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COLLEGE of CHARLESTON

UNDERGRADUATE RESEARCH AND CREATIVE ACTIVITIES

Application Cover Page

Application for Summer Undergraduate Research with Faculty (SURF) Grant

Applicants should refer to the SURF Program Description and Guidelines and the SURF Application Check List to ensure a complete application. Incomplete applications will not be considered for funding.

PROPOSAL TITLE: Neurogenesis and Neural Development in Snapping Shrimp

PRIMARY MENTOR APPLICANT*:

NAME: [redacted]

CofC Email: [redacted]

Department: Psychology

UNDERGRADUATE APPLICANT**:

NAME: [redacted]

CofC Email: [redacted]

CofC ID Number: [redacted]

Primary Major: Biology/Psychology

Faculty Status:
- ■ Tenured/Tenure-track
- ☐ Instructor
- ☐ Visiting
- ☐ Adjunct
- ☐ Other (please specify _____)

Current Enrollment Status:
- ■ Full-time (12 hours or more)
- ☐ Part-time (less than 12 hours)

*Please note in the guidelines the eligibility criteria for faculty mentors and limits on number of grants per faculty mentor.

**If more than one student will work on a project, separate applications must be submitted for each student.

SECONDARY MENTOR APPLICANT:

NAME: [redacted]

Institutional Affiliation: College of Charleston

Department: Biology

Title: Associate Professor

Email: [redacted]

Undergraduate Applicant: [redacted]

Project Information Page

REQUESTED DATES OF PROJECT SUPPORT (mm/dd/yy): From 5/18/15 **To** 8/05/15

TOTAL AMOUNT REQUESTED FROM URCA: $ 6500

1. Does the proposal involve research on human subjects? ☐Yes ■ No
   If yes, status of the **IRB** request (no funds can be awarded without **IRB** approval):
   ☐Submitted ☐Approved
2. Does the proposal involve research with live vertebrate animal subjects? ☐Yes ■No
   If yes, status of the **IACUC** request (no funds can be awarded without **IACUC** approval):
   ☐Submitted ☐ Approved
3. Have student or faculty applicants received URCA support for this or any other project since September 2013 or do they currently hold funding through the URCA program?
   ■Yes ☐No
   If yes, which type? ■SURF ■MAYS ■RPG ☐AYRA
   If the applicant holds funding in the current cycle, specify name of applicant and award amount:

4. Does the **student** have another proposal under consideration by URCA during the current cycle? ☐Yes ■No
   If yes, what type of grant proposal is it? ☐SURF ☐MAYS ☐RPG ☐AYRA

5. Does the **faculty mentor** have another proposal under consideration by URCA during the current cycle? ■Yes ☐No
   If yes, what type of grant proposal is it? ■SURF ☐MAYS ☐RPG ☐AYRA

6. Is there another internal proposal current or pending for this research/creative work?
   ☐Yes ■No
   If yes, please list the source(s) as well as amount of request and dates of award:

7. Is there an external proposal current or pending for this research/creative work?
   ☐Yes ■No
   If yes, please list the source(s) as well as amount of request and dates of award:

8. Does the project involve biohazards or other safety issues? ■Yes ☐No

9. Does the project have potential for copyright or invention? ☐Yes ■No

FERPA WAIVER

The Family Educational Rights and Privacy Act (FERPA) of 1974 establishes the rights of students with regard to educational records. The act makes provision for inspection, review and amendment of educational records by the students and requires, in most instances, prior consent from the student or their parent/guardian if under the age of 18 for disclosure of such records to third parties. The consent must be in writing, signed and dated by the student and must specify records to be released, reason for release, and the names of the parties whom such records shall be released. The act applies to all persons formerly and currently enrolled at an educational institution. Access to educational records does not give permission to make changes to the student's record. For more information visit:
http://www2.ed.gov/policy/gen/guid/fpco/ferpa/index.html

I hereby give permission for the College of Charleston Undergraduate Research and Creative Activities personnel and committee members to obtain

- **information concerning my academic transcript**
- **information concerning my academic advising notes**
- **information concerning my in-class performance and grades**

This waiver will be in effect as long as I am a student at the College of Charleston, or seeking the services of faculty and staff on the College of Charleston campus.

Signatures (Required for All participants): Please read the SURF Guidelines prior to signing this page. Signatures below indicate awareness of and intention to follow appropriate Program, FERPA Waiver, Departmental, School, College and State rules and regulation for conducting projects, travel, and expenditure of funds.

