EASTERN CONNECTICUT STATE UNIVERSITY
FALL 2013/SPRING 2014
APPLICATION FOR REASSIGNED TIME FOR RESEARCH

Name: Barbara Murdoch Date: 26 Jan 2013

Rank: Assistant Professor Department: Biology

Title of Project: **Molecular identification of neuronal progenitors in the olfactory epithelium**

Indicate the number and distribution of credits requested for reassigned time for research. Reassigned time may be up to three (3) faculty load credits in one academic year.

Semester FALL Year 2013 Credit Hours 3 FLCs

Have you been granted previous reassigned time for research? If so, how many faculty load credits and when?
I have not previously applied for reassigned time. This is my first application.

Is the current project directly related to your previous work during reassigned time for research, sabbatical leave, or other paid leave? If yes, please elaborate.
No -this is my first application.

Do you expect any external support for this project?
This is my inaugural application. Presently external funding is not in place, but this project could draw attention from outside agencies once completed.

Submit the following:

1. Completed application form

2. Curriculum vita

3. Narrative that provides the following (no more than five double-spaced pages)

   a. Project Objective: A clear statement of the research question or premise of the creative work as well as the methodology used to complete the project

   b. Expected Outcomes: A clear description of the activities that will be completed with the reassigned time as well as the expected outcome of the project (publication, performance, exhibition, literature review, data collection, data analysis, etc.)

   c. Project Significance: A clear statement of how the project will contribute to the applicant's academic field, to Eastern, or to some other community group

   d. Project Feasibility: A clear statement demonstrating that the applicant possesses the resources (knowledge, skills, facilities, etc.) to complete the project successfully. This should include evidence of previous research or creative work, where appropriate, and documentation that resources required to complete the proposed project successfully are available to the applicant.

If granted reassigned time, I agree to allow my proposal to be viewed as a model by future applicants. **Yes** XX
No _____

a. PROJECT OBJECTIVE: My research interests lie in nervous system development and neurogenesis -how the nervous system produces new neurons. To study neurogenesis, I use genetic, molecular and cell biology approaches in the olfactory epithelium (described below). My research addresses fundamental questions regarding tissue organization and patterning during development, and the molecular mechanisms that lead precursor cells (termed progenitors) to produce neurons. The purpose of this proposal is to answer the following questions: 1) How can we identify progenitors that contribute to neurogenesis? 2) How are these progenitors organized within the olfactory epithelium? 3) How do these progenitors change during development? This initial project lays the foundation for the two broader aims of my research that are i) to determine the function of specific progenitors during chick olfactory development and neurogenesis and ii) to determine the mechanisms by which specific signaling molecules drive neurogenesis.

Background: The olfactory epithelium is a nervous system tissue that allows organisms, including humans, to sense smell. Neurons within the olfactory epithelium are continually dying and without their replacement, the sense of smell would be lost. The olfactory epithelium is ideal for the study of neurogenesis as it i) generates new neurons, not only during embryonic development, but also throughout adult life, and thus maintains a continuous sense of smell ii) within a single tissue contains cells at varying stages of development, and iii) contains a simple neuronal population, unlike the more complex populations found within the brain (Schwob 2002).

We used to think that neurogenesis did not occur in the adult nervous system. Now it's known that there are discrete regions within the adult nervous system, including the brain and the olfactory epithelium, where new neurons are produced (Graziadei and Graziadei 1979; Weiss,

