

FY2000
Proposal for a Shared Use
Sutter Laser Based Pipette Puller, Model P-2000

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This is a proposal requesting \$11,450 from the FY2000 KUMC Shared Biomedical Research Equipment Funds.

Introduction

The overall goal of this proposal is to acquire a Laser based, quartz micropipette puller to permit more accurate, reproducible, and efficient preparation of reliable and high quality microelectrodes with fine, adequate tip sizes and properties for electrophysiological recordings or stimulation of biological tissues.

A micropipette puller is an indispensable piece of equipment in research labs that use microelectrodes. Conventional pullers employ metal heating elements adequate only for materials of lower melting points, such as borosilicate or aluminosilicate glass. The tip properties of the microelectrodes made from these glass materials are often susceptible to conditions in the ambient environment such as temperature and humidity, even during storage. The electrodes, therefore, need to be made freshly for each experiment. In addition, during fabrication of the microelectrodes, it is often necessary to change the heating and pulling parameters as ambient conditions change in order to get a desired tip size and property for a specific purpose of recording, micro-stimulation, or micro-injection. The process, therefore, requires skills, experience, and significant preparation time before each experiment. Furthermore, due to the nature of the material properties, the tips of the microelectrodes made from borosilicate or aluminosilicate glass often are unable to withstand the torsion created when penetrating tough connective tissue, causing them to break.

Quartz is a pure silicon dioxide material possessing superior qualities to that of borosilicate or aluminosilicate glass. It has, however, a higher melting point. Using Laser technology, quartz microelectrodes can be fabricated by heating quartz tubings with, for instance, a CO₂ LASER, and pulling them with a mechanical system of high precision and accuracy. The fine tips of the quartz microelectrodes not only have a *higher torsional strength*, but also are less susceptible to changes in ambient conditions and are able to *maintain the desired tip property* for extended storage times of up to weeks or months. Because they last longer, either when stored or in use, fewer would need to be made, resulting in *significant time and material savings* for the investigators.

1. Description of Equipment and Price

The Sutter Laser-Based Micropipette Puller, Model P-2000

The attached printout from Sutter's website provides a detailed description of the equipment and the quoted price. Briefly, this is a classic Flaming/Brown type puller of high mechanical precision, in combination with a powerful CO₂ Laser and sophisticated programmable microprocessor controller. It is capable of pulling electrodes with a tip diameters measuring less than 0.01 μm, and is also quite effective in fabricating fine electrodes that have a larger pore size (therefore lower resistance) from thin-wall quartz

tubings for dye or current injection. Because of the programmable microprocessor, the fabrication can be made with a higher reliability and efficiency than other commercial pullers.

Rationale for selecting the Sutter:

Sutter is currently one of the few major vendors that specialize in providing a range of equipment for fabricating and manipulating microelectrodes in electrophysiological recordings, and is the only vendor that makes the classic Flaming/Brown type puller. There have been consistent and extensive efforts by the company in research and development of new products, and in providing high quality maintenance service and technical support.

The total cost of the equipment is \$14,950. This includes the basic unit quoted at \$12,250 (see attached price list), \$2,000 for quartz tubings of various sizes, and \$ 700 estimated for the shipping/handling/installing/in-service cost.

2. Statement of Equipment Need

Currently, Drs. Nudo and Chen are collaborating on a study in which we examine the potential neural structures that serve as substrates for compensatory or adaptive post-injury motor strategies. In this study, we need to physiologically characterize both the primary motor and the ventral premotor areas of squirrel monkey cortex by intracortical electrical microstimulation. At a grid resolution of about 250 μm , the procedure often requires over 400 penetrations into the surface of the cortex. Since the procedure is performed multiple times in the same animal over a period of months, the connective tissues (e.g. pia mater) on the surface of the cortex often becomes more tough, and therefore makes penetration with the stimulating glass micropipette more difficult. The tips of the glass micropipettes either break, or dimple the cortical tissue to the extent that long-lasting depression of neural activity occurs. *Either outcome can significantly slow the progress of the experimental procedure, and seriously compromise the results.* The quartz microelectrodes made by the proposed Laser Puller should overcome that difficulty.

Dr. Chen has considerable experience using a prototype version of Sutter P-2000 when it was first launched as a test model in the early '90s. Quartz micropipettes played a crucial role in his successful intracellular recordings of cortical neurons from awake behaving primates (see the attached reprint). The fine but resistive tip of the quartz micropipette proved to be more effective than that of borosilicate or aluminosilicate glass in penetrating the tough connective tissue such as the regenerated pia at the cortical surface. The desired fine tip size of the quartz micropipette also survived better after the penetration.

In recent years, quartz microelectrodes also have been found to have superior quality in patch-clamp recording either for single channel, multiple channels, or whole-cell patches (see the cited publications in the attached reference list from the Sutter website). KU Research projects using these approaches such as in Drs. Stehno-Bittel, Valenzeno, and Michaelis' labs will benefit from availability of this equipment. Since it is possible to fabricate the quartz microelectrodes and store them for an extended period of time without compromising their tip properties, it is feasible for a shared use of the equipment between KUMC and KU-Lawrence campuses.