Undergraduate Applicant: [redacted] Signature — 1-26-15 Date

Faculty/Mentor Applicant: [redacted] — 1-30-15 Date

Faculty/Mentor Applicant: [redacted] — 1/26/15 Date

Chair: I acknowledge that the above student and faculty mentor(s) are applying for URCA Funding and that the funds for successful proposals will be transferred into the departmental R & D account for dispersal based on the budget included in this proposal.

Chair: [redacted] Signature — 1/30/15 Date

2/2/15

Proposed Budget Table

	I	II	III	IV	V
	SURF Funding Requested*		**Dept/School Other Internal Support for Project**	**External Support for Project**	**Total Cost of SURF Project (Add Columns I-IV for each row)**
	For Use Prior to June 30	For Use After July 1			
A. Student Salary (taxable amount)	1750	1750			3500
B. Faculty Salary (taxable amount)		1000			1000
C. Student Travel**	0	0			0
D. Faculty Travel**					0
E. Supplies & Materials	1500	500			2000
F. Other					0
G. Total Costs Per Column (Add values from cells A-F for each column)	3250	3250	0	0	6500
TOTAL SURF REQUEST: (Add values from cells G-I + G-II)	6500				

COLLEGE of CHARLESTON

UNDERGRADUATE RESEARCH AND CREATIVE ACTIVITIES

Summer Undergraduate Research with Faculty (SURF) Grant Application

PART II

To be submitted electronically as a PDF to urca@cofc.edu by 5pm, February 2, 2015

Part I and Part II of the application must be submitted in order to be considered for funding. Part I of the application can be accessed at www.urca.cofc.edu.

PROPOSAL TITLE: **Neurogenesis and Neural Development in the Snapping Shrimp**

PRIMARY MENTOR APPLICANT: [redacted]

UNDERGRADUATE APPLICANT: [redacted]

SURF Application Instructions

A complete description of the application requirements and instructions can be found on the URCA website (www.urca.cofc.edu) in the SURF Description and Guidelines handbook.

Undergraduate Applicant: [redacted]

1. STUDENT STATEMENT OF INTENT

I've always found it easier and more interesting to learn concepts and information by imagining the applications they could have in reality. Whenever I study Biology, or any subject for that matter, I enjoy making connections to what I experience myself everyday. Whether it is learning how organisms interact, or what the purpose is of every little part of a creature. But, having knowledge is useless unless you know how to apply it. Before I began working in the lab, I could only imagine all the possibilities. However, even after my first day I began to hear terminology and techniques that I had only ever read about in textbooks. College has become an academic dream for me with the help of research, because I have the opportunity to not only learn, but also be able to use that knowledge.

I began my undergraduate research career in the second semester of my freshman year by participating in the FYE research rotation course where I had the opportunity to visit 6 labs at the college and contribute to the professors' research. In connection with that experience I joined Dr. [redacted] lab to begin work on snapping shrimp behavior. With Dr. [redacted] going on a year sabbatical, I continued in my sophomore year by participating in an independent study through a collaborative project between her lab and Dr. [redacted] that is more focused on cellular neuroscience. Our work has led to a new summer collaboration with Dr. [redacted] that is the focus of this proposal. Throughout this process I have seen my research experience benefit from my previous academics and I have no doubt that it will aid my future studies also. Even though it seems to be in the distant future, I am looking forward to being able to pull all my ideas and hard work together to yield a conclusion and present my results to other researchers at Neuroscience conferences. Ultimately, this path will lead to the completion of my Bachelors Essay for the Honors College.

After college, I am planning to continue my studies in medical school to obtain an MD and explore clinical research. As I mentioned earlier, working in the lab has taught me how to apply classroom knowledge, and discover new knowledge, a skill that will be useful when transitioning from a medical student to a practicing doctor. This research opportunity will support my future research endeavors, enhance my studies, and eventually lead to valuable skills and experience needed for my career choice.

Undergraduate Applicant: [redacted]

2. NON-TECHNICAL PROJECT ABSTRACT

The ability of an adult nervous system to change in response to environmental changes, such as damage to the system, is unique and challenging when scientists are studying vertebrate systems. This is because to observe a response, permanent damage must often be done to the organism's nervous system. Therefore, invertebrates that are known for their regenerative abilities are an ideal system to explore and study the changes an organism goes through to recover or adapt to its environmental needs. The snapping shrimp, or *Alpheus angulosus*, is a small crustacean with two claws, one big and the other small. If a shrimp loses its big claw, its primary defense, it transforms its small claw into a big claw while growing a new small claw. The claws have different purposes and thus different sensory neuron inputs and outputs. We study the neural changes involved in transformation by tracking and studying the sensory hairs' (setae) distribution on the claws throughout the duration of this process. Past studies have revealed that setae change, both in composition and number, mostly on the cutting edge of the claw. This proposal focuses on the development of the sensory hairs, particularly how and where the sensory neurons in the sensory hairs are created and how they develop

