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To be presented at the Meeting of the Society for Neuroscience 2008

Hippocampal dendritic branching and spine changes precede β -amyloid plaque formation in the young ArcA β transgenic mouse model of Alzheimer's Disease

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Abstract:

The arcA β transgenic mouse expresses human APP695 with the combined Swedish and Arctic mutations. This results in a transgenic mouse model of brain β -amyloid pathology caused by both increased production of A β and enhanced formation of oligomeric A β species. Small intraneuronal A β aggregates occur as early as 3 months of age and precede β -amyloid plaque formation in the neuropil by several months. Furthermore, severe behavioral deficits and impaired long-term potentiation also manifest before plaque deposition is detectable, suggesting a toxic role of small oligomeric A β species in this mouse model. The purpose of this study was to determine if hippocampal dysfunction in these young mice (e.g., LTP deficits) correlates with alterations in dendritic branching and spines in hippocampus neurons. Methods: Formalin fixed brain blocks from 3.5 month-old transgenic mice (N= 5) and age-matched non-transgenic controls (N = 5) were stained using the rapid Golgi method. This technique shows the soma, dendritic branching and spines of a small randomly-stained population of neurons. All slides were coded. Dendritic branching and spine analyses of randomly selected hippocampal CA1s and granule cells of the dentate gyrus were carried out. Results: Sholl analysis of the basilar tree of CA1 pyramids in the 3.5 month-old ArcA β mice had significantly less dendritic branching (-35%, $p < 0.0002$) and complexity (-30%, $p < 0.009$) than age-matched non-transgenic littermate controls. There was also a significant loss of dendritic spines on the apical (-15%, $p < 0.02$) and basilar (-20%, $p < 0.004$) trees of the transgenic mice. For the granule cells of the dentate gyrus, the 3.5 month-old arcA β tg mouse again showed a significant loss of spines (12%, $p < 0.01$). Conclusions: The reduction in hippocampal dendritic branching and spines in the arcA β transgenic mice occurs before the deposition of extracellular β -amyloid plaques, but coincides with the occurrence of small intraneuronal A β aggregates. This present data therefore suggests that small A β species might damage neuronal dendritic and synaptic circuitry. This disruption of neuronal networks may, in turn, underlie the subsequent functional impairment seen in these arcA β tg mice.

Click the following Link to View the Article
Published in Nature Neuroscience 2008

Activation of Estrogen Receptor Beta Regulates Hippocampal Synaptic Plasticity and Improves Memory

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Estrogens have long been implicated in influencing cognitive processes, yet the molecular mechanisms underlying these effects and the relative roles of the estrogen receptors alpha (ER α) and beta (ER β) remain unclear. Utilizing pharmacological, biochemical and behavioral techniques, we demonstrate for the first time that the effects of estrogen on hippocampal synaptic plasticity and memory in rodents are mediated through ER β . Selective ER β agonists increased levels of key synaptic proteins in vivo including PSD-95, synaptophysin and the AMPA-receptor subunit GluR1. These effects were absent in ER β knockout mice. In hippocampal slices ER β activation enhanced long-term potentiation (LTP), an effect that was absent in slices from ER β knockout mice. ER β activation induced morphological changes in hippocampal neurons in vivo including increased dendritic branching and density of mushroom-type spines. An ER β agonist, but not an ER α agonist, also improved performance in a variety of hippocampal-dependent memory tasks. Taken together, our data suggest that activation of ER β can regulate hippocampal synaptic plasticity and improve hippocampal-dependent cognition.

Presented at the American Society for Neural Transplantation and Repair, Clearwater, FL
2008

NEUROSTRUCTURAL CONSEQUENCES OF DEVELOPMENTAL EXPOSURE TO MODERATE DOSES OF CANNABINOIDS IN A RAT MODEL

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Even though marijuana is the most widely used illegal drug among women of reproductive age, reports dealing with the effects of prenatal exposure to this substance of abuse are still controversial. More complex and less understood is the scenario concerning the possible long-term consequences of in utero exposure to cannabis derivatives on cognitive functions.

In this study the synthetic CB1 agonist WIN 55,212-2 (WIN) was administered daily to pregnant rats from gestational day 5 to 20 (0.5mg/kg). This dose is equivalent to a moderate or low exposure to marijuana in humans and has no overt toxic effects. The treatment with WIN did not affect gestational and reproduction parameters and WIN-exposed pups did not show any sign of malformations or malnutrition. However, a deeper investigation revealed that prenatal treatment with WIN altered pup performance in homing behavior and produced a decrease in the rate of separation-induced ultrasonic vocalizations. Behavioral deficits that resulted were long-lasting, since prenatal WIN exposure caused a disruption of memory retention in young and adult offspring subjected either to a passive or an active avoidance task. Moreover, an altered dendritic morphology of hippocampal CA1 pyramids was detected in young (40 day-old) rats. In particular, in the prenatally WIN-exposed rats, there was a significant 12% increase in estimated total dendritic length and a highly significant increase in branching complexity in the middle-third of the dendritic tree. These findings suggest that moderate exposure to cannabinoids during crucial periods of brain development can cause dysmorphic maturation of the hippocampus. Such subtle morphological alterations and commensurate changes in brain circuitry would be, in turn, a factor underlying the behavioral deficits observed both in early and late postnatal life.

Presented at the Society for Neuroscience, San Diego, November, 2007

A Chronic Stress Regimen with Unpredictable Stressor Presentation Decreases the Density of Spines on the CA3 Pyramidal Neurons of the Hippocampus in the Adult C57BL/6J Male Mouse

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The neural changes associated with chronic stress are of great interest to those interested in brain health as they may be linked to the cognitive impairments and neurodegeneration observed in many brain diseases. To examine how chronic stress impacts brain structural elements adult C57BL/6J male mice (N=10) were exposed to 21 consecutive days of the following stressors in random order: (1) 8 hrs restraint; (2) 3 mins forced swim in ice water; (3) 8 hrs of light-cycle disruption; (4) 8 hrs at 15o; (5) social reorganization or remained in their home-cage. Immediately following the last stressor mice were anesthetized, perfused with paraformaldehyde, the brains removed and maintained in fixative until sections were prepared and examined for alterations in neuronal plasticity. This chronic stress regimen, designed to minimize habituation by presenting the stressors in an unpredictable random order, produced a measurable but non-significant change in body weight. It also caused a 27% decrease in thymus weight indicating a sustained activation of the HPA axis throughout the stress period although this decrease is minimal compared to the 93% reduction observed in animals exposed to supra-physiological levels of corticosterone through pellet (200 mg) implant for the 21 days. Dendritic spines were quantified for spine density along terminal tip and internal branch segments of the apical tree of the CA3 pyramidal neurons of the hippocampus. Spines were also categorized into "L-" (lollipop), "N-" (nubby), and "D-type" (dimple) configurations. Five to six randomly selected Golgi-impregnated CA3 neurons were evaluated from each subject and spines quantified along 30 um Golgi-impregnated sections. Findings revealed a ~ 15.5% decrease in L-and N- but not D-type spines on the apical dendrites. A lesser but still significant decrease in all spine types was also observed on the internal segments of the apical dendrites. A less dramatic loss of spines was observed in mice exposed continuously to supra-physiological levels of corticosterone. Reduced spine density may be indicative of reduced neuronal plasticity. Thus, our data provides evidence that the CRS regimen alters plasticity in the hippocampal area of the mouse as it does in the rat and provides a chronic stress model that can be used in future studies utilizing this rodent species to examine the signaling pathways suspected to mediate the effects of stress on plasticity.

Presented at the Society for Neuroscience, San Diego, November, 2007

Neurotoxic Consequences of Chlorpyrifos Exposure on Dendritic Circuitry in the Adult Rat

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Chlorpyrifos (CPF) is a common organophosphate (OP) insecticide. It has extensive use in agriculture as a pesticide. The primary mechanism of action is inhibition of acetylcholinesterase (AChE). Exposure to OPs may have deleterious neurobehavioral consequences. This has been well documented in neonatal and developing animals; however, the impact on the adult brain is more poorly understood. Analysis of Golgi stained neurons was chosen for morphological assay which allows for complete visualization of entire dendritic branching arbor and dendritic spines and quantification of the morphometric changes. Adult CPF-treated rats were given 18mg/kg for 14 days. There were six CPF and six control subjects in each group. Five randomly-selected neurons were evaluated from each region of each subject: the cingulate gyrus of the frontal cortex, CA1s in the hippocampus, and granule cells of the dentate gyrus. Dendritic branching was assessed by preparation of camera lucida drawings and subsequent Sholl analysis which profiles the amount and distribution of the arbor. Dendritic spines were counted along 30um long dendritic segments.

Results: Whereas the cingulate and CA1 neurons showed no effects of the CPF-exposure on dendritic branching or spine density, in the dentate gyrus, analysis of the dendritic arbor of the granule cells revealed a significant reduction of branching across the entire tree in the chlorpyrifos exposed animals ($p < 0.0001$, Wilcoxon Test). This corresponded to a ~16% decrease in dendritic material. Branch point analysis of these animals revealed a trend toward less complex dendritic arbors in the CPF treatment group. Dendritic spine analysis of the granule cells showed a significant ~7% ($p < 0.01$, unpaired T-test) decrease of spine density in the middle third of the dendritic tree of the granule cells in the CPF treatment group.

These results show that a 14 day exposure to CPF causes a significant reduction in dendritic branching, along with spine loss in the dentate gyrus of the hippocampus. Relative to the cortical neurons and the hippocampal CA1s where no neurotoxic effects were observed, the granule cells appear to be selectively sensitive to CPF-exposure. Damage to granule cells -- and to the related hippocampal circuitry -- may be the underlying neuroanatomical basis for cognitive dysfunction observed in OP-exposed subjects.

Presented at the Society for Neuroscience, San Diego, November, 2007

Heavy Particle Irradiation and Neuronal Damage: A Potential Risk to Future Interplanetary Exploration?

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In future deep space missions, astronauts will be exposed to heavy particle radiation over lengthy periods. Potentially, this exposure could result in neuronal damage, impair brain circuitry, and compromise the astronaut's cognitive and behavioral capabilities which would threaten mission success. At this time, relatively little is known of the extent of this exposure on neuronal circuitry. The purpose of this study was to evaluate the effects of high-energy (56)Fe particles on dendritic branching and spine parameters in layer II-III cortical neurons in the adult rat. These parameters are critical to the integrity of brain circuitry: dendritic branching comprises about 95% of total neuronal volume and the vast majority of synapses occur on the dendritic spines.

4 month-old Sprague-Dawley rats were exposed to a single exposure of (56)Fe radiation (1.5Gy of 1GeV/n). 28 days later the animals were sacrificed. Formalin fixed coronal tissue blocks were stained using the rapid Golgi method and, from coded slides, randomly selected layer II-III pyramids of the frontal cortex were evaluated for dendritic branching and spines. Camera lucida drawings of the basilar tree were assessed using Sholl analysis. Results showed that there was a statistically significant loss of dendritic material (~10%) in the dendritic arbor ($p = 0.008$, Wilcoxon test) of the neurons exposed to the heavy particle irradiation.

