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Article

Development of a Neuroscience-oriented "Methods" Course for Graduate Students of Pharmacology and Toxicology

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To provide graduate students in pharmacology/toxicology exposure to, and cross-training in, a variety of relevant laboratory skills, the Duquesne University School of Pharmacy developed a "methods" course as part of the core curriculum. Because some of the participating departmental faculty are neuroscientists, this course often applied cutting-edge techniques to neuroscience-based systems, including experiments with brain G protein–coupled receptors. Techniques covered by the course include animal handling and behavioral testing, bacterial and mammalian cell culture, enzyme-linked immunosorbent assay, western blotting, receptor binding of radioligands, plasmid DNA amplification and purification, reverse transcriptase-polymerase chain reaction, gel electrophoresis, and UV-visible and fluorescence spectroscopy. The course also encompasses research aspects such as experimental design and record keeping, statistical analysis, and scientific writing. Students were evaluated via laboratory reports and examinations, and students in turn evaluated the course using a detailed exit survey. This course introduces the graduate student to many more techniques and approaches than can be provided by the traditional graduate "rotation" format alone and should serve as a template for graduate programs in many basic research disciplines.

INTRODUCTION

In the experience of the authors, most academic science departments require their first-year graduate students to participate in experiential laboratory "rotations." At least two and perhaps four rotations, research stints in a given laboratory lasting less than a semester, are conducted during the first academic year. The rotations are selected by the student with the consent of the professor of that laboratory and are meant to assist the student in deciding on a thesis laboratory. Thus, the student is exposed to two to four research projects and a limited number of laboratory techniques before exclusively pursuing thesis research for the remainder of his/her graduate education. One approach to broadening the methodological exposure and training of the student would be to institute a mandatory "methods" course; however, few departments would tolerate the additional time and effort required of the students and faculty instructors for such a course while trying to maintain competitive research programs.

In contrast, a research methods course should be quite useful to both the faculty and students of small academic science departments. For these departments, new graduate students might only be admitted to replace outgoing students in order not to exceed the number of available teaching assistant stipends. By necessity, the incoming student would probably be assigned to a thesis laboratory from the outset, defeating the purpose of a rotation system. A methods course would not only compensate for omitting the research experiences normally afforded by rotations, but

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provide a greater variety of bench techniques encountered. The course is also a logical way to utilize the research expertise of the collective faculty without demanding more than 1–3 wk of teaching from any one professor.

A Web site screening of graduate neuroscience, pharmaceutical science, or other biomedical science programs of 15 colleges or universities sought to determine if similar methods courses were offered and if this correlated with the absence of a laboratory rotation format in selecting a thesis laboratory. The 15 institutes were either 1) comparable to Duquesne University regarding faculty teaching versus research emphasis, 2) peer group pharmacy schools, or 3) within Pittsburgh. The rotation format was expressly or apparently used at Boston College Department of Biology (2006), Carnegie Mellon University Department of Biological Sciences (2006), Fordham University Department of Biology (2006), Georgetown University Department of Pharmacology (2006), the University of Maryland at Baltimore School of Pharmacy (2006), the University of Minnesota School of Pharmacy (2006), the University of North Dakota Department of Pharmacology (2006), Purdue University School of Pharmacy (2006), the University of Pittsburgh Department of Neuroscience (2006), and Temple University School of Pharmacy (2006); no rotation format was obvious from the University at Buffalo School of Pharmacy (2006), University of Cincinnati College of Pharmacy (2006), Creighton University College of Pharmacy (2006), Long Island University-Brooklyn College of Pharmacy (2006), and Wayne State University School of Pharmacy (2006) Web sites. Methods courses were found at the Buffalo, Creighton, Fordham, Georgetown, Maryland, North Dakota, Temple, and Wayne State Web sites; however, the syllabus was accessible only for the Georgetown program. The subject matter within this syllabus was similar to that described in the present work, but the Georgetown course contained no laboratory component. No correlation between methods course offerings and the lack of a rotation system was apparent from this survey, and no evidence suggested that a strategy of replacing laboratory rotations with a comprehensive methods course was used by these institutes.

The pharmacology/toxicology department of the Duquesne University School of Pharmacy (DUSOP) is a small department of the nature described above. To best serve the needs of both its graduate students and faculty, a neuroscience-oriented methods course in pharmacology and toxicology was created and is described herein. At least some of the skills and methods taught in this course are applicable to most biomedical science graduate programs and even advanced undergraduate programs (Caldwell *et al.*, 2004; Novo *et al.*, 2005), and different techniques or skill sets could easily be substituted in adapting the course to a particular department.

