MEETING REPORT

The 15th Annual Meeting of the Winter Conference on Neural Plasticity Guadeloupe, French Antilles February 23 – 28, 2003

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The 15th Annual Meeting of the Winter Conference on Neural Plasticity was held in Guadeloupe, French Antilles from February 23 – 28, 2003. The Conference was attended by approximately 80 participants from Canada, United Kingdom, Germany, Hungary, Italy, and the United States. Most of the participants were neuroscientists from universities and basic research departments of applied institutions. The primary focus of the conference is to annually review recent advances in the area of neural plasticity from multi-disciplinary and lifespan perspectives. There were nine scientific sessions, each lasting two hours.

GENES, GENE EXPRESSION AND LONG-TERM MEMORY

Long-term memory formation requires gene expression. In the last decade, we have witnessed the discovery of genes involved in memory formation. To understand the biological changes occurring when memories are formed we need to have a clear picture of

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which molecules and molecular mechanisms are activated and/or required during the memory process. Intriguing new findings are constantly being unraveled in this area. In this session the participants discussed recent exciting results concerning molecular candidates and mechanisms involved in memory formation, as well as the use of methods for identifying molecules involved in the memory process.

Josh Dubnau (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA) presented evidence that a convergence from a behavioral and a micro-array screen reveals a role for the *staufen* mRNA localization machinery in *Drosophila* memory. He described a parallel use of micro-array and forward mutagenesis screens to characterize genes involved in Pavlovian learning and memory. Using additional (extant) mutations in *staufen* to transiently disrupt *staufen* function, he identified a role for this conserved pathway in long-lasting memory.

Sandra Peña de Ortiz (University of Puerto Rico, San Juan, Puerto Rico) presented data on gene expression profiling using cDNA microarrays. She discussed the importance of statistical analysis and molecular validation using laser capture microdissection coupled to real time PCR as well as of immunohistochemistry and Western blotting analysis to identify and characterize candidate genes in hippocampal-dependent spatial discrimination learning. Moreover, she described findings on temporal gene profiling, gene clustering, and bioinformatic gene promoter analysis to study the dynamics of gene regulation in the amygdala during consolidation of conditioned taste aversion.

Cristina Alberini (Mount Sinai School of Medicine, New York, NY, USA), who chaired the session, discussed advantages and disadvantages of screening approaches for the identification of genes regulated in the hippocampus during inhibitory avoidance memory. She presented evidence that changes related to synaptic morphological modification supported by activation of differentiation and growth pathways are present 20 hours after training.

Karl Peter Giese (University College London, UK) presented evidence that transcriptional activation from hippocampal memory formation differs between the sexes. Signaling by calcium/calmodulin kinase kinase (CaMKK) α is required for male but not female contextual memory formation. Furthermore, CaMKK β signaling is required for spatial memory formation in males but not females. Males require the CaMKK signaling to activate the transcription factor CREB in hippocampus, which appears to be essential for the establishment of LTP at CA1 synapses. Thus, the CaMK cascade appears to be key for male hippocampal memory formation.

THE EMERGENCE OF ORDER IN THE DEVELOPING BRAIN

The adult brain is wired with fascinating precision, reflecting the need for specificity of neural connections associated with specific behaviors. Sensory inputs are organized in topographic networks, which means that the spatio-temporal relationships between neurons are preserved in their projections. The emergence of this topographic order in the developing brain occurs in two major steps. Under genetic and molecular control, connections initially follow an approximate wiring diagram. Once neurons have migrated to their final destination, they send their axon to a specific target to build topographic maps with the help of guidance molecules. Precision is then achieved by the elimination of inappro-

priate inputs and the stabilization and growth of appropriate connections based on experience. In this symposium, chaired by **Evelyne Sernagor** (University of Newcastle upon Tyne, UK) the participants discussed both early (genetic, molecular) and late (activity-dependent) aspects of map formation in the developing brain.

Zoltan Molnar (Oxford University, UK) presented results on transgenic mice identifying several genes involved in the control of thalamic axon growth, pathfinding and cortical target selection. He discussed how both pre- and postsynaptic elements influence the way in which thalamic projections impose cytoarchitectonic differentiation on the developing cortex.

