**CVM 2012 Summer Veterinary Scholars Research Grant Competition**

*Note: This grant application is to be prepared by the student in collaboration with their faculty mentor. By submitting this proposal, the faculty mentor assures the review committee that the student has participated substantially in the preparation of this proposal.*

<table>
<thead>
<tr>
<th>1. <strong>Project Title:</strong> The impact of heart failure on the expression levels of corticotropin releasing hormone (CRH) Type 1 and Type 2 receptors in the brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. <strong>Student</strong></td>
</tr>
<tr>
<td><strong>Name:</strong> Freshman Vetstudent</td>
</tr>
<tr>
<td><strong>E-mail:</strong> please don’t email me</td>
</tr>
<tr>
<td><strong>Telephone:</strong> please don’t call me</td>
</tr>
<tr>
<td>3. <strong>Mentor</strong></td>
</tr>
<tr>
<td><strong>Name:</strong> Dr. Linda Hayward, PhD</td>
</tr>
<tr>
<td><strong>E-mail:</strong> <a href="mailto:haywardl@ufl.edu">haywardl@ufl.edu</a></td>
</tr>
<tr>
<td><strong>Telephone:</strong> 352-294-4048</td>
</tr>
<tr>
<td><strong>Department (and College if not CVM):</strong> Physiological Sciences</td>
</tr>
</tbody>
</table>
The effects of stress have been a known contributor to heart failure in both humans and animals. Corticotropin releasing hormone or CRH is the protein in the brain that has been linked to the perception of stress, elevated blood pressure and heart rate responses during stress, and stress hormone release in the periphery from adrenal gland (cortisol). CRH produces its effects via binding to one of two receptors subtypes: Type 1 and Type 2. Both receptor subtypes are found throughout the body, including heart and blood vessels and in the brain. In general, when activated these two receptors work in an oppositional manner to each other. Selective activation of CRH Type 1 receptors generally produces excitatory effects, including high blood pressure, excess stress hormone release, and an increase in heart rate. Alternatively, activation of Type 2 receptors produces effects that may be associated with recovery following activation of Type 1 receptors, including improvements in heart function and lowering blood pressure. Unfortunately, during many cardiovascular diseases, such as heart failure, it appears that the levels of Type 2 receptors may decrease, not only removing the beneficial effects, but also allowing the Type 1 receptors to exert their effects in an unsuppressed manner.

This reduction in Type 2 receptors in individuals with heart failure has been well documented in heart tissue. It is not yet known if Type 2 receptors in the brain also decrease in heart failure and contribute to the disease or if Type 1 receptors increase. Thus, in this study we will evaluate for the first time the impact of heart failure on the expression levels of both Type 1 and Type 2 receptors in the brain compared to normal age matched animals. Changes in heart muscle expression of Type 2 receptors will also be evaluated to confirm previous observations. If there is indeed a decrease in CRH Type 2 or a change in the ratio of Type 1 vs Type 2 receptors in the brain, the brain could be targeted to increase Type 2 or re-establish the balance of these receptors and therefore respond to circulating hormones as a treatment of chronic cardiovascular disease. Targeting the loss of the brain Type 2 receptors instead of the heart Type 2 receptors should prove to be more effective, as the brain will produce “entire body” regulatory results, rather than the local results associated with heart receptors.
Complete the following sections, expanding each section as necessary, but do not exceed the stated page limitations. Do not use a font size smaller than 10 point.

5. **Hypothesis:** It is hypothesized that the downregulation of corticotropin releasing hormone (CRH) Type-2 receptors, but not CRH Type 1 receptors, occurs in select regions of the brain during heart failure and this is associated with a similar down regulation of CRH Type 2 receptors in cardiac tissue in heart failure.

6. **Specific Aims (not to exceed 1 page, single-spaced).**

   Urocortin is a relatively new peptide that is structurally related to corticotropin releasing hormone (CRH). The specific role of urocortin in normal and abnormal physiology remains to be completely understood. In prior studies however, a consistent rise in the peripheral urocortin levels was identified in patients with cardiovascular disease, including heart failure. This compensatory increase in urocortin has been shown to be consistent with a decrease in peripheral urocortin receptors: corticotropin releasing hormone type-2 receptor (CRH R2). The normal activation of these receptors mediates beneficial consequences, such as vasodilatation, positive inotropic, and diuretic effects. The loss of these receptors is thought to contribute to further deterioration of the cardiovascular system in heart failure (20). Interestingly, supplemental stimulation of CRH R2 receptor has been shown to have a therapeutic effect on patients with heart failure. This suggests that therapeutic approaches targeting the urocortin/CRH receptor system may be beneficial in combating cardiovascular diseases.

