

**School of Engineering and Technology, IUPUI
Multidisciplinary Undergraduate Research Institute (MURI)**

MURI Mentors Project Application Form

For Fall 2005

- *To be completed by a Mentor Faculty Member or Researcher from the School of Engineering and Technology.*
- *To fill in this form place your cursor in the form field and type. Tab from field to field.*

Date: July 2005

Name of Proposer: **John H. Schild, PhD**

Title of Proposer: **Associate Professor**

Department of Proposer: **Biomedical Engineering Department**

Proposed Project Title: **System for recording of single nerve fiber activity**

Approximate Duration: **2 semesters, only one semester of support requested**

Number of Students Requested: **3**

Disciplines or Majors Involved (at least two disciplines):

**Biomedical Engineering - 1
Electrical and Computer Engineering - 1 or 2
Mechanical Engineering Technology - 1**

Support Needed from MURI for Supplies and Equipment Usage (\$2,000 limit per team):

\$1,550 (see budget justification)

Project proposal with sections for the following information (please attach or cut and paste into this form):

1) Objectives, 2) Research Methodology, 3) Team Organization, 4) Expected Outcomes, 5) Benefits, 6) Time-Table, 7) Justification of Budget for Equipment and Supplies, 8) Short Resume. No more than five pages, excluding resume.

See following pages.

Please submit applications to MURI Scholars Program, Kelly Koher at kkoher@iupui.edu or 723. W. Michigan St., Rm. SL 260, Indianapolis, Indiana 46202, Tel: 317-274-9717, Fax: 317-274-9744. Electronic submissions are preferred.

Abstract

This project will bring together a team of undergraduate researchers (1 BME, 1 ECE and 1 MET) to develop an experimental setup for recording and preliminary analysis of electrical activity recorded from single afferent nerve fibers. Fine threads of biological membrane, or axons, are the primary source of information transmission to (afferent) and from (efferent) the central nervous system. There are two basic physiological types of afferent axons, myelinated and unmyelinated. Those with an insulating sheath of myelin are generally between 2 - 7 μm in diameter while unmyelinated axons are generally less than 2 μm in diameter. The electrical activity of these fibers have been studied for many years using electronic instrumentation capable of recording the extracellular field potentials produced as bioelectric signals traverse the length of these fibers. These signals are generally in the range of 20-100 μV and require a low noise, high impedance bipolar instrumentation amplifier for reliable recording. All equipment necessary for this task is presently available in the mentor's laboratory. The primary objective of the team will be to customize the instrumentation for a particular experimental application.

Project Objectives

There are three specific project objectives that are spread out across three team members. Additional details for each objective can be found under **Research Methodology**. While each member will have explicit tasks and expectations, all will have the opportunity to join in the work of other team members as interest dictates.

1. (BME) Surgical isolation of the vagus nerve from an adult (> 150 g) male rat and preparation of the tissue for neurophysiological recording. This team member will carry out the nerve stimulation and recording procedures.
2. (ECE) Design and fabrication of JFET based biopotential preamplifier for low noise detection of extracellular action potentials.
3. (MET) Design and fabrication of nerve recording chamber.

Research Methodology

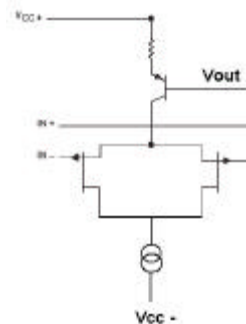
1. (BME) Surgical isolation of the vagus nerve

Adult male rats will be deeply anesthetized through free inhalation of halothane in an induction chamber. Euthanasia will be carried by cervical transection. The rat will be secured to a surgical station. Under stereo microscopy the vagus nerve will be dissected from the submandibular region down to the heart (~2 cm). The nerve tissue will be placed in a recording chamber containing and perfused (at 37°C) with a Krebs-Henseleit bicarbonate (KHB) solution containing (in mM): 118 NaCl, 4.7 KCl, 1.25 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 25 NaHCO₃, 10 HEPES and 11.1 glucose and equilibrated to a Ph of 7.35 with a bubbled gas mixture of 5% CO₂ - 95% O₂. The perfusion rate will be maintained between 2-4 ml per minute. The tissue will be cleaned of any gross connective tissues. Bipolar stimulation and recording electrodes will be positioned at opposite ends of

the nerve. These electrodes will be connected to a bipolar stimulus isolator (ISO-Flex, AMPI) and a commercially available bipolar recording amplifier and data acquisition system (LabScribe, ADI). Brief pulses (< 500 usec) of constant current will be used to evoke compound action potentials that will be recorded by the downstream bipolar recording electrode. The mentor believes that this use of animals in teaching and research is covered under his existing animal use protocol (SC143R). If not, a teaching protocol will be obtained before the surgical aspects of the project are initiated.

