinhibition of the two rhythms. The circuit is based off of work by Gutierrez [58], who tried to make an electrical synapse-based conductance model that loosely resembled the competition between the slow pyloric and fast gastric mill rhythm. The circuit is entirely symmetrical, and makes use of a relatively small number of parameters compared to its complexity (Figure 3.19). The design is simple: a fast rhythm (F1 and F2) and a slow rhythm (S1 and S2) each produce more or less antiphase oscillations. However, F1 and S1 are both activator coupled to a hub well, and F2 and S2 are inhibitor coupled to the hub. The slow wells receive significant light inhibition to modify their frequency ($k(I) > 1 \times 10^{-4}$), and the hub received a lower level of inhibition ($k(I) = 5 \times 10^{-5}$). Thus the relative “influence” of each well determines which rhythm profile
Our goal was to create a two-speed BZ circuit that could modify the output of the hub through competition between two stable rhythms, while maintaining a degree of stability. We found evidence for firing order stability in our five-well circuit. Prinz et al. found that across a wide range of conductances and temperatures, the simple order of cells firing in the pyloric rhythm is maintained [57]. In the pyloric rhythm, LP bursts, and as the burst continues PY begins its own burst. After LP ends, AB/PD begins a burst, and after a brief pause where all cells are silent, the LP begins again, as shown in Figure 3.21. Many of our simulations of the five-well circuit had similar results: a five-part rhythm that maintains a consistent order of firing regardless of the parameters we use. One such rhythm is shown in Figure 3.14. Our parameters of interest were the dimensions of the channels, which defines

the hub will follow and try to synchronize with.
the strength of coupling, and the level of light inhibiting the slow wells.

The stronger we make activator coupling, the more the system descends into two pairs of entrained oscillators: the three activator-coupled wells synchronize, as do the other two. However, at a low enough level of activator coupling, we are able to observe a $k(I)$-dependent switch in behavior. In Figure 3.22, we see that increasing the light inhibition on the slow wells encourages the hub to entrain with slow 1. We also find that as coupling to slow 1 increases, the frequency of the hub decreases (Figure 3.23).

The main goal of creating this five-well model was to perform analysis similar to that of Gutierrez et al. [17], who searched a parameter space based on the synaptic conductances associated with the circuit. As in Gutierrez et al., we assumed all coupling strengths to be symmetrical. We defined three quantities: the strength of activator coupling, the strength of inhibitor coupling of wells onto the hub, and the strength of inhibitor coupling between the wells on the sides. We then varied the coupling strengths of the activator and inhibitor channels that connect to the hub, and fixed the value of the inhibitor coupling of the side channels not connected to the hub. We found a significant change in the firing rate of the cell, as shown in Figure 3.25. At more extreme values of the coupling constants, we found that the phase relationships between the pairs of fast and slow cells were no longer as robust,
Figure 3.16: Successful use of light to switch pacemaker. Here we increased the light constant suppressing the slow well from 0 to $4.5 \times 10^{-4}$, inducing a switch in synchrony. The hub well became entrained with the inhibited well, and fell out of step with the uninhibited well. The black arrow denotes the time at which the light was turned on.

and that as we increased activator coupling, the frequencies of the three wells with activator coupling all increased. We still have a very interesting finding: for a fixed geometry, changing the dimensions of the channels even slightly can lead to significant changes in firing rate.

The Five-well model provides a robust, complex circuit with two interesting properties. Not only does the output of the circuit (as defined by the hub firing rate) change in relation to coupling constants, but also the oscillator
Figure 3.17: The four-well circuit, inspired by the STG. On the top is the traditional circuit, as shown in [56], and on the bottom is our four-well model. Note the structural similarities between the two systems.
Figure 3.18: As we increase the photoinhibition on the slow well, we find its firing rate declines. This contrasts earlier models, which found that increasing photoinhibition could stop oscillations, but often had an otherwise negligible effect on firing rate.