These results suggest that exposure of the rat brain to heavy particle irradiation equivalent to that which astronauts might encounter on deep space missions has a deleterious effect on neuronal morphology and hence, on brain circuitry. Neuroprotective strategies may need to be devised to minimize brain damage and assure mission success.

Presented at the Society for Neuroscience, San Diego, November, 2007

The Effect of Chronic Lithium Chloride on Dendritic Branching in the Adult Rat Neocortex and Hippocampus

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Lithium may serve as a treatment for bipolar disorders and other psychiatric conditions by counteracting both mania and depression. Lithium can affect neurotransmitter activity by increasing serotonin levels and decreasing noradrenaline discharge. Lithium may also influence neuroplasticity and the dendritic parameters that are the underlying neurostructural basis for the cognitive and behavioral manifestations.

We evaluated the effects of chronic administration of lithium chloride to adult rats on the morphology of the dendritic arbor of Golgi stained granule cells of the dentate gyrus and layer II-III pyramids of the prefrontal cortex. The dendritic arbor comprises about 95% of the volume of the typical neuron. Adult rats were injected with lithium chloride (1mEq/kg/day IP injection for 14 days) or saline. Formalin fixed blocks of tissue were Golgi stained and camera lucida drawings were prepared from randomly selected neurons. Sholl analysis was used to assess amount and distribution of the dendritic arbor.

Lithium treatment resulted in a neuroplastic remodeling of the dendritic arbor of the granule cells: relative to the controls, there was significantly increased branching in the proximal region ($p=0.0028$, Wilcoxon test) and decreased branching in the distal portion of the arbor ($p = 0.0024$, Wilcoxon). There was also a significant increase in branching complexity in the medial portion of the tree. (Previous studies have indicated that neurophysiological changes in dentate gyrus occur in lithium treated rats.)

In the prefrontal layer II-III pyramids, the lithium treatment also resulted in a significant neuroplastic increase of the basilar dendritic arbor which was most pronounced in the medial portion of the tree. Conversely, in the apical dendritic tree, the lithium resulted in a decrease in the amount of dendritic material in the proximal region of the arbor.

Although the specific mechanisms for these neuroplastic dendritic alterations are not currently known, the changes in the dendritic arbor due to the chronic lithium can clearly modify the transfer of information in cortical and hippocampal brain circuits and this can, in turn, influence behavior.

Presented at the Nutrisciences and Health Conference, Charlottetown, PEI, Canada, July, 2007

Dietary Supplementation in Aged Rats with Blueberry Extract, a Nutritional Antioxidant, Enhances Dendritic Neuroplasticity in Cortical Neurons

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Aged-related oxidative stress results in the formation of reactive oxygen species (ROS) which can damage neurons and may an important component associated with disruption of brain circuits and concomitant age-related cognitive impairment. This study attempts to characterize the potential beneficial effects of the nutritional antioxidants (e.g., polyphenols) found in blueberries (BB), on morphological indices of brain circuitry in old rats. Nineteen month old male Fischer 344 rats were given either a standard NIH-31 rat chow (controls) or an NIH chow enriched with 2% BB extract for 2.5 months. Following behavioral testing, the 21.5 mon-old rats were killed and their brains stained using the Golgi method (N = 3 subjects/group).. Golgi staining permits microscopic visualization and quantitative analysis of the dendritic parameters of the impregnated neurons. From coded slides, randomly selected layer II-III cortical neurons of frontal cortex in the 21.5 month old rats (7 neurons/subject) were evaluated for the extent of their dendritic branching. Two and half months of the BB-enriched diet resulted in a significant increase in dendritic branching, primarily in the proximal half of the basilar dendritic arbor of the layer II-III neurons (a 14% increase). This suggests that even in old subjects, BB dietary supplementation appears to have a neuroplastic impact on neuronal morphology: it can mitigate normal age-related dendritic atrophy. This would suggest that the BB-extract can exert anti-aging neuroprotection in maintaining or enhancing the integrity of brain circuitry in the old rats.

Click the following Link to View the Article
[Published in Environmental Health Perspectives 2007](#)

Ontogenetic Alterations in Molecular and Structural Correlates of Dendritic Growth After Developmental Exposure to Polychlorinated Biphenyls.

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OBJECTIVE: Perinatal exposure to polychlorinated biphenyls (PCBs) is associated with decreased IQ scores, impaired learning and memory, psychomotor difficulties, and attentional deficits in children. It is postulated that these neuropsychological deficits reflect altered patterns of neuronal connectivity. To test this hypothesis, we examined the effects of developmental PCB exposure on dendritic growth. **METHODS:** Rat dams were gavaged from gestational day 6 through postnatal day (PND) 21 with vehicle (corn oil) or the commercial PCB mixture Aroclor 1254 (6 mg/kg/day). Dendritic growth and molecular markers were examined in pups during development. **RESULTS:** Golgi analyses of CA1 hippocampal pyramidal neurons and cerebellar Purkinje cells indicated that developmental exposure to PCBs caused a pronounced age-related increase in dendritic growth. Thus, even though dendritic lengths were significantly attenuated in PCB-treated animals at PND22, the rate of growth was accelerated at later ages such that by PND60, dendritic growth was comparable to or even exceeded that observed in vehicle controls. Quantitative reverse transcriptase polymerase chain reaction analyses demonstrated that from PND4 through PND21, PCBs generally increased expression of both spinophilin and RC3/neurogranin mRNA in the hippocampus, cerebellum, and cortex with the most significant increases observed in the cortex. **CONCLUSIONS:** This study demonstrates that developmental PCB exposure alters the ontogenetic profile of dendritogenesis in critical brain regions, supporting the hypothesis that disruption of neuronal connectivity contributes to neuropsychological deficits seen in exposed children.

Presented at the 2006 Annual Meeting of the Society for Neuroscience. Atlanta, GA

Dendritic atrophy and spine plasticity in frontal cortex neurons in Mild Cognitive Impairment: A quantitative Golgi analysis.

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Disruption of dendritic branching and/or spine components likely play a role in the neuropathology underlying the onset of cognitive impairment seen in Alzheimer disease (AD). Whether disruption in these components of brain circuitry is altered in elderly people with a clinical diagnosis of mild cognitive impairment (MCI) without frank AD remain unknown. To evaluate these dendritic alterations, formalin fixed frontal (area 9) cortical tissue blocks were Golgi stained from individuals who died with a clinical diagnosis of either No Cognitive Impairment (NCI), MCI, or AD obtained from the University of Kentucky. Layer II-III pyramidal neurons (6/subject) were randomly selected from coded slides. This data represents the initial phase of a larger on-going Golgi investigation of neo- and limbic brain circuitry in the progression of AD. Sholl analysis revealed significant dendritic atrophy in the MCI compared to AD and controls. However, there was a significant increase in pyramidal neuron spine density in MCI as compared to controls and AD (which did not differ from each other). The underlying molecular mechanisms are unknown: mutations in amyloid beta (A β) (and/or tau) genes may enhance dendritic pruning (microtubule depolymerization in the shafts of dendrites) by promoting enhanced calcium influx from the endoplasmic reticulum, or A β could promote spine formation via actin polymerization. Similar dichotomous findings were seen in the 3xtg triple AD transgenic mouse model of AD, further implicating genetic mutation in APP and tau genes in this process. These findings may represent compensatory neuroplastic responses, which may assist in maintaining cortical circuitry and delay severe cognitive dysfunction in MCI.

Presented at the 2006 Annual Meeting of the Society for Neuroscience. Atlanta, GA

Dichotomous Dendritic Changes in the Aging Triple Transgenic Mouse Model of Alzheimer's Disease: Dendritic Spines Increase and Branching Decreases in Layer V Pyramidal Cells.

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The triple transgenic mouse model of Alzheimer's disease (3xTgAD) was generated by expressing three different mutant genes (APP, PS1 and tau) linked to inherited forms of dementia. 3xTgAD mice exhibit age-related amyloid and tau pathology that is associated with synaptic dysfunction and memory impairment. From randomly selected Golgi stained neurons, we evaluated neocortical layer V dendritic spine density (basilar tree and main apical branch) and basilar dendritic branching in aging (15 mon-old) 3xTgAD male and female mice and age-matched controls. Branching analysis of the basilar dendritic arbor of both 3xTg males and females showed significant mild-to-moderate branching atrophy in the distal 2/3rds of the tree (~15%). Conversely, for both aging males and females, there was an overall significant average increase in spines (~ 50%) for these layer V cortical neurons. A possible mechanism for the contradictory dendritic findings is that Abeta, the PS1 mutation (and tau mutations as well) promote enhanced calcium influx and release from the ER; therefore, this would tend to cause dendritic pruning (microtubule depolymerization in the shafts of dendrites), but could also promote spine formation (actin polymerization). We have also seen a similar dichotomous dendritic response in cortical neurons from autopsied humans diagnosed with mild cognitive impairment (a prodromal stage of AD); this lends additional strength to the 3xtg mouse as a valid model of Alzheimer's. These dendritic findings would suggest that alterations in cortical circuitry are contributing to the cognitive dysfunction associated with the progression of the disease.

Presented at the 2006 Annual Meeting of the Society for Neuroscience. Atlanta, GA

Human Umbilical Cord Blood Cell Treatment Mitigates Loss of Dendritic Branching and Spines in the Aged Rat Brain.

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Human Umbilical Cord Blood (HUCB) cells are enriched for stem cells that have the potential to initiate and maintain tissue repair. The potential neuroprotective role of HUCB on the circuitry of the aging brain is unknown. However, HUCB may release certain neurotrophic factors that protect the dendritic and synaptic circuitry from age-related deterioration. This study was designed to provide new insight into the neuroprotective role of HUCB in aging rats. The brains from 22 month-old aging rats and 4 month-old young controls were evaluated for dendritic branching and spine morphology from several cell populations. The old rats were administered a single treatment of HUCB either i.v. or directly into the hippocampus. Cortical and hippocampal neurons were assessed using Golgi impregnated preparations which can reveal the amount of dendritic material and the numbers of dendritic spines of randomly stained neurons. In the neocortex and hippocampus, layer II-III pyramids and granule cells, respectively, showed age-related spine loss which was largely attenuated by the single HUCB cell treatment. Also, age-related granule cell dendritic branch atrophy was reversed by HUCB. These results will have significant impact on future applications of umbilical cord therapies

Presented at the 2006 Annual Meeting of the Society for Neuroscience. Atlanta, GA

Disruption of Normal Cortical Dendritic Growth in the Akt3 KnockOut Mouse.

