ARTICLE
Modular Digital Course in Undergraduate Neuroscience Education (MDCUNE): A Website Offering Free Digital Tools for Neuroscience Educators

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We are providing free digital resources for teaching neuroscience labs at http://mdcune.psych.ucla.edu/. These resources will ultimately include materials for teaching laboratories in electrophysiology of neuronal circuits (SWIMMY), a Neuroinformatics/Bioinformatics module, and two modules for investigating the effects of hormones on early CNS development—one focusing on the development of the song system and one focusing on sex differences in spinal cord motor neurons. All of these modules are inquiry based—students gain from genuine experiences in doing actual studies rather than just simulations. These materials should provide instructors the ability to provide good quality laboratory experiences regardless of resource limitations. Currently, modules on sex differences in the spinal cord and virtual neural circuits (SWIMMY) are available on our website. More will be available in summer 2009 and 2010. SWIMMY was demonstrated at the Faculty for Undergraduate Neuroscience (FUN) Workshop—The Undergraduate Neuroscience Education: Interactions, interdisciplines, and curricular best practices at Macalester College in July 2008.

Key words: digital laboratories; computer assisted instruction; web-based instruction; QTL analysis; central pattern generators; hormonal effects on CNS

Digital labs offer some advantages over traditional wet labs. First and foremost, digital labs are less expensive to conduct. Because the equipment consists entirely of computers, there is no need for a specialized physical space or specialized equipment. Thus, equipment costs are substantially reduced. Animal care costs are eliminated, and little or no supplies are needed. These advantages make digital labs feasible at just about any institution, especially those that lack the resources to provide a course with traditional wet-labs.

Digital labs can also expand the vistas of undergraduate neuroscience education. Digital labs allow instructional modules that are absolutely not practical at the scale of undergraduate laboratories. One specific example is the Bioinformatics module detailed below. Large numbers of researchers worked for years to create the databases and analysis tools now available free on the web. Now, using these databases and analysis tools, students can formulate new, tractable questions, as well as obtain and interpret data in a few weeks in an undergraduate setting. Another specific example is the birdsong module detailed below—the original experiment took a year to perform, but the digital format allows students to replicate the data collection, analysis, and interpretation in a few weeks.

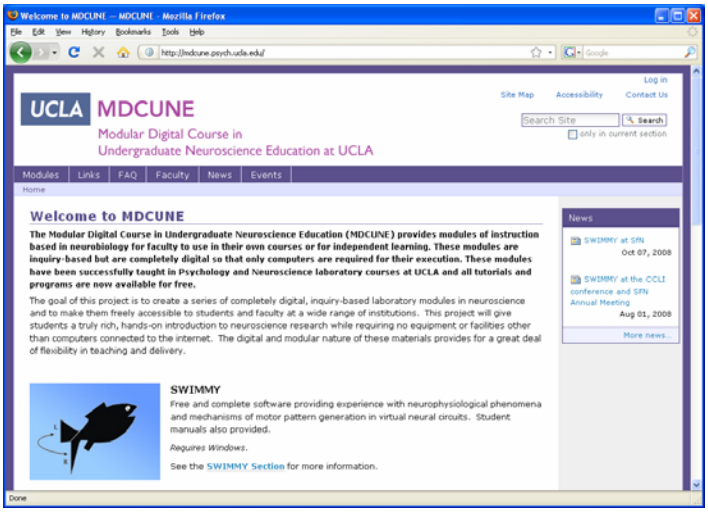

Figure 1. Splash page from MDCUNE where complete materials for digital laboratories can be downloaded for free.

Digital labs need not be demonstrations, simulations, or cook-book exercises. Rather, digital labs can and should be inquiry-based. The digital labs described below provide students with good-quality, inquiry based, edifying experiences, which have been successfully tested at the undergraduate level. We are providing digital lab teaching tools described below for free at http://mdcune.psych.ucla.edu/ (Figure 1).

Prototype Project: RatSCIA Our prototype project was described in a paper published in JUNE, "Sex differences and organizational effects of androgen in spinal cord motor nuclei" (Grisham et al., 2003), along with a collection of digital photomicrographs of SNB spinal cord motor neurons (see Figure 2). This collection of digital images came from a study that examined sex differences in these motor neurons and the effect of an anti-androgen drug on motor neuron development (Grisham et al., 1992).

