The Journal of Undergraduate Neuroscience Education (JUNE), Fall 2015, 14(1):A46-A55

ARTICLE
Using Tinbergen's Four Questions as the Framework for a Neuroscience Capstone Course

John Meitzen

Department of Biological Sciences, W.M. Keck Center for Behavioral Biology, Center for Human Health and the Environment, Center for Comparative Medicine and Translational Research, North Carolina State University, Raleigh, NC 27695.

Capstone courses for upper-division students are a common feature of the undergraduate neuroscience curriculum. Here is described a method for adapting Nikolaas Tinbergen's four questions to use as a framework for a neuroscience capstone course, in this case with a particular emphasis on neurotoxins. This course is intended to be a challenging opportunity for students to integrate and apply knowledge and skills gained from their major study, a B.S. in Biological Sciences with a Concentration in Integrative Physiology and Neurobiology. In particular, a broad, integrative approach is favored, with emphasis placed on primary literature, scientific process and effective, professional communication. To achieve this, Tinbergen's four questions were adapted and implemented as the overarching framework of the course. Tinbergen's questions range from the proximate to ultimate/evolutionary view, providing an excellent base upon which to teach students an integrative approach to understanding neuroscientific phenomena. For example, a particular neurotoxin can be examined from the proximate level (i.e., mechanism: how does this toxin specifically impact neural physiology) to the ultimate/evolutionary level (i.e., adaptation: why and to what extent did this toxin evolve naturally or the reason that it was initially invented by humans). The mechanics, goals, and objectives of the course are presented as we believe that it will serve as a flexible and useful model for neuroscience capstone courses concerning a wide variety of topics across multiple types of institutions.

Key words: Neuroscience capstone course, Nikolaas Tinbergen, Tinbergen's four questions, neurotoxins, pedagogy

---

Capstone courses are a recommended component of an undergraduate neuroscience curriculum (Wiertelak and Ramirez, 2008), and are increasingly prevalent not only in neuroscience but in many other fields as well. Capstone courses are typically organized around a specific subject, ranging from research topics such as "drugs of abuse" or "color vision" to broad courses that range from incorporating collaborative learning to preparing students to attend the annual Society for Neuroscience meeting (Bucci and Falls, 2007; Kennedy and Hassebrock, 2012; Kurczek and Johnson, 2014; Esteban and Holloway, 2015). What these courses tend to have in common is an emphasis on critical analysis, an opportunity to deeply study one particular topic, readings of primary literature, and presentation skills. Capstone courses are typically taught at the junior/senior level, with student learning outcomes that typically involve integrating, synthesizing, and presenting methods and information gained over the course of his or her undergraduate studies. Given this emphasis on scientific practice over particular scientific content, the opportunity to teach a capstone can trigger a great deal of reflection in the instructor about exactly what aspects of scientific practice to convey in the class, and how to best convey those methods. For the capstone class presented in this article, it was decided to incorporate the scientific questions as a key visible component. To successfully accomplish this, relevant overarching questions needed to be articulated and presented. For that an approach that is core to behavioral neuroscience and especially neuroethology was applied: Nikolaas Tinbergen's four questions.

Nikolaas Tinbergen (1907-1988) was a Nobel prize-winning ethologist who made a number of contributions to behavioral biology, but is most remembered for his articulation of four fundamental questions that he argued should be incorporated into the study of any animal behavior (Tinbergen, 1963), and have since been applied to many other fields as well. These questions focus on four basic biological levels, namely: causation (mechanism), development (ontogeny), function (adaptation), and evolution (phylogeny). Since Tinbergen's original work, these four questions have been repeatedly adapted and modified to create meaningful intellectual frameworks for a number of disciplines and research questions, including neuroscience (Okanoya, 2004; Bateson and Laland, 2013; Gonzalez-Liencres et al., 2013; Stewart and Kalueff, 2015). Here it is described how Tinbergen's four questions were adapted to be the framework of an integrative neuroscience capstone course. This general approach of using modified versions of Tinbergen's questions is widely applicable across a variety of neuroscience-related capstone course topics, and serves as a model by which to incorporate scientific process as a highly visible and key component of course structure.

PRE-COURSE PREPARATION

Student Admission/Level. Capstone courses in the Biological Sciences Department at North Carolina State University are a challenging opportunity for students to integrate and apply knowledge and skills gained from their preliminary studies. Unlike some styles of capstone (Bucci and Falls, 2007), students are not interviewed prior to the course to determine sufficient interest and qualifications. Instead, a fairly rigorous set of prerequisite courses are required, including neurobiology, endocrinology, and either cell biology, statistics, biochemistry, or genetics. These prerequisites create a pool of qualified students that are mostly seniors, with the occasional junior and post-baccalaureate. While many students are participating in undergraduate research, this is not a pre-requisite for the course and is not formally considered in entry decisions. Completing a capstone course is also not currently mandatory for graduation, which helps limit the entry pool to motivated students. To facilitate discussion, provide ample opportunity for multiple student presentations, and create a seminar-style feel, the course enrollment is usually limited to 12-15 students. The course timeline presented here is that used for 12 students.

Elucidating the Learning Objectives. This course's emphasis on scientific communication, reading of original scientific papers and reviews, and developing an intellectual approach to information evaluation and synthesis led to elucidating five specific learning objectives:

1. Integrate concepts, theories, and methods across different areas within neuroscience using the framework of Tinbergen's Four Questions, and apply the knowledge and/or skills acquired through previous coursework in these areas to the specific issues addressed in this course.
2. Evaluate how scientific knowledge is applied in addressing questions about neuroscience.
3. Demonstrate an ability to engage productively in collaborative projects.
4. Work with the instructor to identify topics and areas of interest within the scope of the course to explore and discuss with fellow classmates.
5. Communicate the results of their work in a professional manner, in both spoken, visual, and written formats.

Following the framework of Bloom's Taxonomy (revised), the learning objectives of this capstone course rests firmly in developing the following skills in the Cognitive Domain: Application, Analysis, Evaluation, and Creation/Synthesis (Krathwohl, 2002).