David Willshaw (University of Edinburgh, UK) discussed what happens when the models for the formation of retinotectal connections by means of the induction of molecular markers are confronted with the more recent findings of gradients of Eph receptors and their associated ligands, the ephrins. The induction of counter gradients from retina to tectum can underlie the formation of ordered patterns of connections and various abnormal visual maps are produced in genetically manipulated animals.

Martha Constantine-Paton (Massachusetts Institute of Technology, Cambridge, MA, USA) discussed the interface between molecular mechanisms of glutamate receptor regulation and activity-dependent synaptic refinement in the developing visual pathway. Controlled eye-opening is used to demonstrate that both synaptic refinement onto collicular neurons and a highly stereotyped sequence of glutamate receptor current changes are time-locked to eye opening and not chronological age. The signaling complexes scaffolded beneath the glutamate receptors via association with the PSD-95 family of membrane associated guanylate kinases are altered with experience.

Barry Stein (Wake Forest University School of Medicine, Winston-Salem, NC, USA) discussed how multisensory integration gradually develops in postnatal neurons from the superior colliculus. These neurons must receive functional inputs from a specific region of cortex and obtain sufficient experience with sensory cues. Depriving an animal of these cortical inputs or raising animals in complete darkness precludes the maturation of multisensory (i.e., visual-nonvisual) integration. Animals reared in environments in which visual and auditory cues are always derived from different spatial locations develop multisensory neurons with altered visual-auditory receptive fields and multisensory integration.

CONTRIBUTIONS OF NEUROTRANSMITTER TRANSPORTERS TO SYNAPTIC SIGNALING

Speakers in this symposium described recent advances in the study of neurotransmitter transporters, emphasizing how these proteins might participate in inhibitory and excitatory synaptic signaling and various forms of synaptic plasticity.

Michael Quick (University of Southern California, Los Angeles, CA, USA) described experiments on recycling of the GABA transporter GAT1, showing that GAT1 trafficks on a vesicle similar to small synaptic vesicles, undergoes endocytosis via clathrin, and recycles to the plasma membrane on the order of minutes in a calcium-dependent manner.

Linda Overstreet (Vollum Institute, Portland, OR, USA) focused on the roles for GABA transporters in synapse specificity at inhibitory synapses in hippocampus. Her

work suggested that GABA transporters limit postsynaptic interactions between release sites, thereby affecting the time course of synaptic inhibition.

Jeffrey Diamond (NINDS, Bethesda, MD, USA) discussed the use of electrophysiological data and modeling to estimate the distance that glutamate diffuses following release from hippocampal synapses and the regulation of that diffusion by glial glutamate transporters.

Thomas Otis (UCLA, Los Angeles, CA, USA), who chaired the session, presented evidence that postsynaptic glutamate transporters limit activation of co-localized metabotropic glutamate receptors (mGluRs) at parallel fiber and climbing fiber synapses in cerebellum. The implications of such regulation for various forms of mGluR-dependent synaptic plasticity were discussed.

STRUCTURAL BASIS OF PLASTICITY, LEARNING, AND MEMORY

Learning is assumed to cause some morphological alteration in neural structure such that information can be stored in long term memory. A great deal of evidence has accumulated over the last two decades indicating that dendritic and synaptic structure are altered following learning/memory and its many model systems, such as long-term potentiation (LTP). However, recent advances in morphological techniques have greatly improved our ability to examine and characterize these changes, while similar advances in mathematical modeling have begun to help us understand exactly how these anatomical changes translate into increased synaptic efficacy and power. This session reviewed recent evidence gained from a number of sources shedding light on the structural basis of learning and memory, and model systems.

Ted L. Petit (University of Toronto, Toronto, Canada), who chaired the session, began with an overview of recent advances in the anatomy of memory storage. He reviewed the work of **Gang-Yi Wu** (Baylor Medical School, TX, USA) and discussed the development and plasticity of dendritic spines in cell culture, including rapidly reversible dendritic swelling, as well as the role of the Ras-MAPK pathway in the regulation of cell size and dendritic morphogenesis, particularly the shapes of dendritic spines.

