PART I *(Form Pages 1 to 6, 9)*

Form Approved Through [illegible]

Department of Health and Human Services

Public Health Service

Council: 05/2005

PI: PONCE, CARLOS R

IPF:3212902

Rt 9 8 5 3 7 4 vice Aw: NOV 16 2004 ation

1 F31 NS052926-01

Dual: GM

IRG: ZRG1 DIG-B(29) L

Received: 11/16/2004

Follow instructions carefully.
Do not exceed character length restrictions indicated.

1. TITLE OF RESEARCH TRAINING PROPOSAL *(Do not exceed 56 characters)*
Minority Predoctoral Fellowship Program

2. LEVEL OF FELLOWSHIP	3. PROGRAM ANNOUNCEMENT/REQUEST FOR APPLICATIONS	
Predoctoral	PA-00-069	
4a. NAME OF APPLICANT *(Last, First, Middle Initial)*	4b. EMAIL ADDRESS	4c. HIGHEST DEGREE(S)
Ponce, Carlos R.	Personal Info	B.S.

4d. PRESENT MAILING ADDRESS *(Street, City, State, Zip Code)*	4e. PERMANENT MAILING ADDRESS *(Street, City, State, Zip Code)*
MD/PhD Program Personal Info Boston, MA 02115	Personal Info

4f. OFFICE TELEPHONE NO. *(Area Code, No. and Ext.)*	4g. HOME TELEPHONE NO. *(Area Code and No.)*	4h. PERMANENT PHONE NO. *(Area Code and No.)*	4i. FAX NUMBER *(Area Code and No.)*
Personal Info	Personal Info	Same as 4g.	N/A

4j. Personal Info

5. TRAINING UNDER PROPOSED AWARD *(See Fields of Training)*		6. PRIOR AND/OR CURRENT NRSA SUPPORT *(Individual or Institutional)*
Discipline No.: 2920	Subcategory Name: Systems/Integrative Neuroscience	☒ NO ☐ YES *(If "Yes," refer to Item 24, Form Page 5)*

7a. DATES OF PROPOSED AWARD		7b. PROPOSED AWARD DURATION	8. DEGREE SOUGHT DURING PROPOSED AWARD	
From *(MM/DD/YY)*:	Through *(MM/DD/YY)*:	*(In months)*	Degree:	Expected Completion Date:
07/01/05	06/30/10	60	MDPh	06/30/10

SPONSOR COMPLETES ITEMS 9 THROUGH 14

9. HUMAN SUBJECTS	9a. RESEARCH EXEMPT	9b. HUMAN SUBJECTS ASSURANCE NO.	9c. NIH-DEFINED PHASE III CLINICAL TRIAL	10a. VERTEBRATE ANIMALS	10b. ANIMAL WELFARE ASSURANCE NO.
☒ NO ☐ YES	☒ NO ☐ YES If "Yes" Exemption No.:		☐ NO ☐ YES	☐ NO ☒ YES	A3431-01

11a. NAME OF SPONSOR (Last, First, Middle Initial)	11b. NAME OF PROPOSED SPONSORING INSTITUTION
Personal Info Telephone: Fax: Email:	Harvard Medical School Address: 25 Shattuck Street Boston, MA 02115
11c. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT	
Department of Neurobiology	
11d. MAJOR SUBDIVISION	12. ENTITY IDENTIFICATION NO. / DUNS NO.
Harvard Medical School	EIN / 047006379
13. NAME AND TEL. NO. OF ADVISOR IF DIFFERENT FROM 11a. Telephone: Name and address of institution where research training will take place if different from Item 11 b. Address:	14. NAME OF OFFICIAL IN BUSINESS OFFICE Personal Info Telephone: Fax: Title: Asst Director of Sponsored Research Address: Personal Info 25 Shattuck St. Boston, MA 02115 Email: spa_award@hms.harvard.edu

15. APPLICANT CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete, and accurate to the best of my knowledge, and I agree to comply with the terms and conditions of award if an award is issued as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I certify that I have read the Ruth L. Kirschstein National Research Service Award Assurance, that I will abide by the Assurance if an award is made, and that the award will not support residency training.

