Laboratory in Neuroscience Manual

INTERDISCIPLINARY
NEUROSCIENCE
MINOR

Neur 302 - Spring 2008

Neuroscience Lab Manual: Table of Contents

Lab in Neuroscience (NEUR 302) - Spring 2008 - brief Syllabus

Jan. 17 Class rescheduled due to instructor's absence as an alternative assignment, please read the chapter in McCall on research design on the Neuroscience/Parmly website (www.parmly.luc.edu/neuro/.)
Jan. 24 Introduction to Research in Neuroscience
Jan. 31 Faculty presentations and student's selection of Independent Research Projects (IRPs)
Feb. 7 Students meet with each other to informally present their IRPs
Feb. 14 Research Proposal due to faculty advisors. (faculty provide feedback)
Feb. 21 Final research proposal due
Feb. 28 in Lab
Mar 13 in Lab
Mar. 27 in Lab
Apr 3 in Lab
Apr. 10 in Lab
Apr. 17 Poster preparation
Apr. 24 Poster presentation to faculty advisors
May 5 Final Research Report Due by 4:00 pm

PARTICIPATING FACULTY (as of Dec. 10, 2007):

Lakeshore faculty:

Dr. Duke Han dhan2@luc.edu

Dr Stan Sheft, auditory processing of modulation in sound-source segregation, ssheft@luc.edu

Dr Raymond Dye, Binaural processing and sound lateralization, rdye@luc.edu

Dr Richard Fay, Auditory perception by fishes, rfay@luc.edu

Strich Faculty:

Dr Karie Scrogin, role of central serotonin in reflex control of circulation after hemorrhage, kscrogi@lumc.edu

Dr. Scrogin's research: We have a project examining the role of hindbrain serotonergic neurons in the autonomic responses to acidemia produced by hypovolemic shock, high ambient CO^2 exposure or metabolic acidosis. A student could be involved in these studies by watching surgery, helping with post-op animal care, participating in the experiments which involve exposing the animals to the above stimuli while collecting cardiovascular and respiratory data. Our models are rats and mice. Students could participate directly in perfusion and extraction of the brain, cutting of brain sections and subsequent immunohistochemical analyses.

Dr Michael Collins, Dept Pharmacology, LUMC.

COURSE REQUIREMENTS AND GRADING: Performance on independent research projects will be evaluated in terms of : (1) a written research proposal, (2) a poster presentation of the experimental results to faculty and students, (3) a final written report and (4) evaluation by faculty advisor. Students may work by themselves (or in teams, depending on class size and as be determined by the faculty advisor) and research interest, with a key faculty member serving as an advisor for each project. **Only one research proposal and poster presentation per team is required and each will be evaluated as a team effort (i.e. each person in the team will receive the same score). Final written reports, however, must be done independently by each student and will be evaluated as individual efforts.**

Grading will be based on a total of 200 points:, 50 points for the written research proposal (teams submit this as a group effort), 50 points for the poster presentation (team effort), and 100 points for the final written report (individual effort). Final grades will be assigned on the following basis: A = 91 - 100%, A- = 87 – 90%, B+ = 84 - 86%, B = 80 - 83; B- = 77 – 79%, C+ = 74 - 76%, C = 70 - 73 etc.). A grade of C or better is needed on the final report (70 points or higher) in order to complete the requirements for the neuroscience minor.

Although we encourage students to use each other as resources for discussing concepts and clarifying issues, **the final report must be done independently and conform to Loyola's standards of academic integrity as published in the undergraduate Handbook**. **Plagiarized reports based on fabricated data will receive a failing grade (no points) and may lead to a failing grade in the course and/or suspension from the university.**

GROUND RULES FOR USING THE LAB:

1. Key Card Access: LUC ID cards should be used to gain access to all LSC and LUMC labs.

2. Authorized Users: Only authorized students and faculty are allowed to use the lab. These include students enrolled in NS 301/302 and in Psych 311. Friends and family are not allowed in the lab for safety and security reasons and out of consideration to other students using the lab.

3. Safety Policies: Adhere to the safety policies of the lab. Food and drinks are not allowed in the lab at any time.

4. Lab Maintenance: Take good care of the lab and equipment. Remember that this is a shared facility used by different faculty and students and that different courses are taught in this facility during the same semester. When you are finished for the day, make sure that microscopes are covered, the power to equipment is turned off, surgical tools and working surfaces are cleaned, and all supplies, cables, tools etc. are put away. Take special care in cleaning up after working with saline preparations. Saline is nothing more than salt water and will cause equipment, surgical tools and the physiology platform to rust. Rinse all tools and equipment that comes in contact with saline thoroughly and carefully.

5. Lab Security: Keep equipment and the lab secure. When you use the lab during non-class hours, please keep the lab door closed and locked at all times. Do not let anyone into the lab who isn't authorized to be there.

INDEPENDENT RESEARCH PROJECT

Required Readings:

Chapter 12, Introduction to Research Design, pp. 299 - 321 in: McCall, R.B. (2001) *Fundamental Statistics for Behavioral Sciences*, Wadsworth/Thompson Learning, CA.

Sample Research Proposal (Appendix B)
Sample Poster Lay-Out (Appendix C)
Introduction to Research Design (Appendix D)

Planning Phase

The planning phase of the independent research project consists of 3 steps that will take place over a 4-week period. First, you will be given a general introduction to research design and methods. Next, Loyola neuroscientists, who have volunteered to serve as faculty advisors, will give brief presentations and/or lab tours to describe various research options for your independent project. Based on your research interests, you will then select a faculty advisor and work with that advisor to develop an independent research project. At this stage, you will meet (in coordination with Dr Lucas) with fellow students at a convenient time for a feedback session to help you determine if you are on the right path to preparing a research proposal (Progress report #1). After searching the literature in the area of your research topic, you will write a research proposal that will summarize in a concrete and specific way what you intend to do during your independent project, how you will do it and why you are doing it. As an intermediate step in the preparation of the research proposal, you will prepare a first draft of the proposal to give to your faculty advisor. This will help you to zero in on the essential elements of your proposal and give faculty an opportunity to give you rapid feedback early on in the planning phase. As a supplement to the research proposal, you will also write a complete list of ALL equipment, animals and supplies that you will need for your project. Be sure to include how many of each you will need. It is ESSENTIAL that this list be complete down to the last nitty-gritty detail, as it will help faculty to plan for your needs and will ensure that you have what you need in order to get your project done on time. Finally, you will conduct some pilot studies to further develop your methods and evaluate the feasibility of your proposal. This is the time to refine your methods, solve unanticipated problems and to see if your plans work well enough so that you can actually get some results.

In order to increase the probability that your research project will be successful, it is **STRONGLY** recommended that you adhere to the following schedule during the planning phase.