The original study required a rat breeding colony, microtomes, hoods for staining, a good quality microscope, a digital camera, image-capturing software, and a year's work from two graduate students and a postdoc. In its current digital format, faculty using this image collection need only have computers at their disposal, so the costs of running this lab are minimal. Students start with data collection followed by analysis, and interpretation. This

image collection allows students the opportunity not only to replicate a study, but also to extend the original study by investigating another motor neuron pool (RDLN—see Figure 2).

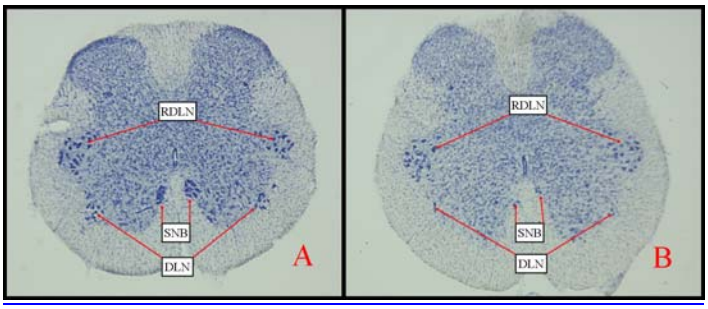

Figure 2. Sexually dimorphic spinal cord nucleus (SNB) and non-sexually dimorphic nucleus (RDLN) on which the prototype project (RatsCIA) was based. (*A*) Cross section of a male. (*B*) Cross section of a female.

Subsequent to the publication in JUNE, this image collection was downloaded over a thousand times, indicating the need and interest for these types of teaching tools. The image library and relevant support materials (including a PDF of the JUNE article) can be downloaded at http://mdcune.psych.ucla.edu/modules/ratscia. This success inspired a NSF-funded project, the Modular Digital Course in Undergraduate Neuroscience Education (MDCUNE), which is a free, online resource for disseminating digital materials for laboratory instruction http://mdcune.psych.ucla.edu/.

Modular Digital Course in Undergraduate Neuroscience Education This project is generating three more modules of digital tools that will provide genuine, inquiry-based instruction in neuroscience: a virtual neurophysiology lab module, a neuroinformatics /bioinformatics module, and a bird song module.

1) SWIMMY: Circuit Neurophysiology and Analyses of Virtual Central Pattern Generators SWIMMY has been more thoroughly described in a previous issue of JUNE (Grisham et al., 2008b) and was presented at the Faculty for Undergraduate Neuroscience Workshop at Macalester College in July 2008. SWIMMY was developed to circumvent the many problems in teaching neurophysiology as a wet lab. SWIMMY eliminates the need for expensive electrophysiology equipment and time spent troubleshooting recording problems. Also, SWIMMY does not use live preparations, which some students find objectionable.

Students first use SWIMMY to review the basics of neurophysiology. Subsequently, students ascertain the neurons involved in the swimming behavior and determine their interconnections by examining the time relationships between neuronal events and by devising and executing virtual experiments. Once students determine all the connections, they will arrive at a schematic that looks something like Figure 3. Students then are challenged to discover which cells are the Central Pattern Generator

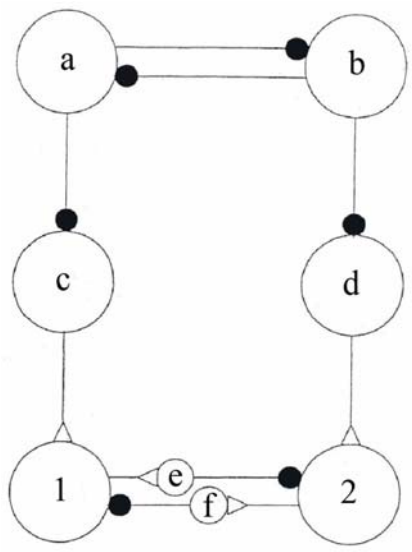

Figure 3. Schematic of the neural circuit that SWIMMY uses to move its virtual tail. Neurons 1 and 2 are the motor neurons.

