Beyond the CURE: addressing the needs of undergraduates through an advanced independent research program that examines the effects of gene regulation in neurodegenerative diseases

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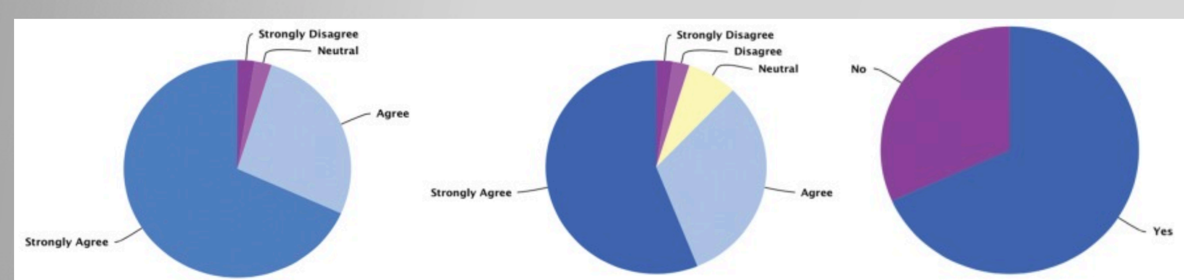
Support for Course-based Undergraduate Research Experiences

Traditional laboratory-based undergraduate curricula focus on exposing students to a variety of techniques and model organisms that may reinforce concepts learned in a partner lecture course. This approach has several advantages including exposure to a variety of techniques. However, significant disadvantages include the relative lack of discovery and limited experience of failure, which is inherent to research. Additionally, brief experiences with techniques lack the power of iteration that allows students to think in greater depth about how to optimize techniques and interpret results. (Auchincloss *et al.*, 2014)

Students who engage in research during their undergraduate education exhibit greater long-term retention in science, deeper understanding of the scientific method, and are more scientifically literate (Russell *et al.*, 2007, Lopatto, 2009, Mervis, 2010). However, most departments at large universities are unable to offer students opportunities for research in a faculty laboratory because mentoring relationships are not available for the large number of undergraduates. Course-based undergraduate research experiences (CUREs) provide an alternative to one-on-one mentored research opportunities in a classroom setting. By definition, CUREs provide most of the components of "real" research, however, the challenges of designing and supporting curriculum on a large scale limit their implementation.



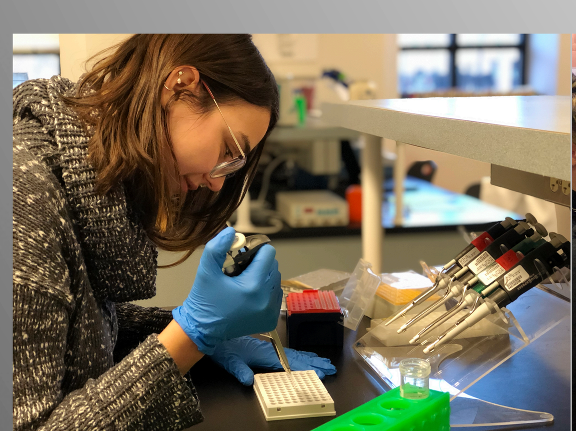
Students who completed he Python Project, a CURE offered in the Department of Molecular, Cellular, & Developmental Biology, were surveyed five years after graduating. Left: 58% "strongly agreed" with the statement "My decision to engage in independent research was positively influenced by my participation in The Python Project." Center: 56% "strongly agreed" with the statement "My participation in The Python Project increased my understanding of performing novel research." Right: 68.3% responded "yes" to "After taking The Python Project course, I worked in a research laboratory during my undergraduate education (paid, internship;, volunteer, or independent/honors credit)."

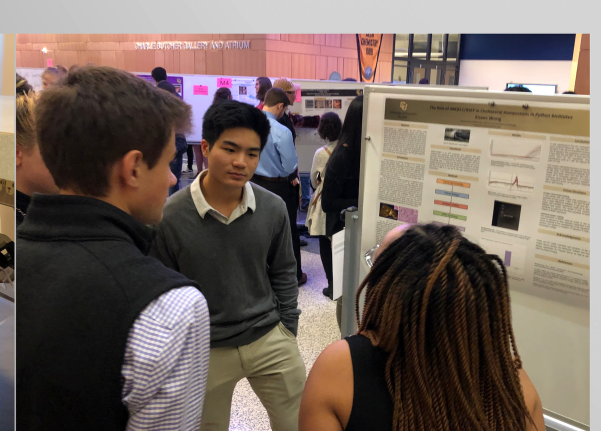
Course-based Undergraduate Research Experiences at CU Boulder