Phase 1

- meet with faculty advisor to discuss experiments and experimental design
- search the literature for relevant papers

Phase 2

- read background literature and make an outline of the main points to be covered in the significance section of the research proposal

- write first draft of the specific aims and methods sections of the research proposal
- submit list of needed equipment and supplies to faculty advisor

Phase 3

- submit first draft of specific aims and methods sections to faculty advisor for his/her feedback
- begin pilot study to identify potential problems and to determine feasibility of experimental design
- determine how you will record your results and keep track of various experimental variables;

Phase 4

- get feedback from faculty advisor on research proposal and experimental design
- Continue pilot work as needed and prepare final draft of research proposal.

Independent research projects by former students

Lavrakas, N. J. (2000) Interaction of word frequency with place of articulation categorization - 2000

Azuogo, C. and Lazzaro, M. (2000) Neuronal tract tracing in the goldfish optic tectum using horseradish peroxidase

Jimenez, R. and Hernandez, R. (2000) Rheotaxis in crayfish.

Cisneros, S. and Khemmani, M. (2000) Hemispheric lateralization of melodic perception

Selas, G. and Taglia, L (2001). A study of d-wave origin in *Rana pipiens* using electroretinograms.

Kutis, S. and Scroggins, K. (2001). Afferent and efferent nerve fiber tracing in the goldfish corpus cerebelli using horseradish peroxidase.

Gearhart, S. and Randazzo, J. (2001). The effects of tetraethylammonium chloride on the presynaptic potentials of the crayfish motor nerve 3.

DeLa Torre, R. and Lukaszczyk, B (2002). Flicker Fusion: The effect of flicker frequency on the electroretinogram (ERG) of the frog, *Rana pipiens*.

Gastevsky, D. and Lekostaj, J. (2002). The effects of duration on pitch discrimination.

Koval, J. (2002). Stimulus-response properties of tonic stretch receptor organ in crayfish, *Procambarus clarkii*

Mrejeru, A. and Yuen, J. (2002). Encoding of pulse repetition rate in goldfish saccular nerve fibers.

Farmer, L. and Jovanovich, S. (2002). Characterization of motor neurons within tailfan nerve 4 of *Procambarus clarkii*.

Jeka, N. and Murphy, N. (2002). Dark adaptation in *Rana pipiens*.

Buck, C. and Sigafus, P. (2003). Exploring the startle response of larval zebrafish to electric stimuli.

Andriusis, J. and Gunaratnam, M. (2003) Comparison of the medial octavolateralis nuclei volume of the lateral line system in blind cavefish and sighted goldfish.

Hepler, S. and Thornton, K. (2003). Projections of the olfactory tract in goldfish (Carassius auratus): A tract tracing study using horseradish peroxidase.

Dervin, K. (2003). Envelope modulation and detection of the interaural time difference.
Plovanich, M. (2003). Habituation in zebrafish.

Research Proposal

The purpose of a research proposal is to ensure that your experiment will produce results that are interpretable and of significant scientific merit. In other words, it forces you to think critically and carefully about your experiments before you do them. Your written research proposal will be based on the same guidelines that scientists use when submitting research proposals to outside granting agencies, such as the National Institutes for Health or the National Science Foundation. It consists of four main sections, as follows:

TITLE: **A short descriptive title that communicates what you'll be doing**

A. Specific Aims: **3 - 5 sentences**

This section should be short and sweet. You should enumerate the specific goals of your experiment - what is the point of the experiment? Make sure that you clearly state the main independent and dependent variables. For example, in the lab exercise that you did on resting membrane potentials, the specific aim was to determine the effects of different extracellular potassium concentrations on the resting membrane potential of crayfish superficial flexor muscles. Another way to state the same aim is: to determine the role of potassium in establishing the resting membrane potential. Remember that the specific aims for any particular experiment are just that, *concrete* and *specific*; they differ from what may be the overall goal of a research program, such as to understand how the nervous system controls postural reflexes in crayfish.

B. Background and Significance: **1 - 2 pages**

For this section, you will need to do a literature search to determine how your own research question fits into the general scheme of research in this area. You can think of this section as preparation for the introduction you will write in your final research paper and it should contain all of the formal elements of an introduction published in most scientific journal papers (e.g. literature citations throughout the text). The important part of this section is the *significance* part. Refrain from doing an exhaustive review of all possible papers remotely related to your research topic. Rather, you must pick and choose past studies (be sure to cite them) that are most directly related to the research question you are addressing and you must identify the particular findings in those studies that are relevant to your proposal. In other words, emphasize those details of the earlier work that will best help the reader understand why you are proposing this particular experiment and why your experiment is important. What new information will you provide and how will this help to answer outstanding questions in your field of investigation?

C. Experimental Design and Methods: **1 - 2 pages**

This section should contain the same information as a methods section in a published research report and should cover several areas, best delineated with subsections, as follows.

1. General Experimental Design: Start with a general, overall description of what you will do to test your hypothesis (e.g. *To test the hypothesis that potassium concentrations are important to*

the establishment of the resting membrane potential, intracellular recording techniques will be used to measure the resting membrane potential of crayfish superficial flexor muscles exposed to different extracellular concentrations of potassium). Make sure that this description includes the main dependent (i.e. resting membrane potential) and independent (i.e. extracellular potassium concentrations) variables that were measured or manipulated.

2. Experimental Animals: Give information on the experimental animals that you used (e.g. species, size, and sex, if important). Indicate how animals were housed and cared for prior to, during and after experimental manipulations. State that you followed IACUC-approved protocols for the handling and treatment of experimental animals.

3. Experimental Set-up and Procedures: Describe all of the relevant details of your experiment, including surgical approaches, how you will make your measurements (*e.g. "membrane potentials will be measured with an intracellular glass micropipette electrode (1 - 10 MΩ impedance) and a silver wire reference electrode placed in the extracellular saline bath"*), how you intend to manipulate various variables (*e.g. "preparation will be bathed in crayfish saline of three different concentrations of potassium: 5.4, 26.8 and 60 mMoles/liter"*), and the equipment you will use to control stimulus variables or to measure results *(e.g. "membrane potentials will be amplified by an intracellular amplifier (brand and model) and measured by oscilloscope and digital read-out on the intracellular amplifier*"). Although it is important to indicate the equipment and in some cases, the supplies you will use, don't just list them; rather explain why and how you will use them. Be sure to indicate how you will ensure that your results will be both valid and reliable. For example, how many repeat measurements at each potassium concentration are needed to get statistically significant results? Should you make repeated measures in the same or different preparations? Should you use different preparations for measuring different potassium concentrations? Describe any possible uncontrolled variables that may present themselves and how you will control for them (e.g. *"to control for order effects, the order in which potassium concentrations of the saline bath are changed and tested will be randomized*").

Be specific about only those details that are important.. For instance, it may be important to say that you will use a micro-pipette electrode with a 1 - 10 mΩ tip impedance, because you can't measure DC resting potentials very accurately with higher tip impedances and because the impedance range may be critical to the success of these particular experiments. However, it's not so critical to say how you will actually make the electrode tip (e.g. with a David Kopf Vertical Pipette Puller, Model 720). It could be important to say how the tip will be pulled if the pulling technique were somehow critical to the success of the recording technique or if it were a new technique that you were developing for the proposal. In this case, however, it doesn't matter if the electrode is pulled with a vertical pipette puller made by David Kopf or a horizontal puller made by Narishige as long as the tip impedance is in the right range. As a fledgling scientist, it may be difficult for you to make some of these judgments, so you may want to seek advice from a faculty member on some of these finer points. Most of all,THINK about it. Is it important information? Does a knowledgeable reader (i.e. someone in the same field) need to know this level of detail in order to evaluate the results and to understand what was done?