(CPG) via further experiments, and discover how the swimming rhythm is generated by the CPG. Finally, students use an animation feature that "reads out" the output of the motor neurons to describe the behavior produced by various lesions, and to explain the behavioral phenomena via their understanding of the circuit (Figure 4). Thus, to arrive at a thorough understanding of the circuit, students must compare and contrast the results obtained from the experiments that they design to create well-reasoned, logical arguments supporting their deductions.

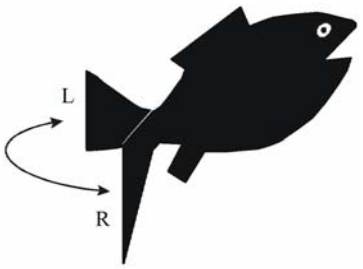

Figure 4. Animated figure that “reads out” the activity of the motor neurons and moves its tail in accordance with their output.

2) Bioinformatics: QTL Analysis of Mouse Olfactory Bulb Morphology There is a clear need for instruction at the undergraduate level in the use of bioinformatics tools, but few good resources are available. Our module will remedy this situation by offering a cohesive package that integrates several bioinformatic resources in a way that is accessible to undergraduate students.

This module brings together information gathered from brain anatomy and bioinformatics websites, including the Mouse Brain Library (Rosen et al., 2000), GeneNetwork’s WebQTL (Wang et al., 2003), UCSC Genome Browser (Kent et al., 2002), The Allen Brain Atlas (Lein et al., 2007), the National Center for Biotechnology Information’s Entrez Gene, and NCBI’s PUBMED.

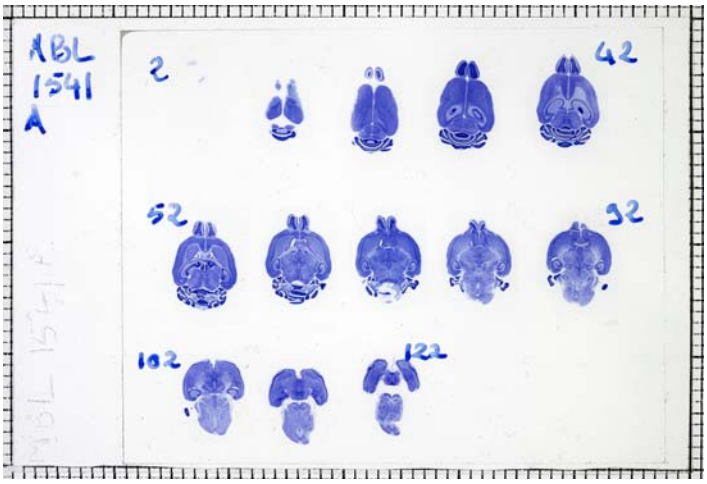

Figure 5. Slide from the Mouse Brain Library showing horizontal sections from a single recombinant inbred mouse. (Published with permission from Rob Williams of the Mouse Brain Library.)

In this module, students quantify a phenotype using NIH Image (see References for URL) to analyze digital images that are downloaded from the Mouse Brain Library (see references for URL)—Figure 5. The Mouse Brain Library provides digitized images of sectioned and stained mouse brains from individuals of different recombinant inbred strains and allows the quantification of many brain phenotypes. We quantify olfactory bulb size since it is relatively easy to operationally define, and there is a published study with which to compare the data (Williams et al., 2001). After students "clean-up" their morphometric data by statistically controlling for variables, they enter their data into a web-based QTL analysis (GeneNetwork's WebQTL—see References for URL). QTL analysis relates variations in phenotype to loci on chromosomes where genes impacting the phenotype can be found (Figure 6).

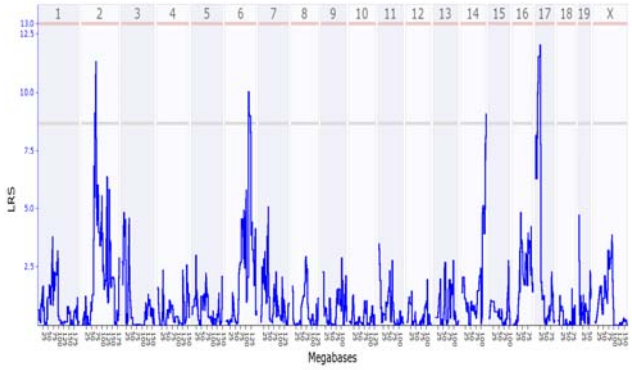

Figure 6. Screenshot of one output level of WebQTL on GeneNetwork. This graph displays the likelihood ratio statistic (blue squiggly line) as a function of megabases on the various chromosomes, which are numbered at top. Here a portion of Chromosome 2, 6, 14, and 17 are identified as loci where there may be genes affecting the phenotype.

