Spring 4-1-2017

The Evolution of Senses: My Research Journey into the Nervous System of Cnidaria

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INQUIRY journal

www.unh.edu/inquiryjournal SPRING 2017

Research Article

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I began my journey in biological research the summer after my first year at UNH, when I was selected to participate in the in the Research Experience and Apprenticeship Program (REAP). I still remember walking into the Plachetzki laboratory for the first time. Just like me, the laboratory was brand new. It felt as if I was walking into a different world, one filled with shiny equipment and foreign reagents that could unlock the answers to scientific questions. My mentor, Dr. David Plachetzki, gave me a tour and told me about all the research ideas he planned to pursue at UNH. His excitement about research in sensory evolution was contagious. The more time I spent in this laboratory, the more passionate I became about this field of study.

Molly Hartley

I wanted to better understand when senses evolved and what mechanisms early evolving animals used to process and interact with their surroundings. The Hamel Center for Undergraduate Research at the University of New Hampshire awarded me funding to conduct my evolutionary biology research through the Research Experiences and Apprenticeship Program (REAP) in 2013, with Undergraduate Research Awards (URA) from 2014–2016, and with Summer Undergraduate Research Fellowships (SURF) in 2015 and 2016. I also received a Research Presentation Grant to present my findings at an international conference in 2017.

In the Plachetzki laboratory, I used animal sensory systems as a model to help characterize how diverse and complex traits evolved from a common ancestor. I researched the sensory systems of animals in the phylum Cnidaria, a group of aquatic animals that all share a common specialized cell type called the cnidocyte. Cnidocytes are neurons that are specialized for stinging and capturing prey. Cnidarians are an evolutionarily ancient group and are among the earliest animal groups to possess a nervous system. Although they do not have many of the structures most people associate with a nervous system, such as a brain or spinal cord, their very basic nervous system allows them to respond to environmental stimuli like light and chemical attractants, and their simplicity makes them easy to study. Conducting research on these animals not only made me more knowledgeable about evolutionary genomics and the origins of complex sensory systems but also allowed me to understand the process of making scientific discoveries.

Evolutionarily Ancient Animals

Cnidarians are the closest evolutionarily sister to bilaterians, the group of animals that includes humans. Bilaterians have bilateral symmetry, or symmetry across their left and right sides, while Cnidarians have radial symmetry, with no identifiable front or back. Cnidarians also contain far fewer known species than bilaterians. Although Cnidarians and bilaterians look vastly different, they actually express similar genes and have many sensory systems in common for processing and responding to their environments. By better characterizing processes in Cnidarians that are also known to occur in bilaterians, we can shed light on how and when the process evolved. Dr. Plachetzki's work creating phylogenetic trees of sensory genes provided evidence that Cnidarians have the ability to respond to light and to taste specific chemicals, something that was previously unknown. Much of my research aimed to provide further evidence to support this inference.