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In the CNS, the insulin signal transduction pathway regulates many diverse processes ranging from metabolism to memory formation. Binding of insulin to the insulin receptor results in activation of phosphatidylinositol 3'-kinase (PI3K), generation of phosphatidylinositol-3,4,5-trisphosphate (PIP3), and activation of the serine/ threonine kinase Akt / protein kinase B. In mammals, three highly conserved proteins, Akt1, Akt2, and Akt3, comprise the Akt family. Akt3 is the predominant isoform, representing about half of total Akt protein and is the most prevalent isoform in the cortex and hippocampus. Akt3 is required for normal brain growth and mice deficient in Akt3 demonstrate a selective 20% decrease in overall brain size. Signaling through the PI3K-Akt pathway can modulate dendritic branching. Here, we demonstrate the *in vivo* requirement of Akt3 for regulation of dendrite growth. Using Golgi-stained neurons, compared to the wild-type controls, analysis of the basilar tree of parietal layer V pyramids of Akt3-deficient brains showed a 20% reduction in the amount and distribution of dendrite branching ($p < 0.0001$, Wilcoxon test). There was also a trend toward reduction of dendritic spine density on the terminal tips of these neurons in the Akt3 deficient mice (-9%, $p = 0.07$, unpaired t-test). Thus, the serine/ threonine kinase Akt3 influences normal brain neuronal branch growth and development of normal brain circuitry.

Presented at the 2006 Annual Meeting of the Society for Neuroscience. Atlanta, GA

Effects of Hyper- and Hypoglycemia on Dendritic Branching and Spines in the Young Adult Diabetic Rat.

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Childhood diabetes and related medical issues are a growing health problem. Children with diabetes onset before 5 years old may have reduced neurocognitive function. Typically, this problem has been attributed to hypoglycemia, a complication of insulin therapy. However, hyperglycemia is much more common than intermittent hypoglycemia during early childhood diabetes. Relatively little is known about the effects of hyperglycemia on development of neural circuitry. The purpose of this study was to evaluate the effects of chronic hyperglycemia and intermittent hypoglycemia on dendritic branching and spines in the young rat neocortex. Starting at four weeks of age, experimental rats were exposed to 4 weeks of either chronic hyperglycemia or intermittent (3 hours, 3x/week) hypoglycemia. Tissue was stained using Golgi impregnation methods to assess dendritic parameters. Compared to age-matched controls, evaluation of the basilar tree of layer II-III pyramids in the parietal cortex showed that although the intermittent hypoglycemia did not affect dendritic branching, the hyperglycemic paradigm resulted in significantly smaller (-16%, $p < 0.05$) and less complex dendritic arbors relative to the controls. There was also a 9% loss of dendritic spines on the internal branch segments of the basilar tree ($p < 0.004$). These findings indicate that chronic hyperglycemia in the developing brain – which is associated with early childhood diabetes – may result in damage to cortical neurons, compromise brain circuitry and could be the neuroanatomical basis for the neurocognitive impairment found in this disorder. Other studies on hippocampal neurons and using additional dosages are currently in progress. *(Supported by a grant from the Juvenile Diabetes Research Foundation)*

Presented at the Annual Meeting of the Society of Toxicology, San Diego, March, 2006

Stress and Depleted Uranium Exposure Alter Dendritic Morphology in the Rat

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Studies of rats with intramuscular implantation of depleted uranium (DU) reveal that in addition to the well-described accumulation of this metal in kidney and bone, it is also increased in brain (Pellmar et al, 1999). In addition, Gulf War I veterans with long-term residual DU shrapnel had lowered performance efficiency in selected neurocognitive tests (McDairmond et al, 2000). This study was undertaken to additionally assess the neurotoxicologic effects of implanted DU in rats, and to see if stress altered this response, focusing on changes to the hippocampal pyramidal cells. Adult male Sprague-Dawley rats each had 20 DU or tantalum (control) pellets (1mm x 2mm) implanted in the gastrocnemius muscles, for a six month period. Stress was applied 5 days/week for the entire exposure period in a random pattern of handling, restraint, and swimming to minimize habituation. At terminal sacrifice, cerebral regions from animals exposed to the stress, DU, the combination of stress and DU and the tantalum controls were removed and stained by the Golgi impregnation procedure. The basilar dendritic arbor of hippocampal CA1 pyramidal cells was assessed using Sholl analysis. Preliminary data shows that relative to the negative controls, there was an increase in dendritic material in the inner 1/3 of the arbor in rats administered DU alone. This diminished in the outer 2/3 of the arbor, a region where the combination of stress and DU elicited increase in dendritic material. There also was decrease in dendritic material in animals only exposed to stress in this outer region. Thus, 24 weeks exposure to a high dose of implanted DU pellets alters the dendritic tree of CA1 pyramidal neurons which may influence cognitive behavior. (Supported by DAMD17-01-1-0775, US Army Medical Research and Materiel Command)

Presented at the annual meeting of the Society for Neuroscience, Washington, DC,
November, 2005

The Increase in CA1 Spines Produced by Spatial Learning is Blocked by Pre-Training, but Not Pre-Retrieval, Predator Stress.

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Rats exhibit strong long-term spatial memory when they are trained in the radial arm water maze (RAWM). However, when rats were exposed to a cat after learning the location of the hidden escape platform, their spatial memory was impaired (Hippocampus, 9:542-552, 1999; Learning & Memory, 10:326-336, 2003). Here, we investigated the influence of spatial learning and predator stress on long-term (24 hr) memory and dendritic spines in CA1. In addition, we have tested the hypothesis that there will be a differential expression of learning and stress-induced amnesia on well-developed, lollypop-shaped (L-type) spines, compared to stubby, nubbin-shaped (N-type) spines.

Adult male SD rats were trained to learn the location of a hidden platform in the water maze, and then their spatial memory was tested 1 and 24 hr later. Rats were exposed to a cat for 30 min either before they learned the platform location (Stress Day 1), before the 24 hr memory test trial (Stress Day 2) or not at all (No Stress), with brain extraction immediately after the 24 hr memory test trial. The three groups showed equivalent learning and 1 hr memory on Day 1, but both stress groups showed impaired performance on the 24 hr memory test. Thus, the Stress Day 1 group exhibited impaired storage of the spatial information, and the Stress Day 2 group exhibited impaired retrieval of stored information. The No Stress and Stress Day 2 groups both exhibited a significant increase in N-type spines, with no change in the number of L-Type spines. There was no increase in either type of spine in the Stress Day 1 Group.

These findings indicate that rats that had 24 hr to consolidate spatial information (the "No stress" and "Stress Day 2" groups) exhibited increase in spines. Rats that were stressed before learning (Stress Day 1 group) had impaired consolidation and lacked an increase in spines. Moreover, the spine changes were specific to N-type spines, which are known to be more plastic than L-type spines. Overall, these findings support the idea that increases in N-type spines are involved in the storage of information. We have also provided the novel observation that morphological evidence of memory storage (spine changes) remain when acute stress interferes with the retrieval of a stored memory.

Supported by the VA

Presented at the Annual Meeting of the Society for Neuroscience, Washington DC,
November, 2005

Hyperglycemia Induced Alteration of Cortex and Hippocampus in the Rat

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Children with diabetes onset before 5 years of age have reduced neurocognitive function. This problem has been attributed to hypoglycemia, a complication of insulin therapy. The eye, kidney and nerve complications of diabetes have been reduced by intensified Insulin therapy which is associated with a 3 fold increase in severe hypoglycemia and therefore was not recommended for children less than 13 years of age. Since hyperglycemia is much more common than intermittent hypoglycemia during early childhood diabetes, it is important to determine if hyperglycemia affects brain growth and development. Rats were exposed to 4 weeks of either chronic hyperglycemia or intermittent (3 hours, 3 times/week) hypoglycemia from 4 to 8 weeks of age. The brains of these animals were compared to those of similarly aged normal control animals. Overall cell density, and the density of Map-2-positive neurons and S-100b-positive astrocytes in the entorhinal cortex and hippocampus of these animals were compared between groups. The number of cells was increased and the cell size reduced in the cortex of diabetic animals as assessed by DNA/wet weight of brain and protein/DNA content. Reduced amounts of protein, fatty acids, and cholesterol/µgram DNA also indicate smaller cells with reduced myelin content in the cortex of the diabetic animals. Histologic evaluation of these brains confirmed that there was an increase in the number of small neurons in these areas with a slight decrease in the number of astrocytes. These observations require further confirmation and evaluation, but indicate that chronic hyperglycemia may be more damaging than intermittent hypoglycemia to the developing brain.

Supported by a grant 1-2004-751 from the Juvenile Diabetes Research Foundation

Presented at the Society for Neuroscience NIDA Satellite Symposium "Frontiers in Addiction Research", Washington, DC, November, 2005

Neurofunctional Consequences of Developmental Exposure to Moderate Doses of Cannabinoids in a Rat Model.

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Even though marijuana is the most widely used illegal drug among women of reproductive age, reports dealing with the effects of prenatal exposure to this substance of abuse are still controversial. More complex and less understood is the scenario concerning the possible long-term consequences of in utero exposure to cannabis derivatives on cognitive functions.

In this study the synthetic CB1 agonist WIN 55,212-2 (WIN) was administered daily to pregnant rats from gestational day 5 to 20 (0.5mg/kg). This dose is equivalent to a moderate or low exposure to marijuana in humans and has no overt toxic effects. The treatment with WIN did not affect gestational and reproduction parameters and WIN-exposed pups did not show any sign of malformations or malnutrition. However, a deeper investigation revealed that prenatal treatment with WIN altered pup performance in homing behavior and produced a decrease in the rate of separation-induced ultrasonic vocalizations. Behavioral deficits that resulted were long-lasting, since prenatal WIN exposure caused a disruption of memory retention in young and adult offspring subjected either to a passive or an active avoidance task. Moreover, an altered dendritic morphology of hippocampal CA1 pyramids was detected in young (40 day-old) rats. In particular, in the prenatally WIN-exposed rats, there was a significant 12% increase in estimated total dendritic length and a highly significant increase in branching complexity in the middle-third of the dendritic tree. These findings suggest that moderate exposure to cannabinoids during crucial periods of brain development can cause dysmorphic maturation of the hippocampus. Such subtle morphological alterations and commensurate changes in brain circuitry would be, in turn, a factor underlying the behavioral deficits observed both in early and late postnatal life.

Presented at the meeting of the Neurobehavioral Society, St. Pete Beach, FL, June, 2005

This Is Your (Child's) Brain on Drugs: in utero Exposure to a Cannabinoid Agonist Affects Dendritic Morphology of Hippocampal CA1 Neurons in the Young Rat.

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Among women of reproductive age marijuana is the most widely used illegal drug. The impact of cannabis exposure on the developing brain is poorly understood. A specific cannabinoid receptor (CB1) is highly expressed in many brain regions, including the hippocampus. The synthetic CB1 agonist WIN 55,212-2 was administered daily to pregnant rats from gestational day 5 to 20 (0.5mg/kg). This dose is equivalent to a moderate or low exposure of marijuana in humans and has no overt toxic effects. The behavioral consequences in 40 day-old (do) rats included hyperactive behavior and memory impairment. To assess the effects of this prenatal exposure on dendritic morphology of CA1 pyramids of the hippocampus, fixed brain tissue from WIN-exposed 40 do offspring (N= 6) and age-matched controls (N=5) was Golgi stained. The basilar dendritic arbors of CA1s were quantified. In the prenatally WIN-exposed 40 do rats, there was a significant 12% increase in estimated total dendritic length and a highly significant increase in branching in the middle-third of the dendritic tree. This finding suggests that hippocampal circuitry was affected by the cannabinoid agonist; perhaps due to failure of normal developmental pruning back of the dendritic tree. Dymorphic maturation of the hippocampus would be a factor underlying behavioral and cognitive changes seen in the young 40 day-old rats.