Selecting the Theme. Capstone experiences lend themselves to a wide variety of themes, and one based upon Tinbergen's four questions is no exception. On one side of the spectrum are very general and diffuse themes, for example, "Recent Advances in Neuroscience." On the other side are much more specific themes, for example, neuroethology, addiction and drugs of abuse, animal communication, ion channels, neurotoxins, etc. Any one of these themes would fit well with using Tinbergen's four questions as a method. For this particular course's first and second iterations, a "neurotoxin" theme was chosen. Neurotoxins was chosen as it was a broad enough theme to allow students to pursue individual interests, while still providing enough of a focus to bind the course together. Furthermore, the study of neurotoxins is able to be approached from multiple levels, from the proximate mode of action and differences across development/ontogeny, to higher order, ultimate questions such as the toxin's role in an animal's natural behavior or the evolution of mechanisms of resistance to a toxin. This topic lends itself well to straightforward analysis using the framework of Tinbergen's four questions.

Adapting Tinbergen's Four Questions. Tinbergen's questions have been re-formulated multiple times since their first formal articulation in the early 1960s (Tinbergen, 1963; Bateson and Laland, 2013). As a starting point for modification, the following simplified version of the questions was used:

1. Causation (mechanism): How does the feature/behavior work at the proximate level?
2. Ontogeny (development): How does the feature or behavior develop within an individual across the lifespan?
3. Phylogeny (Evolution): Why and to what extent has the feature/behavior evolved in and across species over many generations?
4. Adaptation (function): In what way (if any) does the feature/behavior serve an adaptive function?

These four basic questions were then modified by the instructor to build a framework for investigating neurotoxins. Some questions, such as the one dealing with mechanistic causation required very little modification. Others were subdivided into two related questions, such as ontogeny. Other questions required more intense revision, given that the class covered both natural and man-made toxins. The final version used was this:

1. Causation (mechanism): How does the neurotoxin work at the cellular and molecular levels?
2. Ontogeny (development): How does production of the neurotoxin vary by an organism's lifespan? Does sensitivity to a neurotoxin vary across an organism's lifespan? This question is particularly pertinent to compounds that compromise fetal development.
3. Evolution/Adaptive Function/Original Function: Why and to what extent did a species evolve this neurotoxin? What adaptive significance does the neurotoxin have? In the case of a man-made neurotoxin, why was this neurotoxin originally invented or used?
4. Application: How can the neurotoxin be used, either commercially or not, for the betterment of society?

Collectively, these four questions guided the students in their investigations of particular neurotoxins. While in this case the questions were articulated by the instructor prior to the beginning of the course, students could also potentially work in groups to modify the four basic questions to apply to neurotoxins (or whatever the course topic).

COURSE FORMAT

Four distinct course phases. The course was organized into four distinct phases, the introductory phase, the paired oral presentation phase, the solo oral presentation phase, and the poster phase (Figure 1). The first phase was an introductory period that focused on three learning objectives: 1) that students knew and understood who Nikolas Tinbergen was, his four questions, and how they can be adapted to study neurotoxins and other subjects, 2) that students had an adequate background knowledge in basic neuroscience (i.e., the ionic mechanisms underlying the action potential, synaptic transmission, etc.), and 3) that students understood the difference between a scientific manuscript and review and could find them using databases such as Pubmed, Web of Science and Google Scholar. These goals were accomplished via short lectures, study questions, and in class group activities. For example, to reinforce the differences between a scientific manuscript and a review, a stack of intermixed papers were distributed to student pairs. The students then had to decide whether each manuscript was a manuscript or a review, and then support this with verbal argumentation to the rest of the class. At the end of this period achievement of learning objectives was assessed via an in class small group project on basic neuroscience. This was the equivalent of a final exam given during one of the pre-requisites of the course, Neurobiology. Students were allowed to work together, but not use outside sources. The first phase typically lasted ~2 weeks across four class meetings.

The second phase consisted of oral presentations by student pairs on neurotoxin topics pre-selected by the instructor, which will be described in more detail below. In the original version of this course students were immediately required to do individual presentations. However, it was found that many of the students had never performed solo hour-long presentations, and that a training period when students presented in pairs was helpful. The two students together selected the manuscript and review, created the presentation, and lead class discussion. Students who were not presenting were required to turn in a one paragraph reading analysis of the assigned literature. This could be typed or handwritten. A regurgitation of the study's findings was not acceptable. Rather, the students were encouraged to write about whether the paper's findings were convincing, whether it sparked the student to do further research on a particular topic, or how the paper fit into Tinbergen's four questions. Overall, the reading analysis was a necessary mechanism to help ensure that students accomplished the required readings during the two oral phases of the class. This first oral phase typically occurred over ~3 weeks across six class meetings.

The third phase was solo oral presentations. This followed the same format as the presentations given by student pairs, except that a single student was responsible for all aspects of the presentation. Reading analyses by non-presenting students were still required. This phase typically lasted ~6 weeks across 12 class periods.

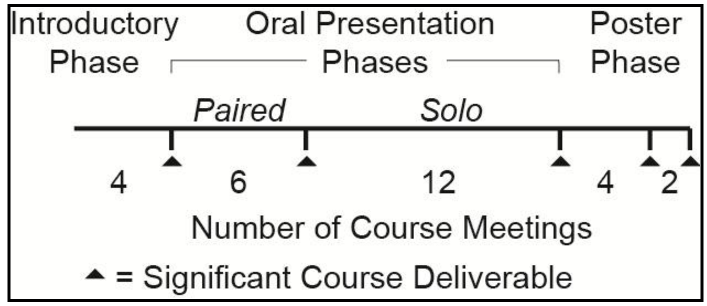

Figure 1. Course Format: four distinct phases. The first phase was introductory, and emphasized basic neuroscience and Tinbergen's four questions. The second two phases were the paired oral presentation and solo oral presentation. Here the students gave the class a one hour presentation on the neurotoxin of their choice, based around Tinbergen's four questions and incorporating a "journal club" style approach to presenting a research article. Students first presented in pairs and then solo. The last phase was the poster phase, where students prepared and presented a poster on their topic at a large symposium, and finished the course by submitting an analysis paper. Triangles indicate major course deliverables, including (in chronological order) an in class small group project on basic neuroscience, paired and solo oral presentations, poster presentation, and final analysis paper.