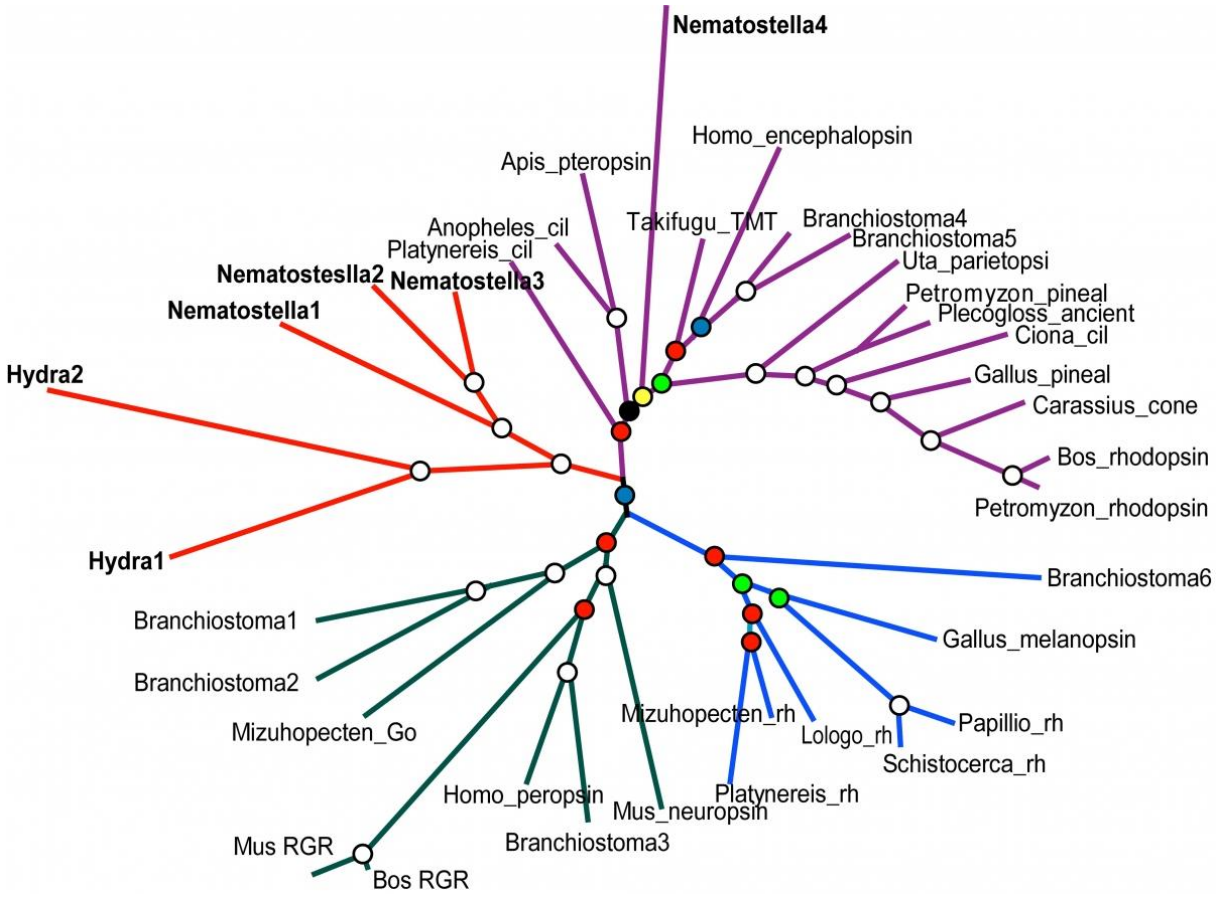

Figure 1. Dr. Plachetzki's phylogenetic tree based on the presence and similarities of the opsin genes expressed in a diverse set of species. The color of each group of branches represents a clade, or a group of organisms that evolved from a common ancestor. The longer the branch is, the more mutations occurred, which further separate it from its ancestor. The colors of the circles indicate how likely the branching is to be correct, with white being most confident, followed by red, blue, green, and yellow, and with black being least confident.

Within the phylum Cnidaria, most of my work was on the animal *Hydra magnipapillata*. Hydra are small, freshwater polyps, composed of a mouth and body surrounded by a ring of tentacles. When I first started in the laboratory, the only thing I knew about hydra was that they are small animals about the size of a fly and known to be immortal; their stem cells continuously generate new cells to replace old ones. Hydra's regenerative ability allows it to regrow complete body parts that become injured or amputated. Because hydra reproduce asexually, the colony of hydra clones we conduct research on in our lab all theoretically express the same genes, making our results from this model organism highly reproducible.

My Introduction to Molecular Biology Research

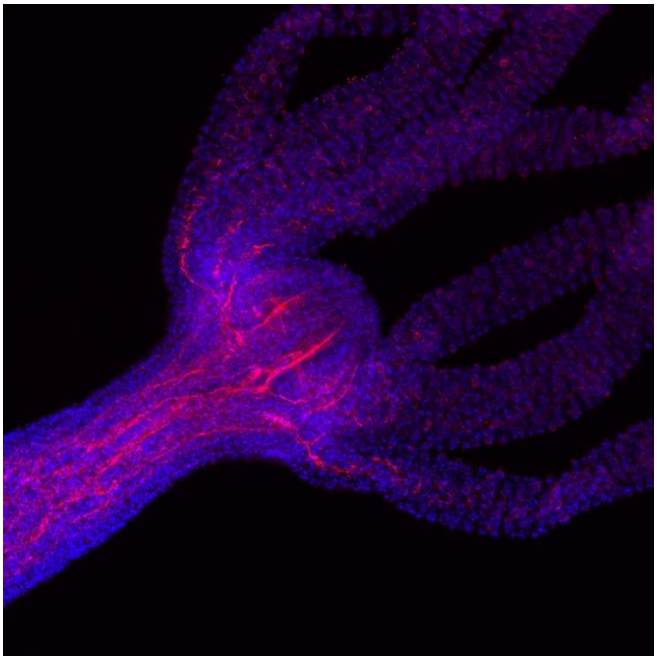

Figure 2. Image of hydra displaying the net-like nervous system stained in pink.