SIGNATURE *(Required of each applicant)*	DATE
[signature]	11/09/04

Stipends	$20,772
Other Fellowship Expenses	$11,913
Institutional Allowance	$2,750
Federal Direct Costs	$35,435
TOTAL AWARD	$35,435

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project, is as follows.
02 $35,435
03 $35,435

FISCAL INFORMATION:
CFDA Number: 93.853
EIN: EIN
Document Number: FNS052926A

IC/	CAN /	FY2005 /	FY2006 /	FY2007
NS/	8426313/	35,435/	35,435/	35,435

NIH ADMINISTRATIVE DATA:
PCC: EDWARECN / OC: 41.2L /Processed: MYRECKE 050727 0232

Fellow's e-mail: Personal Info

SECTION II - PAYMENT/HOTLINE INFORMATION - 1 F31 NS052926-01

For Payment and HHS Office of Inspector General Hotline Information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III - TERMS AND CONDITIONS - 1 F31 NS052926-01

This award is based on the application submitted to, and as approved by, the NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Grant Award.
b. The restrictions on the expenditure of federal funds in appropriations acts, to the extent those restrictions are pertinent to the award.
c. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
d. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(3) Research Design and Methods

General aims: To perform the following experiments, we will train a monkey to sit in a primate chair, using water and fruit as positive reinforcements. After a period of approximately ten days, a scleral coil and headpost will be implanted using standard surgical procedures. The monkey will be allowed to recuperate for seven to ten days. After this period, Proprietary Info

Proprietary Info The chamber will allow the insertion of recording electrodes through the anterior bank of the superior temporal sulcus and into MT. Both devices will be fixed to the skull using dental acrylic. We will perform these procedures in collaboration with Steven Lomber.

There are several Proprietary Info First, the retinotopy of V2 in the lunate sulcus in macaques has been well-defined (Zeki and Sandeman, 1976, Van Essen and Zeki, 1978) and it represents only an area of 4x10° below the horizontal meridian. Prior studies have shown that a) neuronal activity is silenced at temperatures of 20° or less, b) the 20° thermocline is usually localized to 1-2 mm from the actual loop (Lomber *et al.*, 1999) and c) most MT-projecting neurons in V2 actually reside in the top layers (II and III), which would be most vulnerable to the cooling (Maunsell & Van Essen, 1983; Shipp & Zeki, 1989). Therefore, the most important factor to determine the region of inactivation is the shape of the loop itself. To prove that V1 input to MT remains viable during activation of the cryoloop, we will also implant an electrode on the opercular side of the posterior bank of the lunate, a region of V1 that corresponds to the retinotopical area of V2 inactivation. We will measure field potentials from this region during the control, cooling and recovery phases of our experiments.

Behavioral task: The monkey will be trained to fixate on a small target, which will be placed on a screen position that brings the reversibly-inactivated region to the center of the monitor. Liquid rewards will be administered if the animal maintains gaze within a small window (3°-diameter) for a variable time interval (1-3 seconds).

General experimental strategy: At the start of every recording session, we will find MT by measuring the depth of electrode penetration and quantifying the size and direction-selectivity of the receptive fields (RF) encountered during penetration. Once we confirm our desired location, we will select RFs that are located within the corresponding V2 scotoma for the inactivation experiments.

Every isolated neuron will be initially characterized by its 1) direction-, 2) speed- and 3) disparity response properties as well as 4) its area summation curve. All stimuli within this initial experimental block will consist of circular patches of random dot fields (RDFs) of constant density and luminance, which will be presented in a continuous, interleaved random fashion. To quantify direction selectivity, each RDF will move in one of eight directions (0-315° in 45° intervals) at a fixed speed and with zero disparity. After the neuron's preferred direction is identified, we will measure the speed tuning by displaying a series of RDFs moving at seven different speeds (2, 4, 8, 16, 32, 64 and 128°/s). Area summation curves will be plotted by presenting RDFs with a range of aperture sizes determined by the neuron's measured RF size, moving at the neuron's optimal parameters.