The fourth phase focused on poster presentations and, to a lesser extent, the final student-written analysis paper. During this period, poster design was taught; students practiced and critiqued each other's posters during class, and made short reports on the progress of his or her final analysis paper. This phase culminated in a shared poster symposium that included all of the capstone-style courses offered by our department (Figure 2). In 2015 the symposium was 2 hours long and included 68 students from 7 different classes. Many students brought friends and family members, and the symposium included refreshments and the taking of class pictures. Overall the fourth phase, including the final symposium, typically lasted ~2 weeks across 4 class periods. We will now cover the oral presentation and poster phases of the class in greater detail.

ORAL PRESENTATION PHASES

Class Meeting Format. During the oral presentation phases, the presenting students were encouraged to arrive early for setting up and troubleshooting his or her presentation. Class always began with an activity that focused on communication skills, the student's written reading analyses, or Tinbergen's four questions. For example, since the meeting room was equipped with two large dry erase boards and a smartboard, students might be placed in groups of 2-4, sent to the boards and challenged with questions ranging from very broad (i.e., "What makes a good scientific experiment?") to more

Figure 2. Poster Symposium. At the end of the semester students presented their projects in a poster symposium. This symposium incorporated multiple capstone classes, with attendance from other departmental members, college-level administrators, student friends and parents.

specific (i.e., "What is the proximate mechanism of tetrodotoxin?"). Other starting activities included each student presenting a 30 second summary of their reading analyses, accompanied with 20 second responses from other students, or neural jeopardy. The intention was to rapidly focus the student's attention onto the class at hand while practicing their communication skills, a learning objective of the course. This activity typically took ten minutes to complete. After this any course business was addressed. This typically involved reminding students of approach due dates and discussion topic/paper submissions. Finally, the discussion leader(s) were introduced and began his or her presentations.

Selecting Topics and Leaders for Presentation, Readings, and Discussion. The primary challenge for the oral presentation phase was devising a way to assign topics and student discussion leaders. An important goal of the course was to allow students to select topics that directly built on their previous coursework and were relevant to their personal interests and career goals. Thus, it would not be appropriate for the instructor to select all topics for discussion. On the other hand, "neurotoxins" is a general topic, and many students did not have previous experience with selecting scientific papers and reviews, or with the hour-long presentations expected from them. To balance these demands, a hybrid model for the selection of topic, discussion leaders, and scientific papers was developed. This model incorporated a list of topics pre-approved by the course instructor for the paired student's first oral presentation. It also allowed the students to select their own topics for the second solo presentation.

NC State operates on ~16 week semester academic calendar, which permits ample course time for ~6 initial paired student presentations, and ~12 presentations that are presented by a single student. This is based on the course meeting twice a week for 75 minutes per meeting. Students signed up for their first and second oral presentation dates on the second course day. This was done in case students dropped after the first course session (although no students dropped the course in either Spring 2014 or 2015 semesters), and to give the students time to pick a peer presentation partner for the first presentation. It was also important to the students to have adequate time to investigate the various topics, and to receive other class and work schedules to determine the optimal presentation dates. For both paired and solo student presentations, the topic and readings were required to be pre-approved by the instructor at least one week in advance of the presentation. Most students opted to have their readings approved several weeks before the presentation. Readings were one scientific review and one scientific research paper. Most students selected several potential manuscripts and solicited instructor advice on which one to present to the class.

For the paired presentation, the two student presenters chose a topic from a pre-approved list, and were provided with example manuscripts. Most students worked with the provided manuscripts, although a few students selected different papers which were then approved by the instructor. These topics were chosen by the instructor prior to the start of the course. These initial toxins had all been extensively studied, were easily investigated using Tinbergen's four questions, and, most importantly, show-cased the wide variety of topics available within the overall theme of the class. For Spring 2014 and 2015, these included: tetrodotoxin (TTX) to illustrate the use of toxins in fundamental research (Lopez-Santiago et al., 2006; Chau et al., 2011; Bane et al., 2014; Itoi et al., 2014; Dorris et al., 2015), botulinium toxin (Botox) to illustrate biomedical applications (Favre-Guilmard et al., 2009; Wheeler and Smith, 2013), accidental Parkinson's Disease via MPTP (Przedborski et al., 2004; Hare et al., 2013; Aguiar et al., 2014), conotoxins to link animal behavior to biomedical research (Sircar and Kim, 1999; Lewis et al., 2012; Liu et al., 2013), scorpion toxins and resistant grasshopper mice to illustrate evolutionary neurotoxin arms races (Momin and Wood, 2008; Rowe et al., 2013), and "zombie caterpillers and voodoo wasps" to illustrate the numerous toxins involved with parasite manipulation of a host's nervous system (Beckage and Gelman, 2004; Grosman et al., 2008; Gal and Libersat, 2010; Weinersmith and Faulkes, 2014). Please note that all paper references both here and in the following paragraph are to those manuscripts chosen by students to discuss in class. Some of these manuscripts were outstanding, and some were not. All provided excellent opportunities for students to learn about reading, presenting, and critiquing primary scientific literature.