4. Data Analysis: Finally, you must include a plan for how you will analyze your results. For example, " *the mean and standard deviation of 5 repeat measures of resting membrane potential*

for each potassium concentration will be plotted against the log of the external potassium concentration and compared with the Nernst equation predictions". A good proposal (and good planning) also includes a discussion of several possible outcomes - e.g. the case where the results match the predicted or hypothesized outcome (e.g.the Nernst equation predictions) and cases where the results do not match the predicted outcome. Try to think ahead of all the possible outcomes and what they might look like in the final data analysis. This will help you to think more clearly about how you want to conduct your experiment and analyze your results.

If you do a good job on the methods section on your final written research proposal, then the methods section of your final written report is essentially done.

D. Literature Cited

The full citation for all the papers you reference in your research proposal should be listed, following the same format used in published papers. See *Literature Cited* section in *How to Write a Scientific Paper* for further detail on when and how to cite the literature.

Data Collection Phase

The execution phase of the independent research project is the time that you will spend collecting data. By this point in time, all methodological problems encountered in your feasibility studies should have been solved and you should be ready to go full speed ahead to collect as much data as possible. You should also have a good record-keeping system in place. How will you record your results and keep track of different experimental variables? It is generally a good practice to keep a notebook with copious comments on exactly what you did, when you did it and what happened after you did it. Do not assume that you can collect all of the data you need in one experimental session or that you will be successful in getting data each time you try an experiment. Data collection is frequently a slow and tedious process beset with frustrating problems. You must be patient and persistent. **MAKE BACK-UP COPIES OF ANY DATA YOU COLLECT AS SOON AS POSSIBLE AFTER COLLECTING THEM.** There's nothing more frustrating than spending an entire day collecting data only to find out later that your one and only computer file of the results was inadvertently deleted.

Data Analysis and Poster Preparation Phase

During the 3-week data analysis and poster preparation phase, you will analyze your results and prepare figures, tables or charts to clearly and concisely summarize your main findings. The data analysis phase is NOT something that can be completed in one day. Even though you have already decided and described how you will analyze your results in your research proposal, unexpected results often take you in new directions requiring different ways of analyzing the data. Be flexible, but thorough, meticulous and very careful. Artifactual results can easily crop up if you don't know how to use a statistical software package correctly or if you inadvertently delete or relocate part of your spreadsheet. Ask yourself if the final results make sense. If they don't, look for silly mistakes you may have made in data entry or analysis before assuming that the results are un-interpretable. Try different ways of analyzing and displaying the results to determine which is best. Seek feedback from faculty at the beginning of the data analysis phase - don't wait until the very end!

Poster Format

Posters are similar to abstracts in research reports or publications in that their main purpose is to summarize the key elements of your study. Like most scientific communications, posters have discrete sections. They should include a brief (one paragraph) introduction to communicate what you did and why you did it, a brief (one paragraph) methods section to communicate how you did it, a results section (generally graphs only) and a summary and conclusions section. For your introduction, you can draw from what you have written for the background and significance section of your research proposal, but remember that you will need to pare this down so that you summarize only the key elements. Likewise, your methods section should briefly summarize how the experiments were conducted. The best way to do this is to give a sequential summary of the main steps taken to collect the data, leaving out many of the details that might be included in a cookbook-like version of the methods. For example, steps 1 - 9 in Section A of the Lab Procedures (week 1) for Motor Innervation of a Crayfish Postural Muscle could be summarized in one sentence as follows: *Animals were anesthetized by cold immersion in ice and the 1st abdominal ganglion and its nerve roots were surgically exposed*. Figures illustrating your experimental set-up or flow-charts showing the sequence of experimental procedures are other effective ways for communicating methods in a poster.

Keep in mind that you will have limited (3.5' x 4') space for your poster. Rather than trying to cram a lot of small text and figures into your poster, narrow down the material you present to a few key elements, using bold numbers and headings to guide the reader, as illustrated by the sample poster in Appendix C. As this sample shows, the majority of the poster should be devoted to the results section where you will graphically or visually display your main findings. There should be at least one graph, table or chart to illustrate each key finding. Finally, posters should have a brief, preferably enumerated summary and conclusions section to verbally describe your main findings and what you conclude from these. This should be short and to the point - i.e. one sentence per each main finding and conclusion. Remember, posters are not designed to cover everything that you did or found out, but only the essentials. They are used most effectively as a visual aid to a verbal presentation, which is best done as an informal 'story' of what you did rather than a recitation of a paper that you might have written on the topic. The best poster presentations evoke constructive feedback and lively, engaging discussion.

Be sure to allow yourself plenty of time to prepare the poster - it is definitely NOT something that can be done in a few hours. You need to think carefully about what you want to communicate. In general, this means that you need to have a clear understanding of what you did, why you did it, how you did it and what you learned from it.

Poster Presentation and Evaluation

Be prepared to present your work with this in mind. If you work with a lab partner, you should discuss ahead of time what you will say and which sections of the poster each of you will present. Your poster presentation will be evaluated by several different faculty and by your class peers for your final grade. They will rate the following areas on a scale of 1 to 10 with (10) being very successful and (1) indicating the need for considerable improvement. Because the same criteria will be used in evaluating final reports, this is your chance to get good feedback from different faculty on how you can improve the presentation and interpretation of your experiment.

Introduction: Provided a clear statement of the purpose of the experiment or the hypothesis being tested. Placed the work into an overall context that cultivated interest in the questions being addressed ________

Methods: Provided a clear explanation of what was done during the experiment and how the experiment was conducted ________

Results: Provided a clear picture of the main results. Figures were easy to read, interpret and understand relative to the stated purpose of the experiment_______

Summary and Conclusions: Provided a clear statement of the essential findings and the conclusions that could be drawn from them._______

Overall Impression: ______

Summary of Poster Guidelines

- The space provided for the display of posters will be 3.5 by 4 ft (half of one bulletin board space in hallway outside the lab). All of the poster materials must fit in this space.
- It is recommended that the poster be prepared by creating a PowerPoint presentation, printing out the PowerPoint pages, and mounting these pages onto the poster background.
- The poster must include the following pieces of information prominently displayed:
    - the title of the research project
    - the student's name
    - the name of the student's research advisor.
- Each poster should be organized to include the following sections:
    - Introduction
    - Methods
    - Results
    - Conclusions
    - References
- It is recommended that the text for the Introduction, Methods, and Conclusion sections be presented as bullet points to make it easier for those reading the poster.
- All text should be presented in a font of at least 22 point so that it can be read from a distance of a few feet.
- The results section may include some or all of the following: bulleted text, tables, figures, images, photographs, and illustrations.
- Posters should stimulate discussion. Therefore, keep text to a minimum, emphasize graphics, and make sure every item in your poster is necessary.