GeneNetwork's WebQTL provides a direct link to the University of California, Santa Cruz Genome Browser (URL in References), which provides a list of the genes that are in that portion of the chromosome (see Figure 7). Students can search through this list and find a gene that is highly expressed in the olfactory bulb from the data provided by the UCSC Genome Browser.

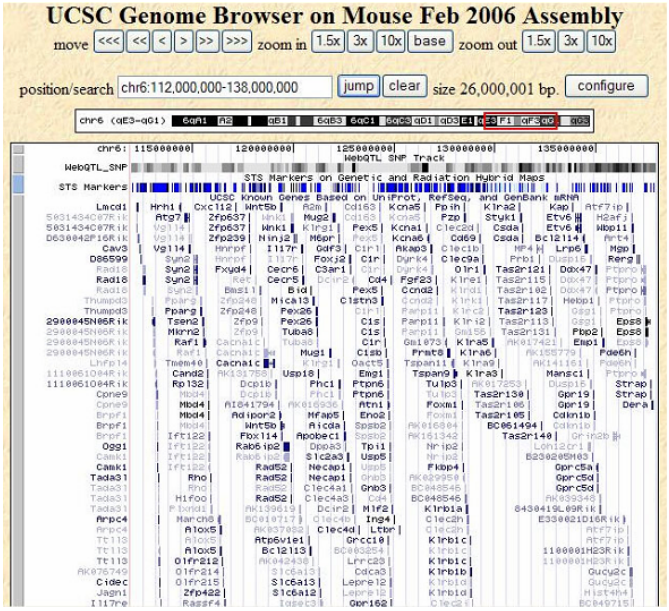

Figure 7. Screenshot of the UCSC Genome Browser displaying the known genes on the distal region of chromosome 6. These gene labels are hot-linked to further information about them.

Once students identify a gene that is highly expressed, they also can link to the Allen Brain Atlas (URL in References), which displays the expression pattern of that specific gene via *in situ* hybridization views along with an atlas (c.f. Ramos et al., 2007, for a more complete description). Thus, students can discover the cell layers where their gene of interest is expressed (Figure 8).

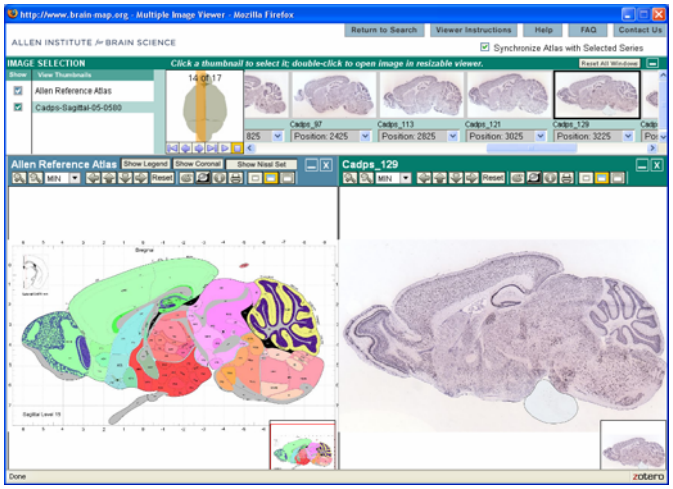

Figure 8. Screenshot from the Allen Brain Atlas displaying both the *in situ* hybridization (right) and corresponding atlas plate (left) for a sagittal section of a mouse brain.

The UCSC Genome Browser also provides links to the National Center for Biotechnology Information resources

such as Entrez Gene and PUBMED (URLs in References). These resources allow the students to discover more information about their highly expressed gene including its nucleotide and amino acid sequence, as well as find articles about their gene that provide a deeper intellectual involvement in this exercise.