In the experiment blocks measuring disparity tuning, our stimuli will be RDFs containing dot pairs, one colored red and the other colored cyan, offset horizontally by a variable distance. The monkey will view this stimulus through a pair of monocular filters colored red or cyan, so only one set of dots is visible to each eye. This resulting retinal disparity creates a sense of depth to the observer. We will present a range of stimuli with spatial disparities ranging from –1.6° to +1.6° at intervals of 0.4°, moving at the optimal speed and direction.

Specific Aim 1. *To determine whether recovery of the true direction of motion in MT neurons is* Proprietary Info Proprietary Info

Integration of contour and terminator signals. The two-dimensional integration of conflicting motion signals (like those contained in barber poles) is a time-dependent process that has been observed at the neuronal, oculomotor and perceptual levels Personal Info Masson *et al.*, 2000; Lorenceau *et al.*, 1992). Using fields of bars oriented at different angles with respect to their direction of motion, Personal Info observed that MT neurons initially respond to the direction of movement defined by the contours. Over the following 70-80 ms, the neurons' direction-tuning represents the true global direction, represented by the line terminators. Proprietary Info

Proprietary Info

von der Heydt R, Peterhans E. (1989a) Mechanisms of contour perception in monkey visual cortex. I. Lines of pattern discontinuity. J Neurosci. May; 9(5): 1731-48.

von der Heydt R, Peterhans E. (1989b) Mechanisms of contour perception in monkey visual cortex. II. Contours bridging gaps.J Neurosci. May; 9(5): 1749-63.

von der Heydt R, Peterhans E. (1993) Functional organization of area V2 in the alert macaque. Eur J Neurosci. May 1; 5(5): 509-24.

Zar, JH (1999) Biostatistical Analysis, 4th Ed. Prentice-Hall, Inc.

Zeki SM. (1969) Representation of central visual fields in prestriate cortex of monkey. Brain Res. Jul; 14(2): 271-91.

Zeki SM. (1971) Cortical projections from two prestriate areas in the monkey. Brain Res. Nov; 34(1): 19-35.

Zeki SM. (1974) Functional organization of a visual area in the posterior bank of the superior temporal sulcus of the rhesus monkey. J Physiol. Feb; 236(3): 549-73.

Zeki SM. (1978) Uniformity and diversity of structure and function in rhesus monkey prestriate visual cortex. J Physiol. Apr; 277: 273-90.

Zeki SM. (1980) The response properties of cells in the middle temporal area (area MT) of owl monkey visual cortex. Proc R Soc Lond B Biol Sci. Feb 29;207(1167):239-48.

Item 10b.

(7) Vertebrate Animals.

1. All surgical procedures will be done in an inspected and approved operating room using aseptic technique and general anesthesia. The procedures for handling alert monkeys have been described in detail in the Methods section and are standard practice in many laboratories. All behavioral training is accomplished through operant conditioning techniques in which the animal is *rewarded* for desired behavior. No punishment will be used. Since the animals work for liquid rewards, daily water intake is regulated to maintain the animal in a motivated, but healthy state. Each animal always has the opportunity to work for as much water as it desires. In addition, a minimum daily water intake is established during the training period, and the animal's intake is supplemented by the experimenter if necessary. The animal's hydration status is monitored each day by measurement of body weight, inspection of the feces and assessment of skin turgor. All experiments will be performed on adult macaque monkeys, male and female, weighing between 4 and 15 kg.