I embarked on the first phase of my long-term research experience the summer after my first year at UNH, when I began to learn about the research process through the Research Experience and Apprenticeship Program (REAP). That summer I worked to obtain portions of various sensory gene sequences of the hydra. I carried out simple protocols, such as polymerase chain reaction, or PCR (to make many copies of a gene); gel electrophoresis (to visualize the copied gene); and molecular cloning techniques (to insert one copy of the gene into a plasmid to be used in later protocols). At the time, these simple experiments appeared daunting. I put pressure on myself to learn the protocols quickly and carry them out successfully in order to move the research project forward. I would spend hours, if not days, working on a project that never appeared like anything more than a small test tube full of liquid. There was often no way of checking that my experiment was working properly until it was over and nothing could be done to fix any mistakes in the many biochemical manipulations performed. This aspect of molecular biology research can cause great stress and frustration, but it is also the reason I love conducting my own experiments in the lab. The concentration and critical thinking skills required make it much more gratifying when an experiment is completed properly.

I still remember the first time I got confirmation that the genes I had cloned into plasmids were actually the genes I had intended to capture. This small finding that the genes predicted to be in the hydra were in fact expressed was exciting and groundbreaking for me, and I knew I wanted to continue being a part of the research that was on the forefront of sensory evolution. By the end of the summer I was using a technique called rapid amplification of cDNA ends (RACE) to amplify an entire taste gene of the hydra. RACE is very similar to PCR, but it allows for longer pieces of DNA to be amplified so the entire 1,500 base pair gene could be obtained from the hydra.

In Situ Hybridization Experiments

By the fall of my sophomore year, I transitioned from completing basic experiments to conducting meticulous staining procedures on the hydra's body with the goal of finding taste and sight sensory receptors on their outer surface. This would provide evidence that hydra can "taste" amino acids and confirm that they can "see," or sense, shadows. Dr. Plachetzki had previously published a paper revealing that hydra have surface opsin receptors. These opsins are light-sensitive proteins found in cells on the exterior of the animal that allow the organism to sense and react to light and/or shadows without having complex structures like eyes (1). Based on recent phylogenetic analyses not yet published, Dr. Plachetzki also found evidence that cnidarians have the same taste receptor that is found in humans (T1R receptor). This was also the taste receptor for which we had obtained the full-length sequence using RACE. Dr. Plachetzki now wanted me to conduct fluorescent in situ hybridization (FISH) experiments to determine if the taste receptors and light receptors are co-localized in the same neuronal cell types. In other words, we wanted to determine whether the same cell could contain both the taste receptor and light receptor. FISH experiments mark where the gene is expressed on the hydra using fluorescent colored tags that bind to the genes. An overlap of red and green within a single cell-type provides evidence that the two proteins are in that cell.

Based on previous research, our working hypothesis in the Plachetzki laboratory was that hydra have a polymodal sensory-motor (PSM) neuron that is able to respond to both light and to taste. Specific sensory receptors detect light and chemical attractants, and this information is passed on to other neurons by a signaling cascade that ultimately leads to the opening or closing of an ion channel within the cell. Such ion channels similarly modulate neurons in both humans and hydra and can be thought of as large doors that cause different responses to occur in the cell based on whether the channels are open or shut. We hypothesized that in the presence of amino acids that leak out of prey animals, a cascade of protein interactions cause the stinging cells in the hydra to fire to try to catch the prey. When one of the hydra's light receptors senses a photon of light, it initiates a cascade of events in the same cell that cause the stinging cell not to fire (2). This simple system explains how a single stinging cell can coordinate light and chemical information from the environment into a feeding behavior in the absence of a complex brain. The stinging response is tuned to dim light conditions and is inhibited at midday when there is the most light and prey are scarce. In this way the hydra's energy is saved for when there will be more prey, usually at dusk or dawn.