Final Written Report

Remember that although you may work in teams during the planning, execution and analysis phases of the research project, the final written report must be done independently by each student. Your final written report will follow the same format as published journal articles, as summarized in the following section.

How to Write a Scientific Paper

Scientific papers published in peer-reviewed journals (e.g. Journal of Experimental Biology) typically have six sections presented in the following order: abstract, introduction, materials and methods, results, discussion and bibliography. Peer review means that the published papers have been criticized (and altered as a result, usually) by other scientists in the field, chosen by the editor of the journal. As with any form of writing, the main purpose of a scientific paper is to communicate. Ideas and questions should be expressed clearly and logically. Results and data should be communicated within a logical structure and fully explained. Any conjectures or analysis should also be communicated coherently and always with reference to the problem or data set at hand. The writer must always take responsibility to:

-Summarize the key elements (problem, methods, results, conclusions) of your study (Abstract)

-Formulate the problem (Introduction).

-Present the methods used to address the problem (Materials and Methods)

-Describe the results of the experiments (Results).

-Place those results in some perspective, both in relation to the main problem the paper addresses and to the results of other scientists (Discussion).

-Give credit where credit is due (citing papers within the text) and give the full reference of any paper you cite (Bibliography)

In general, if a reader can't understand what you have written, it's usually your fault. Your goal should be to write so that anybody, from your grandmother to the experts in your field, can understand the essentials of your experiment, providing they are willing to take the time to try to learn some basic vocabulary. Take it as your responsibility to be logical and clear. Explain what you are doing and why. Writing a clear and well-organized paper takes lots of work and effort - even for the most experienced scientists, who frequently write several drafts of each paper, asking others to evaluate preliminary drafts for clarity and completeness. So, allow yourself plenty of time to write and re-write your paper to ensure that it is clear and well-organized. In addition to the information summarized in your lab manual, the following IBRO web site has excellent advice on writing papers: http://www.ibro.info Click on the 'Education and Training' icon on the Main Menu at the top of the screen and select 'Map and Compass'. You can also download the same material (as pdf files) from Ganglia/public/homework/independent research projects or view the printed files in a bound notebook in the lab.

- **Part 1** What is a peer-reviewed article:
- **Part 2** Determining authorship:
- **Part 3** Selecting a journal:
- **Part 4** Anatomy 1: The Introduction (intro.pdf)
- **Part 5** Anatomy 2: Methods (methods.pdf)
- **Part 6** Anatomy 3: Results (results.pdf)
- **Part 7** Anatomy 4: Discussion (discussion.pdf)

- **Part 8** Selecting a title (including keywords)
- **Part 9** Writing the abstract (abstract.pdf)
- **Part 10** The process: Outlining, writing, and editing (process.pdf)
- **Part 11** English as a second (or third!) language (writing in english.doc)
- **Part 12** Designing tables and figures (tables and figures.pdf)

Abstract

Nearly all forms of scientific writing start with some sort of abstract or summary of the paper. This is usually a half-page (~ 200 - 500 words) that allows a reader to decide if he/she wants to read your paper or that refreshes their memory about the contents of the paper after they have read it. An abstract should summarize the entire paper. It should include a brief statement of the problem, question or hypothesis that is at the heart of the paper (a 1 - 2 sentence summary of the Introduction). It should also include a brief statement of how you approached the problem (summary of the methods) and a summary of the results and conclusions reached. It is not acceptable to say "*these findings are discussed.*" You can say something concrete, even if you say it briefly. For instance "*The resting membrane potential was found to be a function of extracellular potassium concentration, as predicted by the Nernst equation*." Don't describe your methods in the abstract, unless they are critical to your particular approach.

Introduction

This section should be rather short (2 - 3 pages), with three main goals: (1) to identify the problem or question that you will address or the hypothesis that you will test, (2) to provide the rationale for asking this particular question or doing these particular experiments, and (3) to place your work in an overall, historical context of published papers on your topic.

The most important aim of an introduction is to clearly state the central problem or any hypotheses that you will test. The introduction not only tells the reader what question (or questions) you're asking, but also why you are asking those particular questions. The 'why' of the experiment usually makes sense only after you have explained what others have done (citing the literature) and what we already know and don't know - i.e. what gaps in our knowledge need to be filled? Thus, you MUST do a literature search to determine how your own research question fits into the general scheme of research in this area. Refrain from doing an exhaustive review of all possible papers remotely related to your research topic. Rather, you must pick and choose past studies (be sure to cite them) that are most directly related to the research question you are addressing and you must identify the particular findings in those studies that are relevant to your proposal. In other words, emphasize those details of the earlier work that will best help the reader understand why you are proposing this particular experiment and why your experiment is important. What new information will you provide and how will this help to answer outstanding questions in your field of investigation? The last few sentences of a good introduction (or the first few sentences of the methods section, see below) will also include a brief preview or summary of the general experimental design - i.e. how you addressed the question posed in your introduction.

Materials and Methods

The materials and methods section is where you describe the techniques you used, what experiments you did, how you controlled for possible confounding variables and how the data were collected and analyzed (including statistical tests). Sometimes, it is easier to communicate certain details of how you analyzed the data in the results section rather than the methods section because figures often show the end point of your analysis rather than the raw results. Clarity should be your guiding rule as to whether you include this information in the results section or the methods section. The material and methods section should be broken down into three or four general subsections, each with their own subheading, as outlined below.

1. General Experimental Design: Start with a general, overall description of what you did to test your hypothesis (e.g. *To test the hypothesis that potassium concentrations are important to the establishment of the resting membrane potential, intracellular recording techniques were used to measure the resting membrane potential of crayfish superficial flexor muscles exposed to different extracellular concentrations of potassium*). Make sure that this description includes the main dependent (i.e. resting membrane potential) and independent (i.e. extracellular potassium concentrations) variables that were measured or manipulated. This general description can be placed at either the end of the introduction or the beginning of the methods section, but it should always be in one of the two places.

2. Experimental Animals: Give information on the experimental animals that you used (e.g. species, size, and sex, if important). Indicate how animals were housed and cared for prior to, during and after experimental manipulations. State that you followed IACUC-approved protocols for the handling and treatment of experimental animals.

3. Experimental Set-up and Procedures: Describe all of the relevant details of your experiment, including surgical approaches, how you made your measurements (*e.g. "membrane potentials were measured with an intracellular glass micropipette electrode (1 - 10 MΩ impedance) and a silver wire reference electrode placed in the extracellular saline bath"*), how you manipulated various variables (*e.g. "preparation was bathed in crayfish saline of three different concentrations of potassium: 5.4, 26.8 and 60 mMoles/liter"*), and the equipment used to control stimulus variables or to measure results *(e.g. "membrane potentials were amplified by an intracellular amplifier (brand and model) and measured by oscilloscope and digital read-out on the intracellular amplifier* "). Although it is important to indicate the equipment and in some cases, the supplies you used, don't just list them; rather explain why and how they were used in each case. Be sure to indicate how you ensured that your results were both valid and reliable. For example, how many repeat measurements at each potassium concentration were necessary in order to get statistically significant results? Did you make repeated measures in the same or different preparations? Did you use different preparations for measuring different potassium concentrations? Describe any possible uncontrolled variables that may have presented themselves and how you controlled for them (e.g. *"to control for order effects, the order in which potassium concentrations of the saline bath were changed and tested were randomized*").