DESIGN

Expected Outcomes

DUSOP pharmacology/toxicology graduate students are expected to learn the rationale behind several cutting-edge neuropharmacological and toxicological techniques during the lecture component of the course, "Methods of Evaluation of Drug Action and Toxicity" (GPHSC 572). Through the laboratory component, students are expected to identify specific types of research design, determine the appropriate statistical method for a given experimental design, and demonstrate proper record keeping via laboratory notebooks, reports, and computer spreadsheets. Students are expected to demonstrate competence in performing the various "wet bench" techniques and to comprehend, analyze, and evaluate their results. Overall, the students should 1) develop at least rudimentary skills in several methods that underpin basic research in the fields of pharmacology and toxicology and 2) gain sufficient exposure to new techniques that can be potentially incorporated into their thesis research or career path.

Educational Environment

The pharmacology/toxicology department is a component of the Division of Pharmaceutical Sciences of the DUSOP. All eight participating faculty members are members of the division; five members have pharmacology/toxicology primary appointments. A senior graduate student served as the teaching assistant (TA) for laboratory exercises, but all laboratories were led by the appropriate faculty member. The faculty preparation time averaged 2 h/wk. The TA was responsible for preparing the experiment in the laboratory of the faculty member teaching that day, using the facilities and equipment of that faculty member. All expenses were covered by the Division. The preparation occasionally began the day before the experiment, if cell growth, transformation, or transfection was required. One methodology was typically covered per week in two 1-h lecture sessions and one 3-h laboratory session. The number of M.S. and Ph.D. pharmacology/toxicology graduate students enrolled in the course ranges from six to eight, half of the number of departmental students because the course is offered every other year. The Spring 2005 course contained six students: two from the United States, three from India, and one from Nigeria. All of the foreign students held student visas. Only one of the six possessed the M.S. degree and was thus a Ph.D. candidate. The remaining five were automatically M.S. candidates, although some indicated their intent of continuing toward the Ph.D.

Course Components

Section 1: Introduction to Science. *Objectives.* To outline the components, design, and assessment methods used in the course and to discuss aspects of science itself.

Design. The first class included an introduction to the course, detailing the rationale for offering the course and justifying its designation as a degree requirement, rather than an option. Details were presented regarding the class schedule, expectations for laboratory experiences, class participation, and examinations. The second half of the class was devoted to a summary discussion of the character of science as an approach for discovering the properties of the natural world. The role of experimentation was emphasized as distinguishing science from other philosophical approaches to knowledge.

Section 2: Ethics. *Objectives.* To become familiar with the classical ethical dilemmas associated with science and to be able to recognize appropriate courses of action if exposed to such dilemmas in their careers.

Design. A 90-min lecture discussed morals, ethics, and ethical dilemmas that may arise in the laboratory and elsewhere in the current and future professional experience of the student. Topics of discussion included the use of animals in research, proper data recording as well as its falsification, and plagiarism. The role that the cultural experience or religious beliefs of a scientist plays in making moral or ethical judgments was also discussed. Each student was given a packet of reading materials that described historical cases of scientific fraud as well as dilemmas that scientists encounter when publishing their data. The students were required to answer questions associated with the readings and give their opinions on specific ethical dilemmas.

Section 3: Experimental Design. *Objectives.* To introduce the students to several standard approaches to designing a scientific study.

Design. Four 90-min lectures were devoted to this topic, reinforced with assigned readings from *Principles of Experimental Design for the Life Sciences* (Selwyn, 1996). Approaches included the "completely randomized design," "randomized block design," "cross-over studies," and "sequential designs." Appropriate statistical analyses for each experimental design method were identified and discussed. The lectures additionally addressed types of data, sample size, accuracy, precision, bias, significance, and the various types of experimental controls. Experiments utilized parametric and nonparametric data. The goal of the parametric data study was to determine if rats repeatedly exposed to pentobarbital would experience a reduction in induced sleep time. Aspects of the experiment especially relevant to the experimental design were intersubject variability, the use of controls, and the application of a repeated-measures design and appropriate statistical analyses. For the study utilizing nonparametric data, the goal was to determine if the frequency of blond hair between men and women on campus differed. The students needed to define "blond hair," design the study in such a way as to avoid bias, determine the number of experimental subjects needed to provide adequate statistical power, and perform the appropriate statistical analyses.

