

15th Annual Spring Brain Conference, March 10–13, 2004, Summary Report^{*}

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The 15th Annual Spring Brain Conference (SBC) at the Radisson Poco Diablo Resort in Sedona, AZ over 4 days (March 10–13, 2004). This was SBC's 9th meeting in Sedona. Eighty-three scientists attended, 43 for the first time. SBC issued Travel Awards to the following 4 Young Investigators: Dwight Bergles (Hopkins), Stephen Smith (Oregon) Erika Piedras-Renteria (Loyola-Chicago) and Kristin Anstrom (Wake Forest); and to 7 Trainee Fellows: Kelley Savoie (Loyola-Chicago), Laurie Cestnick (MIT), Yuqing Cao (Stanford), Nathan Pakratz (Indiana), Susi Lee (Rockefeller), Eric Ullian (Stanford) and Kathrine Bradley (Minnesota). (The full details of this and past meetings are available at <http://www.springbrain.org/>. The 16th Annual Spring Brain Conference will be held in Sedona, AZ from March 16 to March 19, 2005. Check the web site for details.)

The meeting was organized into 12 plenary sessions, 2 lectures, a poster session, and a special NIH information session/workshop. The topics and the presenters are given below, followed by a narrative of session highlights.

Wednesday March 10, 2004

Keynote Address. The Neurobiology of Emotion: Classical and Novel Molecular Candidates. Huda Akil (Michigan).

Thursday March 11, 2004

Plenary Session I. Tauopathies and Synucleinopathies: The Two Largest Groups of Human Adult Onset Neurodegenerative Diseases. Michael L. Hutton (Mayo Clinic, FL) organizer; Dennis Dickson (Mayo Clinic, FL) and Matthew Farrer (Mayo Clinic, FL) presenters. *Plenary Session II.* Synaptic Modulation by Extracellular Ions. Stephen Smith (Oregon Health and Science University – OHSU) organizer; Stephen Smith (OHSU), Henrike von Gersdorff (Vollum Institute) and Edwin McCleskey (Vollum) presenters. *Plenary Session III.* Mechanisms of Recovery and Novel Reparative Strategies Following Traumatic Brain Injury. Douglas Smith (Southern Illinois) organizer; Dalton Dietrich (Miami), David Hovda (UCLA) and Niklas Marklund (Penn) presenters. *Plenary Session IV.* Genetic Approaches to Neurological Diseases. Katherine Woodbury-Harris (NINDS) organizer; Tatiana Foroud (Indiana), James Meschia (Mayo Clinic, FL), Louis Ptacek (UCSF), Katrina Gwinn-Hardy (NINDS) presenters.

Friday March 12, 2004

Plenary Session V. Dyslexia and Brain. Glenn Rosen (Harvard) organizer; Laurie Cestnick (MIT), Thomas Zeffiro (Georgetown) and Glenn Rosen

^{*}Supported by grants from Advanced Targeting Systems, Janssen Pharmaceuticals, Ortho-McNeil Pharmaceutical, Inc., Pfizer Pharmaceuticals and NIH Grant R13 NS048177. Reporting organized by Erika Piedras-Rentería⁷; report edited by Erika Piedras Rentería⁸, Robert Yeziarski⁹ and Thomas A. Woolsey¹⁰.

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ISSN 0899-0220 (print)/ISSN 1369-1651 (online)/00/000001-7 © 200? Taylor & Francis Ltd

DOI: 10.1080/08990220410001721284

(Harvard) presenters. *Plenary Session VI*. Glia, an Essential Element of the Synapse. Eric Newman (Michigan) organizer; Eric Newman (Michigan), Erik Ullian (Sanford) and Dwight Bergles (Hopkins) presenters. *Plenary Session VII*. Neuro-imaging in Normal Aging, MCI and Alzheimers Disease. Molly Wagster (NIA) organizer; Dae-Shik Kim (BU), Charles DiCarli (UCD), Ron Killiany (BU) and Douglas L. Rosene (BU) presenters. *Plenary Session VIII*. Developmental Specialization of Auditory Signaling. Lance Zirpel (Minnesota) organizer; Thomas Parks (Utah) and Dan Sanes (NYU) presenters. *Poster Session. Special Session*. Funding Opportunities and Review Procedures for Brain Research at NIH. *Plenary Address*. The Perception of Space – Notre Dame de Paris. Henry J. “Peter” Ralston (UCSF).