2. (ECE) Design and fabrication of JFET bipolar preamplifier

While the mentor has a commercially available biopotential amplifier for these recordings, the input impedance and electrical noise floor are not adequate for recording the microvolt level extracellular signals produced by the nerve fibers. This team member will be charged with identifying suitable transistors (likely JFET) for use in a standard differential recording mode (see schematic). The circuit will be first simulated in pSpice and eventually fabricated using matched pairs of transistors on a custom circuit board for low noise operation.



3. (MET) Design and fabrication of nerve recording chamber.

Nerve recording chambers are available commercially (see figure). However, these are general purpose devices that are less than optimal for our application. Successful implementation of this project will require machine fabrication of the chamber from a Plexiglas block and inclusion of mounts for stimulating and recording electrodes specific to our application.



Team Organization

An initial meeting of team members will be held with the faculty mentor in order to clarify project objectives, allocate specific subprojects and review laboratory facilities (SL140) and resources (SL140).

Over the duration of the project, team members are expected to attend the weekly meetings of postdoctoral fellows and graduate students in the mentor's laboratory. Each will give a brief report (< 5 min) and field questions from lab members regarding their progress toward meeting project objectives during the previous week.

Expected Outcomes

There will be two central outcomes of this project, 1) a custom piece of instrumentation that will become a regularly used research tool in the mentor's laboratory (see **Project Objectives** and **Research Methodology**) and 2) experience working as an interdisciplinary engineering team with well defined deliverables. In addition to such practical experience team members will gain and/or refine the following skills:

1. The initial team meeting w/the mentor will establish individual responsibilities w/in the team and expectations for sharing of information between team members. A list of

individual objectives will be established along with an initial timeline that all members will agree upon.

2. Weekly meetings will repeatedly enforce the need to keep good records of activities and the expectation by other lab members for a brief, concise and clear summary of progress and problems.
3. Weekly meetings will also require the student to “think on his/her feet”. As questions will be coming from lab members with varying experiences in the engineering and life sciences, team members will have to learn how to tailor their responses according to the skill set of the particular member asking the question.

Benefits

Each team member will have a well defined subproject and a clear understanding of how their particular deliverable must mesh with those of other team members. A time line for completion of each project will be established at the initial meeting along with expectations for a final demonstration of the complete system. In addition to use in his own research, the mentor intends to evaluate the potential for using the system in a series of neurobiology laboratory experiments for BME 331 Biosignals and Systems and BME 411 Quantitative Physiology.

Time-Table

All three projects can be initiated concurrently. As satisfactory materials and resources exist in the mentor’s laboratory to carry out the life science aspects of this endeavor the BME student will immediately begin training w/the mentor on the surgical procedures used in the nerve isolation and training w/the laboratory instrumentation. As prototypes of the preamplifier and recording chamber become available, these will be incorporated into the nerve recording protocols. It is anticipated that the entire project can be completed over the course of one semester: One month devoted to the initial design of the devices, one month devoted to fabrication and bench testing and one month devoted to laboratory testing and, if necessary, redesign.

Budget Justification

There are major expenses for this project.

1. \$750 – Animal purchase (30 Sprague Dawley adult male @ \$14/ea + S/H), husbandry (\$0.50/animal/day) Expendable laboratory supplies (syringes & filters, surgical supplies, recording solution supplies)
2. \$500 – Electronics purchase, fabrication of mini printed circuit board and shielded cables
3. \$300 – Materials purchase for fabrication of nerve recording chamber (Plexiglas, insulated Pt-Ir wire for stimulating and recording electrodes, luer lock connectors).

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format on preceding page for each person. **DO NOT EXCEED FOUR PAGES.**

| | | | |
|--------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------|---------|-----------------|
| NAME | POSITION TITLE | | |
| John Henry Schild | Associate Professor, Biomedical Engineering | | |
| <i>EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training)</i> | | | |
| INSTITUTION AND LOCATION | DEGREE <i>(if applicable)</i> | YEAR(s) | FIELD OF STUDY |
| Case Western Reserve University, Cleveland, OH | BS | 12/1983 | Biomedical Eng. |
| Case Western Reserve University, Cleveland, OH | MS | 05/1988 | Biomedical Eng. |
| Rice University, Houston TX | Ph.D. | 05/1994 | Bioengineering |