   CRH R2 receptors are also found in the brain. Studies comparing normotensive and hypertensive patients have provided insight to a link between exaggerated brain activity in regions containing CRH neurons and the progression of stress-related high blood pressure (7,16,17). Depression and anxiety often occur with heart failure and are correlated with a chronic increase of sympathetic drive. Sympathoexcitation is mediated in part through activation of the CRH type 1 (CRH R1) receptors in the brain which work antagonistically to CRH R2 receptors. Thus, changes in sympathetic drive and elevated anxiety in heart failure patients may be due to an increase in R1 reactivity, a decrease in CRH R2 reactivity or some overall shift in the balance of these two receptor systems. The impact of heart failure on brain expression of CRH R1 or R2 receptors however has not been previously evaluated. Based on the peripheral changes known to occur in R2 receptors in this disease, the brain may undergo parallel changes in CRH R2.

   The specific aim of this experiment is to determine if there is a loss of CRH R2 receptor expression in the brain in animals with heart failure consistent with the loss of CRH R2 receptors in the periphery. If this is the case, then both central and peripheral CRH R2 receptors could be targeted for upregulation. The exact role of the CRH R2 system in the brain is currently not known, but it may be involved in regulatory function in the hypothalamus and the medulla. Therefore, correction of the central R2 system may provide better effects than correction of the peripheral system. Central correction may also help mediate the increased effects of R1 (sympathetic) excitation in the brain that contributes to changes in psychological well being in heart failure.
7. Summary review of pertinent literature (not to exceed 1 pages, single spaced).

The amygdala, a complex group of subnuclei located in the forebrain, plays a critical role in both inherent reactivity and learned responses to stressful stimuli (15). The central nucleus of the amygdala (CEA) coordinates stress-related behavioral responses (i.e., freezing or escape) via projections to the medial amygdala (MEA) with changes in blood pressure (BP), heart rate (HR) and cortisol release (or corticosterone in rodents; CORT) via projections to the different subnuclei within the hypothalamus and brainstem. Chronic changes within the amygdala, induced by either learning, genetic programming, or changes in circulating hormone levels have the potential to play an important role in heightening stress reactivity (18).

Currently, little is known regarding the role of the amygdala in mediating heightened cardiovascular responses to mental stress, despite evidence that this region of the brain may be hyperactive in both human (3, 10) and animals (6) with cardiovascular disease. The outcome of the study proposed will significantly advance our understanding of how the dysregulation of the amygdala alters autonomic responses to stress and contributes to cardiovascular disease with a specific focus on the role of CRH receptors and thus are both innovative and translational since CRH receptors are currently being investigated as potential targets for certain types of psychological disease (4).

CRH acts on two types of receptors, CRH-R1 and CRH-R2. Chemical blockade of CRH-R1 receptors in CEA has been reported to significantly reduce behavioral signs of anxiety in normotensive animals (13, 14) and CRH-R1 knockout mice also have reduced levels of anxiety (1, 2). Alternatively, local infusion of CRH into the CEA elevates measures of anxiety (9) and induces sustained increases in HR (19), indicating that CRH release within the CEA (either from intrinsic or extrinsic sources) is anxiogenic and modulates autonomic drive via stimulation of CRH-R1 receptors. Alternatively CRH-R2 knockout mice have significantly elevated levels of behavioral anxiety and are hypertensive (5, 8, 12), suggesting that selective activation of CRH-R1 vs CRH-R2 receptors can trigger opposing cardiovascular effects. This raises the new possibility that exaggerated responses to stress in hypertension may be mediated by an imbalance in receptor expression within either the CEA (elevated CRH-R1 to R2 ratio) or specific efferent projection sites (9).