Saturday, March 13, 2004

Plenary Session IX. Images of Addiction. Teresa Levitin (NIDA) and James Smith (Wake Forest) organizers; Linda Porrino (Wake Forest), Dean Wong (Hopkins) and Anna Rose Childress (Penn) presenters. *Plenary Session X*. Calcium Homeostasis and Neurological Diseases. Erika Piedras-Rentería (Loyola Chicago) organizer; Stefan McDonough (Amgen), Yu-Qing Cao (Stanford) and Maureen McEnery (Case) presenters. *Plenary Session XI*. The $\delta 2$ Glutamate Receptor and the Lurcher Mutant: What Happens When a Good Receptor Goes Bad? Michael Vogel (MD Psychiatric Research Center) organizer; Michael Vogel (MD Psychiatric Research Center) and Michisuke Yuzaki (St. Jude). *Plenary Session XII*. Stroke: Clinical Trials. Katherine Woodbury-Harris (NINDS) organizer; Susan Fagan (Medical University of Georgia), Yuko Palesch (MUSC) Jeffrey Saver (UCLA) presenters.

Summary of Presentations

Keynote Address. Dr. Akil reviewed two approaches to study molecular mechanisms underlying emotion, stress and pathogenesis of mood and anxiety disorders. The first is the hypothesis-driven approach to study the classic stress circuit: the limbic-hypothalamic-pituitary-adrenal (LHPA) axis. She presented evidence that the LHPA stress circuit is a complex system with multiple control mechanisms and that these mechanisms are altered in pathological states. Individual rats exhibit marked differences in behavioral responses to a novel environment. Rats that exhibited increased locomotor activity and sustained exploration in such an environment (high responding rats, HR) also had a greater propensity to self-administer drugs (amphetamine and cocaine) as compared to their less responsive animals (LR). HR rats exhibited high concentrations of stress-induced plasma corticosterone, higher basal level of corticotrophin-releasing hormone (CRH) mRNA in the

hypothalamus but lower in amygdala as well as lower basal level of glucocorticoid receptor (GR) mRNA in the hippocampus. Thus HR rats were more prone to novelty seeking despite the fact that this elicited higher stress responses relative to LR rats. Administration of a GR antagonist into the hippocampus of LR rats resulted in an anxiety level comparable to that of HR rats, indicating that hippocampal GR levels were responsible for the level of anxiety in exploring novelty. This hypothesis was further tested in transgenic mice that over expressed GR in forebrain regions. These mice exhibited increased anxiety level in an elevated open maze test, as well as increased responsiveness to antidepressants in the forced-swim test. The level of various molecules important for stress/anxiety response was also elevated, including CRH in amygdala, norepinephrine in locus coeruleus, dopamine transporter in VTA as well as the 5-HT_{1A} receptor in hippocampus. Therefore GR-over-expressing mice could be regarded as a new model of emotional-lability.

The second discovery based approach used DNA microarrays in the analysis of the organization and regulation of the brain in both illness and health. Dr. Akil described an international effort to discover candidate genes and pathways associated with psychiatric disorders, including schizophrenia and mood disorders, through microarray assay on tissue from postmortem human brains. Preliminary analyses showed that tissue from bipolar patients tended to have more up-regulated genes while tissue from major depression patients tended to have more down-regulated genes. However, only 68 genes were affected in their expression level in both illnesses (representing 19% and 27% of the total altered genes in bipolar and major depression patients, respectively). In addition, it has been found that individuals who suffered prolonged agonal states, such as with respiratory arrest, multi-organ failure or coma tended to have lower brain tissue pH. With lower pH, brain samples showed a decrease in expression of genes involved in energy metabolism, proteolytic activities but had an increase in expression of genes encoding stress-response proteins and transcription factors.