Presented at the 7th International Conference on Alzheimer's and Parkinson's Diseases,
Sorrento, Italy, March, 2005

Cortical and Hippocampal Dendritic Spine Alterations in the Triple Transgenic Mouse Model of Alzheimer's Disease.

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The triple transgenic mouse model of Alzheimer's disease (3xTgAD) was generated by expressing three different mutant genes (APP, PS1 and tau) linked to inherited forms of dementia. 3xTgAD mice exhibit age-related amyloid and tau pathology which is associated with synaptic dysfunction and memory impairment. Memory impairment in AD correlates strongly with synaptic loss. In the present study we evaluated the density and configuration of dendritic spines in neocortex and hippocampus of male 3xTgAD mice. From coded slides, dendritic spines of Golgi stained neurons were evaluated on randomly selected layer II/III pyramids of the parietal neocortex and on the basilar tree of hippocampal CA1 pyramids. For 3xTgAD mice (12 months old, n=4) and nontransgenic controls (11.5 months old, n=5) spines were assessed for density and configuration, properties that are known to influence synaptic efficacy. In CA1 neurons, spines on the terminal tip segments of the basilar tree were quantified. Total spine density was reduced in the aged 3xTgAD mice. Most strongly affected were spines lacking well-defined spine heads. On neocortical layer II/III pyramids, spines were assessed throughout the dendritic arbor. Compared to controls, there was no significant total spine loss in the 3xTgAD mice. However, there was a significant increase in small-headed spines, a form of spine believed to be dysfunctional. These initial findings suggest that both hippocampal and cortical circuitry in the aged 3xTgAD mice are altered, but in different ways. In the hippocampus spine loss occurs, whereas in the parietal neocortex an increase in dysfunctional spines may occur.

Presented at the meeting of the Society for Neuroscience, San Diego,
November, 2004

Alterations in the Morphology of Dendrites in the Nucleus Accumbens and Frontal Cortex Following Repeated Neonatal Isolation Stress.

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Newborn rat pups isolated from the dam and littermates for 1 hr. per day (PN 2-9) display behavioral sensitization when tested as juveniles and adults and also have a hyper-responsive mesolimbic dopamine system to either a drug or stress challenge. We have analyzed fixed brain tissue from adult male rats using the rapid Golgi method in order to quantify changes in dendrites and synaptic spines. In addition, some animals were given memantine, an NMDA receptor antagonist, at the time of isolation to test whether blockade of NMDA receptors will attenuate stress-induced sensitized behavior and morphological changes. Memantine (10 mg/kg) was given by oral gavage at each animal's 1-hr isolation on each day from PN 2-9. Isolation-only (ISO), non-isolation only (NON-ISO) and memantine only (MEM) groups were included in the design. The drug was tolerated well and had no effect on body weight over time for any treatment group. Examination of Golgi-stained sections using the Sholl analysis reveals that, compared to NON-ISO controls, ISO animals had a 23% increase in the branching of dendrites in medium spiny neurons of the nucleus accumbens shell. There were no significant alterations in spine number or type, or in cell soma size. ISO-MEM and MEM animals had dendritic branching levels intermediate between the ISO and NON-ISO levels, consistent with behavioral data that indicated memantine could attenuate the effects of isolation, but had other effects of its own. In the layer II/III pyramidal cells of the frontal cortex, there were no differences between ISO and NON-ISO animals in dendritic branching, but both groups receiving memantine were significantly lower than controls. There was a trend to increase in spine number (14%) but it was not significant. However, soma size of pyramidal cells was smaller in isolated groups, regardless of memantine treatment.

Presented at the meeting of the International Society for
Developmental Neuroscience, Edinburgh, Scotland, August, 2004

Cortical and Hippocampal Dendritic Alterations in the ASMKO Mouse A Knockout Model of Niemann-Pick Disease, Type A.

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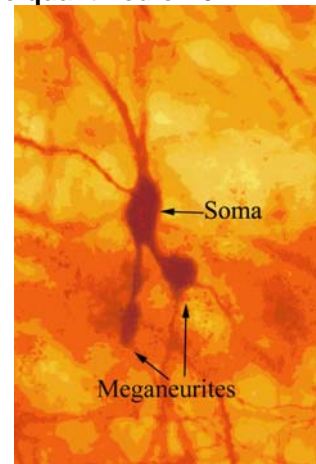
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Type A Niemann-Pick Disease (NP-A) is caused by deficiency of acid sphingomyelinase (ASM) which results in sphingomyelin accumulation. NP-A is a severe neurologic disease that begins in the first few months of life, is accompanied by progressive psychomotor retardation, and leads to death by 2 to 3 years of age. The NP-A knockout mouse model was created by targeted disruption of the gene encoding acid sphingomyelinase.

Formalin-fixed brains of 18 week-old ASMKO(-/-) mice (N=8) and normal littermate controls (N=8) were stained using the Rapid Golgi method. This reveals the soma, dendritic arbor, and spines of randomly stained neurons.

From coded slides dendritic branching, spines and soma size were quantified on 6 randomly selected neurons from parietal neocortex layer V pyramids, CA1 pyramids, and granule cells of the dentate gyrus of each brain. Grossly, nearly all CA1 pyramids of the hippocampus of the ASMKO mice were distinguished by the widespread appearance of "meganeurites" neuritic structures near the soma swollen by accumulation of sphingomyelin.

Results. Parietal Layer V Pyramids: In KO mice, dendritic length was significantly reduced (-19% ; $p = 0.01$) but without significant dendritic spine loss (-7%, NS); soma size was significantly increased (+15%, $p=0.02$). **Granule cells of the Dentate Gyrus:** branching was reduced by 14% ($p=0.03$), spines were reduced by 9% ($p=0.02$), soma size was unaffected. **CA1 Pyramids:** the distribution of dendritic branching was not significantly reduced; there was a significant increase in spines (+10%, $p=0.02$), and a 25% increase in soma size ($p=0.0001$).



Conclusion: In ASMKO mice Layer V pyramids and granule cells showed evidence of damage; however, despite the widespread presence of large dysmorphic meganeurites on all CA1 pyramids, these neurons showed no loss of dendritic arbor and, indeed, an increase in spines. This suggests that meganeurite formation in ASMKO mice may sequester accumulated sphingomyelin in a less toxic format and, therefore, meganeurites may have some neuroprotective function.

Presented at the meeting of the International Behavioral Neuroscience Society, Key West, Florida, June, 2004

Abnormally Excessive Cortical Dendritic Branching in a Knockout Mouse Model of the Fragile X Mental Retardation Syndrome.

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Fragile X syndrome is the most common cause of inherited mental retardation and is caused by a mutation in the FMR1 gene leading to absence of the fragile X mental retardation protein (FMRP). Knockout of the gene associated with Fragile X, *Fmr1*, results in mice with abnormalities analogous to human symptoms. Neuroanatomical findings include an excess of abnormally thin, immature dendritic spines. FMRP may play a role in the normal process of dendritic spine growth and pruning of excess immature synapses. The impact of *Fmr1* deletion on development of dendritic branching is poorly understood and is the focus of this study. Using Golgi impregnated neurons, we evaluated dendritic arborization of layer II/III pyramids of the parietal cortex of 10 week-old fragile X mice. Coronal blocks of the parietal cortex from *fmr-1* knockout mice and WT controls (FVB/NJ) were stained using the Rapid Golgi method (N=5 per group). From coded slides, 5-6 layer II/III pyramidal neurons were randomly selected. Camera lucida drawings of the basilar tree were made of each neuron. Sholl analysis showed that the Fragile X mice had significantly more dendritic material throughout the extent of the basilar tree ($p = 0.0002$, Wilcoxon test). Estimated total dendritic length was 28% longer ($p=0.001$, unpaired T test) and branching complexity was greater. These findings suggest that Fragile X mice may have abnormalities of developmental pruning of both branching and spines which would result in anomalous processing of information in cortical circuits. This would contribute to the cognitive dysfunction and behavioral problems associated with Fragile X syndrome.

Presented at the annual meeting of the Society for Neuroscience, New Orleans, November, 2003

ANTEROGRADE AMNESIA IN CARDIAC ARREST SURVIVORS: A RODENT MODEL

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Progress in medical technology has led to an increased number of cardiac arrest survivors resulting in a population of patients with behavioral and physiological impairments. The hippocampus is particularly sensitive to arrest-induced global hypoxia and clinical evidence suggests that cardiac arrest and cardiopulmonary resuscitation (CA/CPR) survivors report memory deficits. The current study used the Morris water maze to characterize the learning and memory deficits elicited by 8 minutes of cardiac arrest in a mouse model. The Morris water maze has been widely used as a test of spatial discrimination and place navigation in rodents, and is sensitive to alterations in the function of the hippocampal formation. Over the 8 days of pre-surgical water maze training, all animals showed a reduction in the mean distance traveled and latency to reach the hidden platform, indicating a mean improvement in performance. Following CA/CPR, mice were allowed to recover for 7 days. CA/CPR and SHAM animals performed the same on probe trials, indicating that the animals were able to identify the spatial location that previously contained the hidden platform. There also were no post-surgical differences observed in the visible platform trials, indicating both groups were motivated to find the platform. CA/CPR animals exhibited a performance decrement during the reversal training (when platform is moved to a new location), suggesting that CA/CPR may have impaired their ability to learn new spatial tasks. Ultimately, a better understanding of the behavioral deficits that result from CA/CPR and the dendritic alterations that underlie these changes may provide insight into the memory deficits reported by CA/CPR survivors. *Support Contributed By: NIH NS40267*

Published in Journal of Neuroscience Research 73: 1-9 (2003)

Insulin-like Growth Factor 1 Is Essential for Normal Dendritic Growth

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This study evaluated somatic and dendritic growth of neurons in the frontoparietal cortex of Igf1^{-/-} brains. Pyramidal neuron density was increased by approximately 25% (P =.005) and soma size reduced by approximately 10% (P <.001). Golgi staining revealed that cortical layer II-III neurons exhibited a significant reduction in dendritic length and complexity in Igf1 null mice. Dendritic spine density and presumably synaptic contacts were reduced by 16% (P =.002). Similar findings were obtained for cortical layer V and piriform cortex pyramids. Supporting a reduction in synapses, synaptotagmin levels were reduced by 30% (P <.02) in the Igf1 null brain. Investigation of factors critically involved in dendritic growth and synaptogenesis showed an approximately 50% reduction in cortical CDC42 protein expression (P <.001) and an approximately 10% reduction in brain cholesterol levels (P <.01) in Igf1 null mice. Evidence is presented that Igf1 deletion causes disruptions in lipid and microtubule metabolism, leading to impaired neuronal somatic and dendritic growth. Published 2003 Wiley-Liss, Inc.