Jeff Kleim (University of Lethbridge, Lethbridge, Alberta, Canada) reviewed recent work showing the specificity of learning-dependent synapse formation within the cerebellum during eye blink conditioning. In addition, evidence was presented that synaptogenesis within the motor cortex following motor skill learning is related to motor map reorganization.

Diano Marrone (University of Toronto, Toronto, Canada) summarized a series of experiments on the time-course of changes in synaptic curvature and perforations that occur in the rat dentate gyrus following long-term potentiation and lesion recovery. The issue of whether this is a universal model of synaptic plasticity where synapses may move towards or away from a maximally effective profile was also discussed.

Ted Berger (University of Southern California, Los Angeles, CA, USA) spoke about mathematical models of synaptic structure, showing the relationship between alterations in synaptic morphology and projected alterations in synaptic electrophysiology. Dr. Berger's work showed that the changes in synaptic morphology discussed by the other

speakers in the session would have a direct effect of both increasing post-synaptic potentials and changing the dynamics of synaptic transmission due to changes in the axon terminal's ability to sequester calcium.

THE DIVERSE FUNCTIONS OF HYPERPOLARIZATION-ACTIVATED (IH) CHANNELS IN THE CNS

Hyperpolarization-activated channels are widely expressed in various CNS regions in a cellular and sub-cellular dependent fashion. These channels have a profound impact on both cellular physiology and many aspects of behavior. This session explored the anatomy, physiology and behavioral impact of hyperpolarization-activated channels.

Zoltan Nusser (Institute of Experimental Medicine, Budapest, Hungary) presented recent data on EM immunogold localization of HCN1 in cortical and hippocampal nerve cells. He also described some rules governing the cell surface distribution of HCN1 in hippocampal and neocortical pyramidal cells (cellular domain-dependence, distance-dependence and subcellular compartment-dependence). These rules are cell-type specific, as HCN1 has completely different subcellular distributions in distinct types of GABAergic interneurons.

Stephen Williams (MRC-LMB, Cambridge, UK) discussed experiments where they have identified dendritic expression patterns of HCN channel classes in thalamic, neocortical and cerebellar neurons. Electrophysiological investigations have revealed that HCN channels play key cell-type specific roles in the control of neuronal excitability and function with two principle modes of operation — referred to as phasic and tonic. The dynamic activation/deactivation of HCN channels has been found to control the axo-somatic efficacy of dendritically generated synaptic potentials, whereas the tonic activation of channels has been found to regulate the global level of neuronal excitability by the control of membrane potential bistability.

Bob Zucker (University of California, Berkeley, CA, USA) discussed his results examining the crucial role that HCN channels play in modulating transmitter release at crayfish glutamatergic neuromuscular junctions. Serotonin enhances transmission several fold, by activating a cyclase to produce cAMP, which binds to HCN channels. The mechanism of HCN modulation of transmission is unknown, somehow involving actin, but apparently not ion flux through the channels. Long-term facilitation also increases transmitter release through an indirect activation of I_h channels. This long-lasting enhancement, unlike the hormonal modulation, involves local presynaptic protein synthesis, and numerous kinases and phosphatases probably regulating protein synthesis. Once LTF is induced, subsequent cAMP enhancement is I_h -independent, and once cAMP opens I_h channels, continued cAMP action is I_h -independent, implying a separate cAMP target that is not protein kinase A.

Matt Nolan (Columbia University, New York, NY, USA) examined the contribution of HCN1 channels to motor learning and integrative properties of cerebellar cortical neurons. HCN channels have biophysical properties suitable for control of neuronal output; however, investigation of the function of specific HCN channels is hindered by the absence of selective tools. Therefore, Nolan's group generated and analyzed mice with

knockout of the HCN1 gene. HCN1 knockout mice have profound learning deficits in the rotarod task and modified timing of the conditioned eye-blink reflex. In the absence of HCN1, the input-output relationship of cerebellar Purkinje cells is modifiable by hyperpolarizing inputs. These data are accounted for in a model according to which HCN1 in Purkinje cells is required for reliable and accurate decoding of cerebellar inputs important for certain forms of motor learning.