In contrast to the first oral presentation, the second presentation featured a student-selected topic and readings, and was solely presented. These topics had to be approved by the professor a minimum of one week in advance of the presentation, and featured a variety of topics driven solely by student interest. The breadth of the student-driven research topics was outstanding, and featured topics that the instructor would have never dreamed of tackling. Over the two iterations of the course these included lytico-bodeg disease (Wilson et al., 2002;

Steele, 2005), toxiplasmosis (Webster, 2007; Prandovszky et al., 2011), various drugs of abuse such as ethanol, nicotine, and methamphetamine (Dani et al., 2001; Krasnova and Cadet, 2009; Brust, 2010; Broadwater et al., 2014; Huang et al., 2014; Rau et al., 2014), metals such as mercury and lead (Ventura et al., 2005; El-Sherbeeny et al., 2006; Jelliffe-Pawlowski et al., 2006; Korbas et al., 2008), arsenic (Smedley and Kinniburgh, 2002; Karsy et al., 2014), latrotoxin (Ushkaryov et al., 2004; Mesngon and McNutt, 2011), fish-related venoms and toxins (Benoit et al., 1996; Church and Hodgson, 2002a; Church and Hodgson, 2002b; Stewart et al., 2010), cnidarian venoms (Cuypers et al., 2006; Tibballs et al., 2011), venoms from snakes (Schweitz et al., 1990; Sampaio et al., 2010; Diochot et al., 2012; Wang et al., 2012; Baron et al., 2013), nerve agent VX (Joosen et al., 2010; Joosen et al., 2013), sarin (Abu-Qare and Abou-Donia, 2002; Allon et al., 2011), the role of microglia in neurotoxicity (Block et al., 2007; Loane et al., 2009), rabies virus (Ugolini, 2011; Zampieri et al., 2014), applications of excitotoxicity (Pape and Pare, 2010; Nabavi et al., 2014), fracking waste (Huerta-Rivas et al., 2012; Webb et al., 2014), and disease-induced neurotoxicity such as HIV and Parkinson's Disease (Kramer-Hammerle et al., 2005; Thomas and Beal, 2007; Liu et al., 2013; Zhao et al., 2013). Overall these topics generated an exciting course that catered to individual student strengths and interests, with the common intellectual approach of employing Tinbergen's Questions to unify the course, as emphasized in the presentation format.

Presentation Format. Student oral presentations were expected to last ~1 hour and included slides presented using programs such as Powerpoint or Prezi, movies, and class discussion questions. The presentations were typically divided into two parts. First, background material on the neurotoxin in question was presented, using Tinbergen's four questions as the overall framework and usually drawing heavily on scientific reviews. This was typically ~10 minutes of the presentation. The second part of the presentation revolved around the scientific paper assigned by the student to the class to read. The students were expected to establish why he or she picked the particular paper, how the paper fit into Tinbergen's four questions, the paper's central hypothesis and present an argument as to why the other class members should care about the papers topic and findings. The student then presented and lead discussion on each figure. Some students presented the paper's methods as a separate section, but most opted to discuss the methods relevant to each figure. As students presented each figure, they were encouraged to analyze what hypotheses the authors were testing, to understand how the data were generated and how the data is being visually depicted, and finally, whether the authors successfully tested the hypothesis. It was important to emphasize that no figures in a scientific paper could be skipped, unless there was a specific, defendable reason that was clearly stated. Not understanding the figure was not an acceptable reason. After each figure was presented, the student then presented the discussion section, with a strong emphasis on whether the authors actually tested the stated hypothesis of the paper. Communicating these expectations to the students was facilitated by the distribution of guidelines and a sample rubric the first day of class (please see supplemental materials for sample rubrics).

The most successful presentations were those when the student was obviously enthusiastic about their subject material, incorporated movies or interactive contact during the background phase of the presentation, and specifically incorporated discussion questions into the presentation. Another important component of a successful student presentation was the instructor carefully limiting his or her own discussion. The best classes were when the instructor limited comments to fixing factual errors, to pointing out controls, to provide other supporting or contradictory evidence, to ask the students questions to help make conceptual connections, or to answer specific student questions. Of course, a class like this depends on students actually discussing. In our experience, the combination of an introductory activity coupled with a student leader was sufficient to stimulate meaningful discussion. This potential challenge is discussed in more detail below.

POSTER PRESENTATION PHASE

Poster format, practice, and presentation. After the oral presentation phase, students then presented his or her project via a scientific poster. A poster presentation was included as part of the class to help fulfill the fifth learning objective, "Communicate the results of their work in a professional manner, in both spoken, visual, and written formats." Students were encouraged to present on the same overall topic that they chose during the oral presentation phase of the course, and topics typically ranged from a critical analysis of a single scientific paper to presenting a specific neurotoxin within the context of all four of Tinbergen's four questions. The subject of the poster was kept flexible to allow students to present their best work within the context of their project. Whatever the particular topic, students were expected to present research from the primary literature, critically evaluate whether that research successfully tested experimental hypotheses, the overall conclusions, and the student's proposed future directions for that topic or research line. Resources for making posters (i.e., written guidelines, templates, useful websites, etc.) were provided on the class webpage, and sample posters from previous classes were made available to the students. Poster drafts were practiced and critiqued during the last two class periods before the symposium.

During the first iteration of teaching this course we discovered that this relatively short turn-around time only works if a complete and graded poster draft in Powerpoint, Illustrator, or Pages format is due the first day of the poster critiques. This was graded on a simple pass/fail basis, i.e., if the student came to class with a draft then he or she received a pass. To ensure this occurred, on the first poster practice day student presenters were randomly selected (literally picking names out of a hat). Students then gave their poster presentation with a strictly enforced three minute time limit. Student posters and presentations were then peer-critiqued, with the instructor giving an occasional comment. Typically six students presented their draft posters during a single class period. After this presentation, most students were required to make revisions to their poster and the revised poster was emailed to the instructor for final approval. Students were then responsible for printing their poster using large-format printers located in the NCSU libraries. The cost of this printing was covered by the department. Overall, this mechanism worked well to ensure high-quality posters with concise presentations at the large poster symposium at the end of the semester. The first time the poster symposium was held it was relatively informal. Due to student feedback, in 2015 the symposium became shorter, better organized, and much more formal (Figure 2). The symposium combined all capstones and other relevant classes. The entire department, college-level administrators, students, friends and parents were invited to attend. Students typically took this assignment very seriously, most likely because of the public nature of this symposium, along with the realization of presenting along with their peers from other classes.