Be specific about details that are important. For instance, it may be important to say you used a micro-pipette electrode with a 1 - 10 mΩ tip impedance, because you can't measure DC resting potentials very accurately with higher tip impedances and because the impedance range may be critical to the success of these particular experiments. However, it's not so critical to say how the tip was pulled (e.g. with a David Kopf Vertical Pipette Puller, Model 720). It could be important to say how the tip was pulled if the pulling technique were somehow critical to the success of the recording technique or if it were a new technique being reported for the first time. In this case, however, it doesn't matter if the electrode is pulled with a vertical pipette puller made by David Kopf or a horizontal puller made by some other company as long as the tip impedance is in the right range. As a fledgling scientist, it may be difficult for you to make some of these judgements, so you may want to seek advice from a faculty member on some of these finer points. Most of all, THINK about it. Is it important information? Does a knowledgeable reader (i.e. someone in the same field) need to know this level of detail in order to evaluate the results and to understand what was done?

4. Data Analysis: Finally, you must describe how your results were analyzed and any statistical tests that were used. For example, " *transverse sections of the brain were viewed under a compound microscope and the number of filled cell bodies were counted for each specimen in each age group. A 1-way ANOVA was used to test for significant differences between age groups* ".

Results

This section should be an explicit description of your results, which for the most part should be presented as figures or tables. Each figure and table should be numbered (e.g. Fig. 1, Table 1) consecutively in the order in which they are described in the text. Each figure should have a caption and each table should have a title. The text of the results section is designed to 'walk' the reader through the results and to tell the reader what to see in the figures. In other words, state the main take-home message that you want the reader to get from each figure. For x,y plots, this is usually a simple statement of the relationship between the dependent and independent variables (e.g. "*Fig. 2 shows that evoked spike rate doubles for every doubling of stimulus amplitude."*). For single micrographs, it might be a description of what is shown - i.e. the number of cell bodies that are filled and their size and shape or the extent of dendritic branching etc.).

Do not draw any conclusions in the results section; just present and describe your data. You may talk about any findings you have that relate to what method you used, or why this method is good or bad, but you should not describe any methods or techniques; that belongs in the methods section. Likewise, you should not cite other people's work in the results section, or attempt to place your results in a larger context; that belongs in the discussion. Just present your results (as graphs, tables, micrographs etc) and describe your findings, along with any statistical test results that show e.g. significant or insignificant differences between treatment groups.

Discussion

The main goals of this section are to identify your key findings, to state if or how they helped solve the problem posed in the introduction, and to place them in the larger context of the literature that already exists. Here is where you are free to say what you think it all means. Although you may favor a particular meaning or interpretation, a good discussion explores all possible interpretations, using logic and experimental evidence to rule out less likely ones and to support the most probable. In some (actually many) cases, it is not possible to rule out two or more different interpretations without further experiments. If this is the case, you should say so in the discussion, indicating which experiments need to be done. You should also discuss possible confounding variables and how your results from control experiments have ruled these out.

A common mistake that beginners often make when writing the discussion is to include too much. You should be very careful to stick to your data, only writing about things that are strictly relevant to the experiment at hand. For example, if your experiment was designed to test the effects of external potassium concentrations on the resting membrane potential of crayfish superficial flexor muscles, you should review the related literature and talk about what others have done to determine how resting membrane potentials are established. In this case, it would be appropriate to talk about similar experiments in different species and/or muscle cells (e.g. frog sartorious muscle) or even different cell (e.g. nerve) types. It might also be informative to talk about the relative importance of different ion species (e.g. potassium vs. sodium) in establishing membrane potentials. However, it would NOT be appropriate to launch off into a discussion of how superficial flexor muscles are used to maintain body position in crayfish, even though you did your experiments on these muscles and what you say may be true.

When comparing your results to those from similar experiments, you should try to offer some interpretation of why they are different (or similar). Be sure to cite other peoples' work correctly and fairly. Try to keep your discussion approximately the same length as your result section. This will keep you from going overboard on a literature review or excessive speculation.

Literature Cited

Citations should be used whenever you refer to specific results, general findings ('facts') or ideas that are not your own. Citations are also used to substantiate any claims you make that are not directly supported by the results presented in your current paper. These include claims based on the work of others as well as those based on your own work published in previous years. Thus, if the reader wants to verify your claim or to learn more about it, he/she can go to the reference you cited.

<u>Guidelines for citing references in the text of your paper</u>

When citing (referring to) references in the text of your paper, you must use the standard format in published papers, always citing the authors' last names and date of publication, as illustrated in the following examples:

One author: The lateral line system has long been regarded as a short-range system, capable of detecting nearby animate and inanimate bodies (Dijkgraaf 1934). This ability was first demonstrated experimentally by Hofer (1908) and later coined 'ferntastsinn' or 'touch-at-a distance' by Dijkgraaf (1934). Dijkgraaf (1934) also demonstrated that....

Two authors: The operating range of the lateral line system is thus considered to be a function of fish length; the longer the fish, the further away a given source can be detected (Denton and Gray 1983). The distance range or 'active space' (Bossert and Wilson 1963) of the lateral line system is likely to depend on a number of factors besides fish length, including the size of the source and its amplitude of motion at the source, as recently demonstrated by Montgomery and Milton (1993).

Three or more authors (First Author + "et al."): Furthermore, unlike experiments on treefrogs in which unilateral occlusion of one ear causes frogs to turn towards the side of the unoccluded ear regardless of source location (Feng et al. 1976), experiments on sculpin with unilaterally denervated lateral- line systems did not cause sculpin to turn in circles towards their intact sides.... OR.... Feng et al. (1976) showed that.......

Several references in a row (in order of publication date, from earliest to latest; if same dates, then alphabetical): The operating range of the lateral line system is thus considered to be a function of fish length; the longer the fish, the further away a given source can be detected (Denton and Gray 1983, 1988, 1989; Kalmijn 1988, 1989; Coombs 1996).

<u>Guidelines for listing references at the end of your paper</u>
The full citation for all the papers you reference in your research proposal should be listed, following the same format used in published papers. List all single or multiple-authored papers in alphabetical order according to the last name of the first author. Last names of first authors should always come first, followed by the initials of the first and middle names. For papers having the same first author, single-authored papers should come before dual-authored papers, followed by multiple (3 or more)-authored papers. Dual-authored papers with the same first author should be alphabetized according to the last name of the second author. Multiple-authored papers with the same first author should be listed in chronological order (from earliest to latest). Note difference in format for a journal article [e.g. Denton and Gray (1983] vs. a chapter in a book [e.g. Denton and Gray (1988)]. Guidelines for other details (e.g. whether there should be a 'period' following each author's initial, whether the title of the journal should be italicized or bold, whether there should be a comma or colon after the volume no. etc) vary between journals and are enough to drive even the most seasoned scientist crazy! Just pick a style (any style you like in a reputable scientific journal) and use it consistently throughout your entire list of references!