To address concerns expressed by University of Colorado Boulder, the National Academies of Science, Engineering, and Medicine, and others about the quality of scientific training at the undergraduate level, MCDB made a commitment to providing opportunities for research-based experiences early in our students' education. Three Course-based Undergraduate Research Experiences (CUREs) were introduced to replace the traditional labs previously required for the MCDB major: Introduction to Molecular Biology Lab and Introduction to Genetics Lab. Students choose one of the two "Discovery Labs" that screen compound libraries in *Salmonella* and Drosophila melanogaster to identify novel therapeutics or the phage genomics lab (SEA-PHAGES). In addition to learning technical skills and receiving training in experimental design, students learn about model systems, drug development, data analysis, research ethics, and basic biostatistics.

The progress of the courses mirrors that of a graduate program in the life sciences. Students spend the first five weeks of the semester learning about the scientific problem and focusing on recently published research that attempts to address this problem. Students explore the weaknesses of the work and think critically about the impact of the problem. Finally, students develop the technical skills required to perform the research.

During the following eight weeks, students acquire and compile data. They develop expertise in the techniques and in analyzing data while maintaining laboratory notebooks that summarize their hypotheses, methods, findings, and interpretation of data. Regular lab meetings provide opportunities for scientific discussion and research planning. In the final three weeks of the semester, students focus on finalizing their data, presenting it in a public symposium where they defend their work, and planning research proposals and papers. The written submissions include analysis of the quality of the data, commentary on the potential impact of the findings, and recommendations for future experimentation that would help lead the compounds to the clinical trials stage.





CUREs: Solutions with a Longer-term Need

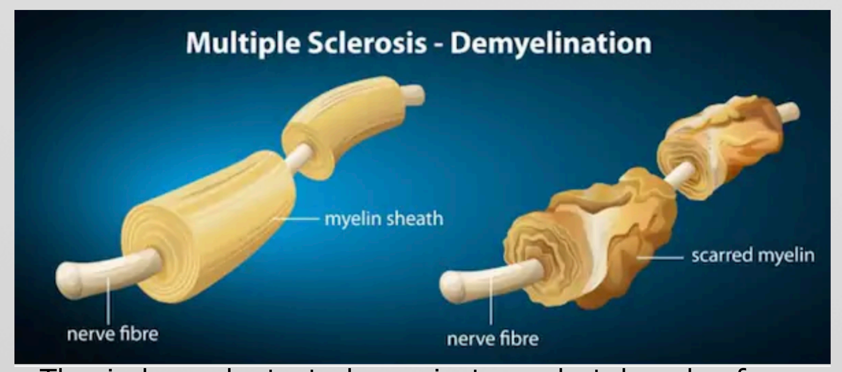
Students who enroll in CUREs both at CU Boulder and elsewhere experience higher rates of retention in science, greater gains in depths of biology concept understanding, and greater feelings of membership in the scientific community. As personal interest increases, so does the desire to join a faculty member's research laboratory to continue growth as independent and creative scientists. However, at large universities like CU Boulder, the availability of such opportunities remains limited.

A resource that is often not utilized is the research expertise of teaching faculty who have often completed post-doctoral training in research, have a great deal of experience mentoring undergraduates in the laboratory, and may have have research interests of their own that could form the basis of a small, undergraduate-drive research group.

Under the supervision of a senior instructor, 8-12 students have developed a sophisticated, multi-semester research project.

The educational aims of the project are to:
1. Provide an opportunity for research "ownership"
2. Encourage independent thinking, creativity, and resourcefulness
3. Create an environment in which undergraduates are free to explore and optimize