Presented at the meeting of the Society for Neuroscience
New Orleans, November, 2003

EFFECT OF PILOCARPINE-INDUCED TEMPORAL LOBE EPILEPSY ON DENDRITIC MORPHOLOGY IN THE ENTORHINAL CORTEX OF ADULT RATS: A QUANTITATIVE GOLGI ANALYSIS.

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Many clinical and neuropathological features of human temporal lobe epilepsy (TLE) are reproduced using the lithium-pilocarpine (Li-Pilo) rat model. Status epilepticus is followed by a "silent" seizure free stage (14-25 days) and then by life-long spontaneous recurrent seizures (SRS) (2-3/weeks). This TLE model results in necrosis-related neuronal damage to the hippocampus, neocortex, and piriform and entorhinal cortices. We evaluated the effects of TLE on dendritic morphology of stellate cells in the entorhinal cortex.

METHODS: Adult S-D male rats were administered lithium and pilocarpine or saline (controls). Subjects were sacrificed about 2 months after the appearance of the SRS. Coronal and horizontal blocks encompassing the entorhinal cortex were stained using the Rapid Golgi method. From coded slides, camera lucida drawings of dendritic arbors of randomly selected layer II stellate cells were drawn and Sholl analysis was used to assess the amount and distribution of dendritic material. Dendritic spine number and configuration were also evaluated on terminal tip segments. **RESULTS:** Sholl analysis showed that the stellate cells from the Li-Pilo rats had significantly less dendritic material ($p < 0.0001$, Wilcoxon test). Estimated total dendritic length was reduced by 18%. TLE also significantly reduced dendritic spine density by 29% ($p = 0.0002$, Mann-Whitney test). All spine types were compromised. **CONCLUSIONS:** These dendritic changes would compromise entorhinal circuitry, alter normal signalling patterns to the dentate gyrus, and could contribute to epileptogenesis. TLE damage to stellate cells may thus be intimately involved in the widespread epileptic activity seen in this model. (Supported in part by INSERM)

Presented at the 6th International Conference on Neuroprotective Agents
Hilton Head, September, 2002

The Ultra Low Weight Glycosaminoglycan, C3, Protects Against Loss of Dendritic Branching and Spines of Pyramidal Cells Following Cortical Damage in the Rat

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Introduction and Methods

The group of glycosaminoglycans (or GAGs) is composed of heparin, heparan sulfate, dermatan sulfate, and chondroitin sulfate, and their analogs, including a novel GAG, C3. C3 is an ultra-low molecular weight analog of heparin produced by fractionation of heparin via γ -irradiation, followed by gel filtration. It is comprised mainly of 6-10 oligosaccharides, with a molecular weight of 2.1 kD, and a USP value of 12 U/mg. C3 can cross the blood brain barrier, and in previous studies has been shown to have neurotrophic properties resulting in an increase in dendritic branching and spines in young adult rats. Other investigations have indicated that it may be beneficial in neurodegenerative disorders such as Alzheimer's disease.

As part of a larger series of studies investigating C3, one group of adult rats had previously been subjected to saline injections into their lateral ventricles (ICV), with the needle cannulae inserted bilaterally through the frontal cortices (saline group, N = 5). Another group of rats, which also had an ICV saline injection, had been given C3 orally (once daily, by gavage, 25mg/kg) beginning one week prior to the ICV saline and continuing for one week following saline administration (C3 group, N = 4). Total treatment time for C3 was thus 14 days.

Initial observations of Golgi-stained neurons from the region adjacent to the needle track showed damaged cortical neurons as assessed by dendritic atrophy and spine loss. In view of findings from earlier studies suggesting the neurotrophic potential of C3, we decided to assess whether oral C3 treatment may have a neuroprotective role against the cortical stab wound-like damage caused by the needle inserted into the ventricle.

Dendritic branching and spines were quantitatively evaluated from Golgi stained tissue. As dendritic material of the cortical neuron comprises over 95% of its volume and the vast majority of synapses occur on spines, these dendritic parameters mirror the integrity and sophistication of cortical circuitry as well as the general health of the neurons.

Following 14 days of C3 treatment or saline, the rats were euthanized, their brains removed and immersion fixed in 4% paraformaldehyde for subsequent Golgi staining. 3mm coronal blocks of frontal cortex encompassing the needle cannula track were stained using the Rapid Golgi method and all slides were coded for neuronal analysis by an observer blind to treatment. In addition to the layer V pyramidal neurons selected adjacent to the needle track from the C3 treated or untreated animals, a third group of layer V pyramids were evaluated from saline treated subjects (N = 5): randomly selected neurons located in the same region of frontal cortex but distal from the needle track. (and hence, undamaged). These neurons represented a baseline control by which to compare with the damaged neurons.

For assessment of dendritic branching, camera lucida drawings were made of the basilar tree of randomly selected, well-stained layer V pyramidal cells (n = 5 per subject). The basilar trees were evaluated for extent and distribution of their arbors by the Sholl method of concentric circles. This also provides an estimate of total dendritic length. Dendritic spines were counted along 30 micron segments from approximately 4 terminal tips of the basilar dendritic branches of each neuron (5 neurons per subject).

Results and Conclusions

Compared to the (undamaged) layer V pyramids distal from the needle track, e.g., baseline controls, neurons adjacent to the needle track from the untreated (saline) subjects showed a significant loss of both dendritic material (-15%, Sholl analysis: $p < 0.001$, Wilcoxon) and dendritic spines (-21.4%, $p < 0.0001$, Mann-Whitney). This indicated that the needle cortical stab wound resulted in significant damage to these neurons. By contrast, in comparison to the damaged neurons from the saline-treated subjects, the neurons from

rats given C3 orally (for 7 days prior to, and 7 days after, the cortical stab wound) had significantly more dendritic material ($p < 0.001$, Wilcoxon) and 26% more spines ($p < 0.0001$, Mann-Whitney). Combining branching and spine data together (a so-called Circuitry Index reflecting estimated total synaptic connections), the cortical stab wound-induced neurotrauma resulted in a 33% reduction in circuitry in nearby neurons compared to neurons distal from the wound ($p = 0.0079$, Mann-Whitney). The C3 treatment protected neurons adjacent to the stab wound from this damage, the Circuitry Index now being the same as the baseline neuronal values ($p = 0.9$, N.S.).

In conclusion, C3 treatment has clearly exhibited a strong neuroprotective role maintaining normal cortical circuitry for neurons that would otherwise show significant neurotrauma-related injury. Further studies will be required to determine if C3 achieved this result primarily by means of minimizing initial damage and/or by enhancing neuroplastic recovery of the neurons from the cortical stab wound injury.

(Supported in part by NIA STTR Grant # 1-R41-AG15740-2 awarded to RFM and IH)

Presented at the meeting of the Society for Neuroscience,
Orlando, FL, November, 2002

Rapid Nonhuman Primate Model for NeuroAIDS: Evidence for Neuronal Damage.

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CNS dysfunction induced by HIV infection, known as neuroAIDS, continues to be a significant problem in the AIDS pandemic. We have obtained a reproducible model of neuroAIDS in rhesus macaques, utilizing a CD8-depletion regimen at the time of SIV inoculation. Most of the monkeys show cognitive, motor, neurophysiological, and neuropathological abnormalities within four months after infection. To more fully characterize the neuronal changes accompanying the CNS disease, we utilized Golgi-impregnation staining to evaluate pathological alterations in dendritic branching and spines from multiple brain regions. Randomly selected pyramidal neurons located in layers II/III and V in the Anterior Cingulate Gyrus (ACG), the Middle Frontal Gyrus (MFG), the Superior Temporal Gyrus (STG) and in CA1 hippocampus were evaluated (3 subjects/group; 6 neurons/region/subject). From coded slides, camera lucida drawings were made of the basilar pyramidal trees. Sholl analysis was used to quantify the extent and distribution of neuronal dendritic arbors. Dendritic spines were counted along 30 micra lengths of terminal dendritic tip segments. In the SIV-infected monkeys the presence of damaged neurons was often in spotty patches and was characterized by dendritic varicosities, spine loss, and dendritic branch atrophy. Most areas showed significant loss of branching and/or spines with an average reduction of neural circuitry throughout each of these brain regions of almost 17%. These findings validate this reproducible nonhuman primate model for neuroAIDS. (Supported by NIH grants MH59468, MH62261, MH61692 and DA12444)

Presented at the meeting of the Society for Neuroscience,
Orlando, FL, November, 2002

IGF1 IS ESSENTIAL FOR NORMAL DENDRITIC GROWTH

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IGF1 gene deletion results in reduced brain growth and mental retardation. We have previously shown that brain glucose utilization is profoundly impaired during early postnatal development in the *IGF1* null mouse. Given that IGF1 is normally selectively expressed by projection neurons that grow the most extensive dendritic arbors, we hypothesized that absence of IGF1 would result in attenuation of dendritic growth and synaptogenesis. Sholl analysis of the basilar trees of Golgi stained fronto-parietal cortical layer II-III pyramids in *IGF1*^{-/-} and wild-type (WT) 50 day-old mice shows that *IGF1*^{-/-} neurons have significant deficits in both dendritic length and complexity. *IGF1*^{-/-} neurons have fewer dendritic intersections, particularly at increasing distances away from the soma ($P=0.0002$) and fewer branch points at each branch order measured indicating reduced branching complexity. *IGF1*^{-/-} dendrites are ~ 25% shorter than WT ($P<0.001$). Dendritic spines are reduced by 16% ($P = 0.002$) and synaptotagmin levels are reduced by 30% ($P = 0.01$) in the *IGF1*^{-/-} brain, suggesting decreased synaptogenesis. Similar findings were obtained in cortical layer V and in the piriform cortex. Cholesterol and phospholipid are each reduced by ~10% in *IGF1*^{-/-} brains ($P<0.01$ and $P<0.05$ respectively), suggesting impaired lipogenesis in the *IGF1*^{-/-} brain. These data show that IGF1 is essential for normal dendritic growth and synaptogenesis, probably through its insulin-like, anabolic effects on glucose utilization and lipogenesis.