Figure 3.19: The five-well Gutierrez circuit. Our BZ schematic is pictured below.
Figure 3.20: Voltage traces of the pyloric rhythm. Image from Tang et al. [56]

Figure 3.21: Constant order of firing in the five-well circuit: the five wells fire in a specific order for the duration of the simulation. All have the same firing rate and keep a constant phase difference with one another.
Figure 3.22: $k(I)$-dependent synchronization in the five-well model. The top figure has no light inhibition on the two slow wells, while the one on the bottom has a strong ($k(I) = 5 \times 10^{-4}$) inhibition. In the first plot, the hub well is strongly synchronized with both F1 and S1, due to activator coupling. In the second plot, the hub well is now coupled to S1, and F1 is oscillating more independently. In other words, strong enough light inhibition can overtake activator coupling.
Figure 3.23: All firing rates depend on inhibition of the slow wells. As the inhibition on the slow wells increased, the coupling between the hub and S1 increased, and the firing rate of the hub decreased. This tells us that the five-well model can produce a circuit that through the use of photoinhibition, can change both entrainment patterns and the frequency of the hub well.

Figure 3.24: Mid-experiment photinhibition of five-well model. We began an experiment with no photoinhibition on the slow wells and found the hub and F1 entrained quickly. However, halfway through (black arrow) we increased $k(I)$ to $5 \times 10^{-4}$. The hub firing rate fell quickly as it received less excitatory input from S1.
Figure 3.25: We varied the connection strengths of the wells along the lines of Gutierrez et al [17]. The color of each square on the grid corresponds to the firing rate of the hub for a given pair of coupling strengths. As we can see from the plot, the coupling strengths can have a very strong effect on the frequency of the hub oscillator.
Chapter 4

Discussion

We have successfully demonstrated the feasibility of a number of potential models for use in future BZ circuits. Although any and all of these could be explored ad infinitum, we have demonstrated a few important properties of each. Beginning with the three-cell linear model, we have entire ensembles of BZ wells whose group firing properties will change significantly in response to photoinhibition or high concentration gradients. Using photoinhibition, we can alter the pattern of synchrony across the whole circuit just by targeting a single well.

One limitation we have struggled with in BZ systems is ineffective control of frequency through photoinhibition. As shown in the single well model, light inhibition can easily extinguish a reaction, but it cannot provide a great deal of control over the frequency. Our four-well model provides a sort of stability for one oscillator, allowing us to change its frequency and expose it to light conditions impossible in isolation, simply because reagents can diffuse to and from the well. The five-cell model provides a degree of control over both frequency and synchrony of the wells, yet provides an oddly robust system. The same degeneracies and connections that allow these complex BZ circuits to preform unique behaviors also limit their responsiveness to outside perturbations.

Across all circuit designs, we found initial concentrations of dynamic variables had a negligible impact on frequency. Even when initial concentrations favored one well by a factor of ten, frequencies typically did not move by more than a few percentage points. On the other hand, in all models, asymmetries in initial concentrations were found to affect the relative phase of the wells. Highly biased starting conditions prevent the wells from synchronizing, and change the profile of the circuit. We also have quantifiable estimates of the magnitudes of these effects, and how to precipitate or avoid them, depending on the system. In addition to continuing to explore these existing models, we will continue to explore other systems. The animal kingdom has many unique neural circuits we have not yet explored. We foresee BZ systems making use of the circuits that govern flight control, bipedal motion, tentacle
contraction, many more. These simple models are simply a first step.

Now that we have identified a number of key circuit elements and good design parameters for them, the next step is to develop chips with physical properties capable of producing this behavior. The physical principles that govern BZ oscillators in these chips are not yet fully explained, but based on the success of the lamprey-like CPG, we seem to have the ability to translate our models into physical systems. Once we have demonstrated these properties experimentally in chips, we can begin the difficult task of integrating mechanically oscillating gels into the design. These gels will in turn produce new complications, which will have to be dealt with in time. Nevertheless, with this paper we are ever so slightly closer to our goal of realizing a non-biological synthetic soft robot.

In summation, this project has characterized a number of new potential neuroscience-inspired well geometries, given some suggestions for future designs, and characterized possible behaviors of these systems. Future development in this area will hopefully lead to a wider repertoire of circuit elements and their successful implementation into BZ systems.