Our website has already been populated with some of these materials http://mdcune.psych.ucla.edu/. A detailed instructor's manual should be available in Summer 2009.

3) Bird Song System: Developmental Effects of Hormones The bird song system is an important model system in neurobiology. Adult neurogenesis (Alvarez-Buylla et al., 1988) and dramatic brain sex differences (Nottebohm and Arnold, 1976) were first described in this system, and it is still the focus of ongoing investigations on the neural basis of learning and memory (Nordeen and Nordeen, 1988; Troyer and Doupe, 2000), as well as on the genetic and hormonal bases of sexual differentiation in the nervous system (cf. Agate et al., 2003; Grisham and Arnold, 1995).

The rich literature base on the song system, the robust difference between the sexes in the size of song nuclei (Grisham and Arnold, 1995), and the dramatic masculinizing effect of hormones on this system's development (Grisham and Arnold, 1995) make the zebra finch song system ideal for use in undergraduate laboratories. Nevertheless, studying this system requires significant investments: supporting a bird colony, purchasing good quality microscopes, microtomes, histological supplies for processing brains, and digital microscope cameras. Such extensive facilities and equipment requirements make studying the bird song system out of reach for many institutions.

We are surmounting these obstacles by providing a collection of images for undergraduate students to replicate a published experiment that examined the doses of hormone required to masculinize the song system (Grisham et al., 2008a—Figures 9, 10). Further, students can extend this study--we are adding images from untreated males, so that students can explore whether or not any of the treatments fully sex reverse the female song system

At this point in time, the digital images are being collected, and we anticipate that they will be fully available in Summer 2010. So far, our experience with students using a prototype library of digital images has proved to be highly successful (see Figure 10). Anecdotally, the digital images are better at focusing students on the relevant aspects of the task relative to using tissue on slides. When we tried using the actual tissue, one student looked for the telencephalic song nuclei in the lower brainstem.

STUDENT EXPERIENCES

Digital labs are highly successful teaching tools. We have used SWIMMY for several terms, and students have invariably responded positively. We obtained data from a cohort of students that had experienced both in vivo electrophysiology labs as well as our digital lab, SWIMMY. Students experiencing both conditions rated the virtual lab

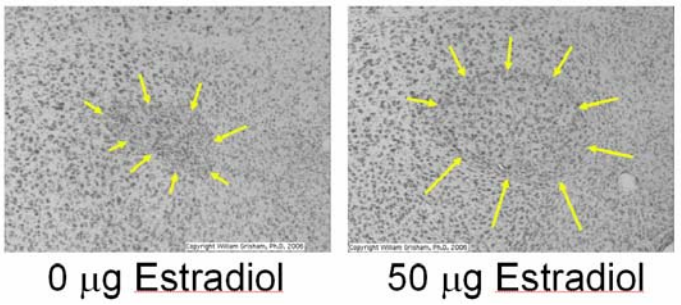

Figure 9. Nucleus RA in female zebra finches that were either treated with 0 μg or 50 μg in early development. These images are prototypes for the forthcoming Bird Song System module.

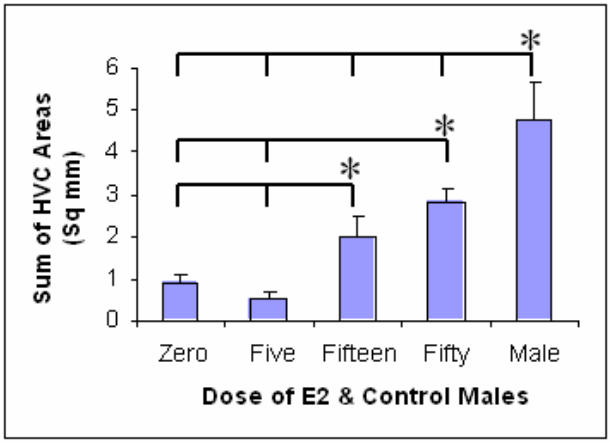

Figure 10. Summed HVC areas as a function of estradiol (E2) dose (in micrograms) given to four female groups at hatching and one control male group. Actual data produced by UCLA undergraduates. Asterisks and brackets indicate significant differences. Comparison between 15 μg and 0 μg doses significant at $p < .06$, all others significant at $p < .05$.

experience as having some definite advantages (see Figure 11). Students show gains in their knowledge of neuroscience and their ability to reason their way through problems based in neuroscience (cf. Grisham et al., 2008b).