Presented at the meeting of the Society for Neuroscience, Orlando, FL, November, 2002

NEUROPATHOLOGY OF THE NIEMANN PICK A (ASMKO) MOUSE

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This study describes the nature, distribution and progression of neuropathology in the Niemann Pick Type A (NP-A) mouse that was created by targeted disruption of the gene encoding acid sphingomyelinase (ASM). ASMKO (-/-) and normal littermate (+/+) animals from 4 to 28 weeks of age were prepared by fixation perfusion and in most cases the brains were processed for paraffin histology. In H&E stained specimens, lysosomal accumulation was readily apparent in the cytoplasm of ASMKO animals, particularly in larger neurons throughout the brain and spinal cord. Sensory neurons within dorsal root

ganglion were also severely affected with massive lysosomal accumulation giving the cells a foamy appearance classically associated with visceral NP-A pathology. Mild lysosomal accumulation was present as early as six weeks and progressively worsened with age. Brain regions most severely affected included pyramidal cells in the cortex (layers II, III, V) and hippocampus (CA1), most subnuclei of the thalamus, and magnocellular brainstem structures such as the trigeminal nucleus. Despite advanced disease in older animals, the only region observed to undergo neurodegeneration was an almost complete depletion of Purkinje cells from the cerebellum (see poster by Stewart et al). Silver impregnation staining did reveal evidence for more widespread degenerating structures (see poster by Switzer). However, the use of retrograde tracers demonstrated that diseased neurons were able to efficiently transport dyes and that distant brain structures retained normal, intact connections (e.g. striatum to nigra). Further structural analysis using Golgi staining revealed relatively preserved fine neuronal morphology, although there was evidence for stripping of dendritic spines as well as meganeurite formation within some CA1 and cortical pyramidal neurons.

Presented at the meeting of the Society for Neuroscience,
Orlando, FL, November, 2002

**TETRACHLOROAZOBENZENE (TCAB)-INDUCED DEVELOPMENTAL
NEUROTOXICITY IN CORTEX AND CEREBELLUM IN THE RAT:
A Quantitative Morphometric Golgi Analysis.**

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3,3',4,4'-tetrachloroazobenzene (TCAB) is a dioxin-like organochlorine compound formed as a byproduct in the manufacture of herbicides. It impairs thyroid function, and thus controls critical aspects of brain development. The potential neurotoxic consequences of TCAB exposure need to be determined. TCAB was administered by gavage to SD female rats starting two weeks before pregnancy until the pups were weaned. TCAB dose levels were 0.1 mg/kg/day (low dose) or 3.0 mg/kg/day (high dose). Controls received corn oil. Male pups were sacrificed when 22 days old. Neocortex was stained using the Rapid Golgi method and cerebella, using the Golgi-Cox variant. Using coded slides, from each subject 6 randomly selected layer V pyramids and 6 Purkinje cells (PCs) were evaluated. Analysis of dendritic arbor of the V pyramids by Sholl analysis showed that control arbor > low dose TCAB > hi dose TCAB. Significant dendritic spine loss was seen only in the high dose TCAB. The Neuronal Circuitry Index (branching/spine index) showed that low dose TCAB reduced pyramidal cell circuitry by 18%, and high dose TCAB exposure, by 32%. In cerebellum, PC dendritic area was significantly reduced only in the high dose TCAB group. However, both low dose and high dose groups had small ectopic dysmorphic PC-like neurons located in the molecular layer. The results suggest that the impaired development of neocortical and cerebellar neurons may be mediated by thyroid dysfunction due to the TCAB exposure.

Presented at the meeting of the Society for Neuroscience,
Orlando, FL, November, 2002

**DEVELOPMENTAL EXPOSURE TO HEXACHLOROBENZENE (HCB)
IN THE RAT RESULTS IN ECTOPIC AND DYSMORPHIC
CEREBELLAR PURKINJE CELLS**

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Hexachlorobenzene (HCB) is a dioxin-like organochlorine compound that binds to the aryl hydrocarbon receptor (Ah). HCB may impair thyroid metabolism. HCB is widespread in the environment and accumulates in biological systems. It is transplacentally transferred from mother to fetus during pregnancy and through breast milk. Neurotoxic consequences of HCB exposure on the developing brain are, however, poorly documented. HCB (2.5 mg/kg/day) was administered by gavage to Sprague-Dawley female rats starting two weeks before pregnancy until the pups were weaned. Controls received corn oil. Pups were sacrificed when 22 days-old and cerebella were stained using the Golgi-Cox method. There were seven male subjects per group. Using coded slides, from each subject seven randomly selected Purkinje cells (PCs) were evaluated for soma size and areal extent of their dendritic arbor. There were no differences between groups for PCs that had correctly migrated and were located in the Purkinje cell layer. However, there were also a significant number of neurons with Purkinje cell-like characteristics that had improperly migrated into the molecular cell layer. These highly dysmorphic PCs had dendritic areas 90% smaller than normal, and somas which were 75% smaller than normal. Since thyroid function influences critical aspects of cerebellar development, the abnormal migration of PCs and their dysmorphic development may reflect the neurotoxic impact of HCB exposure on thyroid function.

Presented at the meeting of the Endocrinology Society
San Francisco, CA, June, 2002

Endogenous Brain IGF1 is Essential for Normal Dendritic Growth.

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IGF1 deficiency results in reduced postnatal brain development in both human and animal models. Our previous studies on IGF1 gene deleted mouse brain (IGF1^{-/-}) have shown that despite the reduction in brain size, the IGF1^{-/-} brain anatomy and cell numbers are normal for the most part. Given that the dendritic neuropil occupies a very large percentage of the brain parenchyma, the attenuation in IGF1^{-/-} brain size may result from the deficit in the growth of dendritic processes in the absence of endogenous brain IGF1. To test this

hypothesis, we used Golgi histological staining and biochemical analysis to evaluate the dendritic growth and complexity of cortical neurons in the IGF1^{-/-} and wild-type (WT) brains of 50 day-old mice. Morphometric analysis of fronto-parietal cortical pyramidal layer II-III neurons shows that the soma size of IGF1^{-/-} neuron is reduced by 10% (P<0.001), and that the cell density is significantly higher by 25% compared to that of WT (P=0.005). Analysis of camera lucida drawing of the basilar trees of the Golgi stained II/III pyramids shows that IGF1^{-/-} neurons have less dendritic material than WT s as reflected by deficits in both dendritic length and complexity. Sholl analysis of the basilar trees reveals that IGF1^{-/-} neurons have fewer dendritic intersections with the measuring concentric circles, particularly at the increasing distances away from the soma (P=0.0002). Branch point analysis shows that IGF1^{-/-} neurons have fewer branch points at each branch order measured indicating less branching complexity. IGF1^{-/-} neurons have an average of 25% shorter dendrites than WT s (P<0.001). Supporting this finding, brain total lipid, the major substance for constructing dendrites, is decreased by 29% in the IGF1^{-/-} brain homogenates (P=0.059). Cholesterol and phospholipid, the two primary components of the brain lipid, are reduced by 9% in IGF1^{-/-} brains individually (P<0.01 for cholesterol and P<0.05 for phospholipid). Further investigation of the enzymes involved in neuronal morphogenesis demonstrates that the cdc42, a member belonging to the Rho GTPase family, is reduced in IGF1^{-/-} brains by 30% compared to WT s (P<0.005). Rac1 and RhoA, the other two members in the Rho family, show no change in IGF1^{-/-} brains. Our results suggest that the normal growth of dendritic processes is significantly impaired in the absence of endogenous brain IGF1 and that the cdc 42 is one of the major modulators for the action IGF1 in regulating brain dendritic development.

Presented at the forthcoming meeting of the International Society for the Study of Fatty Acids & Lipids, Montreal, May, 2002

ALTERATIONS IN BEHAVIOR AND NEUROANATOMY IN DHA DEFICIENT RATS

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If docosahexaenoic acid (DHA) is essential for optimal nervous system function, then diets that lead to a loss in brain DHA would be expected to result in functional deficits. Brain function is usually assessed using behavioral tasks of varying complexity. In this work, rats were given diets with only safflower oil as their source of essential fats (n-3 Def group) or safflower oil plus sources of alpha-linolenate and DHA (n-3 Adq group) for two generations. Male pups (behavior) and female pups (anatomy) were weaned to the same diets that their mothers consumed and then tested at various ages. In spatial tasks, animals in the n-3 Def group acquired the Morris water maze more slowly and performed poorly on the retention trial. In a go, no-go olfactory discrimination task, n-3 Def rats again acquired the task more slowly and failed to acquire a learning set. Anatomical studies indicated smaller neuronal areas of cell bodies in the hippocampus as well as other brain areas. Golgi staining and Sholl analysis indicated that granule cells in the dentate gyrus had significantly less dendritic arborization in the n-3 Def animals at both 21 and 68 days of age than the n-3 Adq group. A similar loss of dendritic arborization was also observed in cortical pyramidal neurons in 21-day old n-3 Def rats, however, this difference was no

longer found in 68 d old animals apparently due to greater dendritic pruning in the n-3 Adq brains. Persistent alterations in hippocampal circuitry in the n-3 Def animals may be the neurostructural basis for the observed behavioral deficits.

Presented at the Seventh International Geneva/Springfield
Symposium on Advances in Alzheimer Therapy, April, 2002

GLYCOSAMINOGLYCANS AS A POTENTIAL THERAPY FOR ALZHEIMER'S DISEASE

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Over the past decade a mixture of glycosaminoglycans (GAGs) has been shown to improve cognition in aged rats and to reverse cognitive and neurochemical deficits in patients with Alzheimer's disease (AD). This mixture of GAGs (called GAP or GAG-polysulfate), which is composed of heparin (10-20%), heparan sulfate (40-60%), dermatan sulfate (20-35%) and chondroitin sulfate (2-8%), has earned a use-patent in several European countries. The effects mentioned above may be attributable to the ability of GAGs to compete with endogenous proteoglycans (PG), such as heparan sulfate PG, and to prevent their tendency to: a) induce amyloid formation via an increase in aggregation rate of β amyloid (Ab) into β -pleated fibrils and b) increase polymerization of tau into paired helical proteins, which make up the neurofibrillary tangles of AD. During the past three years we have been working to identify the active component(s) of the GAP mixture, by fractionating each of the components of GAP into smaller molecular weight fractions, and testing their respective protective activity on several animal models of brain neuron degeneration.

C3 is an ultra-low molecular weight analog of heparin produced by fractionation of heparin via γ -irradiation, followed by gel filtration. It is comprised mainly of 4-10 oligosaccharides, with an apparent molecular weight of 2.1 kD, and a USP value of 12 U/mg. The fractionation procedure has been standardized; thus we can manufacture a reproducible product in large quantities (kgs). This substance has been found to be the most active of several products obtained via fractionation of the above-mentioned components of GAP).

To date we have observed, using C3, that: a) it can cross the blood brain barrier (BBB); b) it prevents tau-2 immunoreactivity and reactive astrocytosis in both the AF64A, and the Ab(25-35) treated rat; c) its protective effect is obtained following either oral or subcutaneous administration of C3; d) it has neurotrophic effects in the brain, in vivo, as measured by Golgi impregnation technology; e) it is devoid of significant anticoagulant activity; and f) it is safe at high doses.

Thus, C3 is a promising candidate for further development and clinical testing in patients with AD and other central neurodegenerative disease states. It will soon be employed in Phase I clinical studies.

