NANOSCALE MEASUREMENTS OF VESICLE CONTENT IN SOLUTION, IN CELLS, AND IN VARICOSITIES

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Abstract:

Electrochemical cytometry and mass spectrometry imaging with NanoSIMS have been used to peer into the contents and even the substructure of single neurotransmitter vesicles in pheochromocytoma cells. Electrochemical cytometry involves isolating single nanometer vesicles stochastically, and measuring their contents via an electroporated opening, a process taking microseconds. NanoSIMS involves using a tightly focused ion beam to ablate material from a surface, in this case a fixed cell, and carrying out mass spectrometry on the ions ejected. This can be done with 40-50 nanometer resolution. Using electrochemical cytometry and amperometry, we have discovered differences in neurotransmitter release with cisplatin, a chemotherapeautic drug that decreases cognition causing the "chemobrain" effect. We have also discovered that the learning supplement, zinc ion, the dietary supplement, curcumin, and the anesthetic, lidocaine, all either change the amount of dopamine stored in vesicles, or the amount released during exocytosis.

A key aspect to all these findings is the equilibrium between a proteinaceous dense core in the nanometer vesicle and the solution around it. To understand this we have developed protocols with the NanoSIMS to get about 50 nm spatial resolution and determine the relative contents of these compartments in single vesicles observed in fixed cells. Additionally, we have used intracellular cytometry to measure the content of small synaptic vesicles (50-60 nm) in the nerve terminals of the living fruit fly larva and found the amount to be orders of magnitude higher than that measured during exocytosis release leading to the conclusion that release from an actual nerve cell is a very small fraction of the content in each vesicle. This model system is being used to make the transition from model cell systems to the living brain system and the complexity is remarkable.

Short Biography:

Andrew Ewing received his BS degree from St. Lawrence University and a PhD from Indiana University. After a postdoc at the University of North Carolina he joined the faculty at Penn State University for 25 years. He is now Professor at Chalmers University of Technology and the University of Gothenburg, Sweden. His group has pioneered small-volume chemical measurements at single cells, electrochemical detection for capillary electrophoresis, novel approaches for electrochemical imaging of single cells, and new electrochemical strategies to separate individual nanometer vesicles from cells and quantify their contents. His 309 publications have been cited 17279 times with an H-index of 72. He has recently received the Charles N Reilley Award from the Society for Electroanalytical Chemistry (2013), the ACS Analytical Division Award in Electrochemistry (2013), the Norblad-Ekstrand Medal of the Swedish Chemical Society (2014), and the Pittsburgh Conference Award in Analytical Chemistry (2015). He is an Honorary Professor at both Nanjing University of Science and Technology and Beijing University of Science and Technology. He is a member of the Royal Swedish Academy of Sciences (2012) and the Gothenburg Academy of Arts and Sciences (2013).