3. Availability of the proposed Equipment at the KUMC

Is the equipment new or a replacement of an existing item?

This equipment is new, and not available at the KU. It features the Laser technology and a high precision mechanical system controlled by a sophisticated, programmable microprocessor, and provides new research capabilities at KU.

4. Name and department of responsible individual

Daofen Chen, Ph.D.

Assistant Professor, Department of Physical Therapy Education

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Laboratory: Rm. 2008 Smith Building, MRRC, phone: 8-5695, AlphaPager: 917-0141.

Upon acquisition, the proposed equipment will be housed in Dr. Chen's laboratory at 2008 Smith Building, MRRC. The equipment will be made available and accessible to other participating faculty and colleagues *at all times and no cost*.

5. Names of investigators and summary of the research programs

Dr. Chen's research program (Dept. of Physical Therapy Education)

Current funding

American Heart Association, "Neural basis of functional recovery after stroke: Effects of rehabilitative training on cortical neuroplasticity". 1/1/99-12/31/02, \$260,000 total direct costs.

Program summary statement of equipment use

In addition to the collaborative research project with Dr. Nudo described above, the major focus of Dr. Chen's proposed research is to understand the neural mechanism of adaptive changes in cerebral cortex by examining the relationship between the electrical activities and the synaptic plasticity of cortical neurons. The methodology mainly involves simultaneous multi-channel recordings of both intracellular and extracellular (both fast spike and slow field potentials) potentials from cortical neurons, and electromyographical activities in trained behaving monkeys. Event or synaptic potential triggered stimuli will be applied; and their effect on the efficacy of cortical synaptic transmission will be determined. The proposed equipment will be a crucial piece in obtaining the appropriate microelectrodes for both intracellular recordings and extracellular injection of current or dyes.

Dr. Nudo's research program (Center on Aging, Dept. of Physiology)

Current funding

NIH-NINDS, "Reorganization of Motor Cortex Following Brain Injury". 6/1/97-5/30/02, \$1,013,786 total direct costs

Program summary statement of equipment use

Work in this lab focuses on the neural bases for motor skill acquisition and recovery of function after brain injury. Studies utilize neurophysiologic, neuroanatomic, and behavioral techniques to determine the capacity for the primary motor cortex to be altered physiologically and anatomically during motor skill learning in normal and brain-injured animals. Another key element of this research program is to examine local, or intrinsic, connections within the motor cortex. We will determine the specificity of these interconnections and whether they are altered after injury or through use. Microstimulation combined with chronic multi-channel electromyographic recordings will be used to determine the extent of functional plasticity in motor cortex after a stroke-like injury to cerebral cortex. Intracellular injection of dyes in lightly fixed slabs of cortical tissue will be used to describe changes in dendritic branching after injury. The proposed micropipette puller will be indispensable in both the microstimulation studies and in the anatomical studies using intracellular injection of dyes.

Dr. Stehno-Bittel's research program (Depts. Physical Therapy Education and Anatomy)

Current funding

NIH, R01, Brown (PI) Stehno-Bittel (Co-I), "Prehabilitation vs. Rehabilitation for Reducing Bedrest Effects with Aging", 1998-2003, \$476,940, total direct cost.

NIH, R01, Stehno-Bittel (PI), Dunn (Co-I) "Nuclear Ca^{2+} regulates structure/function of pore complex", 1999 – 2004, \$1,065,295 total direct cost.

Plus include the American heart grant – we have an extension on that one.

Program summary and statement of equipment use

Work in this laboratory focuses on the transport of molecules across the nuclear membrane and their regulation by calcium ions. We use patch-clamp techniques to record the activity of

calcium channels on the nuclear membrane. These calcium channels are activated by inositol trisphosphate and are kinetically similar to channels found on the endoplasmic reticulum. We currently use a Sutter Pipette Puller (without quartz capabilities) to fabricate our glass pipettes of borosilicate glass. Our system is built to simultaneously measure both fluorescence from a sample and single channel activity. However, the fluorescent lamp induces too much noise into the current system to allow us to distinguish individual channel activity. *Quartz pipettes have significantly lower noise* and should afford us the ability to measure both parameters simultaneously. I have used quartz pipettes pulled from a Laser Sutter puller as a post-doctoral fellow at Mayo Clinic. The requested equipment is reliable, easy to use, and fashioned for multiple users with the programmable pulling function.

Dr. Cheney's research program (Dept. of Physiology, MRRC)

Current Funding

NIH-NINDS, "Functional studies of brain Infection with neurovirulent SIV in rhesus macaques". 3/1/99-2/28/00 \$195,103. Total project period 4/1/99-2/28/03.

NIH-NINDS, "Corticospinal control of forelimb movement". 9/15/99 – 8/31/00, \$185,079. Total project period: 9/15/99 – 8/30/03.