FINAL ANALYSIS PAPER

Final Paper. The final graded deliverable of the course was the capstone paper. This was an 8-10 page, analysis-driven paper focused on the student's subject of choice. This was usually the same subject the student focused upon during the oral and poster presentation phases of the course. Students typically worked on the manuscript throughout the semester, and it was due ~10 days after the poster presentation. The particular structure of the paper was flexible, in order to give the students the freedom to pursue their particular interest or question. For example, some students chose to equally address each of Tinbergen's four questions in the context of his or her neurotoxin. Others highlighted a particular question, usually within the context of critically evaluating a particular scientific manuscript and proposing a comprehensive future line of research or application. Common to all is the expectation of a critical analysis of at least one research paper, consistent with the emphasis on analysis and critical thinking, not to blindly regurgitate results from particular papers. This assignment had the biggest variability with it came to grade outcomes, which is not surprising since paper revisions received the least amount of class time.

DELIVERABLES AND ASSESSMENT

Deliverables and Assessment. The grading of this course was distributed across assignments: the in class group project on basic neuroscience (5%), oral presentations (5% for the shared presentation, 15% for the solo presentation), reading analyses (20%), poster presentations (5% for the draft poster practice and critiques, 15% for the final poster), final analysis paper (15%), and general class participation (15%). Rubrics were helpful in grading some course deliverables. Also reflected in the assessment of this course was attendance, which was mandatory (discussed further below). Excused absences, and one, no questions asked, unexcused absence did not incur a grade penalty. Additional unexcused absences resulted in the drop of a letter grade (i.e., A+ to A).

OUTCOMES

Students report multiple outcomes for this class, as assessed via student course evaluations. On the whole, student feedback was very positive. In both spring semesters 2014 and 2015, student responders agreed with the statement "Overall, the course was excellent" with a mean of 5.0 (out of a 5-point scale, with 5 being the most positive). Similarly, student responders agreed with the statement "This course improved my knowledge of the subject" with a mean of 5.0. Students routinely praised the small class size and lively discussions. This may partly be due to the nature of the majority of science classes at NC State. Many classes are fairly large and feature didactic lectures, although positive strides have been made with the implementation of freshman seminars similar to other universities (Willard and Brasier, 2014), the biotechnology program (Miller et al., 2009), the research packtrack program (Hawkins and Ferzli, 2014), "scale-up" classes (Gaffney et al., 2008), and incorporating active learning techniques into standard lecture formats. Students also appreciated the exposure to critical analysis, and the application of Tinbergen's four questions to integrate across multiple scientific questions and levels of analysis. Another important outcome was the active development of presentation skills, both oral presentations, via the poster, and in print. For many students, this class was the first time they had the opportunity to thoroughly present an idea and topic of their choice. In addition to these positive outcomes, students also provided several pieces of valuable information about adjusting the course for the next term. One significant weakness identified by the students was the manner in which the poster symposium was initially organized. This led to changes in how the poster symposium was conducted. Several representative student comments are offered here:

> "Small class size is great for this seminar course to better discuss scientific articles and learn from each other."

> "This course was excellent in the fact that it allowed us to us[e] all of our skills we have learned throughout our years at NC State. The small size of the course was also great. I do not see any weaknesses in the course."

> "The only weakness of this course was the poster session we completed at the end. The session needs

to be held in a larger room and be a little more organized. It was difficult to tell what was going on."

"I learned more about the scientific process from [this] class than from the rest of my undergraduate education combined."

[The instructor] "was open to any argument should there be logic behind it. I feel like I've learned more from this class and the discussions/presentations we've had than most other classes I've taken."

"Strength: I was able to apply the class work to my research methods on my personal projects, and apply the information to my daily life. Weakness: Sign ups are better so as to give the presenter more time to prepare for presentations."

"I think that the format was excellent. This is a very different course set up and while I think that initially I wasn't as sure about it, I really liked it by the end. It was kind of hard not to procrastinate the readings, but the daily summaries were good for that reason and did there [sic] job in my opinion. I like that the course wasn't just about the knowledge on each topic, but also teaching us key analytical and presentation skills. I feel much more comfortable presenting these sorts of heavy scientific papers now and do feel that will come in handy later."

POTENTIAL PITFALLS

In the course of teaching this capstone, four basic pitfalls were encountered: attendance, stimulating relevant and balanced discussion, consistent grading across students, and dealing with differences in student skill levels. These pitfalls were not specific to teaching with Tinbergen's four questions or the topic matter of neurotoxins, but rather seem to be common across capstone courses and perhaps discussion-based seminars in general (Bucci and Falls, 2007; Wiertelak and Ramirez, 2008; Kennedy and Hassebrock, 2012; Kurczek and Johnson, 2014). Regarding attendance, in order for a discussion-based class to work, students must attend class. To enforce this, attendance was taken, and participation was included as a component of the overall course grade. Furthermore, an attendance policy was implemented, in that only one unexcused absence was accepted without penalty. After that, each absence resulted in a drop of a letter grade unless a documented excuse is provided (preferably in advance). Coupled to this rather draconian attendance policy was a very liberal and broadly disseminated excused absence policy. Excused absences were given for job interviews, graduate or professional school interviews, research presentations, illnesses, and other similar activities. After all, most of the students in this class were graduating seniors, and it was felt by the instructor that the students should be encouraged to be working towards their next life phase. It is probable that some students took advantage of the liberal excused absence policy, but this risk was felt to be worth the gain.

Stimulating relevant, balanced, and copious discussion is a perennial issue for seminar-style classes. To help create an atmosphere that encouraged discussion, the class always began with a "warm up" activity such as bringing students in groups to the dry erase and smart boards present in the classroom to answer instructor-posed questions, or lightning presentations and responses based upon the students reading analyses. Also, instead of the instructor pointing out a flaw or extension, the students were asked the relevant question (For example, "What's wrong with this graph? Or "How would you do this differently?"). Typically they would be allowed to discuss the question with their neighbor for ~20 seconds for answering. After several class periods discussion occurred spontaneously. The third challenge encountered was consistent grading across oral, poster, and written projects. The development of rubrics facilitated equal grading (Felder and Brent, 2010) (Supplemental Materials). Finally, the last challenge was different student skill levels, especially regarding presentation skills. For example, some students had presented several posters over the course of their undergraduate studies, while some had never presented a poster. With posters, the solid week of class time devoted to poster presentation and practice helped bridge this large variability in preparedness. Regarding oral presentations, this difference in skill level was partially ameliorated by having the students initially present in pairs.