A. Positions and Honors

Professional Experience

- 1983 – 1986 Project Engineer, Rehabilitation Engineering Center, CWRU, Cleveland OH
- 1986 – 1988 Project Engineer, Veterans Administration Hospital & CWRU, Cleveland OH
- 1988 – 1991 Sr. Biomedical Engineer, Div. Restorative Neurology, Baylor College of Medicine, Houston TX
- 1992 – 1994 Teaching Assistant, Dept. Electrical and Computer Engineering, Rice University, Houston TX
- 1994 – 1995 NIH Fellow, Dept. Physiology and Biophysics, Baylor College of Medicine, Houston TX
- 1995 – 1996 NIH Fellow, Dept. Physiology and Pharm., Oregon Health Sciences University, Portland OR
- 1997 Research Asst. Prof., Dept. Physiology and Pharm., Oregon Health Sciences Univ. Portland OR
- 1998 – 2004 Assistant Professor (tenure track), Biomedical Engineering, IUPUI IN
- 1998 – present Adjunct Assistant Professor, Dept. of Biology, IUPUI IN
- 2003 – present Assistant Professor, Stark Neuroscience Research Institute, IU School of Medicine, IN
- 2004 – present Associate Professor (w/tenure), Biomedical Engineering, IUPUI IN

Fellowships

- Shell Foundation Predoctoral Fellow, Rice University, 1992-1994
- NIH Fellow, Institutional Training Grant HL07676, Baylor College of Medicine, 1994-1995
- NIH Fellow, Individual NRSA Award HL09242, Oregon Health Sciences University, 1995 thru 1996

Professional Activities

Reviewer (ad hoc): American J. of Physiology, Annals of Biomedical Engineering, Brain Research, IEEE Trans. Education, IEEE Trans. Biomedical Engineering, J. Applied Physiology, J. Neural Engineering, J. Neurophysiology, J. Neuroscience Methods, Neuropharmacology, Neuroscience

Member: American Physiological Society, Society for Neuroscience, Institute of Electrical & Electronics Engineers, Sigma Xi

Graduate Courses: Experimental Methods for Biomedical Engineers, Bioelectric Phenomena, Methods in Computational Neuroscience, Biosensors and Implantable Devices, Principles of Biomedical Engineering , Probabilistic Methods and Random Processes

School Committees: Graduate Engineering Education (F'01-F'02), Institutional Animal Care and Use Committee (F'01-F'03)

Session Chair: Mathematical Modeling in the Study of Neural Circulatory Control, FASEB Summer Symposium, July 1996

Founding President: International Society for Hybrid Microelectronics, CWRU graduate student chapter

B. Selected peer-reviewed publications

1. Y.H. Jin, T.W. Bailey, B.Y. Li, **J.H. Schild**, M.C. Andresen. P2X and VR1 receptor activation releases glutamate from separate cranial afferent terminals in nucleus tractus solitarius. *J. Neuroscience* Vol. 24(20), 4709-17, 2004.
2. Y.H. Jin, T.W. Bailey, M.W. Doyle, B.Y. Li, S.K. Chang, **J.H. Schild**, D. Mendelowitz, and M.C. Andresen. Ketamine differentially blocks sensory afferent synaptic transmission in medial nucleus tractus solitarius (mNTS). *Anesthesiology*. Vol. 98(1), 121-32, 2003.
3. B.Y. Li and **J.H. Schild**. Patch clamp electrophysiology in the nodose ganglia of the adult rat. *Journal of Neuroscience Methods*, Vol. 115(2), 157-67, 2002.
4. B.Y. Li and **J.H. Schild**. Comparisons of somatic action potentials from dispersed and intact rat nodose sensory ganglia using patch clamp technique. *Acta Pharmacol Sin* Vol. 23(6), 481-9, 2002.
5. P.A. Glazebrook, A.N. Ramirez, **J.H. Schild**, C.C. Shieh, T. Doan, B.A. Wible, D.L. Kunze. Potassium channels Kv1.1, Kv1.2 and Kv1.6 influence excitability of rat visceral sensory neurons. *Journal of Physiology*, Vol. 541, 467-82, 2002.
6. Z. Ben-Miled, D.R. Reitman, R.C. Chin and **J.H. Schild**. Synthesis of Ionic Currents Using Reconfigurable Hardware. *International Journal of Computers and Their Applications*, Vol. 7(3), September 2000.
7. W. Fan, **J.H. Schild** and M.C. Andresen. Graded and dynamic reflex summation of myelinated and unmyelinated rat aortic baroreceptors. *American Journal of Physiology*, 277:R748-R756, 1999
8. **J.H. Schild** and D.L. Kunze. An experimental and modeling study of Na⁺ current heterogeneity in rat nodose neurons and its impact on neuronal discharge. *Journal of Neurophysiology*, 78:3198-3209, 1997.
9. **J.H. Schild**, J.W. Clark, C.C. Canavier, D.L. Kunze and M.C. Andresen. Afferent synaptic drive of rat medial nucleus tractus solitarius neurons: Dynamic simulation of graded vesicular mobilization, release and non-NMDA receptor kinetics. *Journal of Neurophysiology*, 74(4):1529-1547, 1995.
10. Z. Tang, B. Smith, **J.H. Schild** and P.H. Peckham. Data transmission from an implantable biotelemetry by load-shift keying using a circuit configuration modulator. *IEEE Trans. on Biomedical Engineering*, 42(5):1995.
11. A.L. Leis, G.J. Grubweiser, **J.H. Schild**, M.S. West and D.S. Stokic. Control of Ia afferent input to triceps surae (soleus) locomotor nucleus precedes agonist muscle activation during gait. *Journal of Electromyography and Kinesiology*, 5(2): 95-100, 1995.
12. **J.H. Schild**, J.W. Clark, H. Hay, D. Mendelowitz, M.C. Andresen and D.L. Kunze. A- and C-type rat nodose sensory neurons: Model interpretations of dynamic discharge characteristics. *Journal of Neurophysiology*, 72: 338-2358, 1994.
13. A.L. Leis, **J.H. Schild** and D.S. Stokic. Modulation of the tibialis anterior and triceps surae (soleus) H-reflexes during gait. *Muscle and Nerve*, 17(1): 1994
14. **J.H. Schild**, S. Khushalani, J.W. Clark, D.L. Kunze, M.C. Andresen and M. Yang. An ionic current model for neurons in the rat medial nucleus tractus solitarius receiving sensory afferent input. *Journal of Physiology*, 469:341-363, 1993
15. V.L. Delitis, **J.H. Schild**, A. Beric and M.R. Dimitrijevic. Facilitation of motor evoked potentials by somatosensory afferent stimulation. *Journal of EEG and Clinical Neurophysiology*, 85:302-310, 1992