Urocortin is another natural agonist for R2 receptors. When delivered to the brain, urocortin can reduce heart rate and decrease cardiac sympathetic drive. Prior experiments have studied the peripheral administration of urocortin in conjunction with beta-antagonists in order to mitigate the sympathoexcitation associated with cardiovascular disease, resulting in intermediate haemodynamic effects. This suggests a role for urocortin and CRH R2 receptors, given with beta-antagonists for short-term treatment. (11)

If the hypothesis of the proposed experiment proves correct, the upregulation of central R2 receptors may mitigate the sympathoexcitation effects of cardiovascular disease more favorably than the administration of beta-antagonists, especially due to the location of R2 receptors in the amygdala region of the brain. Treating the central R2 region will allow entire body effects as mediated by brain activity, rather than only local effects as mediated by peripheral R2 receptors.
8. Objectives, experimental approach, and analysis (not to exceed 2 pages, single-spaced).

The objective of this study is to evaluate the CRH R2 and R1 receptor levels in central and peripheral tissues of rats with heart failure. All tissues will be removed from adult male, normotensive Sprague Dawley rats that had either undergone procedure to induce heart failure or a sham operation. The experimenter will be blinded to the samples, as the rat samples will come from another laboratory. The brain regions to be evaluated are: medial hypothalamus, lateral hypothalamus, amygdala, lateral pons, dorsal and ventral medulla, and heart (left ventricle). All brain samples will be tested for CRH R1 and R2 levels and the heart sample will be tested for R2 receptors using RT-PCR. In addition, the medial hypothalamus, amygdala and dorsolateral pons will be evaluated for CRH and urocortin mRNA levels. Tissue from 4 controls and 4 heart failure animals will be evaluated. Thus, 8 samples of each of the 6 brain regions and one heart sample, resulting in 56 samples to be tested for 2 genes and 3 brain regions will also be evaluated for CRH and urocortin mRNA or 48 additional samples tested.

For mRNA extraction, brains will be sectioned using a freezing microtome into 400 micron slices and bilateral micropunches of the tissues will be collected, combined within animals. Tissue from the left ventricle will also be collected. Using standard techniques, mRNA will be extracted, quality of the extraction confirmed, and then cDNA will be made from the samples. Next, samples will be processed using RT-PCR for quantification of CRH and urocortin (only some samples), CRH-R1, R2 and 18s mRNA. All samples will be processed in triplicate using standard manufacturer recommended procedures and results will be normalized to levels of 18s mRNA within each sample to normalize cycle threshold (CT) for amplification. Forward and reverse primers to be used are listed in Table 1.

<table>
<thead>
<tr>
<th>Name</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRH</td>
<td>5’-CCGCAGGCGTTGAATTTCTTG-3’</td>
<td>5’-AGATATCGCTATAAAGACACT-3’</td>
</tr>
<tr>
<td>CRH-R1</td>
<td>5’-CGCAAGTGATGTTCTGGTCT-3’</td>
<td>5’-GGGGCCCTGTTAGATGTAGT-3</td>
</tr>
<tr>
<td>CRH-R2</td>
<td>5’-CCCATCTCTCTGGATCTACCTT-3’</td>
<td>5’-CAACATTTTCATTTCCCGATAATCTC-3’</td>
</tr>
<tr>
<td>Urocortin</td>
<td>5’-CCGGATCCATGACCAGGTTG-3’</td>
<td>5’-CCGAATTCTCCAGAACTTC-3’</td>
</tr>
</tbody>
</table>

Following quantification, a two way ANOVA will be used to determine statistical differences between heart failure and normal samples as a function of brain region. P values less than or equal to 0.05 will be accepted as significant.
9. References with citation of authors, titles, journal and dates (not to exceed 2 pages, single-spaced).


Appendix - To Be Filled Out By Faculty Mentor (not included in page limits):

10. Specific source(s) of funding for research project.

The source of funding for this project comes from an ongoing American Heart Association grant to Dr. Hayward

11. Indicate if this application includes a match ($2,500) for student stipend- include the specific source of these funds (include PeopleSoft project number).