Plenary Session I. The pathophysiology and genetics of tauopathies and synucleinopathies was the topic of this session. Dr. Dickenson focused on the pathophysiology of tauopathies and synucleinopathies, with an emphasis on Parkinson’s Disease (PD), multiple system atrophy, Alzheimer’s Disease, and Pick Disease. Dr. Farrer followed with a discussion of the genetics of PD, with a focus on the α synuclein gene SNCA and its promoter polymorphisms that are associated with synucleinopathies. Finally, Dr. Hutton discussed tauopathies, highlighting genetic mutations giving rise to FTDP-17 and tau associated pathogenesis using transgenic

mice. He specifically looked at the transgenic model P301L and its dying back mechanism of neuronal loss, as well as Tg(tau P301L)₄₅₁₀ as an inducible model.

Plenary Session II. It is well established that high synaptic activity in the nervous system leads to decreases in extracellular $[Ca^{2+}]$ and pH and that these changes are most marked at times of acute neuronal injury. The goal of this session was to review recent work describing how falls in extracellular $[Ca^{2+}]$ and pH modulate activity in the nervous system. Dr. von Gersdorff discussed his work describing how increases in extracellular $[H^+]$ in the synaptic cleft modulates presynaptic voltage-activated Ca^{2+} channels and impacts neurotransmitter release. Ribbon-type synapses in the retina transmit via multivesicular release of glutamate. Since synaptic vesicles have a pH of about 5.7, the fusion of vesicles transiently acidifies the synaptic cleft. The pH in the cleft during exocytosis is estimated to decrease from 7.5 to 6.6. Dr. von Gersdorff showed that this pH drop inhibits nearby Ca^{2+} channel activity, which in turn, inhibits neurotransmitter release. This mechanism may be particularly acute in ribbon-type synapses and may influence the light responses of ganglion cells.

Dr. McCleskey described how alterations in extracellular pH, $[Ca^{2+}]$ and lactate combine to stimulate nerve endings of pain fibers under conditions of tissue ischemia. McCleskey's work argues that an ion channel called ASIC3 (acid-sensing ion channel #3) is the critical sensor for lactic acid involved in muscle pain. He reported that ASIC3 senses pH through its ability to bind Ca^{2+} . Protons catalyze release of Ca^{2+} from a site that blocks the pore of the channel, which leads to the pH-activated current. This widely expressed pathway may mediate neuronal damage during stroke and seizure by promoting sustained depolarization following decreases in extracellular pH and Ca^{2+} .

Dr. Smith described how reductions in extracellular $[Ca^{2+}]$ impact ion channels in neocortical nerve terminals. Synaptic strength is very steeply dependent on extracellular $[Ca^{2+}]$ yet paradoxically many synapses continue to transmit signals at high frequency when cleft $[Ca^{2+}]$ falls. To investigate this behavior Dr. Smith's group studied the action of decreasing extracellular $[Ca^{2+}]$ on ion channel activity at single neocortical nerve terminals. Decreases in extracellular $[Ca^{2+}]$ indirectly activated a voltage-dependent, non-selective cation channel. Synaptic transmission is inhibited by the pharmacological block of this pathway suggesting that this novel signaling pathway may compensate for reductions in synaptic efficacy that results from decreases in extracellular $[Ca^{2+}]$.

Plenary Session III. The session considered mechanisms of recovery and repair following traumatic brain injury. Dr. Dietrich presented

endogenous and exogenous reparative strategies. Using a fluid percussion model of TBI with double staining of BrdU and glial markers, neurogenesis was shown in cellular elements expressed GFAP and BrdU. The effects of stem cell transplants were also shown in the fluid percussion model, but the mixed results of these transplants left room for further research. The focus Dr. Markland's talk was dysfunction without death. He showed pediatric TBI in mice and the use of environmental enrichment therapy and BDNF expression benefiting recovery. The last talk was given by Dr. Hovda and focused on myelin inhibitors in repair in axonal injury. He described the hostile axonal environment observed after injury and emphasized that myelin-inhibited axonal outgrowth. He also highlighted Nogo-A as an inhibitory molecule and recent work with the Nogo antibodies to promote outgrowth and repair.

