# **The Neural Dynamics and Nerve Networks of Coral Polyps** Analyzing Anthozoan Action Potentials

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I. Introduction:

Coral neural networks are uniquely situated on the evolutionary timescale as the first evolved and simplest nervous systems in the animal kingdom [1]. Indeed, the emergence of the first long range intracellular communication mechanism can be directly linked to the ancestors of modern day anthozoa--the evolution of voltage-gated sodium channels in these cnidarians allows for action potentials when coupled with voltage-gated calcium channels from earlier evolved porifera [2].

However, although less sophisticated relative to the nervous systems of higher animals, cnidarian neural networks demonstrate complex behavior that still requires much elucidation. In particular, Anthozoa species exhibit periodic pulsation, light sensitivity, and spontaneous nerve net activity that make them particularly compelling to study [3]. Quantification of the neural responses to varying external conditions, background electrophysiological activity, and interneuronal relationships can ultimately motivate the creation of a biophysical and

mathematical model for coelenterate nerve nets, with applications in fields ranging from neuromorphic engineering to machine learning and evolutionary biology.

### **II. Literature Review:**

Zoologist I.D. MacFarlane's 1978 experiments on *Meandrina* meandrites remain the only study of extracellular electrode readings conducted on these organisms and suggest the presence of three different conducting systems [4]. These three systems include two slow action conductive systems--one in the coenosarc (SSc) and the other in the oral disk (SSo)--and one fast-acting colonial nerve net--typically employed in response to predators (*Figure 1*). Probing distinct parts of the colony--the coenosarc and the oral disk separately--allowed for electrode access to the separate networks and manual modulation of their interplay.



*Figure 1.* McFarlane's recorded traces from the slow system coenosarc and slow system oral disk in *Meandrina* [4]

Theoretical research on a "Model of traveling waves in a coral neural network" was conducted in 2008 by Chen et. al, using a generative algorithm and non-specific conductance parameters due to the fact that "there are no intracellular voltage recordings of coral neurons extant" [5]. Though their network's NEURON simulation exhibited coral-like radial contractions, the model was far oversimplified, failing to match MacFarlane's empirical data on repeated spiking and the existence of multiple conductive systems. This research hopes to mitigate this gap, contributing to both the theoretical and empirical fronts of this problem.

Additionally, and perhaps most interestingly, a theoretical paper titled "Period Doublings to Chaos in a Simple Neural Network: An Analytical Proof" as well as a 2018 theory paper in Nature on "Synchronization transition in neuronal networks composed of chaotic or non-chaotic oscillators" suggest that simple neural networks--not wholly dissimilar to the neural networks of corals--can produce chaotic behavior [6, 7]. However, there is still a need for empirical evidence corroborating the theory of these and similar papers in simple animal neural networks--like the neural networks of corals.

#### **III. Methodology:**

Samples of three species of corals were collected with a hammer and chisel while free-diving in the Republic of Palau at depths ranging from 3 to 10 meters, under the auspices of the general use permit of the Palau International Coral Reef Center (PICRC). Colonies were either specifically chosen or cut down to sizes less than 5cm in diameter to match the dimensions of the experimental setup constructed. After collection and between experiments, colonies were kept in water tables at PICRC at 29°C.



Figure 2. Fungia, Goniopora, and Heteroxenia polyps close up (from right to left)

*Fungia* corals were chosen as candidates due to their unique morphology as solitary polyps, *Goniopora* for their large fleshy tentacles, and *Heteroxenia* for their characteristic pulsating behavior (*Figure 2*).



*Figure 3*. Simple suction electrode schematic used for tentacle recordings and stimulation, taken from Johnson et. al [8]

Suction electrodes were constructed according to the methodology proposed by Johnson et. al in their paper "Construction of a Simple Suction Electrode for Extracellular Recording and Stimulation" (*Figure 3*). However, this experiment's design used both PE 90 tubing pulled to a fine diameter and patch clamp glass micropipettes blown and cut to a diameter between 10-50

microns in accordance with tentacle size. Additionally, 1mL syringes were used for more fine control of seal strength. The experimental setup design to house and manipulate these micropipettes consisted of three sets of three axis micromanipulators, with angle brackets allowing for movements and manipulations on a larger scale (*Figure 4*).



*Figure 4*. Experimental setup with all micromanipulators in place and three suction electrodes mounted (Left), a closeup of two suction electrodes, one glass pipette based pulling a *Fungia* tentacle out of the bath, and a pulled PE 90 tubing

## IV. Results - Fungia

The first traces of action potentials in these anthozoa--Fungia, Goniopora, and

Heteroxenia--were recorded, allowing for comparison and quantification of their waveforms,

durations, and other attributes. Additionally, a variety of tests and different stimuli allowed for

an electrophysiological analysis of various behavior in the different species. Although suction electrode readings from numerous parts of the colonies were attempted, these were largely unfruitful for any purpose other than stimulation and the wide majority of readings were conducted on the cnidarian's tentacles--a technique motivated by earlier electrophysiological success reading action potentials in the tentacles of sea anemones [4]. Recordings from tentacles in all species peaked in amplitude approximately 15-20 minutes after initial attachment--given sufficincient seal strength and positioning to overcome the force of tentacle contraction and deflation.