Bossert, W. H. & Wilson, E. O. (1963). The analysis of olfactory communication among animals. *J. Theor. Biol.* 5, 443-469.

Coombs, S. (1996). Interpore spacings on canals may determine distance range of lateral line system. *Soc. Neurosci. Abstracts,* 22, 1819.

Denton, E. J. and Gray, J. A. B. (1983). Mechanical factors in the excitation of clupeid lateral lines. *Proc. Roy. Soc. Lond.* B 218, 1-26.

Denton, E. J. and Gray, J. A .B. (1988). Mechanical factors in the excitation of the lateral line of fishes. In: *Sensory Biology of Aquatic Animals* (Ed. by J. Atema, R. R. Fay, A .N. Popper & W. N. Tavolga); pp. 595-617. New York: Springer-Verlag.

Denton, E. J. and Gray, J. A. B. (1989). Some observations on the forces acting on neuromasts in fish lateral line canals. In *The Mechanosensory Lateral Line: Neurobiology and Evolution.* (Ed. by S. Coombs, P. Görner, & H. Münz. New York: pp. 229-246. Springer-Verlag,

Dijkgraaf, S. (1934) Untersuchungen uber die Funktion der Seitenorgane an Fischen. *Z. Vergl. Physiol. 20*, 162-214.

Feng, A., Gerhardt, H .C. & Capranica, R. (1976). Sound localization behavior of the green treefrog (*Hyla cinerea*) and the barking treefrog (*H. gratiosa*). *J. Comp. Physiol. A,* 107, 241-252.

Hofer, B. (1908) Studien uber die hautsinnesorgane der fische. I. Die funktion der seitenorgane bei den fischen. *Ber. Kgl. Bayer. Biol. Versuchsstation Munchen* 1, 115-164.

Montgomery, J.C. and Coombs, S. (1998). Peripheral encoding of moving sources by the lateral line system of a sit-and-wait predator. *J. Exp. Biol*. 201(1): 91-102.

Montgomery, J. C. and Milton, R.C. (1993). Use of the lateral line for feeding in the torrentfish (*Cheimarrichthys fosteri*). *New Zeal. Jour. Zool*. 20, 121-125.

Montgomery, J., Coombs, S., Conley, R.A. and Bodznick, D. (1995). Hindbrain sensory processing in lateral line, electrosensory and auditory systems: A comparative overview of anatomical and functional similarities. Audit. Neurosci.1: 207-231.

Montgomery, J.C, Baker, C.F. and Carton, A.G. (1997). The lateral line can mediate rheotaxis in fish. *Nature* 389, 960-963.

Strategies for Organizing Your Thoughts and Writing the Final Report

1. Write out of order. Although you have already written a research proposal and hopefully have some idea of what should be included in your introduction, your results may take you in an unanticipated direction. Until you actually write the results section, you may not have a clear idea of how you might want to pitch your introduction or discussion. Also, the introduction and discussion sections are the most difficult ones to write. Therefore, start with the easiest sections first.

One, write the materials and methods section first. This section should be the easiest to write and if you've written a good section on this for your research proposal, it should essentially be done already. You know what you did, and you should have notes or course materials on the protocols used. This is the most straight-forward section and starting with this section gets you rolling, which is often the hardest part of writing.

Two, write the results section next. This is the second easiest section. Here you are just describing your results. Plan your illustrations and make the graphs first. That way you are forced to think about what you actually have to present in terms of data. Take real care to make all the graphs you need, displaying all relevant data (probably most of what you've collected). Once you have made your figures, writing the results section is just a matter of verbally explaining and describing them. Make sure to stick to a logical plan and describe each figure in order. Often, the mere exercise of having to verbally describe the figures will reveal to you the logical plan that is needed.

Three, write the discussion and introduction sections. It's a toss-up as to whether you write the introduction or the discussion section next, as the two are intimately related. If you don't know why you did the experiment and how previous studies in the area led you to this experiment (the introduction), it's difficult to place the results of your own study in an overall context of other, relevant studies (the discussion). On the other hand, your results may take you in an unanticipated direction that only becomes evident as you start to think about and articulate their significance in the discussion section. Thus, the original rationale for doing your experiment, as described in your research proposal, may change, necessitating a change in the main thrust of the introduction as well. What is important to recognize is that the introduction and discussion go hand in hand like book-ends. The introduction needs to introduce the problem and give any background information a reader might need to understand the discussion. The discussion, on the other hand, must refer back to the introduction, and show how the problem was or was not solved and what questions (unsolved problems) remain.

Four, write the abstract last. It's nearly impossible to write an abstract before a paper is written. How can you summarize something before it exists?

2. Write an outline for each section and create subheadings to help organize your thoughts and make it easier for the reader to follow your train of thought. Organize your thoughts for each section by writing an outline and jotting down important points or section subheadings. Think about the ***logical flow of information***. Put yourself in the place of the reader and ask yourself what he/she needs to know before he/she is able to understand a graph or a conclusion that you have reached. Chances are that if you are *thinking* logically, clear and logical writing will follow. Not only does a clear and logical organization help the reader follow your work, but it also increases the probability that the significance of your results will be recognized and appreciated.

Subheadings can be used as both an organizational tool for you and a visual aid for the reader. For example, if the reader needs to review the methods section in order to understand a specific result, the use of subheadings *(e.g. Experimental Animals*, *Surgical Approach, Recording Techniques, Data Analysis*, etc) will make it easier for the reader to find what he/she needs. Likewise, if you are doing a set of related experiments designed to attack a specific problem from different angles or using different methodological approaches (e.g. anatomical and physiological), you might want to report the methods, results and discussion for each approach under different subheadings in each of the respective sections, reserving a separate subheading in the discussion section to address how the anatomical results relate to the physiological results in the context of the problem you are trying to solve. Alternatively, there may be different themes that you wish to talk about in the discussion. For example, you might want to have one subsection on how your results compare to those of similar studies, one on how your results from control experiments have ruled out possible confounding variables and another on the most likely interpretations of your results. Because the interpretation of your results might be affected by confounding variables, you would want to discuss confounding variables first before discussing what you think is the most reasonable interpretation of the results.

Evaluation of Final Report

Your final report will be evaluated on your ability to communicate the purpose, experimental design, results and significance of your research in the context of what is already known from the literature. Your report should be clear and well organized, according to the format and guidelines outlined above. The sample report from a previous student (Appendix B) is a good example of what you should strive for. You will also be evaluated by your faculty advisor (see form below) for your efforts, professional conduct and overall understanding of the research, as evidenced by your performance in lab and on the poster presentation and final report.