Section 4: Dose-Response Curves. *Objectives.* To learn to interpret, analyze, and graph concentration- or dose-response curve data.

Design. A 90-min lecture dealt with construction of concentration- and dose-response curves for drug-receptor binding, emphasizing the difference between the two. The concepts of drug potency and efficacy were thoroughly discussed, and antagonist classes (e.g., functional, competitive, and reversible and irreversible noncompetitive) were differentiated.

Section 5: Sample Solution Preparation. *Objectives.* To learn proper laboratory technique for preparing solutions for experimental assays.

Design. An initial 2-h "refresher" lecture discussed quantitation options including molarity, molality, normality, wt/vol and vol/vol percentages, and suspensions. The discussion covered relevant factors such as drug solubility and choice of vehicle and addressed methods of enhancing solubility. The procedure for preparing serial and other dilutions was demonstrated, and proper preparation of a radioactive solution was addressed as a segue to the "receptor binding of radioligands" section. In the laboratory, the students performed the necessary weighing, calculations, and titration to prepare a solution of a specific concentration and pH.

Section 6: Animal Handling and Pharmacodynamic Screening. *Objectives.* To introduce students to the proper procedures for procuring and handling animals and to examine different routes of drug administration.

Design. Students were given documentation regarding the proper procedures for procuring and handling animals. Guidelines from the Office of Animal Welfare were provided, and the operations and requirements of the Institutional Animal Care and Use Committee were explained. In a single laboratory session, students were asked to detect the onset of action of a hypnotic dose of pentobarbital, a general anesthetic, in rats after administration by five different routes: oral, intraperitoneal, subcutaneous, intramuscular, and intravenous. After the instructor demonstrated proper technique for each of the administration routes, the animal handling and administration skills of the students were observed. The drug onset of action was determined by the loss of the animal's "righting reflex" (ability to bring the body into normal spatial position and to resist forces that perturb this), and the duration of action was determined by noting the time required to regain the righting reflex. Students worked in pairs, and group comparisons of data were analyzed.

Section 7: Behavioral Testing. *Objectives.* To review various methodologies used to assess animal behavior and to provide additional experience in animal handling and drug administration.

Design. Behavioral testing paradigms associated with neurological functions (e.g., "sleep time," presence/absence of righting reflex) were discussed, with emphasis on memory function, neuromuscular coordination, locomotor activity, sedation, sleep deprivation, anxiety, and depression. Rats were intraperitoneally injected with sodium pentobarbital, and the duration of loss of righting reflex was recorded. For the next 2 d, three rats were injected with pentobarbital as on day 1, and the other three rats were injected with saline. On day 4, all six rats were again injected with pentobarbital, and the duration of the loss of righting reflex was recorded. Parametrical statistical tests were used to compare the duration of the loss of righting reflex on day 1 versus day 4 for the chronic pentobarbital exposure group and the saline control group. Students were required to speculate on potential sources of variability in the responses of individual animals and on mechanisms of action that could account for the responses in the two treatment groups.

Section 8: Analytical Methods. *Objectives.* To encourage student understanding of analytical techniques potentially applicable to a thesis research project.

Design. Four lecture hours and two laboratory hours were needed for this section. A single methodology, UV-visible light (UV-Vis) spectroscopy, was utilized as a vehicle for developing questions that the student should ask before data interpretation. The example technology (UV-Vis spectroscopy) was also discussed with respect to limitations, sampling considerations, interferences, and data interpretation. Students generated a calibration curve using acetamin-

ophen as the analyte and were asked to assess the analytical capabilities of UV-Vis spectroscopy for acetaminophen with respect to limit of detection, limit of quantitation, and linear range. After this assessment, students used this method to determine the concentration of unlabeled sample solutions of acetaminophen. Interpretation of the data with respect to the strengths and limitations of the method was emphasized.

Section 9: Formal/Scientific Writing. *Objectives.* To improve student formal/scientific writing skills with respect to poster presentations, manuscripts, theses, grant applications, and curriculum vitae.

Design. Four lecture hours comprised this section. The lectures were unconventional in that the students frequently participated by debating which of two or more alternative versions of a given sentence or paragraph represented formally written prose. Because English was not a first language for several students, it was prudent to spend the large majority of the lecture time on basic English writing skills, leaving much less time for coverage of "science writing." These students occasionally displayed an unorganized, "stream-of-consciousness" writing style (Surratt, 2006). Higher-order writing aspects such as parallel sentence/paragraph structure and proper placement of participles were also discussed. The science writing portion of the section stressed using passive verb tenses to indicate that the data and results are recorded history and not ongoing developments. Avoiding first or second person writing was also emphasized.

