OFFICE OF NAVAL RESEARCH

FINAL REPORT

PUBLICATIONS/PATENTS/PRESENTATIONS/HONORS/STUDENTS REPORTS

for

Grant No. N00014-90-J-1161/P00005

PR Number 96PR0-3596

"Electrochemistry In and At Single Nerve Cells"

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OFFICE OF NAVAL RESEARCH
PUBLICATIONS/PATENTS/PRESENTATIONS/HONORS REPORT

PR Number: 96PR0-3596 000
Contract/Grant Number: N14-90-J-1161/P00005
Contract/Grant Title: Electrochemistry In and At Single Nerve Cells
Principal Investigator: Andrew G. Ewing
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a. Number of papers submitted to refereed journals, but not yet published: 7
b. +Number of papers published in refereed journals (complete citations included): 5
c. +Number of books or chapters submitted, but not yet published: 0
d. +Number of books or chapters published (complete citations included): 1
e. Number of technical reports/non refereed papers: 0
f. Number of patents filed: 0
g. +Number of patents granted: 0
h. +Number of invited presentations (complete citations included): 4
i. +Number of submitted presentations (complete citations included): 1
j. +Honors/Awards/Prizes for contract/grant employees: 0
k. Total number of Full time equivalent Graduate Students and Post Doctoral associates supported during this period, under this R&T project number

Graduate Students: 2.1
Post Doctoral Associates: 0

including the number of

Female Graduate Students: 0.1
Female Post Doctoral Associates: 0

the number of

Minority* Graduate Students: 0
Minority* Post Doctoral Associates: 0

and the number of

Asian Graduate Students: 0.6
Asian Post Doctoral Associates: 0

l. Other funding (agencies, grant titles, amount received this year, total amount, period of performance, and a brief statement regarding the relationship of the research to the ONR grant are included)

The letter and an appropriate title is used as a heading for each list, e.g.:

b. Published Papers in Refereed Journals; or
d. Books and Chapters published.

*Minorities include Blacks, Aleuts, AmIndians, Hispanics, etc. NB: Asians are not considered an under represented or minority group in science and engineering.

Part I

a. Papers Submitted to Refereed Journals (and not yet published)

1. Dopamine Exocytosis from the Neuronal Cell Body Induced by and Increase in Intracellular Sodium Concentration, G. Chen, A. G. Ewing, *J. Neurochem.*, submitted. ONR and NSF support.

2. Electrochemical Analysis in Picoliter Microvials, R. A. Clark, P. Beyer Hietpas, A. G. Ewing, *Anal. Chem.*, submitted. ONR and NSF support.

3. Identification of Multiple Compartments of Dopamine in a Single Cell by Capillary Electrophoresis with Scanning Electrochemical Detection, F. D. Swanek, G. Chen, A. G. Ewing, *Anal. Chem.*, submitted. NSF and ONR support.

4. Vesicular Dopamine Levels of Two Classes of Vesicles Are Differentially Depleted By Amphetamine, G. Chen, A. D. Gutman, A. G. Ewing, *J. Biological Chemistry*, submitted. ONR and NSF support.

5. Characterization of the Effects of Varying the pH and Monomer Concentrations of Poly(oxyphenylene) Insulating Films on Carbon Fiber Electrodes, C. E. MacTaylor, A. G. Ewing, *Electroanalysis*, submitted. ONR support.

6. Chemical Analysis of Single Cells, G. Chen, A. G. Ewing, *Critical Reviews in Neurobiology*, Invited Review, in press. ONR, NSF and NIH support.

7. Electrochemical Monitoring of Bursting Exocytotic Events from the Giant Dopamine Neuron of *Planorbis corneus*, G. Chen, D. A. Gutman, S. E. Zerby, A. G. Ewing, *Brain Research*, in press. ONR and NIH support.

b. Papers Published in Refereed Journals

1.* Observation and Quantitation of Exocytosis from the Cell Body of a Fully Developed Neuron in *Planorbis corneus*, G. Chen, P. F. Gavin, G. Luo, A. G. Ewing, *J. Neurosci.*, 15 (1995) 7747-7755. ONR, NSF and NIH support.

2.* Multiple Classes of Catecholamine Vesicles Observed During Exocytosis from the Planorbis Cell Body, G. Chen, A. G. Ewing, *Brain Research*, 701 (1995) 167-174. ONR support.

3.* The Latency of Exocytosis Varies with the Mechanism of Stimulated Release in PC12 Cells, S. E. Zerby, A. G. Ewing, *J. Neurochem.*, 66 (1996) 651-657. ONR and NSF support.

4. Vesicular Quantal Size Measured by Amperometry at Chromaffin, Mast, Pheochromocytoma, and Pancreatic β-Cells, J. M. Finnegan, K. Pihel, P. S. Cahill, L. Huang, S. E. Zerby, A. G. Ewing, R. T. Kennedy, R. M. Wightman, *J. Neurochem.*, 66 (1996) 1914-1923. ONR and NSF support.

5.* Electrochemical Monitoring of Individual Exocytotic Events from the Varicosities of Differentiated PC12 Cells, S. E. Zerby, A. G. Ewing, *Brain Research*, 712 (1996) 1-10. ONR and NSF support.