Faculty Advisor Evaluation

Please indicate the extent to which you agree or disagree with the following statements about the Neur 302 student you advised. Your evaluation can be based on the student's performance in lab, as well as on his/her performance on the poster presentation and final report.

1. demonstrated active engagement in the planning process by e.g. reading the literature, asking good questions, participating in lab discussions, working with the advisor to solve technical problems.

Strongly Agree Mildly Agree Neutral Mildly Disagree Strongly Disagree

2. made a conscientious effort to complete the research on time by doing the necessary amount of work, by following through on suggested plans of action, and by taking the responsibility to seek help when problems were encountered.

Strongly Agree Mildly Agree Neutral Mildly Disagree Strongly Disagree

3. showed consideration of others and respect for lab policies by following all agreed-upon lab procedures or schedules.

Strongly Agree Mildly Agree Neutral Mildly Disagree Strongly Disagree

4. made some original/new contributions to the research project, whether in terms of background literature searches, methodological developments, or data analysis and interpretation.

Strongly Agree Mildly Agree Neutral Mildly Disagree Strongly Disagree

5. showed a good understanding of the theoretical underpinnings of the research, including the research goals (e.g. hypothesis being tested), the experimental design, and the significance of the results.

Strongly Agree Mildly Agree Neutral Mildly Disagree Strongly Disagree

6. Additional comments:

Appendix A: Required and Suggested Readings

Copies of required readings are available in the manual or as in-class handouts. Copies of suggested readings are bound in two notebooks: one in the neuroscience lab (DH 1011) and the other in the INM office (DH 139). These two locations also house one copy each of Delcomyn's 1997 textbook, *Foundations of Neurobiology*, which is also on reserve at Sullivan Library.

Independent Research Project

Required:

Sample Research Proposal (Appendix B)
Sample Poster Lay-Out (Appendix C)
Sample Final Report (Appendix D)
Chapter 12, Introduction to Research Design, pp. 299 - 321 in: McCall, R.B. (2001) *Fundamental Statistics for Behavioral Sciences*, Wadsworth/Thompson Learning, CA. (Appendix E)

Suggested (General):

Phillips, J. L., Jr. (1992). How to Think About Statistics. W. H. Freeman and Company, New York

Martin, P. and Bateson, P (1993). Measuring Behavior: An Introductory Guide. Cambridge University Press, Cambridge

Part III on Experimental Methods IN: Shaughnessy, J.J. and Zechmeister, E.B. (1990). Research Methods in Psychology, McGraw-Hill, New York

Appendix B: Sample Research Proposal

(Note: The scope of this proposal and the experiment are similar to those we expect for the independent project, but the proposal itself is only a sample and may contain fictitious citations, etc)

Reaction Times to Sounds of Different Loudness

Specific Aims: Sounds of different frequencies, but of the same physical intensity, have different perceived loudness. The aim of this study is to determine if the loudness or the sound intensity of tones of different frequencies determines reaction time.

Background. Sound has the physical dimensions of intensity and frequency, but the subjective dimensions of loudness and pitch (Yost, 1994). A change in physical intensity always results in a change in subjective loudness and a change in physical frequency always results in a change in subjective pitch (Stevens, 1933). However, two sounds presented at the same intensity, but at different frequencies, can also result in a difference in loudness (Fletcher and Munson, 1933). For instance, a very low-frequency or a very high-frequency sound is not as loud as a mid-frequency sound, even if all sounds are presented with the same physical intensity (the basis of the equal-loudness contours, ANSI, 1969). In fact, the frequency of a sound can be so high or so low that it is not audible at all.

The time it takes to react or respond to a sound (reaction time) depends on the sound's physical intensity (Green, 1989). For instance, the time it takes for a listener to press a response button after a tone is presented depends on the physical intensity of the tone. In general, the more intense the tone is the faster the reaction time is. This finding is often explained in terms of the fact that neural conduction times are faster for more intense stimuli (Moore, 1990). Thus, a loud sound will cause neural transduction to be faster than that caused by a softer sound. The neural information resulting from a loud sound will reach the central nervous system sooner than that from a soft sound. Thus, the loud sound can be responded to faster than the soft sound (Green, 1976).

Green and Luce (1987) showed that as the physical intensity of a 1000-Hz tone increased from 20 dB above normal threshold (20 dB SPL) to 80 dB SPL, the reaction time for a button push decreased from 320 to 180 ms. However, Luce and Jesteadt (1988) showed that the reaction time to a 8,000-Hz tone decreased from 510 to 290 ms when the tone's physical intensity increased from 40 dB SPL to 90 dB SPL. Thus, it appears as if reaction time may be slower for a 8,000 Hz tone than it is for a 1,000-Hz tone. It is possible that the different reaction times are due to the different ranges of physical intensities used in the two studies or to other differences (e.g., different listeners) between the two studies. It is also possible that subjective loudness and not physical intensity determines reaction time. That is, a 8,000-Hz tone at 40 dB SPL is judged softer than a 1,000-Hz tone presented at 40 dB SPL (Fletcher and Munson, 1933). Thus, the fact that the reaction time to a 8,000-Hz, 40-dB SPL tone maybe slower than that to a 1,000-Hz, 40-dB SPL tone may be because loudness determines reaction time. That is, the softer the tone the slower the reaction time.

The goal of this study is to present tones of different subjective loudness, but of the same physical intensity, and measure reaction time. The hypothesis is that tones of the same subjective loudness will produce about the same reaction time, even when the tones differ in physical intensity. A basis for this hypothesis is that at the extremes, loudness must determine reaction times. That is, a sound of a very high frequency, even it has a high physical intensity, can be so soft that it will be barely audible. It seems reasonable that in this extreme case, there would be a long reaction time to such a soft sound. If this is true at the extremes, than we are hypothesizing that loudness will also determine reaction times in less extreme cases.

The results from this study may have important practical implications. In some situations, different warning sounds have different frequencies. For instant, a high-frequency warning sound may signify one type of danger, and a low-frequency warning sound may signify a different type of danger. It would be important that people respond equally fast to both types of warning sounds. Thus, it would be crucial to know if the two warning sounds (based on two different frequencies) should be presented at the same physical intensity or alternatively, at the same perceived loudness.

Methods:

Overall Design: Human subjects will be asked to make equal-loudness judgements for tones of different frequencies and physical levels. Reaction times to the same stimuli will then be measured for the same listeners. The reactions times will be compared to physical intensity and judged loudness.

Subjects: Five college students will serve as subjects in this experiment. The hearing of each subject will be determined and only normal hearing subjects will be used. The use of human subjects in these experiments have been approved by Loyola's Institutional Review Board.