Research Background



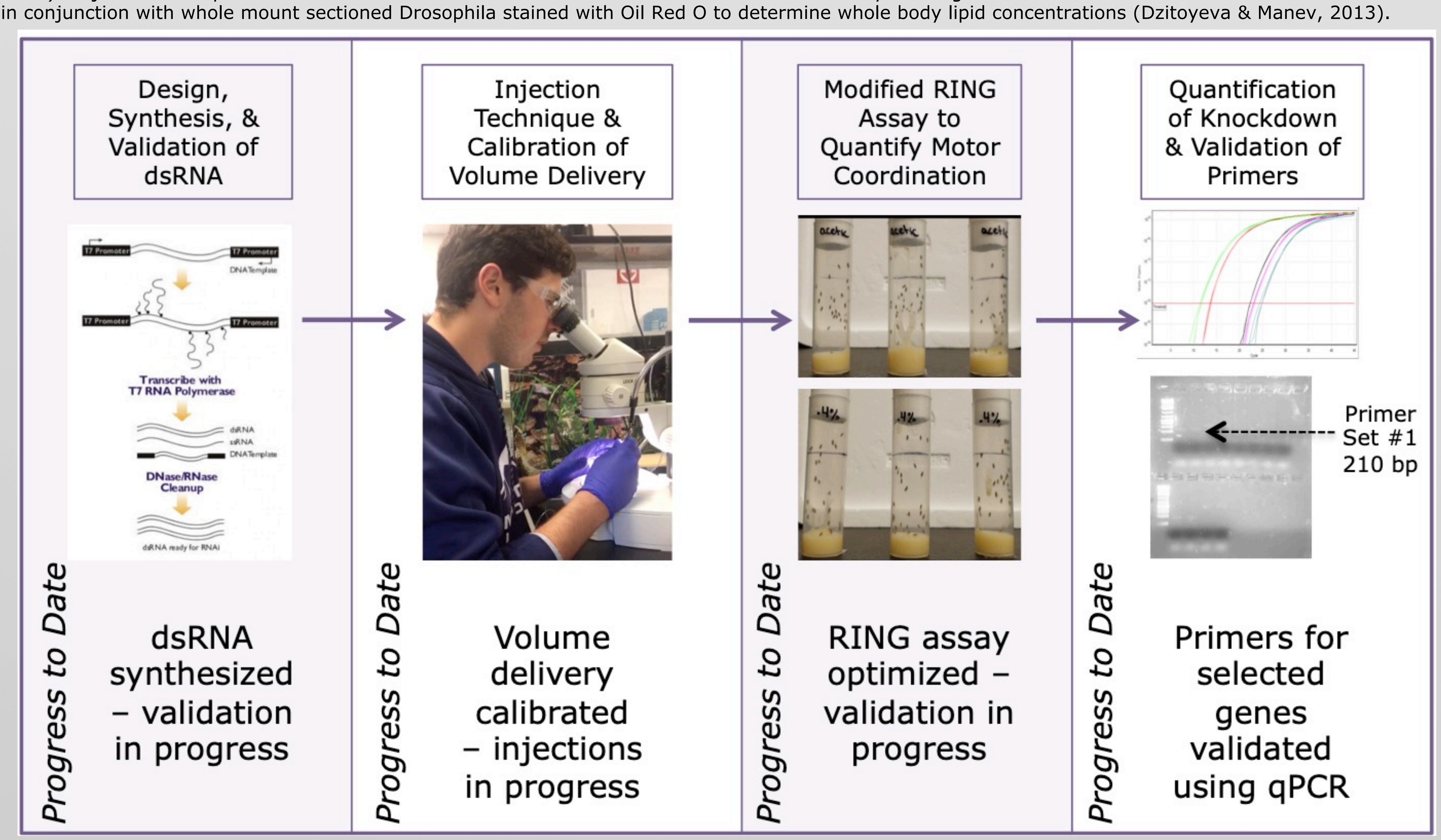
Obesity and elevated serum lipids are associated with a three-fold increase in the risk of developing atherosclerosis, a condition that underlies stroke, myocardial infarction, and sudden cardiac death (Hu, *et al.*, 2001). Strategies that aim to reduce serum cholesterol through modulation of liver enzymes have been successful in decreasing the risk of developing atherosclerosis, slowing progression, and reducing mortality (Janicko, *et al.*, 2013). For example, statins, which inhibit cholesterol biosynthesis in the liver, are considered among the most successful compounds developed for the treatment of cardiovascular disease (Law & Rudnicka 2006). However, recent debate surrounding their effectiveness prompts consideration of alternative cholesterol-lowering therapies, including modulation of liver enzymes responsible for bile acid (BA) synthesis and catabolism (Porez, *et al.*, 2012). For the past three years, students in one of the CUREs in our department, The Python Project, have focused on measuring gene expression related to the production of bile acids in the liver. These studies have been performed in the Burmese python as a model of extreme lipid metabolism after feeding (Riquelme, *et al.*, 2011).