Jeff Magee (LSUHSC, New Orleans, LA, USA), who chaired the session, spoke about behavioral regulation of HCN expression. Long-term exposure (72 hrs) of rats to immobilization stress results in a profound reduction of input resistance, R_{in} , a slight depolarization of membrane potential, V_m , a decrease in excitatory post-synaptic potential, EPSP, half width and a reduction of dendritic action potential duration in hippocampal CA1 pyramidal neurons. Pharmacological blockade of I_h channels indicated that these effects are mediated through an increase in I_h channel activity. Preliminary data from dendritic cell-attached recordings suggest that I_h channel density, but not the voltage-range of activation, is affected by 72-h stress. These data, along with other recent reports, indicate that HCN channel expression is particularly amenable to behavioral or activity-dependent regulation.

FUNCTIONAL IMAGING OF ACTIVITY IN THE NERVOUS SYSTEM

Functional imaging of activity in the nervous system has become an increasingly important tool for dissecting how the nervous system develops and mediates behavioral processes. There have been several approaches developed for the imaging of activity in experimental systems. One successful approach has been to use chemical dyes to detect important functional parameters such as calcium levels within neurons or the voltage across the plasma membrane. A second approach has been to use intrinsic imaging to follow physiological changes. A third and more recent approach has been to use protein derivatives in transgenic animals as reporters of certain types of physiological processes. Many of the protein-based approaches have utilized variants of green fluorescent protein to detect important physiological events within neurons. The studies conducted by the speakers of this session showed the utility of both intrinsic imaging and of using transgenically-supplied reporter molecules to gather important information about the function of the nervous system.

Gero Miesenboeck (Memorial Sloan-Kettering Cancer Center, New York, USA) developed an important new protein reporter named synapto-pHluorin, that can provide an optical readout of synaptic activity in transgenic animals expressing the reporter. He discussed his results in elucidating aspects of olfactory information processing in the antennal lobes of *Drosophila*.

Ron Davis (Baylor College of Medicine, Houston, TX, USA), who chaired the session, has also utilized synapto-pHluorin to monitor synaptic transmission and modifications within the *Drosophila* olfactory system. He discussed results with this reporter and also calcium sensitive reporters for examining synaptic activity and synaptic modifications within the *Drosophila* olfactory system.

Ron Frostig (UCI, Irvine CA) spoke about his experiments that utilize intrinsic signal optical imaging for studying the functional organization of the somatosensory cortex. He showed how sensory representations of whiskers change as a function of the rat's ability to express innate behaviors outside the home cage vs. the standard home cage.

IMPLANTABLE BIOMIMETIC ELECTRONICS: THE NEXT ERA IN NEURAL PROSTHETICS

This session reviewed recent advances in the development of brain-implantable neural prosthetics: microchip models of neural computation that can interact with living neural systems to replace the function of damaged or dysfunctional CNS regions. In general, the session focused on the development and application of "neuromimetic devices," i.e., hardware (Very Large Scale Integrated circuits, VLSI) implementations of biologically realistic neural network models, and "neuron-silicon interfaces," i.e., silicon-based multi-electrode arrays for bi-directional communication between the neuromimetic devices and the brain.

Gislin Dagnelie (Johns Hopkins University, Baltimore, MD, USA) described the development and clinical application of artificial micro-phototransduction systems for the replacement of damaged and/or degenerating retinas. This includes an eyeglass-mounted camera, wireless communication of pixelized information to a subcutaneous receiver, and a multi-site electrical stimulation array for activation of retinal bipolar or ganglion cells. The latest results from clinical trials using this prosthetic and some simulations of performance were described.

James Hickman (Clemson University, Clemson, SC, USA) described the latest developments in neuron-silicon interfacing, including silicon surface chemistry for cell adhesion and patterned/directed neural growth for embryonic and adult neuronal culture. He described his plans for using the control of these variables to create minimalistic *in vitro* models for reproducing circuits for LTP. Hickman also demonstrated these fundamental science and technology principles in the context of realizing a "reflex arc on a chip."