2. The overall goals of this project are to understand the cortical circuitry underlying higher-order motion processing and the role that it plays in perception and behavior. This kind of study can only be performed in a living animal that possesses the biological structures we are interested in understanding and that can be trained to report their perceptions. Computer models are not yet sophisticated enough to serve as subjects in the experiments we have proposed. The macaque monkey is particularly well-suited for these studies, first because behavioral experiments have shown that its visual capabilities are very comparable to those of the human, thus making it a good model for understanding cortical processing in the human brain; second, because a great deal of knowledge about the overall structure of its visual system already exists; and, third, because these monkeys train well and readily adapt to the laboratory routine for behavioral and physiological experiments. Proprietary Info

Proprietary Info

3. Animals will be regularly examined by members of the veterinary staff at the Animal Resource Center at Harvard Medical School. We have found the veterinarians here to be knowledgeable, professional and dedicated, and we are confident that our animals receive the best of care. They are housed in baboon-sized cages, volumetrically 6 times larger than required. We also have access to veterinary staff with extensive primate experience through the New England Regional Primate Research Center.

4. The only potentially painful procedures are those involving surgery, and these will be done under general anesthesia in an approved surgical suite. We use *pre-emptive analgesia* for all surgical procedures. This means

that, in addition to a general anesthetic, the animals receive narcotic anti-pain medication *prior to and during* the surgery, and all incision sites are blocked using a long-lasting local anesthetic (Marcaine HCl). This has been shown in humans to reduce post-surgical pain and speed time to full recovery (Woolf & Chong 1993). Any residual post-operative pain will be treated with narcotics, routinely for 48 hours and as needed thereafter. Animals will be monitored closely in the post-operative period, and signs of distress, such as breathing difficulty, picking at IV sites or weight loss, will be promptly treated in consultation with the veterinary staff.

5. All animals will need to be euthanized in order to allow histological processing of brain tissue for the confirmation of sites of microelectrode recordings. Euthanasia will be carried out by intravenous injection of pentobarbital (50-100 mg/kg) followed by intravascular perfusion with aldehyde fixatives, in accordance with the guidelines of the Panel on Euthanasia of the American Veterinary Medical Association.

The Harvard Medical School animal management program is accredited by the American Association for the Accreditation of Laboratory Animal Care, and Meets National Institutes of Health standards as set forth in the "Guide for the Care and Use of Laboratory Animals" (DHHS Publication No. (NIH) 85-32 Revised 1985). The Institution also accepts as mandatory the PHS "Policy on Humane Care and Use of Laboratory Animals by Awardee Institutions" and NIH "Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training." There is on file with the Office for Protection from Research Risks an approved Assurance of Compliance.

30c. Respective Contributions.

The research training proposal was written and developed by the applicant, with the exception of the Vertebrate Animal section. The sponsor reviewed and edited all remaining sections for accuracy and conciseness.

30d. Selection of Sponsor and Institution.

I am deeply interested in learning about the nature of science and human biology. I also wish to apply this knowledge towards a positive goal in society. I believe that medicine is a great confluence of these intentions, and I was very careful in selecting an institution that would allow me to develop such a career. I chose to attend the Health Sciences and Technology medical program at Harvard Medical School because its focus highlights both my scientific and medical interests. The program has allowed me to learn excellent medical skills while obtaining training in the scientific arena. Because the program makes me a concurrent student at the Massachusetts Institute of Technology, I know I am obtaining an education that is more comprehensive and technology-oriented.

My interest in neuroscience is focused primarily in the underlying mechanisms that give rise to everyday visual phenomena. I have joined Personal Info laboratory because of its solid history of investigating the visual system from a systems-level approach and its outstanding list of publications. I chose the department of neurobiology because of its concentration of systems neuroscience laboratories, which include the teams of Personal Info Personal Info This nucleus meets on a biweekly basis to discuss ongoing work in each lab, which creates a fantastic learning environment for any student interested in how the brain works.

30e. Responsible Conduct of Research

As required by the Division of Medical Sciences at Harvard Medical School, I am currently enrolled in "The Conduct of Science" course (MEDSCI 300). This class provides instruction in the responsible conduct of research as requested by the National Institutes of Health.