December 12, 2003

Cold Spring Harbor Laboratory
Armen Stepanyants, Ph.D.
Computational Postdoc

Biocomplexity Faculty Search Committee
c/o Prof. Rob de Ruyter van Steveninck
Biocomplexity Institute
Indiana University
Swain Hall West 117
Bloomington IN, 47405-7105

Dear Professor Rob de Ruyter van Steveninck:

I would like to apply for the Assistant Professor position at the Biocomplexity Institute, Indiana University, as advertised at naturejobs.com.

I received an education in theoretical condensed matter physics from the Moscow Institute of Physics and Technology and the University of Rhode Island. Later, I became interested in neuroscience and I currently work as a computational postdoc at Cold Spring Harbor Laboratory. At this time, my primary interest is in understanding the principles of synaptic connectivity in complex brain. I have developed a consistent methodology and am collaborating with several internationally acclaimed experimental laboratories to address this fundamental problem. I have extensive teaching experience in a number of undergraduate and graduate courses in physics and mathematics.

Enclosed, please find my application consisting of curriculum vitae, list of publications, summary of research interests, and future research plans, and summary of teaching interests. I also included three resent publications representative of my neuroscience and physics research. Professors Dmitri Chklovskii and Karel Svoboda from Cold Spring Harbor Laboratory, as well as Professor Alexander Meyerovich from the Department of Physics of the University of Rhode Island have agreed to write letters of recommendation in support of my application. I have arranged for these letters to be sent to you as soon as possible.

Sincerely,

Armen Stepanyants

Summary of Research Interests and Future Research Plans

My main research interest can be broadly described as understanding synaptic connectivity principles and changes in connectivity associated with learning and memory in local circuits of the mammalian brain. These problems are among the oldest and most important problems in neuroscience. Without clear knowledge of connectivity principles it is virtually impossible to understand how the brain functions or to understand neural circuitry changes which underlie neurological disorders. One of the main questions in the connectivity field is how neurons organize themselves into highly inter-connected functional circuits. Since neuronal connectivity is not arbitrary, each neuron must somehow find appropriate synaptic partners in the neuropil jungle. This presents a highly non-trivial wiring problem because each cubic millimeter of gray matter contains 10^4 neurons, 10^9 synapses, and 4 kilometers of axons. Although the wiring problem gets solved routinely by developing animals, the underlying wiring mechanisms remain largely a mystery.

In spite of its importance, the local connectivity problem has received very little quantitative attention. This is mainly due to the fact that technology for precise 3D axonal and dendritic reconstructions has become available only recently. It is my belief that with advances in technology, including transgenic labeling, two-photon imaging and accurate neuronal tracing, theoretical and computational studies of connectivity will become a rapidly growing field of neuroscience.

About four years ago, while working as a postdoctoral research assistant at MIT, I became interested in the problem of neuronal connectivity and decided to change my career path by switching to neuroscience. The reasons for this change are the following: I find neuroscience to be a comparatively young and rapidly growing field of science with a large number of opportunities for people with quantitative backgrounds. Neuroscience offers many interesting unanswered questions about brain function and there are a number of applications to be developed for areas of health and disease. It is my belief that with rapid advances in technology many of the breakthroughs will be made by approaching the problems in neuroscience in a conceptual and quantitative manner. My training in theoretical physics has given me the opportunity to be a part of this process.

In the past three years, I have been working in the laboratory of Dr. Chklovskii in Cold Spring Harbor, New York (CSHL). In collaboration with Professor P.R. Hof from Neurobiology of Aging Laboratories, Mount Sinai School of Medicine, we have developed a method to estimate the potential for structural synaptic plasticity in complex neuropil associated with changes in synaptic connectivity patterns through formation and elimination of dendritic spines. To quantify this potential, we estimate the number of different synaptic connectivity patterns attainable without major arbor remodeling. This number is determined by the ratio of actual synapses to potential synapses, i.e. the number of axons that pass within a spine length of a given dendrite. We called this ratio the "filling fraction" and found that it is about 0.2 in the neocortex and hippocampus (Stepanyants et al, Neuron, 34, 275-288, 2002). This finding predicted that many connectivity patterns could be implemented by dendritic spine remodeling. Recently,

studies in the Svoboda laboratory at Cold Spring Harbor provided experimental evidence of activity dependent dendritic spine remodeling in the developing and adult rat neocortex *in vivo* (Trachtenberg et al, Nature, 420, 788-794, 2002).

In addition, I have been working on the problems of evolutionary optimization of brain micro-architecture. I have shown that complex neuropil is optimally designed to store information in synaptic connectivity patterns (Stepanyants, LANL Archive, 0307065, 2003). Optimization methods have been also applied to axon branching. We demonstrated that minimizing conduction delays for signal propagation along axons for constant axonal volume leads to a simple power law for axon branch diameters at a bifurcation point. The power law exponent is 3 for myelinated and 2.5 for non-myelinated axons in agreement with experimental observations (Chklovskii and Stepanyants, BMC Neuroscience, 4:18, 2003).