I used FISH to test our hypothesis that both light and taste receptors are expressed in the same neurons in hydra. During my REAP project I had constructed plasmids of the taste and opsin genes. To prepare for the FISH experiments, which I was conducting with funding from an Undergraduate Research Award, I created probes from these plasmids. The probes were composed of a sequence of nucleotides that binds to the light and taste receptor, as well as a fluorescent molecule that allows the probes to tag where the gene is expressed. I spent much of the fall semester sophomore year synthesizing probes and learning the FISH protocol (3). The FISH staining experiments take a whole week and include approximately twenty hours of washing steps, where you add different solutions to the test tubes containing the hydra. It is critical that great precaution be taken to preserve the integrity of these small animals. The different washing steps use solutions that ensure the hydra body is well preserved and that the gene of interest (for us, the light and taste receptor genes) will be

properly tagged and fluoresce a given color. These complex experiments were difficult to get right. I needed to coordinate properly many parameters to ensure that the fluorescent molecules marked only the genes of interest.

Halfway through the fall semester, I finally preserved the hydra enough during the FISH experiments to analyze my results under a microscope. My mentor and I noticed green and red color all over the hydra in many different cells. This meant that in addition to the probe binding to and staining where the gene of interest was expressed, staining was also occurring elsewhere on the hydra. We realized the experimental protocol had some sort of systematic error. I spent the following spring, summer, and fall of 2015 troubleshooting the protocol. There were endless steps that could have gone wrong, and even more solutions that I could possibly fix for each step. But I could change only one variable in each experiment, otherwise we would not know which variable made a difference. In order to be smart about the troubleshooting, I often discussed the possible solutions with Dr. Plachetzki. He reached out to his colleagues, and we also turned to peer-reviewed scientific articles that discussed various FISH protocols.

In Situ Hybridization Results

Two semesters, two winter break URAs, and a SURF later, I finally obtained significant results during the winter break of my junior year. We varied the concentration of many different reagents, and used a different probe synthesis protocol that led to more specific staining. After applying these changes, we finally obtained promising FISH results that provided evidence that the taste and sight receptors were in the same neuron in the hydra.

The FISH staining experiments established preliminary data supporting the interaction of light and taste sensory systems in hydra PSM neurons. The findings support the hypothesis that the evolutionarily ancient cnidaria have the same taste receptor gene that is well known to be present in the sister taxa of bilaterians, which contains humans. The two groups of animals did not appear on Earth independently of one another. Instead, they evolved from a common ancestor that likely had the taste receptor gene and the opsin receptor gene, and then as the two groups of animals diverged and continued to evolve, they both retained these sensory genes. The retention of the taste gene likely occurred because it is

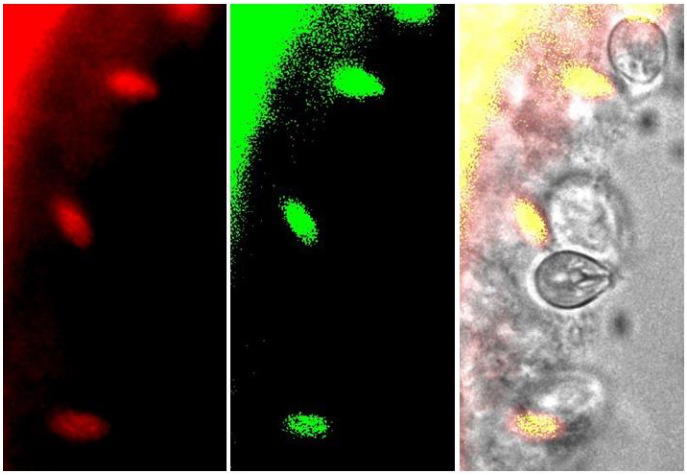

Figure 3. FISH results showing both opsin (red) and taste (green) sensory genes are expressed in the same PSM neuron in the hydra's tentacle. The overlapping gene expression is denoted by the yellow coloring in the third image

evolutionarily advantageous for both groups of animals to be able to detect food. Likewise, it is advantageous for animals to see their surroundings in order to better detect predators and prey.