Ted Berger (University of Southern California, Los Angeles, CA, USA), who chaired the session, described the development of a "cognitive prosthetic," i.e., a biomimetic microchip replacement for the hippocampus to restore lost mnemonic function. The essential elements of this effort are the development of nonlinear systems models of hippocampal neurons and networks, VLSI implementations of those models for miniaturization, and "conformal" multisite electrode arrays for bi-directional communication between the VLSI model and the remaining hippocampus.

THE OTHER SIDE OF THE ENGRAM: USE-DEPENDENT CHANGES IN INTRINSIC NEURONAL EXCITABILITY

Memory storage in the brain and the activity-dependent refinement of neural circuits during development are generally assumed to be mediated by long-term modifications in the strength of synapses. Cellular investigations of memory storage in the mammalian

brain have mostly relied upon model systems, called long-term synaptic potentiation and depression (LTP and LTD). These model systems are considered computationally powerful, in part because they allow for the synapse-specific modification of a large array of inputs (~10,000 synapses for a typical neuron in the mammalian brain). While LTP and LTD are computationally appealing, there are also reasons to believe that they are not the whole story. At this point >6 different mutant mice have been produced which lack LTP or LTD (or both) in particular structures which we know (from lesion experiments) are crucial for certain forms of learning, and yet these forms of learning remain intact. What additional alterations of neuronal function might also contribute to memory storage? One idea, which has received relatively little attention, is that information storage in the mammalian brain may also involve activity-dependent changes in the intrinsic excitability of neurons. This type of plasticity would have the property of affecting postsynaptic signals evoked by synapses impinging on a particular neuron which, depending upon the identity and somatodendritic location of the intrinsic conductances altered, may produce either local or more global changes in signal integration.

There have been scattered results in support of a role for intrinsic, non-synaptic plasticity induced by behavioral training. Studies using *Hermissenda* have shown that associative pairing of light and rotation produces a long-term increase in the intrinsic excitability of type B photoreceptors, due to a reduction of K currents. A similar effect has been seen in CA1 pyramidal neurons of the rabbit hippocampus following trace eyeblink conditioning. In addition, slowly developing changes in the intrinsic excitability of both invertebrate and mammalian neurons have been reported in cell culture systems when activity was blocked with tetrodotoxin. However, until very recently, rapid, synaptically-driven changes in intrinsic excitability of mammalian neurons had not been convincingly demonstrated.

The present symposium, chaired by David Linden (John Hopkins University, Baltimore, MD, USA) brought together cellular electrophysiologists working in diverse cell types of the mammalian brain. These included **Egidio D'Agnelo** (University of Pavia, Pavia, Italy) who spoke on cerebellar granule cells, **Nelson Spurston** (Northwestern University, Evanston, IL, USA) who reviewed the evidence in subicular neurons, **Dan Johnston** (Baylor college of Medicine, Houston, TX, USA) who discussed hippocampal CA1 pyramidal cells, and **David Linden** (Johns Hoipkins University, Baltimore, MD, USA) who spoke on cerebellar deep nuclear neurons. The participants have described and are now beginning to analyze the cellular and molecular underpinnings of persistent, use-dependent modulations of neuronal excitability and, in some cases, to relate them to models of learning and memory.

GENOMICS/PROTEOMICS OF NEUROLOGIC DISORDERS

The completion of the first draft of the human genome gives us a unique opportunity to explore these databases and bring expertise to bear on the complex issues surrounding integration and rationalization of these genomes. Coupled with the proteomic approach which allows for the evaluation of proteins and their post-translational modifications in normal and diseased tissues and whole organisms, these databases have the capacity to illuminate not only how the nervous system works, but to identify novel therapeutic targets

in all forms of neurological disease. Unfortunately, to become of therapeutic or diagnostic value, these proteomic/genomic markers of human disease must be fully evaluated and studied because of the isolation of the brain by the blood brain barrier (BBB). Furthermore, the modern understanding of etiology of neurological disorders has significantly expanded from a purely “neurocentric” view to include other cell types, including glia, endothelium, and white blood cells.

Damir Janigro (Lerner Research Institute, Cleveland, OH, USA), who chaired the session, provided a brief introduction to the proteomics and genomics of neurological disorders. He discussed an anatomical description of “brain-body” barriers, proteomic and genomic approaches to studies designed to understand mechanisms of neurological disorders, as well as new data on peripheral markers useful in the diagnosis of neurological diseases.