Dr. Hayward will provide a match of $2500 for this student from either the above American Heart grant or overhead funds.
# BIOGRAPHICAL SKETCH

## NAME
Freshman Vetstudent

## POSITION TITLE
University of Florida College of Veterinary Medicine Student

## eRA COMMONS USER NAME (credential, e.g., agency login)
n/a

## EDUCATION/TRAINING

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (if applicable)</th>
<th>MM/YY</th>
<th>FIELD OF STUDY</th>
<th>GPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of North Carolina at Wilmington Wilmington, NC</td>
<td>BS</td>
<td>05/10</td>
<td>Biology</td>
<td>3.906</td>
</tr>
<tr>
<td>University of Florida</td>
<td>DVM</td>
<td>05/15</td>
<td>Veterinary Medicine</td>
<td>3.79 GPA</td>
</tr>
</tbody>
</table>

### A. Personal Statement

The purpose of the Merial Veterinary Scholars Program is to expose young students of veterinary medicine to the field of research. Outside of my research experience in lab portions of my undergraduate biology and chemistry courses, I have never performed my own research which is what makes this program so appealing to me. It has always been an interest of mine and I am excited to be completing my own research project. Though I have never done formal research, I am well-versed in what the process entails. While I am organized and detail-oriented which will help me execute the task I have been given, I am also passionate about the work that I will be doing.

The current proposal is to evaluate the role of cardiovascular disease on CRH receptors expression in select regions of the brain of rodents that have undergone surgical procedures to induce heart failure. The hypothesis to be tested is... The methods to be used include molecular analysis of gene expression.

### B. Positions and Honors

- Fall 2006 – Spring 2010 Dean's List
- Fall 2007 – Spring 2010 Chancellor’s Achievement Award
- June 2008 – August 2008 Kennel Technician at Veterinary Clinic Superior
- September 2010 – July 2011 Veterinary Technician, Receptionist, and Kennel Technician at Veterinary Clinic closer to UF

Member of Phi Eta Sigma Honor Society and Phi Kappa Phi Honor Society

### C. Selected Peer-reviewed Publications

I do not have any previous publications.

### D. Research Support

I have not previously had any research support.
NAME
Linda F. Hayward, PhD

POSITION TITLE
Associate Professor

Approximate % FTE (added total should not exceed 100%)
Research_70_% Teaching_29___% Service_1___%

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (if applicable)</th>
<th>YEAR(s)</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of California, Santa Cruz, CA</td>
<td>BA</td>
<td>12/1980</td>
<td>Psychobiology</td>
</tr>
<tr>
<td>University of Washington, Seattle, WA</td>
<td>MS</td>
<td>08/1984</td>
<td>Kinesiology</td>
</tr>
<tr>
<td>Northwestern University, Chicago, IL</td>
<td>PhD</td>
<td>06/1990</td>
<td>Physiology</td>
</tr>
<tr>
<td>University of Iowa, Iowa City, IA</td>
<td>Postdoc</td>
<td>07/1997</td>
<td>Cardiovascular neurophysiology</td>
</tr>
</tbody>
</table>

A. Personal Statement:
The overall focus of Dr Hayward’s research is to investigate the systems physiological and molecular changes which contribute to alterations in cardiorespiratory coupling in health and in disease states such as hypertension, obstructive sleep apnea and perinatal disruption. Dr. Hayward has over 20 years of research experience with a specific focus on investigating how activation of supramedullary brain regions modulate cardiorespiratory function/integration and neuronal activity/protein expression in targeted brain regions. Dr. Hayward’s current research is focusing on the impact of cardiovascular disease on brain CRH receptor expression and the impact on the cardiovascular response to acute interoceptor (hypoxia) or exteroceptive (air jet stress) stress. Dr. Hayward has been PI or co-I on several previous AHA, NIH and university projects, and has the necessary skills to bring about the successful completion of this projection. She has extensive experience with heart rate variability analysis, blood pressure and neural systems analysis, immunohistochemistry and central microinjection/neuronal lesion techniques and molecular analysis. In the last 5 years Dr. Hayward has been the primary mentor for three PhD students and two post-doctoral fellows, and multiple undergraduates, including several NIH sponsored minority summer students, and two veterinary students. Dr. Hayward one of the basic science physiology faculty members in the College of Veterinary Medicine and teaches professional students cardiovascular and musculoskeletal physiology annually, in addition to her involvement in several graduate physiology courses. Dr. Hayward is also Director of the Merial Veterinary Scholar’s Research Program at the University of Florida, a program that provides a 10 week research experience for professional veterinary students following their freshman year of veterinary school.