Section 10: Bacterial Transformation. *Objectives.* To transform bacterial cells with a plasmid cDNA and to amplify, purify, and characterize the plasmid in preparation for transfection of mammalian cells.

Design. The "heat shock" transformation method (Hanahan, 1983) was used to introduce a plasmid cDNA encoding an antibiotic resistance protein and either a wild-type melatonin receptor or dopamine transporter into Ca^{2+}-competent bacterial cells. Discrete colonies of the antibiotic-resistant transformants were isolated by overnight growth on antibiotic-impregnated agar plates. The bacterially harbored amplified plasmid was purified via alkaline lysis and ion-exchange chromatography. Purified plasmid was qualitatively and quantitatively characterized using agarose gel electrophoresis and UV spectroscopy, respectively, specifically assessing percent supercoiled DNA, removal of RNA, nucleic acid-to-protein ratio, and plasmid concentration.

The instructor presented the rationale behind the experiment or a given technique during the several 20–40-min breaks during ongoing incubation, centrifugation, or precipitation steps. Students received detailed lecture notes that corresponded to the short presentations. Alternative transformation methods (e.g., electroporation) were discussed. The overarching theme of these discussions was how bacterial transformation provides unlimited quantities of a cDNA for mammalian cell transfection, allowing characterization of wild-type or mutant brain receptor ligand binding and activation.

Section 11: Mammalian Cell Transfection. *Objectives.* To learn to propagate mammalian cell culture monolayers via trypsinization and to transfect these cells with a plasmid cDNA.

Design. A confluent monolayer of COS-7 (immortalized monkey kidney) cells was detached from the culture dish with limited trypsin digestion; the cell suspension was diluted so that a 30% confluent monolayer would be attained the next day (monitored by light microscopy). Cell monolayers were transfected via the "calcium phosphate" method (Graham and van der Eb, 1973) with the melatonin receptor or dopamine transporter plasmid purified during the bacterial transformation section. Transfections employing a melatonin receptor–green fluorescent protein fusion plasmid were conducted in parallel so that students could see microscopic evidence of a successful procedure within 2 d, in the form of fluorescent green cells expressing the fusion protein.

As with the bacterial transformation section, the allotted lecture hours were spent in the laboratory so that the instructor could discuss the rationale behind the experiment or a given technique during ongoing incubation steps. Detailed lecture notes were disseminated at the beginning of the section. Alternative transfection procedures were discussed, including DEAE-dextran, cationic liposome, and electroporation methods. As explained to the students, the melatonin receptor–transfected cells could have been used in radioligand-binding assays covered in the next section.

Section 12: Receptor Binding of Radioligands. *Objectives.* To learn how to perform and analyze radiolabeled drug saturation of receptor binding sites, in turn learning the skills of cell membrane isolation from tissue, protein quantitation, and radioligand binding data nonlinear regression analysis.

Design. An initial 90-min radioligand-binding lecture covered competition binding of drugs at a receptor, drug-receptor association and dissociation kinetics, and analysis of drug saturation of receptors. The remainder of the section was spent in the laboratory. Students were expected to determine if the affinity (reflected by K_d value) or total binding (reflected by B_{max} value) of [2-^{125}I]iodomelatonin at mouse mammary tissue was altered by various treatments. Students prepared membranes from frozen tissue using a modified version of a published protocol (Witt-Enderby and Dubocovich, 1996). The membrane suspension was added to tubes containing concentrations of [2-^{125}I]iodomelatonin, in the absence or presence of an excess of nonradioactive melatonin to measure nonspecific radioligand binding. Bound radioligand was quantitated by scintillation counting, and receptor affinity and density were determined with GraphPad Prism software (San Diego, CA). Statistics were performed using one-way ANOVA followed by a Newman-Keuls post hoc *t* test.

Section 13: Protein Gel Electrophoresis and Western Blot Analysis. *Objectives.* To learn how to perform western blot analysis, in turn learning the skills of tissue sample preparation, protein analysis, polyacrylamide gel electrophoresis, and densitometric blot quantitation.