(Supported in part by NIA STTR Grant # 1-R41-AG15740)

Presented at the meeting of the Society of Toxicology,
Nashville, April, 2002

**LONG-LASTING NEUROSTRUCTURAL CONSEQUENCES IN THE RAT
HIPPOCAMPUS BY DEVELOPMENTAL EXPOSURE TO A MIXTURE OF
POLYCHLORINATED BIPHENYLS (PCBs).**

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The objective of the study was to assess the effects of developmental exposure to a commercial mixture of PCBs (Aroclor 1254) on neuronal dendritic morphology of hippocampal CA1 pyramidal neurons in postnatal day (PND) 22 and PND 60 male Long-Evans rats. Rat pups were born to mothers who were exposed to Aroclor 1254 (AccuStandard Inc., Lot # 124-191; 0 and 6 mg/kg/day) from gestational day 6 through PND 21. Thus, pups were exposed to PCBs *in utero* and through weaning. Male rats (N = 5-6 per group) were sacrificed on PND 22 and PND 60. Brains were formalin-fixed for Rapid Golgi staining of tissue blocks; coded slides of hippocampus were prepared. For branching and spine analysis, 6 CA1 pyramids were randomly selected from each brain. Camera lucida drawings of the basilar dendritic tree were analyzed using the Sholl method of concentric circles. For spine analysis, counts were made along internal and terminal tip segments of 6-7 neurons from each brain. Results of the branching analysis for the PND 22 PCB-exposed rats showed that, compared to controls, there was significantly less dendritic branching in the outer 2/3rds of the dendritic tree ($p = 0.002$, Wilcoxon test). Spine analysis also showed a reduction in spines on the terminal tips segments of 22 day old PCB-exposed rats ($p=0.005$, T-test). These results suggest that perinatal exposure to Aroclor 1254 resulted in morphometric changes in hippocampus. By PND 60, spine density on terminal tip segments had returned to normal levels. However, branching analysis now showed that compared to controls there was an excessive amount of dendritic material in the distal 2/3rds of the tree ($p=0.001$, Wilcoxon test). This suggested a possible structural "hyperplasticity" in neurons damaged by PCB exposure during the developmental period with a residual long-term dysmorphic impact on hippocampal circuitry. (This abstract does not necessarily reflect USEPA policy).

Presented at the 2nd Annual Neurobiology of Aging Conference
San Diego, November, 2001

**Global Dendritic Neuroplasticity in the Young Adult Rat Brain
Following Administration of C3, an Ultra Low Molecular Weight
Glycosaminoglycan: Implications for Treatment of Alzheimer's Disease.**

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Glycosaminoglycans (GAGs) are linear polysaccharides consisting of a hexosamine, disacchride units, and sulfate substituents. Proteoglycans (PG), such as heparin sulfate PG, have been shown to facilitate the occurrence of classical neuropathological hallmarks of Alzheimer's disease (AD) such as amyloid formation and increased polymerization of tau protein into neurofibrillary tangles. GAGs compete with endogenous PGs to inhibit these occurrences. Therefore, it was thought that treatment with GAGs might attenuate the neuropathological changes associated with AD and would be a useful treatment strategy. We now show that C3 also exerts widespread neuroplastic effects on dendritic branching in young adult rats.

F344 rats (N=5) were administered C3 subcutaneously (s.c.) for 32 days (2.5 mg/kg s.c., b.i.d.). Controls were administered saline. Formalin fixed tissue blocks were Golgi-stained and coded slides prepared. Neurons were randomly selected from three areas for quantitative analysis of dendritic branching: layer V pyramids of frontoparietal cortex; CA1 pyramids of the hippocampus, and granule cells of the dentate gyrus. Camera lucida drawings were made from 6 neurons from each area and Sholl analysis (Method of Concentric Circles) was carried out to assess the amount of dendritic material and its distribution.

In all three populations there was evidence of dendritic neuroplasticity. This was predominately seen by increased arborization which was significant for CA1s ($p=0.001$), for layer V pyramids ($p=0.004$), and for the outer half of the arbor of granule cells of the dentate gyrus ($p=0.001$). Since the vast majority of synapses are on dendritic branches, it is feasible that GAG-mediated structural plasticity would also result in additional synaptic connections. Since Alzheimer's dementia is associated with synaptic loss, C3 treatment may be efficacious in maintaining brain circuitry and minimizing cognitive loss.

(Supported by NIH grant #1 R4 AG15740-01 to RFM, IH, and JML)

Presented at the meeting of the Society for Neuroscience, San Diego,
November, 2001

**DIETARY DEFICIENCY OF DOCOSAHEXAENOIC ACID (DHA) IMPAIRS NORMAL
DENDRITIC DEVELOPMENT IN RAT NEOCORTEX.**

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Docosahexaenoic acid (DHA), an n-3 fatty acid, is a critical structural component of neuronal and synaptic membranes. Since DHA is rapidly deposited during the period of normal brain development, reduced dietary intake of n-3 fats leads to lowered brain DHA that, in turn, may cause losses in brain function. Currently, infant formula in North America is devoid of DHA. Rats were made deficient in brain DHA (DEF group) by limiting n-3 dietary fats through 3 generations. Controls received an n-3 adequate diet (ADQ group) that contained flaxseed oil and DHASCO. Rats were sacrificed at 21 and 68 days of age (N=8/group) and fronto-parietal cortex was Golgi-stained. From coded slides, 40 layer V pyramids were randomly selected from each group and camera lucida drawings were

made of the basilar dendritic trees. Statistical analysis (Sholl Analysis, method of concentric circles) showed that in the 21 d group, there was 15% less dendritic material in the DHA DEF group compared to the ADQ group ($p = 0.0001$, Wilcoxin test). Between 21 to 68 days, dendritic pruning reduced the arbor of the ADQ control group by 25%, and of the DEF group by 13%. Thus, by 68 days the extent of dendritic branching in pyramid neurons from both groups was equivalent. Since DHA deficiency can result in long-term cognitive deficits, our findings suggest possible remaining neurostructural consequences that may result from n-3 deficiency.

Presented at the meeting of the Society for Neuroscience, San Diego
November, 2001

**DENDRITIC AND SOMA SIZE ALTERATIONS IN HIPPOCAMPUS AND CORTEX OF RATS
FOLLOWING NEONATAL BORNA VIRUS INFECTION**

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Altered neuronal morphology and cell losses are reported in postmortem brain of subjects with autism, including small neurons, decreased dendritic complexity in hippocampus, cerebellar Purkinje cell loss, and at least one report of cortical involvement. Neonatal infection of Lewis rats with Bornavirus, a neurotropic RNA virus, results in neuropathologic, behavioral, and neurochemical abnormalities resembling features of autism. Understanding the processes influencing synaptic dysfunction and selective neuronal losses by apoptosis in this animal model may reveal mechanisms important in neurodevelopmental disorders, including autism. Using Golgi-stained tissue, we evaluated changes in neuronal morphology in dentate gyrus and layer V of fronto-parietal cortex in neonatally infected rats. At 3 weeks postinfection, dendritic branching was decreased in dentate gyrus granule cells (Sholl analysis, $p=0.0004$) and in layer V pyramidal cells ($p=0.0006$). Distal dendrites were especially affected. The soma of the granule cells (but not of cortical neurons) were significantly smaller ($p=0.0043$). Other changes included branch atrophy, spine loss, and varicosities. These findings suggest a morphologic substrate underlying processes that disrupt synaptic plasticity or increase vulnerability to apoptosis during brain development, and support the use of this animal model for uncovering fundamental aspects of neurodevelopmental disorder pathogenesis. Supported by: NIH grants K08 MH01608 and RO1 HD37546

Presented at the Yale University School of Medicine Symposium on
Cognitive Enhancers, Anti-Alzheimer and Neuroprotective Drugs
New Haven, October, 2000

**Neurotrophic Effects of the Glycosaminoglycan C3 on Dendritic Arborization and Spines
in the Adult Rat Hippocampus: A Quantitative Golgi Study.**

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Glycosaminoglycans (GAGs) may be effective as a therapeutic strategy in the treatment of Alzheimer's Disease (AD). Previous work from this group using a rat model showed that a single intra-amygdaloid injection of A beta (25-35) could induce abnormal tau-immunoreactive perikarya in the hippocampus. Furthermore, administration of the GAG C3, an ultra low molecular weight heparin mixture of 4-10 oligosaccharides (MW~2.1) could decrease Abeta(25-35)-induced tau-2 immunoreactivity in this model of AD.

In this study, we evaluated the effects of stereotaxic intra-amygdaloid injection of A Beta ((25-35) (5nmol/3ul) and of C3-treatment (administered subcutaneously, s.c.) on dendritic morphology of Golgi-impregnated CA1 pyramids of the hippocampus. Subjects were young-adult, male F344 rats.

There were four groups:

- the controls were rats with intra-amygdaloid (i-a) injection of vehicle (trifluoroacetic acid, TFA, 3ul) followed by saline (32 days, s.c., 1ml/kg, b.i.d.)(N=10);
- treatment groups consisted of:
- i-a injection of Abeta (5nmol/3ul) followed by 32 days of saline (s.c., 1ml/kg, b.i.d.)(N=5); i-a injection of Abeta (5nmol/3ul) followed by GAG-treatment (32 days of C3, 2.5 mg/kg s.c., b.i.d.)(N=5); and i-a injection of vehicle (TFA, 3ul) followed by the C3 GAG (32 days, 2.5 mg/kg s.c., b.i.d.)(N=5).

Formalin-fixed coronal blocks of rat frontoparietal cortex, which included the underlying hippocampus, were stained using the Rapid Golgi method. Slides were sectioned at 120 μm. All brains were coded, and 6 well-impregnated CA1 neurons were randomly chosen from each subject from the hemisphere ipsilateral to the intra-amygdaloid injection of the Aβ(25-35) or the vehicle. For analysis of dendritic branching, camera lucida drawings were made of the basilar tree. The drawings were then quantified by the Sholl analysis (method of concentric circles) which provides a profile of the extent and distribution of the dendritic material. Dendritic spines were quantified on 4-5 randomly selected terminal tip segments from each neuron.

Results. Compared to the controls (TFA vehicle), dendritic branching of the CA1s was not diminished by Aβ; however, dendritic spine density was significantly attenuated ($p < 0.001$). GAG (C3) treatment in the Aβ-treated animals had only a minor effect on increasing dendritic branching ($p = 0.039$, Wilcoxon signed-rank test) and had no impact on spines. However, C3 GAG-treatment alone (e.g., in the TFA vehicle-injected controls) had a remarkable neurotrophic impact on both dendritic branching and spines. C3 treatment increased total dendritic length by 36% ($p < 0.001$) while increasing branching complexity ($p < 0.05$). Simultaneously, dendritic spine density on the terminal tips was increased by 17%. Combining the C3-induced neurotrophic changes in both branching and spines, it was determined that 32 days of C3-treatment resulted in an overall 61% increase in total synaptic contacts on the basilar tree of the CA1 pyramids in these young-adult rats

($p < 0.001$).