* Note: These papers were reported as "not yet published" in last years report.

c. Book Chapters Submitted for Publication

None

d. Books or Chapters Published

1. Contemporary Problems in Biology: Cell Constituent Analysis, P. J. Beyer, S. D. Gilman, R. A. Lee, M. R. Wood, N. Winograd, A. G. Ewing, in "Nanofabrication and Biosystems: Integrating Materials Science, Engineering and Biology", Hoch, H. H., Jelinski, L. W., Craighead, H., eds., Cambridge University Press, New York, (1996), pp. 139-158. ONR, NSF, and NIH Support.

e. Technical Reports etc.

None

f. Patents Filed

None

g. Patents Granted

None

h. Invited Presentations at Topical or Scientific/Technical Society Conferences

1. A. G. Ewing, "Electrochemical and Separations-Based Approaches to Single Cell Analysis," University of Massachusetts Department of Chemistry, Colloquium, Amherst, MA, August 7, 1995.

2. A. G. Ewing, "Neurotransmitted Exocytosis: Is It Quantal? Is It Restricted to the Synapse? Can It Be Manipulated Pharmacologically?" Penn State University, Hershey Medical Center, neuroscience Seminar, Hershey, PA, November 30, 1995.

3. A. G. Ewing, "Understanding Zeptomole Evnets at the Cell Membrane: Electrochemistry and TOF SIMS Imaging," Eastern Analytical Symposium in Honor of Royce Murray, EAS Analytical Award, Somerset, NJ, November 15, 1995.

4. A. G. Ewing, "Separation and Electrochemical Approaches to Single Cell Analysis," University of Wisconsin, Distinguished Speakers in Department of Biochemistry & Molecular Biology, Milwaukee, WI, February 21, 1996.

i. Contributed Presentations at Topical or Scientific/Technical Society Conferences

1. R. A. Clark, P. J. Beyer, A. G. Ewing, "Electrochemical Investigations in Picoliter Microvials," 1996 Pittsburgh Conference on Analytical Chemistry, Chicago, IL, March 5, 1996.

j. Honors/Awards/Prizes

None

k. Number of Graduate Students Receiving Full or Partial Support on ONR Contract

Total 6	Minorities 0	Asian 1

Number of Postdoctoral Associates Receiving Full or Partial Support on ONR Contract

Total 0	Minorities 0	Asian 1

l. Other Funding

Agency: National Institutes of Health
Title: Capillary Electrophoresis - Single Cell Neurochemistry
Amount: $430,070 ($143,521 for 95-96)
Date: July 1, 1995 - June 30, 1998
Support is for capillary electrophoresis development for single cell analysis

Agency: National Science Foundation
Title: Microanalytical Methods in Neurochemistry
Amount: $401,000 ($105,000 for 95-96)
Date: July 1, 1994 - November 30, 1997
This grant deals with development of electrophoresis in small rectangular channels for dynamic separations

Agency: National Institutes of Health
Title: Automated High-Speed DNA Sequencing
Amount: $389,746 ($126,390 First year direct)
Date: September 30, 1995 - September 29, 1998
This grant deals with development of high-speed methods for DNA genotyping and sequencing.

Agency: SmithKline Beecham Pharmaceuticals
Title: Unrestricted Research Support
Amount: $5,000
Date: October 5, 1995 - October 4, 1996

Agency: National Institutes of Health
Title: Molecular Imaging of Biomaterials - Single Cells
Amount: $1,100,649 ($314,555 first year), Co-PI with Nick Winograd (my research group receives approx. one-third of the support from this grant.
Date: April 1, 1996 - March 21, 2000
This grant deals with developing sub-micron mass spectrometry imaging for analysis of the membranes of single nerve cells.

Part II

a. Principal Investigator

Andrew G. Ewing

b. Current Telephone Number

(814) 863-4653

c. Cognizant ONR Scientific Officer

Dr. Robert Nowak

d. Brief (100 - 200 words) Description of Project

This project concerns the development, characterization and application of ultrasmall electrochemical probes for microenvironments. Schemes are under investigation to construct electrodes with 50-nm total tip diameter. These electrodes will be developed to carry out electrochemical experiments in the smallest microenvironments, including single biological synapses. In addition, methods are under development to construct artificial synapses by chemical patterning and directed growth onto an electrode. A large part of our current effort is aimed at monitoring catecholamine release from single exocytotic events as a means to examine cellular pharmacology and chemistry. Major goals of this project are A) to

develop ultrasmall electrodes for placement in the attoliter volume of a single synapse, B) to develop chemical patterning schemes on small electrodes or electrode arrays to guide axonal growth, C) to develop and characterize methods allowing voltammetry in lithographically fabricated picoliter and femtoliter microvials. These methods will allow insights into neurotransmitter exocytosis.

e. Significant Results (50 - 100 words)

Two major advances have been made. First, we have discovered a neuron which exocytosis (single release events) takes place at correlated time intervals indicating that individual nerve cells have an internal clock regulating transmitter release. This startling result suggests that stimulus-release coupling is not in itself the controlling factor in nerve impulses. The second advance is in the area of picoliter (and indeed even femtoliter) microvials. We have succeeded in fabricating clear microvials as small as 350 femtoliters and, to date, have carried out voltammetry in vials with volume as small as 1 picoliter. This advance carries the realm of volumes applicable to voltammetry approximately six orders of magnitude smaller than previously accomplished.