Design. An initial lecture explained the basics of western blot analysis and how this type of analysis is used in research. The majority of the time was spent on a laboratory experiment designed to assess the effect of various drug treatments on uterine progesterone receptor expression. Students prepared nuclear extracts from frozen tissue, quanti-

tated and normalized protein levels for each treatment group using a detergent-compatible protein assay kit, and loaded samples into the wells of a protein-denaturing polyacrylamide gel. The instructor emphasized proper technique in gel loading so as to avoid cross-contamination of wells and provided notes on polyacrylamide gel electrophoresis. The gel-fractionated proteins were electrophoretically transferred to a polyvinylidene fluoride membrane ("blot"), and this membrane was successively exposed to the nonspecific protein-blocking solution, the primary antibody (rabbit anti-progesterone receptor), and the secondary antibody (goat anti-rabbit IgG linked to horseradish peroxidase [HRP]). Blot proteins were visualized using a peroxidase-activated chemiluminescent probe that exposed x-ray film to yield a banding pattern. Individual bands were quantitated by densitometry. Students were expected to know the apparent molecular weight of the receptor on the blot and how to determine this, the significance of band thickness in comparing treatment groups, and how to interpret the blots with respect to effects of drug treatments on uterine progesterone receptor expression.

Section 14. Fluorescence Spectroscopy. *Objectives.* 1) To introduce the concept and basic principles of fluorescence and to discuss the utility of this analytical technique in modern biochemical, pharmaceutical, and neuroscience research. 2) To recognize the factors that affect fluorescence of molecules, to understand the basic concept of fluorescence resonance energy transfer (FRET), and to apply this understanding to the development of assays for monitoring biomolecular interactions.

Design. Three initial lecture hours focused on the basic properties of fluorescence, FRET, bioluminescence resonance energy transfer, and environmental effects on fluorescence. Sample experimental data from several assays that relied on a particular fluorescence method were presented and discussed, along with the relevant mathematical formulas explaining each outcome. The laboratory focused on the basic types of fluorescence instruments, from filter-based plate readers and imagers to research-grade fluorimeters. Students were taught the basic uses, advantages, and disadvantages of each instrument. Finally, students conducted a simple fluorescence displacement assay to learn the basics of setting up the fluorimeter and acquiring and analyzing data.

Section 15. Enzyme-linked Immunosorbent Assay. *Objectives.* To explain the principles behind enzyme-linked immunosorbent assay and design experiments to quantify soluble proteins using this method.

Design. Students working in groups of two conducted enzyme-linked immunosorbent assay (ELISA) experiments using a DuoSet interferon-gamma (INF-γ) ELISA kit to determine the concentration of mouse INF-γ in previously collected cell culture supernatants. On day 1, the instructor presented principles and assumptions of the assay. Particular emphasis was given to the theoretical underpinning of the technique and practical applications of monoclonal antibodies. Data interpretation and its limitations were also discussed. Each group was assigned a different sample of unknown INF-γ concentration. A plate map was constructed to accommodate serially diluted recombinant INF-γ (standards) and samples in triplicate. Plates were coated with INF-γ capture antibody and incubated overnight. Wells without capture antibody served as the negative control. On day 2, standards and samples were loaded onto assigned spots on the antibody-coated plate. Immobilized INF-γ was detected with a biotinylated antibody and a (biotin-binding) streptavidin-HRP conjugate. INF-γ quantitation was achieved using a colorimetric reaction triggered by the addition of an HRP substrate. Reaction intensity was quantified by measuring absorbance at 450 nm using a Perkin Elmer-Cetus microplate reader (Norwalk, CT). Students determined the unknown INF-γ protein levels by interpolating the absorbance readings within those for the linear standard curve.

Section 16: Histological Techniques. *Objectives.* To learn the techniques and steps involved in obtaining stained tissue sections from an animal.

Design. A short (30 min) initial lecture detailed the approach for obtaining hematoxylin and eosin (H&E)-stained sections of mouse tissue; the remainder of the section was spent in the laboratory. Students learned to excise tissues from a killed mouse for fixation or quick-freezing; the latter tissues were used in Section 17. Both sexes of mice were examined to show the different anatomy. The students performed full necropsies, including cardiac punctures to obtain blood and organ weights. The fixed tissues were processed and embedded in paraffin blocks, with each student having the opportunity to trim, process, and embed the tissues they dissected. Thin tissue sections were cut using a microtome and then H&E-stained and mounted for microscopy. To assess the quality of the sections, pictures of microscope fields for each section were captured using an imaging program.