Figure 5. Two traces of a Fungia action potential, showing timescale and event duration.

The action potential train of *Fungia*, one of the evolutionarily oldest anthozoa in a family dating back to the Triassic period, exhibited a single waveform graph across over 100 recordings of events and numerous. Each action potential was measured to be approximately 550 ms in length, and largely subscribed to the analytical solution of the Hodgkin-Huxley model (*Figure 5*).



*Figure 6*. Fungia action potential traces showing discrete and discernible amplitude variations (Yellow thresholds outlining lower amplitude signals, blue outlining higher amplitudes)

A large number of *Fungia* recordings conducted across the entire tentacle exhibited events with varying amplitudes but the same waveform, suggesting that even in one tentacle, electrical signals from more than one spiking cells were present (*Figure 6*). While some cnidarians are postulated to have both sensory and contraction functionality in only one cell, recordings of action potential events of different origins in one tentacle might indicate different or more complex dynamics in *Fungia*.



*Figure 7*. Periodic stimulation of the oral disk, applying a 1ms 9V pulse @ 1Hz, 0.33Hz, 0.2Hz respectively (from top to bottom)

Additionally, when electrically stimulated on the oral disk with a periodic 1ms pulse of 9V, suction electrode readings recorded in a tentacle 1cm from the stimulating electrode exhibited periodic spiking behavior in step with the pulse, as well as multiple firing and sometimes double action potential events (*Figure 7*).



*Figure 8*. Readout trace from repeated mechanical stimulation of the oral disk (using a blunted syringe needle)

Repeated mechanical stimulation of the colony evokes the same waveform each time, with event duration and shape held constant. However, the action potentials exhibited a decreasing amplitude, possibly due to ion depletion in the surrounding tissue (from repeated physical contraction) or sensory numbing to the mechanical stimulus (*Figure 8*).

### V. Results - Goniopora

Although the fleshy polyps and tentacles of *Goniopora*--occasionally extended to over 3cm in length--seem like ideal candidates for suction electrode recordings, their signifcant contraction and deflation immedaitely after the application of any suction or mechanical force made recordings increidbly diffucit to achieve. However, when suction electrodes were applied to contain a fully contracted polyp, action potential events were discernible after a sufficient waiting period. *Goniopora* action potentials had the longest measured duration of 1200ms, potentially corresponding to slower rates of tentacle contraction and movements (*Figure 9*).



*Figure 9*. Recorded action potentials from a 60 polyp *Goniopora* colony

## VI. Results - *Heteroxenia*

*Heteroxenia* corals by far exhibited the most interesting electrophysiological behavior, indicative of the most complex neural dynamics, unsurprising in lieu of their complex periodic tentacle contraction--the only known constant motion in a sedentary animal. While polyps fully retracted in a manner similar to *Goniopora* when suction electrodes were applied, readings were still more readily attainable in these octocorallia, potentially due to the presence of additional non-calcareous supportive and structural tissue. Additionally, polyps were seen to extend after retraction at a faster rate, returning to 2/3rds their fully extended length after approximately 30s, allowing for easier placement of an effective suction electrode seal.

Interestingly--not one, but three--clearly distinct electrophysiological waveforms were exhibited in *Heteroxenia*. The first was seldom seen and only showed up in recordings of the entire polyp (passive aside from mechanical stimulation necessarily evoked by the suction electrodes force on the soft octoroallia tissue) but exhibited longer plateau like feature in its waveform reminiscent of the periodic waveform of a cardiomyocyte action potential (*Figure 10*). Perhaps, this plateau feature is indicative of a periodicity feature--the action potential's 1000ms average duration was unusually slow. Notably these slow events lasted on a similar timescale to the *Goniopora's* tentacle contraction waveform.



*Figure 10*. Side by side comparison of a recorded passive action potential trace from the entire polyp of a *Heteroxenia* (right) and the model action potential of a human cardiomyocyte (left)

The other two action potential waveforms recorded in *Heteroxenia* exhibited their own distinct features. The most common waveform, similar in nature to the analytical action potential trace of the Hodkin and Huley model, had a duration of 600ms (*Figure 11a*). On the other hand, an action potential cascade--originally mistaken for noise in the data--lasted 2600ms on average (*Figure 11b,c*) and either immediately followed electrical stimulation or occurred without stimulation a set amount of time after a pulse was applied (*Figure 12*).