CONCLUSION

Outlined here is a neuroscience capstone course based around Tinbergen's four questions. Inherent in the structure of this approach is an emphasis on scientific method, critical analysis and questions, and an integrative, comprehensive approach to evaluating primary research and literature in neuroscience. While this particular example employed neurotoxins as the subject focus, we believe that a number of different capstone subject topics could be employed, all using specific adaptations of Tinbergen's four questions. Besides the most direct example, behavioral neuroscience, other possible topics benefiting from this approach could be courses focusing on naturally occurring variables in nervous system function such as season or sex, hormones and behavior/ neuroendocrinology, sensory systems, motor functions, and dedicated neural circuits such as those employed with central pattern generators. Overall, it is hoped that the mechanics, goals, and objectives of this course will be helpful as a flexible and useful model for a wide variety of neuroscience capstone courses.

REFERENCES

Abu-Qare AW, Abou-Donia MB (2002) Sarin: health effects, metabolism, and methods of analysis. Food Chem Toxicol 40:1327-1333.

Aguiar AS, Jr., Tristao FS, Amar M, Chevarin C, Glaser V, de Paula Martins R, Moreira EL, Mongeau R, Lanfumey L, Raisman-Vozari R, Latini A, Prediger RD (2014) Six weeks of voluntary exercise don't protect C57BL/6 mice against neurotoxicity of MPTP and MPP(+). Neurotox Res 25:147-152.

Allon N, Chapman S, Egoz I, Rabinovitz I, Kapon J, Weissman BA, Yacov G, Bloch-Shilderman E, Grauer E (2011) Deterioration in brain and heart functions following a single sub-lethal (0.8 LCt50) inhalation exposure of rats to sarin vapor: a putative mechanism of the long term toxicity. Toxicol Appl Pharmacol 253:31-37.

Bane V, Lehane M, Dikshit M, O'Riordan A, Furey A (2014) Tetrodotoxin: chemistry, toxicity, source, distribution and detection. Toxins (Basel) 6:693-755.

Baron A, Diochot S, Salinas M, Deval E, Noel J, Lingueglia E (2013) Venom toxins in the exploration of molecular, physiological and pathophysiological functions of acid-sensing ion channels. Toxicon 75:187-204.

Bateson P, Laland KN (2013) Tinbergen's four questions: an appreciation and an update. Trends Ecol Evol 28:712-718.

Beckage NE, Gelman DB (2004) Wasp parasitoid disruption of host development: implications for new biologically based strategies for insect control. Annu Rev Entomol 49:299-330.

Benoit E, Juzans P, Legrand AM, Molgo J (1996) Nodal swelling produced by ciguatoxin-induced selective activation of sodium channels in myelinated nerve fibers. Neuroscience 71:1121-1131.

Block ML, Zecca L, Hong JS (2007) Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci 8:57-69.

Broadwater MA, Liu W, Crews FT, Spear LP (2014) Persistent loss of hippocampal neurogenesis and increased cell death following adolescent, but not adult, chronic ethanol exposure. Dev Neurosci 36:297-305.

Brust JC (2010) Ethanol and cognition: indirect effects, neurotoxicity and neuroprotection: a review. Int J Environ Res Public Health 7: 1540-1557.

Bucci DJ, Falls WA (2007) An undergraduate neuroscience seminar based on the annual meeting of the Society for Neuroscience. J Undergrad Neurosci Educ 5:A49-A52.

Chau R, Kalaitzis JA, Neilan BA (2011) On the origins and biosynthesis of tetrodotoxin. Aquat Toxicol 104:61-72.

Church JE, Hodgson WC (2002a) Adrenergic and cholinergic activity contributes to the cardiovascular effects of lionfish (Pterois volitans) venom. Toxicon 40:787-796.

Church JE, Hodgson WC (2002b) The pharmacological activity of fish venoms. Toxicon 40:1083-1093.

Cuypers E, Yanagihara A, Karlsson E, Tytgat J (2006) Jellyfish and other cnidarian envenomations cause pain by affecting TRPV1 channels. FEBS Lett 580:5728-5732.

Dani JA, Ji D, Zhou FM (2001) Synaptic plasticity and nicotine addiction. Neuron 31:349-352.

Diochot S, Baron A, Salinas M, Douguet D, Scarzello S, Dabert-Gay AS, Debayle D, Friend V, Alloui A, Lazdunski M, Lingueglia E (2012) Black mamba venom peptides target acid-sensing ion channels to abolish pain. Nature 490:552-555.

Dorris DM, Cao J, Willett JA, Hauser CA, Meitzen J (2015) Intrinsic excitability varies by sex in pre-pubertal striatal medium spiny neurons. J Neurophysiol 113: 720-729.

El-Sherbeeny AM, Odom JV, Smith JE (2006) Visual system manifestations due to systemic exposure to mercury. Cutan Ocul Toxicol 25:173-183.

Esteban DJ, Holloway KS (2015) Mad dogs, vampires, and zombie ants: a multidisciplinary approach to teaching neuroscience, behavior, and microbiology. J Undergrad Neurosci Educ 13:A81-A87.

Favre-Guilmard C, Auguet M, Chabrier PE (2009) Different antinociceptive effects of botulinum toxin type A in inflammatory and peripheral polyneuropathic rat models. Eur J Pharmacol 617:48-53.

Felder RM, Brent R (2010) Hard assessment of soft skills. Chemical Engineering Education 44:63-64.

Gaffney JDH, Richards E, Kustusch MB, Ding L, Beichner R (2008) Scaling up educational reform. J Coll Sci Teach 37:48-53.

Gal R, Libersat F (2010) A wasp manipulates neuronal activity in the sub-esophageal ganglion to decrease the drive for walking in its cockroach prey. PLoS One 5:e10019.