Section 17: Real-Time Reverse Transcriptase-Polymerase Chain Reaction Analysis. *Objectives.* To learn the steps, procedures, pitfalls, and methods of analysis in performing real-time reverse transcriptase-polymerase chain reaction (RT-PCR), including RNA preparation from frozen tissues, RT reactions, and real-time PCR.

Design. A 90-min lecture provided an overview of the procedures and quantification methods; supplementary reading material was disseminated. The problem of cDNA contamination of normal or transgenic tissues was also discussed. Total RNA was prepared from four different frozen tissues obtained from the Section 16 necropsies. The concentration and purity of the RNA samples were determined by 260-nm absorbance readings and determination of the 260 nm:280 nm absorbance ratio, respectively. RT-PCR was conducted using a two-step method, and the reasons for this choice over the one-step method were discussed in the lecture. In Step 1, the students prepared RT reactions as well as the necessary control reactions lacking transcriptase for each tissue. In Step 2, real-time PCR was performed in triplicate for each RT reaction using the necessary controls with cyclophilin, a "housekeeping" gene (i.e., a ubiquitous, constitutively expressed endogenous gene), and target gene primer/probe sets. For each tissue, several dilutions were tested to determine PCR reaction efficiency for each primer/probe set. Each student was required to load some of the samples to understand first-hand the sensitivity of the procedure to small pipetting errors and contamination. Students were

also expected to perform spectrophotometric, dilution, and gene level normalization calculations.

It is a goal for future iterations of the course that the individual sections be even better integrated. For example, the identical cells transfected in Section 11 with the purified plasmid (Section 10) may one day be assessed for radioligand binding (Section 12), western blotting (Section 13), ELISA (Section 15), and RT-PCR (Section 17).

Assessment

Assessment of Students. As stated in the course syllabus, the two examinations accounted for 60% of the course grade; the remaining 40% was assigned to laboratory reports and class participation. The two exams were administered at the midpoint and end of the scheduled classes and were not cumulative. Exam material consisted of short-answer questions regarding methodological theory and specific technical aspects of a procedure, as well as calculations using data taken from the scientific literature. Each instructor was at liberty to choose between "in class" ("open book," but with a time constraint) or "take-home" (open book) options for administering his/her exam portion. An advantage of the take-home option was the ability to ask questions requiring use of online databases. For example, an exam question from Section 17 required each student to use online programs to find the mRNA sequence for a given gene, to create a primer/probe set for real-time RT-PCR, to determine if the primers crossed an intron/exon boundary, and to check the primers and probe for hairpin structures and similar sequences in other genes. The percentage of an exam devoted to a given section correlated with the class/laboratory time allotted to that section, meaning that each section was similarly represented.

The syllabus also allowed instructors to choose whether or not to require a laboratory report or to assess class participation. It was noticed at the end of the course that those sections not employing these assessment options were therefore underrepresented with respect to the final grade (due to fewer grading opportunities for that section). In retrospect, material from course sections not employing a lab report should have constituted a larger portion of the exams, a problem that will be rectified for the next course offering. No instructor elected to grade class participation in the recently completed course.

Laboratory reports were required for Sections 4–8, 12, 13, and 15–17. The reports typically included detailed descriptions of the procedures used and any qualitative observations (e.g., a visual description or photo of a stained tissue or gel banding pattern), plus a record of all quantitative data collected. Drug or reagent dilutions, normalization of protein, or nucleic acid levels between samples, absorbance reading conversions, and other calculations carried out in the course of a laboratory exercise were included in the report. More sophisticated calculations such as statistical analyses or nonlinear regression analyses were occasionally required. In Section 4, for example, students plotted data points to generate a curve used to derive Schild analysis values. In some cases, sample data were provided for calculations as opposed to using data generated during the laboratory exercise. Reports were also expected to contain informed conclusions drawn by the student regarding experimental outcomes (e.g., the effect of drug treatments on melatonin receptor expression in mouse mammary tissue). When there was a discrepancy between the predicted and observed result, the student was expected to offer an explanation. To prompt such higher-order thinking, the instructor occasionally posed questions to be answered in the lab report.