Currently, I am carrying out several projects in an effort to extend my research in the field of neuronal connectivity. I plan to continue this effort into the future. The results of the estimation of the filling fraction for different classes of neurons revealed that, while axons of pyramidal neurons have, in general, high structural plasticity potential, the axons of many classes of GABAergic interneurons have little or no potential for synapse remodeling. These classes of GABAergic interneurons must use precise wiring mechanisms in development in order to insure specificity of connections. To check this hypothesis we, in collaboration with Professor G. Tamás from University of Szeged, Hungary, developed a new method which detects the traces of precise wiring mechanisms in development (Stepanyants et al, Class-specific Features of Neuronal Wiring, submitted to Nature Neuroscience). The method is based on the idea that any wiring mechanism in development will lead to special correlations between overlapping axonal and dendritic arbors in the adult. Our method detects these special correlations from 3D reconstructions of neuronal pairs and determines their significance. We reveal that the layout of axons of many classes of GABAergic interneurons is specific. This specificity is manifested in a relatively high tortuosity, small branch length of these axons, and correlations of their trajectories with the positions of neighboring dendrites. Axons of pyramidal cells show no such specificity, usually taking a relatively straight course through neuropil. However, since wiring patterns among pyramidal cells hold a large potential for circuit remodeling, the specificity can be achieved through growth and retraction of dendritic spines.

I am also working on a project to uncover local potential connectivity of spiny neurons in the cat visual cortex (Stepanyants et al, Domains of Potential Connectivity of Cortical Spiny Neurons, preprint presented at SFN, 2003). This is a large effort in collaboration with the experimental laboratories of Professor J.A. Hirsch from the Department of Biological Sciences at the University of Southern California, and Professor Z.F. Kisvarday from the Department of Physiology at the Ruhr-Universität Bochum, Germany. We developed methods which allow us to determine potential connectivity among neurons depending on their laminar positions and relative separation in the direction parallel to the cortical surface. Based on the morphology of a number of 3D reconstructed spiny neurons from different cortical layers, we are able to produce cortical convergence/divergence maps as well as evaluate the probability for any two neurons to be potentially connected based on their laminar positions. The same technique is now being used to produce potential connectivity maps for the rat barrel

cortex. This is done in collaboration with K. Svoboda's Lab at Cold Spring Harbor, where similar connectivity maps are obtained with glutamate uncaging. Many interesting questions can be addressed by comparing the two methods of assessing cortical connectivity.

In addition, I am working on a project to establish a link between potential and actual connectivity. I am testing a hypothesis that a pair of synaptically connected excitatory neurons has a synapse at all potential synaptic sights. In other words, connectivity between excitatory neurons is "all or none", where if two neurons connect, they convert all their potential synapses into actual ones. I use the Neurolucid system to trace pairs of synaptically connected neurons (determined through electro-physiology), identify all potential synaptic sights and look for a presence of spines and/or boutons at these locations. This project is being done in collaboration with Professor A.M. Thomson from the Department of Pharmacology at London University.

Finally, I am working on a project to identify particular mechanisms involved in establishing subcellular domain specificity by many GABAergic interneurons. In particular, I am trying to analyze the developmental time course of the affinity of basket cell axons towards pyramidal somata. This is done in collaboration with Professor J. Huang at Cold Spring Harbor. The Huang Lab has developed an ideal preparation for investigating this question. By using cell type specific promoters and BAC engineering, they are able to visualize different GABAergic interneurons and postsynaptic pyramidal cells in transgenic mice and in organotypic cortical slice cultures. Confocal images of these systems are being collected for different developmental stages. I am in the process of analyzing these images by using the newly developed correlation analysis. This approach will provide me with the developmental time course of correlations between axons of basket cells and pyramidal somata. By comparing this time course with the time course of morphological changes of the cells, I should be able to identify developmental mechanisms involved in the formation of these correlations.

All my projects have a common theme, which is inference of different aspects of neuronal connectivity through geometrical analysis of 3D reconstructed neurons. Estimation of plasticity potentials for connectivity among different classes of neurons combined with the knowledge of potential connectivity maps and spatial correlations between neuronal arbors describes neuronal connectivity on small and large spatial scales and can be used as a tool for uncovering canonical cortical circuits, their plasticity potentials, and underlying developmental connectivity mechanisms.

Summary of Teaching Interests

During my studies at the University of Rhode Island, I taught recitations and labs for a number of undergraduate courses in physics (throughout seven semesters). In addition, over a period of four years I worked at "Learning Assistance", an organization dedicated to further understanding of sciences among students. During that time I taught and tutored a variety of undergraduate and graduate courses in physics, mathematics, engineering, and business. Later, during my postdoctoral work at MIT, I prepared and taught a course of lectures on Applications of Methods of Quantum Field Theory to Systems with Disorder. This work was done for the lab members of Professor C.C. Mei.

Although I do not have experience teaching courses in Neuroscience, in the last three years at Cold Spring Harbor Laboratory, I acquired a great deal of knowledge in the area of Computational and Theoretical Neuroscience. This is the area where I would be most comfortable participating in teaching activities. I am very interested, in time, to design my own course of lectures addressing important issues of neuronal connectivity and optimal brain design.