Genomics technologies, particularly microarray analysis, are powerful tools for investigation of cellular complexity and the nature of cellular states. **Roy Walker** (National Research Council of Canada, Ottawa, Canada) discussed profiling changes in gene expression during neurogenesis in Human Embryonic Carcinoma cells using Human NT2 EC cells induced to differentiate by the morphogen, retinoic acid. Ultimately, Walker’s group hopes to define and understand complex genetic networks, such as synaptogenesis, with the goal of developing a capability to modulate or replace lost functionality in impaired neurons.

Marianna Sikorska (National Research Council of Canada, Ottawa, Canada) discussed the identification of novel CREB-controlled genes in neurons and their role in synaptogenesis. Numerous studies support the central role of CREB-mediated activity-dependent gene expression in synaptic plasticity and memory formation, and CREB phosphorylation is critical for the generation of new dendritic spines. However, little is known about the identities of the induced genes, the functional properties of their products or how they control dendritic spine formation. Using chromatin immunoprecipitation and bioinformatics approaches Sikorska’s group has probed the identity and functionality of some of these genes.

Reactive gliosis, characterized by astrocyte hypertrophy, is a hallmark of the injury response in the CNS. A wide range of molecular differences in secreted, cell surface and extracellular matrix (ECM) proteins have been described between reactive and non-reactive astrocytes. **Danica Stanimirovic** (National Research Council of Canada, Ottawa, Canada) discussed new approaches to dissect roles of secreted proteins identified using combined genomics and proteomics approaches in forming ECM cues for neuritogenesis and axon growth, including studies of ECM patterning by atomic force microscopy (AFM), and techniques to profile interactions of glycosylated moieties of these proteins (neuroglycomics).

Loss of BBB function is a hallmark of many neurological diseases, whereas its integrity is associated with reduced delivery of pharmacologic substances into the brain. Measuring BBB function by detecting changes in blood composition may be important to diagnose disease progression and monitor when chemotherapeutic penetration may be more effective. **Nicola Marchi** (Cleveland Clinical Foundation, Cleveland, OH, USA) discussed two brain-specific proteins, S100 and neuron-specific enolase (NSE) which are released systemically in a variety of neurological diseases. S100 levels sometimes rise in the absence of neuronal damage, suggesting that S100 is a marker of BBB rather than

neuronal damage. The relevance of these markers to early diagnosis of brain disease was presented.

RESEARCH IN NEURODEGENERATIVE DISEASES

For the past few decades, the participants' independent research groups have been collaborating to investigate the etiology, pathogenesis, and novel therapeutic strategies associated with degenerative diseases such as Alzheimer's disease, Parkinson's disease, and Huntington's disease.

Jeffrey Kordower (Rush Medical Center, Chicago, IL, USA) discussed research showing that stem cell therapy and gene therapy can often have profound effects functionally and structurally in rodent or nonhuman primate models of both Parkinson's disease and Huntington's disease. However, for all this good, success for each of these procedures has yet to be realized clinically. Alzheimer's disease affects 4,000,000 Americans and millions more around the world. The fact that after decades of intense study utilizing billions of dollars, research has yet to generate a consensus regarding which of the constellation of selectively vulnerable regions, or the myriad of different lesions, is critical in manifesting the disease and has resulted in infighting between the talented scientists that comprise this field. Rather than throwing in the towel, AD researchers are using new sophisticated molecular biological techniques to unravel the clues to the pathogenesis of AD.

Elliott Mufson (Rush Presbyterian — St. Luke's Medical Center, Chicago, IL, USA) discussed his research using single cell gene arrays for the study of Alzheimer's disease. Finally, after much optimism in both animal models and open label clinical trials, two double blinded clinical trials testing the hypothesis that fetal transplants are efficacious in patients with Parkinson's disease have failed.

Roy Bakay (Chicago Institute for Neurosurgery & Neuroresearch, Chicago, IL, USA) discussed why double blind clinical transplant studies have failed. Was this predictable when one looks closely at the preclinical data? Do preclinical data predict clinical outcome? The participants in this session discussed these issues in an attempt to understand the present status of the field and set the course for the future.