B. Position and Employment:
1982-1984  Graduate Student, Department of Kinesiology, Univ. Washington, Seattle, WA.
1984-1985  Research Assistant, Rehabilitation Department, VA Medical Center, Seattle, WA.
1985-1990  Graduate Student, Dept. of Physiology, Northwestern Univ., Chicago, IL.
1990-1994  Postdoctoral Associate, Dept. Internal Medicine, Univ. of Iowa, Iowa City, IA
1995-1997  Assistant Research Scientist, Dept. Internal Medicine, Univ. of Iowa, Iowa City, IA.
1997-2004  Assistant Professor, Dept. of Physiological Sciences, McKnight Brain Institute and the Dept. of Neuroscience (Affiliate appointment), Univ. of Florida, Gainesville, FL
2004-      Associate Professor, Dept. of Physiological Sciences, McKnight Brain Institute and Dept. of Neuroscience (Affiliate appointment), Univ. of Florida, Gainesville, FL.
2009-      Associate Chair, Dept. of Physiological Sciences, Univ. of Florida, Gainesville, FL
2010-      Director, Merial Veterinary Research Scholars Program, Univ. of Florida, Gainesville, FL
C. Honors and Professional Memberships:

Honors

1982-present Society for Neuroscience
1992-present Member, American Physiological Society
1996-1997 Central Investment Research Enhancement Award Univ. of Iowa, Iowa City, IA
2000-2003 Grant Peer Reviewer, American Heart Association, Southeastern Research Consortium, Committee 3
2001 Ad Hoc Reviewer, NIH, NHLBI, Respiratory and Applied Physiology Study Section.
2002-2005 Research Committee Member, American Heart Association, Florida/Puerto Rico Affiliate
2003 CE Cournelius Young Investigator Award, Univ. of Florida College of Vet. Med.
2003 Ad Hoc Reviewer, NIH, NHLBI, Cardiovascular and Renal Study Section.
2009-present Reviewer, NIH, NHLBI, F10A Physiology and Pathobiology of Cardiovascular and Respiratory Systems
2010 Ad hoc Reviewer, Louisiana State Board of Regents

D. PUBLICATIONS:

Most Relevant to current application:


Hayward, LF, and von Reitzenstein, M., c-fos expression in the midbrain periaqueductal gray after chemoreceptor and baroreceptor activation., Amer. J. Physiol.; 283(5):H1975-84. 2004


Hayward, LF. Midbrain modulation of the cardiac baroreflex involves excitation of lateral parabrachial neurons in the rat. Brain Res., 1145: 117-127, 2007. PMC1904493


Book Chapters:

Norman, W., and Hayward LF. Neurobiology of Sleep. In: Sleep Medicine, LippincottWilliams & Wilkins, Philadelphia, PA, 2004.

Norman, W., Carney, PR., and Hayward LF. Neurobiology of Sleep. In: Sleep Medicine, LippincottWilliams & Wilkins, Philadelphia, PA, 2011.

**E. ONGOING (ACTIVE) RESEARCH SUPPORT**

**Grant-in-aid (Hayward, PI) 7/1/2011-6/30/2013 15%**

American Heart Association $150,000 direct costs

*Neural mechanism underlying stress-induced sympathoexcitation*

The primary goal of this project is to identify the role of CRH expression in the exaggerated sympathetic response to acute stress in the spontaneously hypertensive rat.

**Research Development Award (Hayward, PI) 5/1/2011-6/1/2012 10%**

*UF-CVM $8,000*

*Impact of salt-sensitive hypertension on brain mechanisms underlying sleep-wake behavior and stress*

This project will evaluate the impact of salt-sensitive hypertension on molecular changes in a novel salt-appetite/stress pathway between the amygdala and lateral hypothalamus.

**Merial Veterinary Student Research Program (Hayward, PI) 4/1/2012-11/30/2012 10%**

Sanofi-Merial $20,000

This project provides stipend support for 8 veterinary students to participate in a summer research program at UF.