We have also taught with the Neuroinformatics module for several terms, and this has similarly proved to be a valuable tool for student learning. In addition to introducing them to Bioinformatics, this module provides instruction in genetics, statistical analyses, neuroanatomy, and molecular techniques. Notably, one of our students reported that the QTL module helped her choose and understand the statistical analyses for her senior thesis.

The Bird Song module has been extensively piloted with undergraduates, and has been successful every time. Students characteristically obtain significant differences among groups—similar to those seen in Figure 10—which they find very gratifying. Further, students benefit not only in learning the facts about hormonal influences on sexual differentiation and comparative neuroanatomy, they also learn about statistical analyses, particularly ANOVA and post-hoc comparisons.

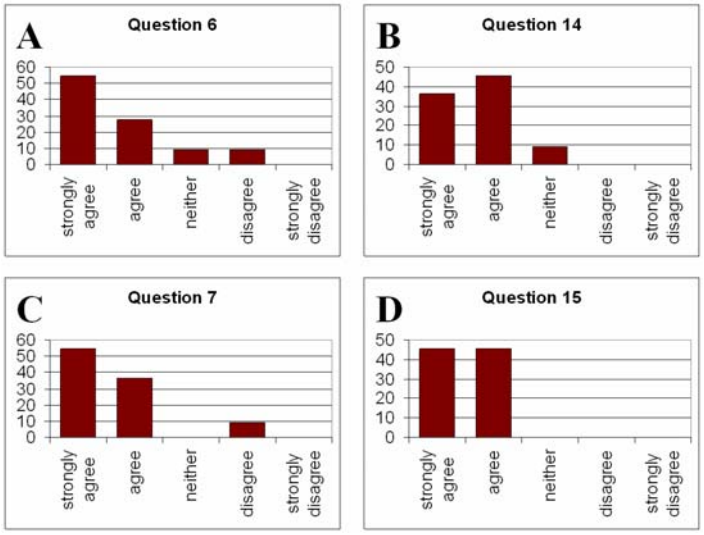

Figure 11. Percent of respondents (n = 11) in the Joint Science Department of the Claremont Colleges agreeing as a function of various scale points. Questions were worded as follows: *A*) Question 6: Using the SWIMMY simulation made learning neurophysiology easier than using the wet-lab preparation. *B*) Question 14: I felt that the SWIMMY application allowed me to focus more on analysis, interpretation, and synthesis than did the wet-lab preparation. *C*) Question 7: I learned more electrophysiology from the SWIMMY module because it was less frustrating than using a live preparation. *D*) Question 15: Using SWIMMY to learn electrophysiology yielded more interpretable results than the wet-lab live preparation. Data courtesy of Dr. Melissa Coleman.

CONCLUSION

In our experience, digital labs can provide valuable educational experiences, but they do offer a different educational experience than traditional wet labs. Digital labs typically sidestep procedure learning so students will not necessarily learn manual skills or technical problem solving required for bench science. Instead, digital labs focus more on data collection, analysis and synthesis. Digital labs restrict the types of procedural errors that can occur, so students lose the opportunity of learning from such mistakes. On the other hand, students sometimes make such disastrous errors in wet labs that their results are uninterpretable, and digital labs prevent such outcomes. Also, mistakes are more reversible in the digital realm than in wet labs, which we find reduces student anxiety. Neither wet labs nor our digital labs always produce the predicted data, which encourages an opportunity for valuable didactic discussion. Nonetheless, our digital labs are likely to yield replicable and interpretable results that allow students to relate their own data to broader concepts discussed in the literature.

Having run both wet and digital labs, we also appreciate other strengths of digital labs. In wet labs, students often had to take turns waiting to share equipment, animals, etc. In contrast, digital labs both allow and demand that each and every student be engaged in all aspects of the process and through all of the instructional time. In addition, digital labs reduce the use of animals in teaching, thus side-stepping potentially thorny ethical objections some students may have.