NIDA "Neuro-AIDS in opiate dependent rhesus macaques". 9/30/99 – 8/31/00, \$404,609. Total project period: 9/30/99 – 8/31/04.

SPINAL CORD RESEARCH FOUNDATION, "Mechanisms of neural compensation for motor disabilities following lesions of the corticospinal system". 3/1/99 – 2/28/00 \$54,750. Total project period: 1/1/97-2/28/00.

Program summary and statement of equipment use

My research focuses on: 1) brain mechanisms underlying the control of voluntary movements, 2) recovery of motor function following brain injury, and 3) motor and cognitive deficits associated with HIV/SIV (simian immunodeficiency virus) infection. We use neurophysiological techniques to investigate the function of neurons in the cerebral cortex and brainstem. The electrical discharges of single neurons are recorded in awake monkeys trained to perform various movement tasks. Computerized signal analysis techniques are used to reveal the functional contribution of a neuron to movement. In another set of projects, we investigate the mechanisms by which SIV infects the brain and injures neurons using neurobehavioral, neurophysiological and neuroanatomical and imaging approaches. We are also investigating the interaction between opiate drugs and neuronal injury associated with lentiviral infection. We will use the equipment in this proposal for producing electrodes that will be used in collecting electrophysiological signals from neuronal cells in normal and SIV-infected monkeys.

Dr. Valzeno's research program (Dept. of Molecular and Integrative Physiology)

Current funding

Departmental support

Pending funding

1. American Heart Association, Grant-in-Aid, Increases in calcium and membrane permeability in preconditioning and cross-preconditioning of cardiac cells in culture 01/01/00 to 12/31/02, requested funding \$195,000 (direct costs).
2. Lied Basic Research Fund Preconditioning in Singlet Oxygen-Mediated Cardiac Cell Death 01/01/00 to 12/31/01, requested funding \$70,000 (direct costs).
3. NIH, R01, Singlet Oxygen and Ca⁺⁺ as Signals in Cardiac Cell Death, 07/01/00 to 06/30/04, \$500,000 (direct costs).
4. Heartland Heart Affiliate, Grant-in-Aid, Importance of bcl-2 in H9c2 rat heart cells, 07/01/00 to 6/30/02, \$80,000 (direct costs).

Program summary and statement of equipment use

Our research program is aimed at understanding how the effects of reactive oxygen species on cell membranes influence cell survival. We have focused on cardiac cells and in particular on the effects of singlet oxygen generated by photosensitizers and light. We monitor changes in membrane permeability by patch clamp electrophysiological techniques in collaboration with department member Dr. Merrill Tarr. The electrophysiological results are correlated with measurements of intracellular calcium levels in collaboration with Dr. Lisa Stehno-Bittel, see above, and with determinations of cell survival. Microelectrode fabrication is a significant component of our electrophysiological studies. The proposed puller will save a considerable amount of preparation time and will provide a higher reliability and quality of patch recording.

Dr. Jeff Radel's research program (Occupational Therapy Education)

Current funding

NIH-NEI, "Transplanted retinæ - Functional integration and efficacy". 4/1//94-3/1/00, \$381,620, total direct costs.

Program summary and statement of equipment use

Much of my research in the next several years will require detailed analysis of neuronal innervation patterns. Of particular importance for my studies will be the ability to create durable electrodes which can then use for precise injections of neuronal tracers and dyes. An important emerging issue in my research is the extent of anatomical proximity between clusters of specific cell types, and the nature of cell-cell contacts in these regions. This sort of analysis would be facilitated greatly with this technology, and the system proposed for shared use by Dr. Chen will meet my need. Because the quartz electrodes retain their tip shape, it will be far more efficient to create a number of pipettes at the same time and then store the prepared pipettes until they are needed. I therefore anticipate that I will use the proposed system occasionally, perhaps once per month, so I do not foresee conflicts arising through its shared use among these investigators. This device will serve a need not presently available on this campus, and I believe its presence in Dr. Chen's laboratory would be a valuable asset to the research community.

6. Outline of commitments for sharing the cost of purchase.

Sources of matching funds are listed as follows:

Cost sharing

Total cost of the Sutter Laser Based Pipette Puller, Model P-2000	\$14,950
Total cost sharing	\$3,500
Dr. Chen's research grant	\$1,500
Center on Aging, Dept. of Physiology (Dr. Nudo's grant)	\$600
Depts. of P.T. Edu. and Anatomy (Dr. Stehno-Bittel's grant)	\$400
Dept. of P.T. Edu. (Fund authorized by the Chair, Dr. Enwemeka)	\$400
MRRC (Fund authorized by the Director, Dr. Cheney)	\$300
Dept. Physiology (Dr. Cheney's grant)	\$200
Dept. Occupational Therapy (Dr. Radel's grant)	\$100
Amount requested from FY99 shared research equipment funds	\$11,450

More than 20% of the total sum of money required for purchasing this equipment will be immediately available as matching funds. Consequently, we are requesting \$11,450 out of the \$14,950 required for purchasing the Sutter Laser Based Pipette Puller, Model P-2000.