Experimental Set Up and Procedures: A computer-controlled, modular hardware system (Tucker Davis Technologies) will be used to generate 500-ms tones of different frequencies (100, 1000, and 10,000 Hz) and different levels (30, 60, 90 dB SPL) for the 1000-Hz tone. Sounds will be played out through head speakers. The physical intensity of the 1000-Hz tone (the standard tone for the loudness stage of the experiment) will be presented at 30, 60, and 90 dB SPL. In the loudness stage of the experiment, the subjects will adjust the physical level of the 100-Hz tone until they judge the 100-Hz tone to be equal in subjective loudness to the 1000-Hz tone. This equal-loudness judgement will be made for the 30,60, and 90 dB SPL 1000-Hz standard tones as well. The experiment will then be repeated for the 10,000-Hz tone. At the end of the loudness stage of the experiment, a set of 100 Hz-and 10,000–Hz tones judged equal in loudness to a 1000-Hz tone will be established for various intensities of the 100-Hz tone. According to past work, the physical intensity of the tones judged equally loud will not be the same. For instance, it is expected that a 100-Hz sound with a physical intensity of 40-45 dB SPL will be needed in order for the subjects to judge this 100-Hz sound equally loud to a 30 dB SPL, 1,000-Hz standard sound (Fletcher and Munson, 1933). In the reaction-time stage of the experiment, the tones at the three frequencies and at the three physical intensities determined in the equal-loudness part of the experiment will be presented in a reaction-time experiment. Subjects will be presented the tones and they will be instructed to respond as soon as they hear the tone. The time it takes for the response button to be pressed from the time tonal onset will be measured to the accuracy of 0.1 ms.

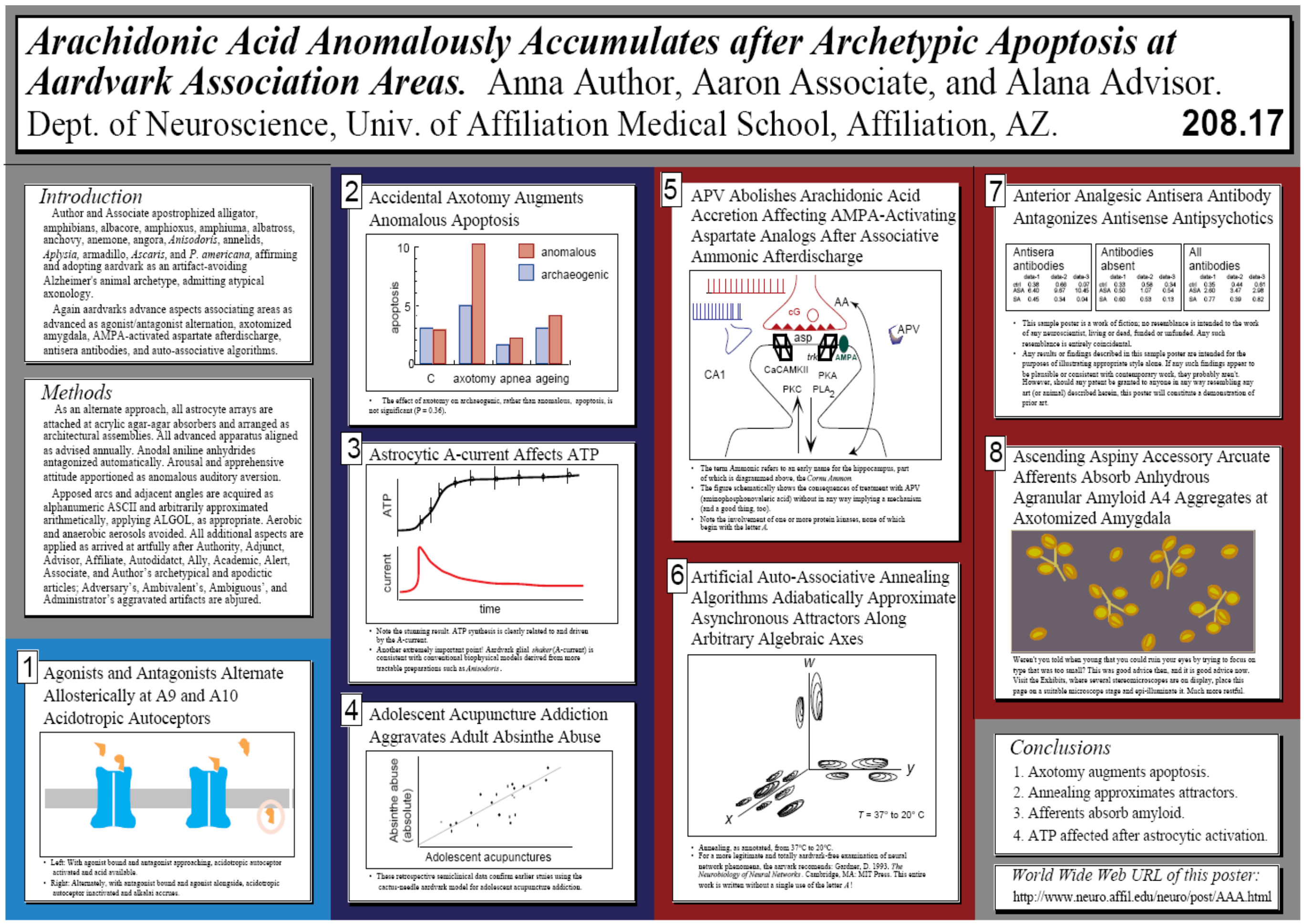
Data Analysis: There will be 25 trials for each combination of intensity and frequency (9 X 25= 225 trials for each of the five subjects). For instance, 25 trials will be presented for the 1,000-Hz tone at 30 dB SPL, at 60 dB SPL, and at 90 dB SPL. The same will be done for the 100-Hz tone when the physical levels are those determined from the loudness stage, as those physical levels judged to yield an equal-loudness match to the 1000-Hz standard tone. It is expected that reaction time will decrease for each tonal frequency as the tone's physical level increases. However, when the tones are presented at those physical intensities such that they were judged to be equally loud, then the reaction time should be similar. For instance, the tone at 100-Hz presented at the physical intensity judged equally loud to the 1,000-Hz, 30 dB SPL tone will have the same reaction time as the 1,000-Hz, 30-dB tone, even though it is expected that the 100-Hz tone will be 10-15 dB more intense than the 1,000-Hz tone. The comparisons will be based on the mean reaction times computed from the five subjects. If the mean reaction-time plus/minus one standard deviation for one condition overlaps that for another condition, then the reaction time for the two conditions will be judged to be statistically the same.

Literature Cited

Fletcher, H. and Munson, W.A. (1933) Loudness: Its definition, measurement, and calculation. *J. Acoust. Soc. Am.* 5, 82-108. (an example for a journal article)

Yost, William A. (1977) Fundamentals of Hearing: An Introduction, Academic Press, New York. (an example for a book)

Appendix C: Sample Poster Lay-Out (available as pdf file on Gateway computer on C: drive file folder: public/homework/independent research projects/poster layout.pdf)



Appendix D: Chapter 12, Introduction to Research Design, pp. 299 - 321 in: McCall, R.B. (2001) *Fundamental Statistics for Behavioral Sciences*, Wadsworth/Thompson Learning, CA. (available as handout from course coordinator). **E-mailed to you in December.**