The independent study projects undertaken by former students in the course under my supervision have extended the focus to include the relationship between lipid homeostasis in the liver and dysfunction of organs like the heart. Recently, we have advanced our studies to consider how serum lipids and abnormal hepatic lipid handling relate to the progression of neurological diseases. Patients with Alzheimer's Disease, Huntington's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis, and Multiple Sclerosis often present with dyslipidemia, and recently, high serum cholesterol in morbidly obese patients was identified as a risk factor for developing early onset dementia (Baumgart, *et al.*, 2015, Dupuis, *et al.*, 2008, Weinstock-Guttman, *et al.*, 2011, Leoni & Caccia 2015). A small number of nutritional studies dating back to the 1980's and a recent study in mice suggest that dietary manipulation of serum cholesterol concentrations can dramatically affect the degree of myelination in the brain and progression of neurodegenerative diseases like multiple sclerosis (Berghoff, *et al.*, 2017). Despite clear evidence supporting a role for bile acids and related serum cholesterol concentrations in other organ systems, little consideration has been given to the link between the liver's ability to regulate serum cholesterol and brain health.

We seek to understand the role of cholesterol homeostasis in neurodegenerative diseases using Drosophila melanogaster as a model organism.

A Student-developed Research Approach – the dsRNA Screen

Using a forward genetics approach, students identify genes involved in regulating lipid metabolism relevant to neurodegenerative diseases. They aim to perform directed or library-based screen of dsRNA libraries available (Iordanou, *et al.*, 2011) and measure the level of transcript expression after dsRNA-mediated knockdown of selected RNAs (Carthew, 2006). Injected Drosophila are screen for motor coordination deficits consistent with demyelinating diseases. Immunohistochemical validation of knockdown is performed in conjunction with whole mount sectioned Drosophila stained with Oil Red O to determine whole body lipid concentrations (Dzitoyeva & Manev, 2013).



Preliminary Outcomes

The project formally began in fall 2018. Since that time, students have refined the goals of the project and have nearly completed optimization of techniques required to begin the dsRNA library screen. Notably, the process of designing and optimizing approaches has provided a great deal of experience with laboratory equipment such that the undergraduates in the group are performing at a level commensurate with graduate students in the department.

Technical achievements:
1. Microscopy – lightmicroscopy & immunofluorescence tissue sample preparation
2. Microinjections – design & construction of apparatus, optimization of volume injection, technique, and quantification of anesthesia effects
3. dsRNA Synthesis - RNA isolation, dsRNA synthesis, PCR validation, gel electrophoresis, qPCR
4. Behavior testing – design, construction, & validation

Among the 16 students involved since fall 2018, four have received funding to support their work, two have started graduate school, and one is attending veterinary school.

Recommendations for Creation of a Non-traditional Laboratory

Over the past five years, we have identified characteristics that are amenable to undergraduate research involvement in a teaching faculty laboratory space:

Experimental Iteration – As with CUREs, engaging students in a small set of techniques allows students to gain expertise.

Flexible Timing – Research projects that are not grant-bound offer students the time to carefully consider the context and significance of the work as well as optimize experiments with as much independence as possible. Additionally, a field that remains relatively understudied such as one that is cross-disciplinary lacks a great deal of scientific competition, which is necessary given the pace of undergraduate research.

Translational Significance – Clinical relevance provides novice scientists with talking points that are relatable and simple to understand. Through surveys of our courses, we've learned that students who understand the translational nature of the research identify themselves more closely with the scientific community and remain in science at a higher rate (unpublished data).

Scalability – Using dsRNA library screens, a larger number of students can participate in the research and is scalable as funding and support become available.

Funding – Identification of modest, stable funding is necessary

Challenges to Implementation

For Students:
1. Coping with uncertainty and unpredictable progression of research
2. Pressure of competing with "real" researchers
3. Acquiring sophisticated skills required to complete the project in a relatively short period of time

For Instructors:
1. Difficulty managing time
2. Selecting students who will benefit from the experience
3. Identifying funding sources
4. Allowing students to think independently and make mistakes
5. Creating a research program with teaching responsibilities

For Departments:
1. Identifying funding sources for opportunities that cost more per student than traditional laboratory experiences
2. Finding laboratory space to accommodate small non-traditional research groups
3. Restructuring teaching responsibilities

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