Reynolds et al. 1996; van der Kooy and Weiss 2000). Unlike the brain, in the olfactory epithelium, newly made neurons reintegrate into the existing circuitry, and continually maintain their function in the adult organism (Graziadei and Graziadei 1979; Farbman 1990; Roskams, Bethel et al. 1996). To understand the mechanisms that drive nervous system development and neurogenesis, we need to establish when and where specific cell types are made, what cell types each progenitor can produce and how this impacts the overall patterning of the nervous system. The biggest challenge in accomplishing these goals is a common problem in stem cell biology, that is, a lack of markers that will identify progenitors capable of producing the cell types of interest (Weissman, Anderson et al. 2001). Once olfactory progenitors are identified, we can begin to unravel the molecular signals that will allow them to generate new neurons (and perhaps other cell types too). My previous research identified a novel olfactory progenitor, the nestin radial glia-like progenitor, which is restricted to the production of neurons and resembles progenitors found in the brain (Murdoch and Roskams 2008). More recently, I identified Pax7 embryonic progenitors that were unique in that they could produce multiple cell types, including neurons, and contribute to the postnatal olfactory epithelium in an unusual spatial pattern (Murdoch, DelConte et al. 2010; Murdoch, DelConte et al. 2012). Further research is needed to i) identify new markers for progenitors and ii) create a more detailed phenotype using multiple markers on previously identified progenitors to further dissect their biological heterogeneity.

Methodology: This project will use chick embryos to study neurogenesis in the developing olfactory epithelium. Although several studies have used transgenic mice to study olfactory neurogenesis (Murdoch and Roskams 2007), chick embryos provide an affordable model organism to identify progenitors, where gene expression can be more precisely manipulated and followed during later development (Bhattacharyya and Bronner-Fraser 2008; Maier, von Hofsten

et al. 2010). To best study the patterning and development of the chick olfactory epithelium, it is first necessary to define markers that will label cells according to their specific type and developmental stage. Few studies have addressed this issue, although limited markers of the neuron lineage have been shown, these lack the resolution required to target single cells (Maier and Gunhaga 2009).

b. EXPECTED OUTCOMES: Activities: I propose to use fluorescence confocal microscopy with immunohistochemistry (after cryosectioning) to test the existence and location of specific cell types and confirm these results using RT-PCR and/or *in situ* hybridization. Chicken embryos will be isolated at various developmental stages and processed according to the specific assay being performed. Example markers to be tested include those for progenitors (PCNA, DNMT3b, nestin, Pax3, Pax6, Pax7, Sox2, Sox3, Otx2, Dlx3, Dlx5), neurons (TuJ1, peripherin, Dcx) and glia (S100β, p75, GFAP, BLBP). The project incorporates a modular design so that, during the academic year when free time is limited, students can participate on smaller aspects of the larger project.

Outcome: During my research reassigned time I will: i) acquire and analyze data, and prepare the data for ii) presentations at appropriate venues and iii) publication in journals such as *Stem Cells and Development* or *Developmental Biology*. Importantly, I will iv) develop and implement a plan for the management of ECSU's confocal microscope, that is critical to ensure the optimal operation of the microscope and proper training of individuals.

c. PROJECT SIGNIFICANCE: Contribution to Academic Field: Completion of this project will contribute novel information to the fields of stem cell biology, development and/or regenerative medicine, regarding the organization, developmental patterning and identification of progenitors in a tissue that continually regenerates neurons. This is a hot topic in science that has

resulted in numerous publications, including from myself (Murdoch and Roskams 2007; Murdoch and Roskams 2008; Murdoch, DelConte et al. 2010; Murdoch, DelConte et al. 2012; Murdoch and Roskams 2013)

Contribution to Eastern: Eastern will benefit in the following ways: i) This research will be published in peer-reviewed journals and presented at scientific meetings with my Eastern Connecticut State University affiliation, bringing attention to ECSU and my department; ii) my research incorporates undergraduate students who will get invaluable cutting-edge training; iii) my teaching is research-informed - as an active researcher I bring the latest technologies to my classroom and demonstrate ECSU's in-house expertise to our students; iv) my results will demonstrate to outside agencies the technical capabilities at ECSU, which may provide opportunities for external funding.

d. PROJECT FEASIBILITY: Eastern has all of the facilities required for the successful completion of this project, including an Olympus Fluoview FV 1000 confocal laser scanning microscope and newly acquired HM 550 VP cryostat. That I have the knowledge and skills required is indicated by my publication record in peer-reviewed journals – of 34 co-authored papers, 11 are as the first author (see bibliography and curriculum vitae).

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