3. PROJECT DESCRIPTION

(a) Technical Project Abstract

The plasticity of the adult nervous system provides a unique challenge to scientists studying vertebrate systems, as drastic changes are rare and often require permanently damaging the system for observation. For this reason, invertebrate systems possessing regenerative abilities provide a unique experimental system for exploring neural plasticity. The snapping shrimp, *Alpheus angulosus,* is a small crustacean with bilaterally asymmetric claws that serve distinct behavioral and sensory functions. If the large claw is lost, the organism switches handedness, transforming their small pincer claw into a large snapping claw while simultaneously developing a small claw on the contralateral side. To better understand how the sensory nervous system adapts to this radical change in body composition, we have examined neural plasticity by tracing changes in sensory hair (setae) distribution on the claws throughout transformation. We have observed only two broad types of setae, simple and plumose. Quantitative analysis across molt stages revealed significant alterations in setae composition and number that occurred primarily on the cutting edge of the claw where the most drastic morphological changes also occur. This proposal focuses on the setae that emerge and proliferate along this edge in order to identify the developmental pathways that are used during setal proliferation and differentiation. In particular, characterizing how the sensory neurons develop inside of these setae during transformation and creating experimental methods that will be able to identify the sites of neurogenesis that create the new sensory neurons. Ultimately, this research will reveal the mechanism underlying the unique transformational ability present in snapping shrimp and provide insight more generally into the processes associated with regeneration and transformation.

(b) Project Objectives and Expected Outcomes

Specific Aim: To characterize the changes in nervous system structure underlying the growth and development of new sensory setae during claw transformation.

- *Short Term Objective 1:* To optimize protocols and generate preliminary data regarding patterns of cell proliferation and neurogenesis in the snapping shrimp

- *Short Term Objective 2:* To characterize the underlying neuroanatomy in new sensory setae during transformation using light microscopy and transmission electron microscopy.

Long Term Objective: The short term objectives outlined above will take the entire summer funding period. Our immediate goal is to employ this technique to reveal more about neural asymmetry and neural regeneration in relation to claw function. However, establishing this technique in this species holds the potential for further investigation into this animal's social system. *Alpheus angulosus* has a relatively unique social system among crustaceans as these shrimp are monogamous. There is a growing amount of evidence demonstrating that neurogenesis may play a critical role in a variety of social behaviors (Gheusi et al., 2009). Further investigations with this technique in this species aim to elucidate the role of neurogenesis beyond peripheral nervous system function to understanding behavioral effects in the central nervous system.

(c) Project Significance

Neural asymmetry is widespread throughout the animal kingdom; this universality suggests that lateralized specialization is adaptive. Nonetheless, the developmental mechanisms underlying bilateral asymmetries in neural structures remain unclear. The ability to regenerate lost structures is also widespread among animals; for organisms with regenerative abilities, generation of laterally specialized neural structures is not limited to early ontogeny, but instead requires significant neural plasticity throughout adulthood. Thus, simple organisms with extreme phenotypic asymmetry combined with regenerative abilities can provide insight into the cellular mechanisms underlying neuroplasticity, regeneration and organization of asymmetric neural systems that traditional model organisms cannot.

The chelae on the first pair of walking legs of snapping shrimp (Alpheus spp.) provide a particularly extreme example of neural plasticity underlying bilaterally asymmetric structures (Figure 1). While both bilateral asymmetry and regenerative abilities are present throughout the phylum Crustaceana, unlike most crustaceans, this phenotypic asymmetry is not fixed in snapping shrimp, and can switch sides or "handedness" throughout adult life (Govind and Pearce 1988; Read and Govind 1997). Snapping shrimp have a large, specialized snapping claw used primarily as a weapon and signal (Hazlett and Winn 1962; Hughes 1996) and a smaller pincer claw used primarily to burrow and feed (Nolan and Salmon 1970). If the larger snapping claw is removed or autotomized, the original pincer will morph over subsequent molts into a snapping claw while a pincer regenerates at the site of the lost snapping claw in a process beginning as early as a week after removal (Mellon 1981). The pincer to snapper transformation requires changes in both sensory and motor systems, as well as changes in gross morphology and underlying musculature, and occurs in a stepwise fashion becoming more like a mature snapper in shape and function after each molt (Pereira et al. 2014). These morphological changes take at least three molts to complete; however, snapping behavior is restored after the first molt (Pereira et al. 2014), indicating that basic snapper motor circuit function is in place despite the fact that complete muscle transformation can take up to eight