Student Assessment of the Course. The six students were e-mailed a two-part survey as a Word file. Part 1 consisted of 30 items to be scored on a 5-point, Likert-type scale of 1 ("strongly disagree") to 5 ("strongly agree"). Part 2 consisted of six "short-answer" questions (items 31–36). To ensure the anonymity of the respondents, students were instructed to type their responses to items 31–36 into the Word file itself, print out all pages of the survey, circle one number for each of items 1–30, and slide their completed, anonymous surveys under the office door of the corresponding author. Five of the six students completed the survey. The means and SDs of all five respondents for each of items 1–30 in Part 1 were calculated (Table 1). The six short-answer items in Part 2 (Table 2) allowed respondents to elaborate on the Part 1 numerical responses and offer opinions and suggestions regarding the course.

The first 15 survey items correspond to 15 of the 17 sections described above. Sections 2 (ethics) and 4 (dose-response curves) were regrettably not explicitly addressed because they were part of Section 3 (experimental design) in the syllabus, which guided construction of the survey. Almost all sections were viewed positively (mean score > 3.0) and some very positively (defined as a mean score > 4.0). The highest scores were associated with the ELISA, fluorescence spectroscopy, and solution preparation sections (Table 1). The results were largely consistent with responses to the short-answer items that queried which sections were strongest or weakest (Item 34) and what was best liked about the course (Item 36). For these two items, three students singled out specific sections as being particularly strong or well liked; these included fluorescence spectroscopy and ELISA, the sections with the highest mean score and lowest SD (Table 1, items 12 and 13). Interestingly, all three students identified the formal/scientific writing section as strong or best liked, even though Item 7 scored slightly lower than items 12 and 13. One possible explanation is that the other two students were already skilled in this area and viewed this section as less critical.

It is notable that the behavioral testing section (item 5) received a neutral mean score, but the SD was unusually high. This may reflect the longstanding observation among the faculty that student views diverge dramatically on conducting actual experiments with live animals. The individual scores for this item were 5, 4, 4, 1, and 1, indicating that students were very positive or negative about this section. Ironically, these bipolar scores yielded the neutral mean score of 3.0. Only Section 17 received a negative rating (item 15), and comments in response to item 34 indicated that the lecture material for this section (RT-PCR) was perceived to be too advanced for the audience; i.e., it was incorrectly assumed that the students possessed a basic knowledge of the technique. Another issue cited as a negative aspect of this section was that the PCR machine was malfunctioning, delaying completion of the section.

Table 1. Responses of Spring 2005 pharmacology/toxicology students to each exit survey item in Part 1

Item no.	Item This course provided a useful introduction to the technique, method, concept, or discipline of:	Responses
1.	Experimental design.	3.6 ± 0.9
2.	Statistical analysis.	3.3 ± 0.7
3.	Sample solution preparation/handling.	4.4 ± 0.9
4.	Animal handling.	3.6 ± 1.1
5.	Behavioral testing.	3.0 ± 1.9
6.	Instrumental analysis.	3.6 ± 0.9
7.	Formal/scientific writing.	4.2 ± 0.8
8.	Bacterial transformation.	4.0 ± 1.2
9.	Mammalian cell transfection.	4.0 ± 1.2
10.	Radioligand binding.	3.6 ± 0.9
11.	Protein gel electrophoresis.	4.2 ± 1.3
12.	Fluorescence spectroscopy.	4.4 ± 0.5
13.	ELISA.	4.6 ± 0.5
14.	Histology.	3.8 ± 1.1
15.	RT-PCR.	2.2 ± 0.8
16.	The course was taught at a level appropriate for a first/second-year graduate student.	3.4 ± 0.9
17.	The course stimulated my interest in new research areas.	3.8 ± 0.8
18.	I learned enough from this course to incorporate these methods and techniques into my thesis research.	3.8 ± 1.3
19.	The lecture sections were useful orientation to the lab work.	4.0 ± 1.4
20.	The lab protocols and experiments worked as advertised.	3.8 ± 0.8
21.	The lab equipment and materials provided were sufficient for conducting high quality experiments.	4.0 ± 1.2
22.	The lab reports enhanced my analytical and record keeping skills and helped me appreciate the overarching goal of the experiment.	3.4 ± 1.5
23.	The examinations were fair in that the tested material was appropriate and enough time was allotted.	3.7 ± 0.8
24.	An appropriate amount of time was devoted to each section.	3.6 ± 0.9
25.	The instructors were knowledgeable in their respective subjects.	4.4 ± 0.5
26.	The instructors were fully prepared to teach their section(s).	3.4 ± 0.9
27.	The instructors were enthusiastic about teaching their section(s).	4.0 ± 1.2
28.	This course should remain a core requirement for a graduate pharmacology/toxicology degree.	4.8 ± 0.4
29.	Overall, this course achieves the goals and competencies stated in the syllabus.	4.2 ± 0.8
30.	I would recommend this course as an elective for a Pharm.D. research track student.	4.0 ± 0.7

Responses were scored on a 5-point, Likert-type scale of 1 = strongly disagree to 5 = strongly agree. Data are shown as mean ± SD.