Gonzalez-Liencres C, Shamay-Tsoory SG, Brune M (2013) Towards a neuroscience of empathy: ontogeny, phylogeny, brain mechanisms, context and psychopathology. Neurosci Biobehav Rev 37:1537-1548.

Grosman AH, Janssen A, de Brito EF, Cordeiro EG, Colares F, Fonseca JO, Lima ER, Pallini A, Sabelis MW (2008) Parasitoid increases survival of its pupae by inducing hosts to fight predators. PLoS One 3:e2276.

Hare DJ, Adlard PA, Doble PA, Finkelstein DI (2013) Metallobiology of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity. Metallomics 5:91-109.

Hawkins MB, Ferzli M (2014) Using a comparative endocrinology model to recruit future scientists. Proceedings of the Association for Biology Laboratory Education 35:6-14.

Huang YY, Levine A, Kandel DB, Yin D, Colnaghi L, Drisaldi B, Kandel ER (2014) D1/D5 receptors and histone deacetylation mediate the Gateway Effect of LTP in hippocampal dentate gyrus. Learn Mem 21:153-160.

Huerta-Rivas A, Lopez-Rubalcava C, Sanchez-Serrano SL, Valdez-Tapia M, Lamas M, Cruz SL (2012) Toluene impairs learning and memory, has antinociceptive effects, and modifies histone acetylation in the dentate gyrus of adolescent and adult rats. Pharmacol Biochem Behav 102:48-57.

Itoi S, Yoshikawa S, Asahina K, Suzuki M, Ishizuka K, Takimoto N, Mitsuoka R, Yokoyama N, Detake A, Takayanagi C, Eguchi M, Tatsuno R, Kawane M, Kokubo S, Takanashi S, Miura A, Suitoh K, Takatani T, Arakawa O, Sakakura Y, Sugita H (2014) Larval pufferfish protected by maternal tetrodotoxin. Toxicon 78:35-40.

Jelliffe-Pawlowski LL, Miles SQ, Courtney JG, Materna B, Charlton V (2006) Effect of magnitude and timing of maternal pregnancy blood lead (Pb) levels on birth outcomes. J Perinatol 26:154-162.

Joosen MJ, van der Schans MJ, Kuijpers WC, van Helden HP, Noort D (2013) Timing of decontamination and treatment in case of percutaneous VX poisoning: a mini review. Chem Biol Interact 203:149-153.

Joosen MJ, van der Schans MJ, van Helden HP (2010) Percutaneous exposure to the nerve agent VX: Efficacy of combined atropine, obidoxime and diazepam treatment. Chem Biol Interact 188:255-263.

Karsy M, Albert L, Murali R, Jhanwar-Uniyal M (2014) The impact of arsenic trioxide and all-trans retinoic acid on p53 R273H-codon mutant glioblastoma. Tumour Biol 35:4567-4580.

Kennedy S, Hassebrock F (2012) Developing a team-taught capstone course in neuroscience. J Undergrad Neurosci Educ 11:A12-A16.

Korbas M, Blechinger SR, Krone PH, Pickering IJ, George GN (2008) Localizing organomercury uptake and accumulation in zebrafish larvae at the tissue and cellular level. Proc Natl Acad Sci U S A 105:12108-12112.

Kramer-Hammerle S, Rothenaigner I, Wolff H, Bell JE, Brack-Werner R (2005) Cells of the central nervous system as targets and reservoirs of the human immunodeficiency virus. Virus Res 111:194-213.

Krasnova IN, Cadet JL (2009) Methamphetamine toxicity and messengers of death. Brain Res Rev 60:379-407.

Krathwohl DR (2002) A revision of Bloom's Taxonomy: an overview. Theory Into Practice 41:212-264.

Kurczek J, Johnson J (2014) The student as teacher: reflections on collaborative learning in a senior seminar. J Undergrad Neurosci Educ 12:A93-A99.

Lewis RJ, Dutertre S, Vetter I, Christie MJ (2012) Conus venom peptide pharmacology. Pharmacol Rev 64:259-298.

Liu H, Liu J, Liang S, Xiong H (2013) Plasma gelsolin protects HIV-1 gp120-induced neuronal injury via voltage-gated K+ channel Kv2.1. Mol Cell Neurosci 57:73-82.

Liu L, Zhao-Shea R, McIntosh JM, Tapper AR (2013) Nicotinic acetylcholine receptors containing the alpha6 subunit contribute to ethanol activation of ventral tegmental area dopaminergic neurons. Biochem Pharmacol 86:1194-1200.

Loane DJ, Stoica BA, Pajoohesh-Ganji A, Byrnes KR, Faden AI (2009) Activation of metabotropic glutamate receptor 5 modulates microglial reactivity and neurotoxicity by inhibiting NADPH oxidase. J Biol Chem 284:15629-15639.

Lopez-Santiago LF, Pertin M, Morisod X, Chen C, Hong S, Wiley J, Decosterd I, Isom LL (2006) Sodium channel beta2 subunits regulate tetrodotoxin-sensitive sodium channels in small dorsal root ganglion neurons and modulate the response to pain. J Neurosci 26:7984-7994.

Mesngon M, McNutt P (2011) Alpha-latrotoxin rescues SNAP-25 from BoNT/A-mediated proteolysis in embryonic stem cell-derived neurons. Toxins (Basel) 3:489-503.

Miller JA, Witherow DS, Carson S (2009) A laboratory-intensive course on RNA interference and model organisms. CBE Life Sci Educ 8: 316-325.

Momin A, Wood JN (2008) Sensory neuron voltage-gated sodium channels as analgesic drug targets. Curr Opin Neurobiol 18:383-388.

Nabavi S, Fox R, Proulx CD, Lin JY, Tsien RY, Malinow R (2014) Engineering a memory with LTD and LTP. Nature 511:348-352.

Okanoya K (2004) The Bengalese finch: a window on the behavioral neurobiology of birdsong syntax. Ann N Y Acad Sci 1016:724-735.

Pape HC, Pare D (2010) Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. Physiol Rev 90:419-463.