Our finding that hydra have taste receptors helps to determine when the taste gene evolved. We now hypothesize that taste evolved hundreds of million years earlier than previous research and literature suggested. Other undergraduates in the Plachetzki laboratory will continue behavioral experiments to shed light on whether the taste and sight systems within the PSMneuron are working together or in parallel. There has been great debate in the literature as to whether the sensory systems work in parallel or as an interconnected network within the PSM neuron (4). We predict that they are working together by causing the cyclic nucleotide gated ion channel to open and close. Our prediction is based on the well-known existence of this signaling system in bilaterians, though in bilaterians the process occurs in two different and highly specialized sensory neurons instead of a single neuron.

Constructing cDNA Libraries

These exciting findings were not the end of my research journey. Other lab personnel took over the FISH staining project when I went abroad the spring semester of my junior year. Upon returning to the States I shifted my focus to constructing complementary DNA (cDNA) and RNA sequencing libraries, which are used to identify all the genes that are expressed in a given tissue or a given organism. These libraries are constructed by synthesizing DNA from the mRNA extracted from whole, adult hydra, so that the genes being expressed are in a stable form that can then be sequenced. This innovative technique, which detects all the genes expressed in a sample, provides important insights into how an organism develops and survives in different environments. I started in this new area of research the summer before my senior year, when I helped a visiting graduate student from Canada construct cDNA libraries on other tissues from different stages of development. Since then I have created libraries of the hydra's head and body to see what genes are being expressed, and libraries of other cnidarians my mentor and his colleagues have collected out at sea. As of this publication, I am working to create DNA sequencing libraries from a species of local marine snail in order to determine the genes responsible for various ecologically important traits. I am in the process of assembling and analyzing all the libraries that I created in the last year.

Research Journey

When I first started my research journey, I had no idea where it would take me. I knew I loved my introductory molecular biology course, but I did not know how much more exciting it would be to carry out laboratory protocols in order to make scientific discoveries. My time in the laboratory allowed me to better understand and truly appreciate hydra's unique qualities. I was amazed by every result I obtained that shed light on how this ancient creature uses a primitive system to sense its environment.

Being able to conduct my own experiments required dedication and good time management skills in order to balance my commitment in lab with my schoolwork, but I am forever grateful to have had this opportunity. It taught me more than I could imagine about how to properly conduct research and communicate my findings with others. I plan to go to medical school after college, and I hope to

incorporate a research component into my profession. My advice to all undergraduates with any interest in research would be to get involved! Luckily for us, the UNH campus is filled with scientists conducting research in many areas of study. Reach out to those conducting research of interest to you, and you never known where it may take you.

There are many people I would like to acknowledge who helped make my research experience possible. I am forever indebted to Mr. Dana Hamel and Ms. Melissa McCoy, whose generous donations allowed me to carry out my research projects. I would also like to thank Dr. Paul Tsang and Mr. Peter Akerman from the Hamel Center for all their help throughout my undergraduate research endeavors. Last, I would like to extend my deepest gratitude to my mentor, Dr. David Plachetzki. Not only did he take the time to introduce me to the field of developmental biology, he also guided me throughout my undergraduate career. His confidence in me even before I even stepped foot in a research laboratory allowed me to accomplish more than I ever could have thought was possible.

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Author and Mentor Bios

When Hampton, New Hampshire native **Molly Hartley** entered her first year at UNH as a biology major, she had not imagined where her journey would lead. Four years and five Hamel Center research grants later, this biomedical sciences/medical microbiology major and University Honors Program member has been irrevocably infected with “the research bug.” Learning about all the biochemical manipulations and enzymatic reactions that can take place in a tiny clear test tube was “astonishing,” she says. Molly hopes that her *Inquiry* article informs readers about the amazing way sensory systems have evolved into the “powerhouses” they are today, a topic she became passionate about during her time at UNH. After graduating in May 2017, Molly plans to work in a molecular biology research lab in Boston for a year before attending medical school. Even after medical school, she hopes her career will include a research component. And she hopes that *Inquiry* readers in any field will be inspired by her experience and will get involved in research of their own.

David Plachetzki is assistant professor in the Department of Molecular, Cellular, and Biomedical Sciences at the University of New Hampshire. He teaches Genetics and Comparative Genomics and manages a lab that uses animal sensory systems as a model to understand how complex traits evolve. He notes that Molly "has done an excellent job on this project and . . . will be difficult to replace once she graduates. I think she has been a great role model for other students." About publishing in *Inquiry*, Dr. Plachetzki says that "being able to write for a general audience is an important skill and is critical for advancing scientific literacy in society."