A review of the remaining items in Table 1 indicates that the students viewed the course positively with respect to building research skills and interests: most item ratings were in the vicinity of "4." The score of 4.2 for item 29 suggests that the course largely achieved its goals. Importantly, the almost perfect score of 4.8 for item 28 indicates that the five respondents recognized the necessity of the course toward a basic graduate pharmacology/toxicology education. Interestingly, the students acknowledged that the instructors were well versed in the material they taught (item 25, score of 4.4 ± 0.5), but were possibly less convinced of instructor enthusiasm (item 27, score of 4.0 ± 1.2) and apparently less impressed by instructor preparation (item 26, score of 3.4 ± 0.9). It should be noted that the relationship between the three means appears to constitute a trend, as opposed to a statistically significant difference. Item 16 is another area of concern, the score (3.4) suggesting that overall lecture content should be simplified. The score for this item isperhaps contradicted, how-

Table 2. Part 2 of exit survey: short-answer items

Item no.	Item
31.	Is there a methodology, technique, or area of research that you believe should be added to the course?
32.	Would you like to have spent more time on a particular section of the course?
33.	Do you believe that too much time was spent on a particular section of the course or even that a portion of the course is dispensable?
34.	Were there sections that you felt were especially strong or weak? If so, which sections?
35.	Do you have other suggestions toward improving the course?
36.	What did you like best about the course?

ever, by the 4.0 score for item 30, the latter score suggesting that the level of course material is suitable for undergraduate pharmacy students pursuing the "research track" concentration option (Surratt *et al.,* 2005).

Respondents were generally satisfied with course content. The only suggested addition (item 31) was a section on high-pressure liquid chromatography. Through items 31 and 32, students also recommended in-depth coverage of cell culture techniques and statistical analysis. No section was considered dispensable (item 33), but it was suggested that the formal/scientific writing, sample preparation, histology, and RT-PCR sections were longer than necessary. Each of these areas was cited only once, and no suggestions were offered on how these sections should be reduced. Regarding other suggestions toward improving the course (item 35), it was mentioned that the instructors could have communicated better among themselves in organizing the sections and in establishing common standards on what was expected of the students. An interesting but unfeasible suggestion was to extend the course to the whole academic year, with each student 1) independently completing the sections at his/her own pace by relying on senior graduate students as mentors and 2) participating in traditional lab rotations. Another intriguing suggestion was for the student to choose to participate in a certain number of the offered sections in fashioning a "mini-thesis" that resembled a research project, with an all-inclusive lab report due at the end of the semester. These suggestions are creative but inconsistent with the course goal of providing the broadest palette of techniques for future research-based career opportunities and decisions.

CONCLUSIONS

By tapping the expertise of eight faculty members of the Duquesne University Division of Pharmaceutical Sciences, a methods course has been created that provides first- and second-year graduate students a unique opportunity to develop a variety of pharmacology, toxicology, and neuroscience skills. This approach allows the student to become acquainted with far more bench techniques than afforded by the traditional laboratory rotation format. Via the responses to Part 2 of the exit survey, students expressed their appreciation of the chance to sample many different research methodologies. The first-hand glimpse of the inner workings of each departmental laboratory was also cited as a positive factor. The participating faculty had hoped that this aspect would not be lost on the students, because this feature of the course justifies in part the lack of conventional lab rotations for first-year students. There were also unexpected benefits of the course, as reflected in the responses to item 36. Familiarity with the different techniques used in the department helped one student to better understand the research seminar presentations of fellow students. Another student stated that he/she learned how to be a more effective teacher and that he/she plans to draw from the strengths of the participating faculty in improving his/her own teaching style. The experimental design, ethics, and scientific writing sections should additionally enhance the student's ability to design, write, and execute competitive grant proposals and manuscripts. The training offered by this course should thus be an asset in shaping an academic, industry, or government research career path. This will be assessed in a future survey of Duquesne pharmacology/toxicology M.S. and Ph.D. graduates. More immediately, the course may identify research area preferences or aversions that steer the student toward the most desirable and appropriate career choices.

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