Perhaps the greatest advantage of digital labs is that they can be adapted for and adopted in a variety of learning environments/communities such as a traditional classroom, in a lab setting, in blended instruction, or even distance learning. Although these labs may be of most interest to institutional settings where traditional wet labs are not possible due to limitations on resources, they have utility in other contexts as well. We have augmented and amplified students' laboratory experiences by using digital labs along with wet labs in our courses. Digital labs also allow easy preservation of teaching materials and tools and easy access to these resources for other faculty. We are pleased to offer these labs to faculty and students at other institutions for free via our website: http://mdcune.psych.ucla.edu/.

REFERENCES

Agate RJ, Grisham W, Wade J, Mann S, Wingfield J, Schanen C, Palotie A, Arnold AP (2003) Neural, not gonadal, origin of brain sex differences in a gynadromorphic finch. Proc Natl Acad Sci USA 100(8):4873-4878.

Alvarez-Buylla A, Theelen M, Nottebohm F (1988) Birth of projection neurons in the higher vocal center of the canary forebrain before, during, and after song learning. Proc Natl Acad Sci USA 85:8722-8726.

Allen Brain Atlas http://brain-map.org/. Accessed 02-13-09.

Entrez Gene http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene. Accessed 02-13-09.

GeneNetwork http://www.genenetwork.org/. Accessed 4-12-09.

Grisham W, Casto JM, Kashon ML, Ward IL, Ward OB (1992) Prenatal flutamide alters sexually dimorphic nuclei in the spinal cord of male rats. Brain Res 578:69-74.

Grisham W, Arnold AP (1995) A direct comparison of the masculinizing effects of testosterone, androstenedione, estrogen, and progesterone on the development of the zebra finch song system. J Neurobiol 26:163-170.

Grisham W, Jones HB, Park SH (2003) Sex differences and organizational effects of androgen in spinal cord motor nuclei. J Undergrad Neurosci Ed 2:A29-A36.

Grisham W, Lee J, Park SH, Mankowski JL, Arnold AP (2008a) A dose-response study of estradiol's effects on the developing zebra finch song system. Neurosc Lett 445:158-161.

Grisham W, Schottler NA, Krasne FB (2008b) Swimmy: free software for teaching neurophysiology of neuronal circuits. J Undergrad Neurosci Ed 7:A1-A8.

Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, Haussler D (2002) The human genome browser at UCSC. Genome Res 12:996-1006.

Lein ES, et al. (2007) Genome-wide atlas of gene expression in the adult mouse brain. Nature 445:168-176.

Mouse Brain Library http://www.mbl.org/. Accessed 04/12/09.

NCBI Entrez Gene http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene accessed 04/12/09.

NCBI PubMed http://www.ncbi.nlm.nih.gov/pubmed. Accessed 04/12/09.

NIH Image http://rsb.info.nih.gov/nih-image. Accessed 04/12/09.

Nordeen EJ, Nordeen KW (1988) Sex and regional differences in the incorporation of neurons born during song learning in zebra finches. J Neurosci 8:2869-2874.

Nottebohm F, Arnold AP (1976) Sexual dimorphism in vocal control areas of the songbird brain. Science 194:211-213.

Pubmed http://www.ncbi.nlm.nih.gov/PubMed/. Accessed 02/16/09.

Ramos RL, Smith PT, Brumberg JC (2007) Novel *in silico* method

for teaching cytoarchitecture, cellular diversity, and gene expression in the mammalian brain. J Undergrad Neurosci Ed 6:A8-A13.

Rosen GD, Williams AG, Capra JA, Connolly MT, Cruz B, Lu L, Airey DC, Kulkarni K, Williams RW (2000) The Mouse Brain Library @ www.mbl.org. Int Mouse Genome Conf 14:166.

Troyer TW, Doupe AJ (2000) An associational model of birdsong sensorimotor learning I. efference copy and the learning of song syllables. J Neurophysiol 84:1204-1223.

Williams WR, Airey CD, Kulkarni A, Zhou G, Lu L (2001) Genetic dissection of the olfactory bulbs of mice: QTLs on four chromosomes modulate bulb size. Behavior Genet 31:61-77.

Wang J, Williams RW, Manly KF (2003) WebQTL: web-based complex trait analysis. Neuroinformatics 1:299-308.

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