Unlike the *Meandrina* corals of the McFarlane study, *Heteroxenia* lack the physiological barriers between multi-polyp continuous oral disks and their coenosarc borders, making it hard if not impossible to truly distinguish which pulse waveforms originated in which system. However, cross comparison with the fact that SSo waveforms were often exhibited as cascades or action potential bursts as well suggests that the cascade event present in these readings originated from a similar slow conducting system. Indeed, even the duration of the cascade events was reminiscent of the lengthy SSo action potential cascade events in McFarlane's *Meandrina (Figure 1)*.



*Figure 11*. a) Spontaneous single action potential event that commonly occurs after a burst eventb) Single burst event c) Burst event immediately following voltage impulse

Additionally, McFarlane suggests that "an SSo pulse can enter the SSc but the reverse does not occur," and many readings of electrical stimulation and recording at the same polyp exhibited single pulse behavior that followed the cascade event that aligned with the SSo.

Interestingly, a large number of traces after single electrical stimulation of 1ms at 9V showed behavior from the two systems on similar timescales. As seen in *Figure 12* below, of seven traces left aligned immediately after the 9V step, after approximately 35 seccords the initial cascade is followed by a single pulse of the other conducting system (in traces 4 and 5) or another cascade (in traces 6, potentially 1, and trace 3 after almost exactly half that time).

Additionally, as seen in traces 1, 6, and 7, after a secondary cascade event, a single pulse would arise 20 seconds later.

Although reasoning behind these set timescales between events and causation across pulses cannot be easily or readily determined, the 35 second timescale between stimulation and solitary pulses matches up to the timescale after which polyps would re-extend after stimulation. Perhaps pulses in the non cascading system correspond to inhibitory events preventing contraction. Indeed, other electrophysiology research on cnidarians suggests recorded events "may in fact be inhibitory" [4]. Either way, the presence of recurring timescales and potential causality here begs further investigation.





Finally, one of the most interesting traces occurred during a constant 9V step wherein repeated spiking behavior was occasionally recorded, but never perfectly periodic--indicating patterns similar to those analytically described in theoretical work on period doubling and chaos in simple neural networks (*Figures 13, 14*) [7]. Further traces and experiments can allow for similar traces to indicate the first empirical evidence of chaos and period doubling as predicted

by the relevant theory. The existence of chaotic behavior arising even in these simple and early evolved neural networks could present more profound implications.



*Figure 13*. Recorded spiking behavior in a *Heteroxenia* polyp during a constantly applied 9V voltage step



*Figure 14.* Theoretically constructed traces of a periodic (top) and chaotic (bottom) oscillating neural network system from Xu et. al. Note similarities between the period doubling system's bottom trace and the voltage step trace recorded in *Figure 13* [7]

#### **VII. Conclusion and Future Work**

The first action potentials in three coral species were recorded, and both periodic and chaotic behavior seem extant, with hints at period doubling in *Heteroxenia* conducting systems during a voltage step. In the future, further *Heteroxenia* chaotic spiking and period doubling experiments will be conducted, as well as attempts to map the conductivity routes of different systems in Anthozoa to allow for improved understanding of the underlying structure of these networks. Intracellular recordings with a patch clamp setup would also allow for the first biophysical model of coral action potential events to be constructed, further informing how theory matches these real world conducting systems.

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#### References

[1] Galliot, B., Quiquand, M., Ghila, L., De Rosa, R., Miljkovic-Licina, M., & Chera, S. (2009). Origins of neurogenesis, a cnidarian view. Developmental biology, 332(1), 2-24.

[2] Nishino, A., & Okamura, Y. (2017). Evolutionary History of Voltage-Gated Sodium Channels. In Voltage-gated Sodium Channels: Structure, Function and Channelopathies (pp. 3-32). Springer, Cham.

[3] Sweeney, B. M. (1976). Circadian rhythms in corals, particularly Fungiidae. The Biological Bulletin, 151(1), 236-246

[4] McFarlane, I. D., & Callan, H. G. (1978). Multiple conducting systems and the control of behaviour in the brain coral Meandrina meandrites (L.). Proceedings of the Royal Society of London. Series B. Biological Sciences, 200(1139), 193-216.

[5] Chen, E., Stiefel, K. M., Sejnowski, T. J., & Bullock, T. H. (2008). Model of traveling waves in a coral nerve network. Journal of Comparative Physiology A, 194(2), 195-200

[6] Wang, X. (1991). Period-doublings to chaos in a simple neural network: An analytical proof. Complex Systems, 5(4), 425-444.

[7] Xu, K., Maidana, J. P., Castro, S., & Orio, P. (2018). Synchronization transition in neuronal networks composed of chaotic or non-chaotic oscillators. Scientific reports, 8(1), 8370.