Plenary Session IV. Many neurological disorders display strong familial inheritance, suggesting a genetic component in the development of these diseases. Gene discovery has shown Mendelian causes for certain disorders; however, neurological diseases are typically genetically complex and influenced by environmental factors. This session emphasized progress in understanding the genetic regulation of neurological disorders, including Parkinson's disease, stroke and channelopathies.

Researchers from the PROGENI study discussed Parkinson's Research and the Organized Genetic Initiative, which has shown that genome wide linkage analyses have uncovered several genes, which may impact an individual's susceptibility to Parkinson's disease. The parkin gene previously thought to only be involved in juvenile Parkinson's disease, may also influence the development of Parkinson's disease in adults. A gene was identified on chromosome two, 2q36-37, which may be important in the development of the disease in families with multiple affected members. Interestingly, interaction of this loci with other genes, including one on the X chromosome, appear to be linked to the age of onset and sex differences in the development of Parkinson's disease.

The Ischemic Stroke Genetic Study (ISGS) is a genetic association study in adults with recent first-ever ischemic stroke that is investigating associations between specific gene polymorphisms and individual subtypes of ischemic stroke. Researchers from ISGS reported that stroke phenotype, such as lesion size, symptom intensity and post-stroke recovery, cannot be predicted by family history. Thus, it appears that genetic determinants of stroke occurrence and symptomatic stroke characteristics differ.

Channelopathies are hereditary disorders clinically characterized by episodes of disturbed excitability of muscle and nerve cells. These disorders are typically caused by malfunctions in ion channel gating.

Research of one specific disorder, Andersen's syndrome, uncovered mutations in the gene encoding Kir2.1. Mutant Kir2.1 channels are nonfunctional, indicating a deficit in membrane repolarization, thus leading to cell inexcitability. Interestingly, many channelopathies, including Andersen's syndrome, share similar phenotypes, suggesting that the molecular basis of these disorders might also overlap.

The National Institute of Neurological Disorders and Stroke (NINDS) recognizes that elucidating the genetic regulation of neurological disorders may uncover the general biological mechanisms underlying the development of more genetically complex diseases. To help support research of the genetic influence in neurological disorders, the NINDS has established a repository of DNA samples, immortalized cell lines and accompanying clinical data for a set of disorders, in order to allow pooling of samples collected from multiple independent sites and facilitating complex gene discovery research.

Plenary Session V. The goal of this session was to make clear that dyslexic individuals have a complex cognitive disorder(s) rather than a simple lack of intelligence. 10–15% of the population is reported to have dyslexia. Laurie Cestnick showed data demonstrating that we read in two different ways: holistically where we recognize familiar words and obtain meaning directly from the orthographic representations of those words, and also via an indirect route where we sound out words that we do not recognize—applying sound units to symbols in order to get a phonological representation of words to get meaning. Dr. Cestnick applied this dual route theory to the brain, demonstrating that there are different neurological paths for reading in these different ways. She discussed what goes wrong when reading fails—behaviorally and neurologically (showing fMRI and MEG brain imaging data on dyslexics). Thomas Zeffiro presented evidence that forms of dyslexia can come about from shared relationships between sensory and motor processing. Dr. Zeffiro discussed remediation components of dyslexia suggesting that often children do not improve after reading remediation programs as a result of a sensorimotor component of the disorder not being fully addressed. If given an opportunity to learn and practice with a set of short words, their performance on recognition tests improve; however, reading fluency worsens. In parallel, dyslexics have problems with tasks that do not involve reading such as balance, line drawing, and rapid finger flexion and motion detection. These deficits suggest that dyslexia may involve a dysregulation of circuits involved in sensorimotor integration and not primary sensory processing. Dr. Rosen discussed animal models of dyslexia. These were paralleled the work that Dr. Cestnick presented in that both research strategies present the same auditory temporal tasks to animals and children respectively and that the