Extramural Research Support (active)

1. **Neurobiology of baroreceptor perikarya and afferentation**

Principle Investigator: John H. Schild, PhD

Agency: The National Institutes of Health, R01 HL072012. Period: 07/03 thru 06/08

Summary: Characterization of voltage- and ligand-gated ion channel properties underlying the differential discharge characteristics of identified aortic baroreceptor neurons in adult rat. A unique aspect of this in vitro study is the use of an intact never-ganglion preparation which makes possible the functional classification of the afferent fiber type associated with the neuron under patch clamp study.

Extramural Research Support (past)

2. **System for Real-Time Functional Assessment of Ion Channel Dynamics**

Principle Investigator: John H. Schild, PhD

Agency: The Whitaker Foundation, Biomedical Engineering Research Award, Period: 01/00 thru 12/03

Summary: A career development award from The Whitaker Foundation with a programmatic goal of integrating the PI's biomedical engineering and life science skill sets. The overarching goal of the research project is to develop a hybrid computational platform capable of using high order mathematical models of ion channel function as real-time investigative tools in cellular electrophysiology.

3. **Neurophysiology of Identified Cardiac Sympathetic Afferent Neurons in the Rat**

Principle Investigator: John H. Schild, PhD

Agency: American Heart Association, Scientist Development Grant (9630277N), Period: 01/97 thru 12/00

Summary: A career development award from The AHA. The overarching goal of the research project was to characterize the electrophysiological properties of fluorescently identified cardiac afferent neurons in the rat. Neurons were enzymatically dispersed from dorsal root and nodose ganglia and studied using the patch clamp technique. Slices of ganglia were prepared for patch clamp study, which enabled identification of fiber type through conduction velocity measurements.

Extramural Research Support (pending)

1. **Neuromechanical basis of baroreceptor function**

Principal Investigator: John H. Schild, PhD

Agency: The National Institutes of Health, R01 HL081819. Period: 02/06 thru 01/11

Summary: The overarching hypothesis for this study is that the regional microanatomy, extracellular tissue matrix and excitable neural membrane of the baroreceptor terminal complex and arterial wall each make functionally distinct contributions to the spatial integration and transduction of localized micromechanical forces associated with arterial pressure. Specific aims center upon the neuromechanical properties of myelinated and unmyelinated aortic BR in the rat as these afferents are accessible for both micro- and macroscopic study using three complementary methodologies: **1)** confocal, electron and fluorescent microscopy in conjunction with immunohistochemical labeling of protein expression at the BR terminal ending, **2)** extracellular recording of aortic BR fiber discharge in response to computer controlled pressure loading of the arterial wall and **3)** reassembly of these disparate microscopy and biophysical data into comprehensive computational models of the

neural and micromechanical mechanisms of mechanosensory transduction that are inaccessible for direct testing and measurement.

2. **Neuromechanical basis of baroreceptor function**

Principle Investigator: John H. Schild, PhD

Agency: The National Institutes of Health, R01 HL081819. Period: 12/05 thru 12/10

Score: 1st submission score of 198 with a percentile of 32.1, 2nd submission planned for 7/05

Summary: An integrative experimental and computational study of the functional contributions to neural encoding of arterial pressure via micromechanical, neuroanatomical and ion channel mechanisms associated with arterial baroreceptors.