Figure 1: An image of *A. Heterochaelis* showing the major snapping claw (left) and the minor pincer claw (right). Image courtesy of www.dosits.org/

molts (Govind 1987). While past work has characterized how the muscles and their associated motor neurons change functionally to support the move from pincer to snapping claw, much less is known about the significant sensory reorganization that occurs in the peripheral and central nervous systems to support these radical changes in wiring (Mellon 1981, Govind et al., 1987)

Our recent analysis of *A. angulosus* claws revealed the presence of two types of setae – simple and plumose (Tracey *et al*, manuscript in preparation). Our quantitation of setae type and number through molt stages during transformation revealed distinct increases in both setae types, including increasing number of sockets containing plumose setae (as well as the appearance of plumose setae in regions where they were previously absent) and increases in the number of setae emerging from individual sockets for both plumose and simple setae, primarily along the edge of the propodus (Figure 2). A notable difference between the plumose setae proliferation as compared to the simple setae changes is that these represent an increase in the number of setae emerging from the claw and the emergence of a new setae type. The plumose setae and any neurons that innervate them represent cell types that are distinct from the majority simple setae that are present throughout the claw, indicating that cell proliferation and the development of new differentiated cell types is occurring to support their appearance.

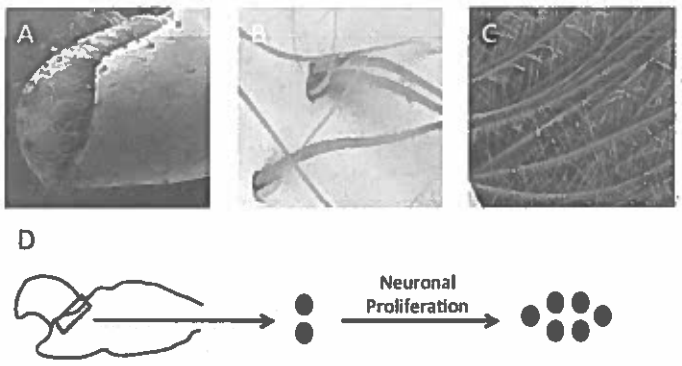

Figure 2: SEM Images of a mature Snapper (A), simple setae (B), and plumose setae (C). **D.** A schematic showing the edge of the claw where most neural proliferation occurs during transformation.

This proposal focuses on the setae that emerge and proliferate along the edge of the propodus in order to identify the developmental pathways that are used during setal proliferation and differentiation. In particular, characterizing how the sensory neurons develop inside of these setae during transformation and creating experimental methods that will be able to identify the sites of neurogenesis. Ultimately, this research will reveal the mechanism underlying the unique transformational ability present in snapping shrimp and provide insight more generally into the processes associated with regeneration and transformation.

(d) Methods of Work

Objective 1: In order to identify dividing cells we will use a well-established marker BrdU (5-bromo-2'-deoxyuridine). BrdU has been widely used to conclusively demonstrate cell proliferation and neurogenesis in the developing and adult nervous system of a wide variety of vertebrate (Kempermann, 2011) and invertebrate (Cayre et al., 2002) animal models. However, this will be our first attempt to use it in our model species *Alpheus angulosus*. Its versatility and success in other animal models make it highly likely to be effective in *Alpheus angulosus*. For example, this same technique has been used reliably in spiny lobsters (Schmidt and Derby, 2011) and crabs (Hansen & Schmidt, 2004). The protocols from these species will serve as a template for our methods. Furthermore, [redacted] has used this technique regularly in mammalian models (Ruscio et al.. 2008) and currently is using it in his research on the California mouse (*Peromyscus californicus*) (Ruscio et al., under review).

The technique will require some modification from the current protocols used in his lab. Specifically the technique involves two main stages 1. Injecting BrdU into the subject. 2. Visualizing the BrdU labeled cells using fluorescent immunocytochemistry. The first stage will involve determining the correct concentration of BrdU for our model species. This will require a high, medium and low dose with 1-2 subjects exposed each dose. Samples from the subjects will be taken 48 hrs. after the dose. The injections will result in BrdU binding to dividing cells. The second stage, fluorescent immunocytochemistry, will provide a fluorescent antibody tag to the BrdU allowing for visualization with fluorescent microscopy. Fluorescent immunocytochemistry involves several steps and takes approximately 3 days. The most critical aspect of this process will be determining antibody concentration. Again we will use a high, medium and low concentration of antibody across all subjects. These concentrations will be based upon research in Dr. [redacted] lab and the appropriate concentration in closely related species. Fortunately, only a small amount of tissue is needed from each subject so these preliminary steps can be performed using a minimal number of subjects (4-6). If these procedures prove efficacious, then we will attempt to double label these cells to provide more information about phenotype (i.e. neuron versus glial cells). We will be able to visualize and quantify our technique using fluorescent microscopy in Dr. [redacted] lab.