findings are similar. He also discussed the role of anomalous neural migration during neural development and how it leads to brain anomalies in dyslexics. Finally, Dr. Rosen showed that dyslexia is correlated with specific neural deficits in the thalamus and cortex that disrupt normal connectivity in the brain. Animals with specific lesions display deficits in tasks that may predict a dyslexic phenotype such as a tone discrimination task. In summary, dyslexia was presented as a disease involving deficits in cognitive processing, that the disease may involve dysregulation of sensorimotor integration and finally, neural targets have been identified that may be involved with these deficits.

Plenary Session VI. The importance of glia as essential elements in the mammalian retinal system was discussed. Dr. Newman discussed how glial cells provide structural and metabolic support for neurons and play an active role in synaptogenesis. Collectively, the pre-, post- and glial-elements form a tripartite synapse, to provide bi-directional communication between glia and neurons. In the mammalian retina, glia participate in information processing; glial-to-glial propagation (evoked by intercellular Ca^{2+} waves throughout network of glial cells) is mediated by both intracellular (calcium) and extracellular (ATP) messengers via gap junctions. Light stimulation evokes increased $[\text{Ca}^{2+}]$ and the frequency of Ca^{2+} transients in Müller cells, the principal retinal glial cell, can directly inhibit neurons by release of ATP and subsequent activation of neuronal adenosine receptor (A1). Thus, glial activation can either facilitate or depress light-evoked EPSC in neurons. Dr. Ullian described the identification of Thrombospondin, an astrocyte-derived molecule (TSP), which plays a role in inducing structural synaptogenesis *in vitro*. TSP, isolated from astrocyte-conditioned medium (ACM) is an extracellular matrix protein (450KD). Although TSP protein is not sufficient to induce electrically active synapses, it produces normal but postsynaptically silent synapses (reminiscent of the developing synaptic profile). TSP increases synapse number and synapse-promoting activity, triggers the formation of structurally silent synapses, providing evidence of a glial role in synaptogenesis throughout the developing brain. Dr. Bergles continued with the theme of synaptic signaling between neurons and glia; his group examined the role of oligodendrocyte precursor cells (OPC) mediating both glutamate and GABA transmission. OPC, abundant in white matter of the optic nerve, are a ubiquitous class of progenitors that express the proteoglycan NG2. OPC express both AMPA and GABA receptors, and engage in rapid signaling with glutamatergic and GABAergic neurons through direct neuron-glia synapses. Although activation of AMPA or GABA_A receptors in OPCs leads to depolarization, these receptors are more likely to serve as routes

for ion flux rather than current sources for depolarization.

Plenary Session VII. This session focused on the growing role of imaging for diagnosis and understanding of cognitive changes with normal aging, mild cognitive impairment and Alzheimer's disease. Dr. Kim provided an update on the basis for several imaging methods—MRI for structural assessment, fMRI for functional changes and Diffusion Tensor Imaging (DTI) for assessing integrity of specific fiber bundles in the brain. His presentation provided a good demonstration of the power of these techniques separately and in combination to assess changes in living patients. Dr. DeCarli discussed combined behavioral and a structural studies (MRI) of elderly patients especially prior to the diagnosis of AD. While noting some overlap between mild cognitive impairment (MCI) and early Alzheimer's disease his principal finding is a significant role for cerebral vascular disease (CVD) in the picture. Current behavioral and fMRI studies are attempting to tease out the regional contributions of CVD and other disorders in functional deficits in the elderly. Dr. Killiany focused on structural changes in the brains of individuals aged 17–88. He described approaches to “segment” white and cortical gray matter. The ratio of white to gray is relatively constant until age 60 when it begins to decline significantly. Current studies are evaluating these changes regionally to determine a relation to behavioral changes. Dr. Douglas Rosene outlined biological bases for some of these changes from structural studies in aged monkeys. In general changes in the structure of myelin and fiber counts support the MRI findings in humans. They also correlate with amplitude but not velocity changes in fiber bundle conduction between different brain regions. The picture that is emerging is one of age-related changes in the brain that can be detected by clinical imaging and correlate with behavioral assays.