Prandovszky E, Gaskell E, Martin H, Dubey JP, Webster JP, McConkey GA (2011) The neurotropic parasite Toxoplasma gondii increases dopamine metabolism. PLoS One 6:e23866.

Przedborski S, Tieu K, Perier C, Vila M (2004) MPTP as a mitochondrial neurotoxic model of Parkinson's disease. J Bioenerg Biomembr 36:375-379.

Rau TF, Kothiwal AS, Rova AR, Brooks DM, Rhoderick JF, Poulsen AJ, Hutchinson J, Poulsen DJ (2014) Administration of low dose methamphetamine 12 h after a severe traumatic brain injury prevents neurological dysfunction and cognitive impairment in rats. Exp Neurol 253:31-40.

Rowe AH, Xiao Y, Rowe MP, Cummins TR, Zakon HH (2013) Voltage-gated sodium channel in grasshopper mice defends against bark scorpion toxin. Science 342:441-446.

Sampaio SC, Hyslop S, Fontes MR, Prado-Franceschi J, Zambelli VO, Magro AJ, Brigatte P, Gutierrez VP, Cury Y (2010) Crotoxin: novel activities for a classic beta-neurotoxin. Toxicon 55:1045-1060.

Schweitz H, Bidard JN, Lazdunski M (1990) Purification and pharmacological characterization of peptide toxins from the black mamba (Dendroaspis polylepis) venom. Toxicon 28:847-856.

Sircar R, Kim D (1999) Female gonadal hormones differentially modulate cocaine-induced behavioral sensitization in Fischer, Lewis, and Sprague-Dawley rats. J Pharmacol Exp Ther 289:54-65.

Smedley PL, Kinniburgh DG (2002) A review of the source, behaviour and distribution of arsenic in natural waters. Appl Geochem 17:517-568.

Steele JC (2005) Parkinsonism-dementia complex of Guam. Mov Disord 20 Suppl 12:S99-S107.

Stewart AM, Kalueff AV (2015) Developing better and more valid animal models of brain disorders. Behav Brain Res 276:28-31.

Stewart I, Lewis RJ, Eaglesham GK, Graham GC, Poole S, Craig SB (2010) Emerging tropical diseases in Australia. Part 2. Ciguatera fish poisoning. Ann Trop Med Parasitol 104:557-571.

Thomas B, Beal MF (2007) Parkinson's disease. Hum Mol Genet 16 Spec No. 2:R183-194.

Tibballs J, Yanagihara AA, Turner HC, Winkel K (2011) Immunological and toxinological responses to jellyfish stings. Inflamm Allergy Drug Targets 10:438-446.

Tinbergen N (1963) On aims and methods of ethology. Z Tierpsychol:410-433.

Ugolini G (2011) Rabies virus as a transneuronal tracer of neuronal connections. Adv Virus Res 79:165-202.

Ushkaryov YA, Volynski KE, Ashton AC (2004) The multiple actions of black widow spider toxins and their selective use in neurosecretion studies. Toxicon 43:527-542.

Ventura DF, Simoes AL, Tomaz S, Costa MF, Lago M, Costa MT, Canto-Pereira LH, de Souza JM, Faria MA, Silveira LC (2005) Colour vision and contrast sensitivity losses of mercury intoxicated industry workers in Brazil. Environ Toxicol Pharmacol 19:523-529.

Wang JH, Xie Y, Wu JC, Han R, Reid PF, Qin ZH, He JK (2012) Crotoxin enhances the antitumor activity of gefinitib (Iressa) in SK-MES-1 human lung squamous carcinoma cells. Oncol Rep 27:1341-1347.

Webb E, Bushkin-Bedient S, Cheng A, Kassotis CD, Balise V, Nagel SC (2014) Developmental and reproductive effects of chemicals associated with unconventional oil and natural gas operations. Rev Environ Health 29:307-318.

Webster JP (2007) The effect of Toxoplasma gondii on animal behavior: playing cat and mouse. Schizophr Bull 33: 752-756.

Weinersmith K, Faulkes Z (2014) Parasitic manipulation of hosts' phenotype, or how to make a zombie--an introduction to the symposium. Integr Comp Biol 54:93-100.

Wheeler A, Smith HS (2013) Botulinum toxins: mechanisms of action, antinociception and clinical applications. Toxicology 306:124-146.

Wiertelak EP, Ramirez JJ (2008) Undergraduate neuroscience education: blueprints for the 21(st) century. J Undergrad Neurosci Educ 6:A34-A39.

Willard AM, Brasier DJ (2014) Controversies in neuroscience: a literature-based course for first year undergraduates that improves scientific confidence while teaching concepts. J Undergrad Neurosci Educ 12:A159-A166.

Wilson JM, Khabazian I, Wong MC, Seyedalikhani A, Bains JS, Pasqualotto BA, Williams DE, Andersen RJ, Simpson RJ, Smith R, Craig UK, Kurland LT, Shaw CA (2002) Behavioral and neurological correlates of ALS-parkinsonism dementia complex in adult mice fed washed cycad flour. Neuromolecular Med 1:207-221.

Zampieri N, Jessell TM, Murray AJ (2014) Mapping sensory circuits by anterograde transsynaptic transfer of recombinant rabies virus. Neuron 81:766-778.

Zhao X, Zhai S, An MS, Wang YH, Yang YF, Ge HQ, Liu JH, Pu XP (2013) Neuroprotective effects of protocatechuic aldehyde against neurotoxin-induced cellular and animal models of Parkinson's disease. PLoS One 8:e78220.

Received June, 15, 2015; revised September 10, 2015; accepted September 21, 2015.

The author thanks Drs. Jane Lubischer, John Godwin, Heather Patisaul, and Lisa McGraw for their encouragement and critiques of this project, and Dr. Lisa McGraw for the photograph in Figure 2.

Address correspondence to: Dr. John Meitzen, Department of Biological Sciences, North Carolina State University, 144 David Clark Labs, Campus Box 7617, Raleigh, NC 27695-7617 Email: jemeitze@ncsu.edu