Short Term Objective 2: In order to characterize the development of new sensory setae and to determine the underlying neuroanatomy, we will isolate claws and section through the setae on the cutting edge of the class (propodus). Mature snapper claws will be obtained by inducing the shrimp to drop the claws. This can be achieved by holding onto a claw with forceps which triggers a predator escape response during which the appendage is cleanly released by the shrimp. Snapper claws from males and females will be collected in this manner and immediately fixed, embedded in plastic blocks, and prepared for sectioning. Thin sections through the setae will allow us to visualize what the mature sensory nervous system looks like in the sensory hairs. Successful completion of this objective will produce a detailed anatomical map of the mature hairs that we can then use to compare to these same setae as they develop during transformation from the small pincer to large snapper. To induce the transformation process, the major snapping claw from individual shrimp will be held to cleanly induce the release of this claw type. Over the next several months, the shrimp will go through three molts (~ 1 molt/month) during which the minor pincer claw will transform into the major snapper claw. Dr. [redacted] lab has successfully maintained large shrimp populations grouped by sex and molt stage for several previously funded SURF proposals. The holding tank system will be reused for this set of experiments. At each molt (3 in total), males and females will be pulled out of the experimental group and have their transforming claw removed to quantify the changes that are occurring to the internal sensory neurons as they transform from small to large. The claws will be fixed, processed, and imaged in the electron microscope in the same manner that we examined the mature snapping claw. The digital images obtained from the transmission electron microscope will be compared within each molt group and across molt stages to create a developmental picture of sensory neuron development through transformation.

Time Line:

May	June	July	August
Collection/	Collection/	Imaging of Setae	Analysis of Setae

Preparation of Claws for Setae TEM; BrdU Labeling	Preparation of Claws for Setae TEM; BrdU Labeling	with TEM; BrdU Labeling	Images; Poster Creation

(e) Faculty Mentor and Student Participant Roles

Faculty Mentor: Drs. [redacted] and [redacted] will help to design the experiments and interpret the data with [redacted] through out the summer. They will also provide direct instruction while [redacted] is learning new experimental techniques. Dr. [redacted] and Dr. [redacted] split their teaching in Germany ensuring that [redacted] will have one mentor working with her early in the summer. They will provide editorial and organizational assistance when [redacted] is creating her posters for Convocation and Society for Neuroscience.

Student Role: [redacted] will be working full time in the lab designing experiments, carrying out experiments, and collecting/analyzing data. Since this will be her first full-time summer in the lab, Drs. [redacted] and [redacted] will be working closely with her while she learns particular experimental techniques. At the end of the summer, [redacted] will design and create the research poster that she will present as first author at Convocation and the Society for Neuroscience.

(f) Current and Pending Support – None

(g) Student Development: Needhee enters this SURF project at its inception and since she is only finishing her sophomore year she will be able to take part in all aspects of an experimental program. This project is a collaborative effort between Dr. [redacted]'s Lab and Dr. [redacted] lab that brings together both the [redacted] lab's work in neurogenesis and behavior with the [redacted] lab's cellular and development neuroscience. This unique collaboration will expose Needhee to the full spectrum of Neuroscience — from development to adult behavior. This summer she will have an immersive research experience that will set the stage for her continued research and growing independence in the lab over the next two years. In addition to her participation in the Convocation Day poster session, we fully expect her to present her summer work at the Faculty for Undergraduate Neuroscience Undergraduate poster session at the Society for Neuroscience meeting this fall. Looking to the future, her ability to continue her work for the next two years will make it possible for [redacted] to participate in the writing of a manuscript based on this work and present her more developed work on the main floor of the Society for Neuroscience in the Fall of her Senior year.

(h) Project Dissemination: [redacted] will present this work as the presenting author at:

- 2015 Convocation Poster Day, College of Charleston
- 2015 Faculty for Undergraduate Neuroscience Poster Session, Society for Neuroscience
- 2015 Neuropalooza Symposium
- 2016 School of Science and Math Poster Day, College of Charleston

(i) Student Involvement in Application Process: [redacted] has been involved in every step of the application process. She wrote the letter of intent and non-technical abstract with Drs. [redacted] providing editorial comments. As part of her independent study, [redacted] has been preparing for the experimental work, discussing literature, and maintaining the shrimp for the summer research work.