Plenary Session VIII. Sound localization requires extremely rapid processing of sensory information from each ear. This discrimination is achieved, in part, by the time of arrival of signals from each cochlea in auditory nuclei within the brainstem. In the avian nucleus magnocellularis (NM), inputs are encoded through glutamatergic synaptic currents mediated by AMPA-type glutamate receptors. In order to discriminate between inputs that arrive with little delay, it is imperative that the time course of the synaptic currents be very brief. This is achieved in the auditory pathway by expressing AMPA receptors with fast kinetics. Indeed, as shown by Raman and Trussell, AMPA receptors in NM neurons exhibit extremely fast desensitization and deactivation kinetics, among the fastest kinetics observed *in vivo*. The Parks lab recently examined the developmental expression of AMPA receptor

subunits in NM neurons of the chick, and compared these to the composition of receptors in brainstem motoneurons (nucleus of glossopharyngeal/vagal nerves; N IX), which exhibit relatively slow synaptic currents. The authors removed NM and N IX tissue from chicks of age E13 to P2, and examined mRNA expression for different AMPA receptor using PCR. They observed a dramatic $\sim 50\%$ decrease in GluR2 expression, and an $\sim 50\%$ increase in GluR3 expression, an effect that will both speed the kinetics of AMPA receptor currents and increase their permeability to Ca^{2+} . In contrast, AMPA receptor subunit expression stayed constant in N IX. These observations were corroborated by Western blot analysis, which revealed a 90% decrease in GluR2 protein in NM from E10 to P2. Analysis of AMPA receptors in heterologous expression indicates that flop isoforms exhibit faster deactivation and desensitization kinetics. This group also examined the relative proportion of GluR3 and 4 transcripts that were in the flip or flop isoforms. In NM neurons, both GluR3 and 4 were found predominantly in the flop isoform, providing a further explanation for the rapid kinetics of their AMPA receptors. These changes in AMPA receptor expression occur prior to the onset of hearing, raising new questions about the mechanisms that are responsible for triggering these processes. Removal of the otocyst, which results in loss of the cochlea and the cochlear nerve, or injection of AMPA receptor antagonists into the embryo, prevented the developmental decline in GluR2 expression in NM. These results suggest that there is developmental plasticity of AMPA receptor expression in the auditory pathway that is induced by spontaneous cochlear activity prior to the onset of hearing.

Plenary Session IX. Repeated use of addictive drugs chemically alters the normal functioning of brain reward mechanisms, producing drug addiction. Addiction is characterized as compulsive, uncontrollable drug use despite serious negative consequences. Research has shown that addiction is a chronic, relapsing, but treatable, disease. One central goal of drug addiction research is to identify the molecular mechanisms underlying addiction, in hopes of developing new treatments. This session provided an overview of the use of brain imaging techniques to elucidate: (1) neuroadaptations following chronic drug use in non-human primates and (2) potential mechanisms underlying drug craving in humans.

Detailed imaging studies of the striatal dopamine system, in parallel with functional studies of glucose utilization, in primates having self-administered cocaine for multiple years were used to examine the temporal progression of neuroadaptations that develop from initial to chronic exposure. Early exposure produces changes solely to the ventral striatum, but with more extended exposure; the response begins to extend throughout the striatum,

engaging areas that are involved with the processing of cognitive and sensorimotor information.

Cue-induced drug desire, or “craving,” is believed to underlie an addict’s tendency to relapse. Through simple Pavlovian conditioning, cues associated with drug taking develop profound incentive properties capable of arousing drug desires, even in recovering addicts. Researchers have begun to use cue reactivity paradigms in conjunction with positron emission tomography in an attempt to identify neuroadaptations for cue-induced drug craving in cocaine-addicted humans. A significant observation in this area of study is the activation of the mesocorticolimbic dopamine system in response to cue-induced cocaine craving. This was revealed by an increase in dopamine in the ventral striatum, amygdala, medial prefrontal cortex, anterior cingulate cortex and orbital frontal cortex. In addition, administration of GABA B receptor agonists blunted both brain activation and craving that was induced by drug cues.

In addition to craving activating reward mechanisms, relapse vulnerability may involve alterations in frontal inhibitory circuitry. Frontal cortical regions, such as the lateral orbital frontal cortex, modulate limbic regions. Changes in these inhibitory circuits augment mesolimbic dopamine signaling. Thus, not only do cues activate reward circuits, deficits in inhibitory circuitry would further enhance the activation of reward mechanisms elicited by drug-associated cues. Additional neuroimaging studies demonstrated activation of the lateral orbital frontal cortex circuitry when addicts attempt to inhibit cue-induced cravings. Recognizing the potential contribution of both the “reward” and “inhibitory” circuits presents multiple targets for intervention, and may help explain why only a small proportion of individuals exposed to rewarding drugs become addicted.

Plenary Session X. The session considered how intracellular calcium homeostasis may be disrupted by calcium channelopathies. Dr. Piedras-Rentería indicated that disruption of intracellular calcium levels severely impacts key neuronal signaling pathways, such as neurotransmitter release, gene expression, and calcium-dependent enzymatic processes. Abnormalities in voltage-gated calcium channel activity, which is a fundamental calcium entry route in neurons, leads to altered calcium homeostasis. Indeed, several calcium channelopathies affecting humans have been described, such as Familial Hemiplegic Migraine (FHM), Episodic Ataxia type 2, seizures, and Spinocerebellar Ataxia type 6. All these diseases arise from the presence of mutations in the P/Q-type calcium channel. The role of voltage-gated P/Q-type calcium channels in neuronal calcium channel function and dysfunction was discussed. Dr. Cao discussed the effects of FHM type 1 mutation on neuronal P/Q-type channel

activity and synaptic transmission. The FHM mutations were introduced in hippocampal neurons *in vitro* and the functional implications of the presence of these mutations were studied by comparing them with wild-type P/Q-type calcium channels. Neurons transfected with the mutations consistently displayed reduced currents and calcium influx when studied under action-potential stimulus protocols. In addition, these mutations displayed impaired contribution of P/Q-type dependent neurotransmission at both excitatory and inhibitory synapses (from ~60% with wild-type channels to ~30% with FHM mutations). Dr. McEnery ended the session by discussing the compensatory mechanisms of voltage-gated channel expression in models of inherited neurological disease. Dr. McEnery discussed the increases of low-voltage gated calcium channels (LVA) found in both the P/Q-type calcium channel knockout animal and the lethargic mouse model. The data presented suggests patterns of convergence of cell dysregulation shared by animals with distinct genetic mutations. Dr. McEnery discussed the possibility that the knowledge of consistent patterns of compensatory expression of non-mutated calcium channels might help design molecular or pharmacological interventions to help correct altered patterns and rescue diseased neurons.

Plenary Session XI. The d2 glutamate receptor (GluRd2) is an orphan receptor that was cloned based on its homology to the glutamate receptor. GluRd2 is predominantly expressed in Purkinje cells and is critical to cerebellar function. Lurcher heterozygous (*Lc*) mice have A654T point mutation in GluRd2 that converts it into a leaky membrane channel that chronically depolarizes Purkinje cells and causes massive cell death in *Lc/+* mice between P525.

Dr. Michael Vogel reviewed the current hypotheses for the mechanisms of Purkinje neuron death in *Lc/+* mice. *Lc/+* Purkinje neuron degeneration was associated with an upregulation in cytochrome oxidase activity as well as an increase in MnSOD and nitrotyrosine expression. The expression of procaspase-3 was increased in many *Lc/+* Purkinje cells and activated caspase-3 was detected in a few scattered neurons. *Lc/+* mice were crossed with Bcl-2 transgenic mice or with Bax knockout mice, respectively. Both delayed Purkinje neuron death. These findings are consistent with the hypothesis that chronic depolarization of *Lc/+* Purkinje neurons causes oxidative stresses that may trigger apoptosis.

Dr. Michisuke Yuzaki proposed that GluRd2 played a role in synapse formation and synaptic plasticity by characterizing GluRd2 knockout mice and hotfoot mice (in which mutant GluRd2 was trapped in ER). Both mutant mice showed ataxia as well as deficits in the rotor rod test. At parallel fiber-Purkinje neuron synapse of mutant mice, there were

increased numbers of “naked” spines, which lacked presynaptic innervation. Mismatch between PSD and active zones have also been observed. He also reported multiple innervation of climbing fibers onto a single Purkinje neuron. Moreover, mutant mice showed impaired cerebellar long-term depression (LTD).

When an antibody against extracellular N-tail of GluRd2 was injected into the cerebellum of wild-type mice, it caused acute ataxia as well as impairment in the rotor rod test. Further analysis revealed that the antibody decreased EPSC size as well as occlusion of LTD, presumably by increased GluR2 endocytosis. Thus it was proposed that GluRd2 normally functions to prevent GluR2 receptor endocytosis. Regarding Lc+/ mice, Dr. Yuzaki provided evidence that the binding of GluRd2 C-terminal with nPIST and Beclin might not be responsible for Purkinje neuron cell death. Rather, autophagocytic pathway could be activated by an excessive Na⁺ influx associated with overstimulation of Na⁺, K⁺-ATPase.

Plenary Session XII. Each year, over 600,000 people in the United States suffer from an acute ischemic stroke episode. Years of research using experimental stroke models have developed many possible neuroprotective agents for acute stroke; however, only one, tissue plasminogen activator (tPA), has made it to clinical use. Speakers in this session discussed procedural changes in the drug development process to enhance the efficiency and success rates in stroke clinical trials.

The drug development process involves three phases. Phase I clinical trials evaluate the toxicity of a new drug to determine the maximum tolerated dose. Phase II trials again monitor the toxicity and side effects of the drug to develop a logical treatment plan. Phase III trials determine in diseased individuals the effectiveness of the treatment. Many

drugs fail throughout these three stages because drug administration parameters determined in non-human models are not directly applicable to humans. For example, doses comparable to the effective dose in animals cannot be achieved in humans due to toxicity. In addition, the therapeutic timeline shown to be effective in experimental models is unsuccessful in humans.

It was proposed that in stroke clinical trials, more extensive preclinical investigation may lead to better translation to human stroke patients. In addition, more attention to the dose and time window in Phase I and II trials may result in more rapid identification of agents likely to fail. Moreover, careful selection of patients for efficacy trials may lead to improved power to detect significant differences in outcome.

It was also proposed that experimental treatments should be administered by paramedics in a prehospital setting for neuroprotective therapies must be delivered early within the first hour after onset to be highly effective for neuroemergencies. Such a prehospital clinical trial is in progress. Researchers at UCLA are testing a new emergency stroke treatment, called FAST-MAG (Field Administration of Stroke Therapy-Magnesium). When trained paramedics reach a patient, they use a checklist to confirm the diagnosis of stroke. Then, the patient is given a high intravenous dose of the neuroprotective agent magnesium sulfate. IV infusion of the drug is continued at the hospital over the next 24 hours in combination with standard therapy (such as clot-busting medications or blood thinners). Thus far, this study has demonstrated that paramedic initiation of magnesium sulfate in the field is an effective and safe treatment for acute stroke. Furthermore, this study has demonstrated that paramedics can safely, effectively, and rapidly start neuroprotective therapies for stroke.

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JOURNAL ID: CSMR 41010

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