Effect of GAG (C3) Treatment on Dendritic Arbor
of Hippocampal CA1 Pyramidal Neurons (basilar tree)



These results indicate that the GAG C3 appears to have a remarkable neurotrophic impact on the hippocampal circuitry of the brain of the normal young-adult rat. The apparent inability of the GAG treatment to produce the same effect in the $A\beta$ -injected AD model may suggest that a more extended time frame (and/or a different dosage) is required for the damaged neurons to respond to C3 treatment. These and other related studies (including the assessment of the C3 treatment on neurons in aged rats) are in progress. In addition, while it is possible that C3 treatment could enhance learning or memory, it is also feasible that the extensive neurotrophic response in the young rat may be deleterious to learning (due to the "noise" induced by the presence of inappropriate synapses). This, too, is the subject of future study.

The present findings demonstrate a clear and dramatic effect of the GAG C3 on inducing an extensive neurotrophic effect on both dendritic branching and spines in the undamaged hippocampus of the adult rat. While awaiting the results of future and more targeted studies, they also suggest that such treatment may be useful in the treatment of AD or in attenuating age-related dendritic atrophy. The study also demonstrates the value of analysis of dendritic branching and spines from Golgi-impregnated tissue to demonstrate and quantify this structural neuroplasticity.

(Supported by HHS STTR grant # AG15740-01 awarded to RFM and IH)

Presented at the Society for Neuroscience, October, 2000, New Orleans

ATROPHIC DENDRITIC BRANCHING OF CORTICAL PYRAMIDAL NEURONS RESULTS
FROM INSULIN-LIKE GROWTH FACTOR 1 (*IGF1*) GENE DELETION:

A QUANTITATIVE GOLGI ASSESSMENT.

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Based on its high level expression in the developing brain and on the finding of mental retardation in the case of human IGF1 gene deletions, IGF1 is thought to play an important role in brain development. The murine *Igf1* (-/-) brain demonstrates preserved cell numbers in most structures, but a generalized reduction in neuropil suggesting a decrease in growth of neuronal processes and compromised circuitry. In this study, we quantified the extent of dendritic branching in cortical layer II-III pyramidal neurons in adult *Igf1* targeted gene deletion mice. Formalin-fixed blocks of frontoparietal cortex of 50 day-old *Igf1* (-/-) mice (N=7) and wild-type littermate controls (N=6) were stained using the Rapid Golgi method. From coded slides, five layer II/III pyramids were randomly selected from each subject. Camera lucida drawings were made of the basilar dendritic tree of each neuron and the dendritic arbor was assessed using the Sholl analysis and other quantitative means. Sholl analysis showed that the dendritic arbor of II/III pyramids from the *Igf1* (-/-) mice had significantly less dendritic material ($p=0.0002$). The estimated total dendritic length of the basilar tree was reduced by 24% in the knockout mice. Branch point analysis confirmed that these neurons also had a less complex dendritic arbor. Conclusions: This study conclusively shows significant dendritic atrophy of layer II/III pyramids in 50 day-old *Igf1* targeted gene deletion mice. Similar, albeit more moderate, dendritic atrophy was also seen in layer V pyramids of these knockout mice. These results support a critical role for IGF1 in the normal maturation of cortical neurons. (Supported by NICHD)

Presented at the Meeting of the Society for Neuroscience, 2000

STRIKING NEUROTROPHIC EFFECT OF CHRONIC GLYCOSAMINOGLYCAN (GAG) TREATMENT ON THE DENDRITIC ARBOR OF HIPPOCAMPAL CA1 PYRAMIDAL NEURONS IN ADULT F344 RATS: A QUANTITATIVE GOLGI ANALYSIS

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In order to test the efficacy of a novel therapeutic strategy in a animal model of Alzheimer's disease (AD), neurostructural assessment of the extent of the dendritic arbor was made of CA1 pyramids in adult rats in which amyloid- β (25-35) (or vehicle) had been stereotaxically injected directly into the amygdala. This was followed by chronic subcutaneous administration of the GAG compound. Previously, amyloid- β was found to result in trans-synaptic cytoskeletal pathology including tau2 immunoreactivity distally in cingulate cortex and hippocampus (Sigurdsson et al, 1996). In this study there were four groups of subjects: (1) Young-adult (3 mon-old) F344 rats (N=8) were given a single intra-amygdala injection of A β (25-35) (5nm/3ul) and killed 32 days post-operatively; (2) Vehicle-injected Controls (N=12); (3) A β + GAG-treated Ss (N=6), and (4) the s.c. GAG-treatment alone (N=5). Formalin-fixed tissue blocks were Golgi-stained and CA1 pyramids (6/brain) were randomly selected from coded slides. The basilar dendritic trees were analyzed using Sholl analysis. While intra-amygdaloid A β + chronic GAG treatment (32 days) resulted in a small increase (+6%) in dendritic arbor above control levels, chronic GAG treatment alone

resulted in a and highly significant increase (+32%) in the amount of dendritic branching in the hippocampal neurons in the young adult rats. These findings lend support the neurotrophic potential of of GAGs and suggests that it may be useful as a therapeutic strategy in AD. (Supported by NIH/SBIR Grant #1-R41-AG15740-01)

Published in the Journal of Neuroscience (2000)

HIPPOCAMPAL ABNORMALITIES AND ENHANCED EXCITABILITY IN A MURINE MODEL OF HUMAN LISSENCEPHALY

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Abstract

Human cortical heterotopia and neuronal migration disorders result in epilepsy; however, the precise mechanisms remain elusive. Here we demonstrate severe neuronal dysplasia and heterotopia throughout the granule cell and pyramidal cell layers of mice containing a heterozygous deletion of Lis1, a mouse model of human 17p13.3-linked lissencephaly. Birth-dating analysis using bromodeoxyuridine revealed that neurons are born at the appropriate time but fail in migration to form a defined cell layer. Heterotopic pyramidal neurons in Lis1^{+/-} mice were stunted and possessed fewer dendritic branches, whereas dentate granule cells were hypertrophic and formed spiny basilar dendrites from which the principal axon emerged. Both somatostatin- and parvalumin-containing-inhibitory neurons were heterotopic and displaced into both stratum radiatum and stratum lacunosum-moleculare. Mechanisms of synaptic transmission were severely disrupted, revealing hyperexcitability at Schaffer collateral-CA1 synapses and depression of mossy fiber-CA3 transmission. In addition, the dynamic range of frequency-dependent facilitation of Lis1^{+/-} mossy fiber transmission was less than that of wild type. Consequently, Lis1^{+/-} hippocampi are prone to interictal electrographic seizure activity in an elevated [K⁺]_o model of epilepsy. In Lis1^{+/-} hippocampus, intense interictal bursting was observed on elevation of extracellular potassium to 6.5mM, a condition that resulted in only minimal bursting in wild type. These anatomical and physiological hippocampal defects may provide a neuronal basis for seizures associated with lissencephaly.

Published in CNS Drug Reviews, 5, suppl 1 (1999)

QUANTITATIVE ASSESSMENT OF NEURONAL DAMAGE IN A TRANSGENIC MURINE MODEL OF ALZHEIMER S DISEASE

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Abstract

Golgi-stained preparations reveal the dendritic arbor and spines of randomly impregnated neurons in exquisite detail. This permits highly quantitative assessments of dendritic parameters (e.g., branching and spines) which reflect the health of the neurons and the extent of circuitry in that brain region.

The PD-APP transgenic (tg) mouse overexpresses human amyloid precursor protein (APP717Và F) and is an animal model of Alzheimer's Disease (AD). These mice develop A-beta plaques in an age-related manner, and show aberrant axonal sprouting. Although increased amyloid deposition has not been demonstrated to result in neuronal loss in these mice, injured neurons should show dendritic atrophy and related alterations. Using Golgi preparations we characterized dendritic changes in the hippocampus of aged tg PDAPP mice and non-transgenic (non-tg) wild-type controls.

Coronal blocks of formalin-fixed tissue encompassing the hippocampal formation from 26 month-old PD-APP mice and age-matched controls were stained using the Rapid Golgi method. From coded slides, CA1 pyramids (n=66) and granule cells (n=67) in the dentate gyrus were randomly selected for dendritic branching analysis. Camera lucida drawings were analyzed using the Sholl Method of Concentric Circles. This analysis produces a profile delineating the extent and distribution of the dendritic field. Dendritic spines along selected branch segments were quantified directly from the microscope at 1,008x.

Results:

- **CA1 Pyramids (basilar tree) Branching:** The basilar tree of the CA1 pyramids from the PD-APP mice showed significant widespread atrophic changes throughout the entire arbor characterized by both loss of dendritic material and simplification of branching. **Spines:** There was a 30% loss of total estimated spines on the basilar tree.
- **Granule Cells -- Branching:** The dendritic arbor of the granule cells showed significant atrophic changes specifically restricted only to the outer 2/3rds of the molecular layer. This portion of the dendritic tree receives afferent input from the entorhinal cortex, an area prominently affected in AD. **Spines:** There was a 45% loss of total estimated spines on the distal outer 1/3rd of the granule cells in the molecular layer. Middle and inner molecular layer segments showed no spine loss.

Summary:

Our findings are in general agreement with previous studies of hippocampal dendritic atrophy in AD brains. Moreover, since these hippocampal neurons receive afferent innervation from the entorhinal cortex and this latter region is widely damaged in AD, our results suggest that amyloid deposition and partial deafferentiation of entorhinal input may both contribute to hippocampal damage with concomitant disruption of brain circuitry. These findings support the use of these PD-APP mice which overexpress amyloid as an animal model of AD. It also serves as validation to the efficacy and sensitivity of quantitative Golgi studies for assessing the extent of neuronal damage and, subsequently, for testing the efficacy of various treatment strategies.

Presented at the Society for Neuroscience, Miami Beach, October, 1999

**HIPPOCAMPAL DENDRITIC ATROPHY IN AGING PD-APP TRANSGENIC MICE
OVEREXPRESSING HUMAN AMYLOID PRECURSOR PROTEIN:
A quantitative Golgi analysis of ca1 pyramids and dentate granule cells.**

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I. Lieberburg², D. Schenk², K. Kahn² and D. Games².

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Abstract

PD-APP transgenic (tg) mice overexpress human amyloid precursor protein (hAPP) V717F, develop A-beta plaques, and mirror other Alzheimer-like pathology. Here we further characterize neurostructural changes in the hippocampus of these mice. Coronal blocks of formalin-fixed tissue encompassing the hippocampal formation from aged (26 months) PD-APP mice and age-matched controls were stained using the Rapid Golgi method. CA1 pyramids (n=66) and granule cells (n=66) in the dentate gyrus were randomly selected from coded slides. Camera lucida drawings were made of their dendritic arbors and analyzed using the Sholl Method. The basilar tree of the CA1 pyramids from the PD-APP mice showed significant atrophic changes including loss of dendritic material and simplification of branching. By contrast, the dendritic arbor of the granule cells showed atrophic changes only in the outer 2/3rds of the molecular layer, e.g., that dendritic region receiving input from the entorhinal cortex, an area severely affected in AD. These findings support the validity of this animal model of AD and provide an anatomical substrate for the cognitive impairment described in these mice. (Supported by Elan Pharmaceuticals)

Presented at the Society for Neuroscience, Miami Beach, October, 1999

**GLYCOSAMINOGLYCANS REVERSE THE DENDRITIC ATROPHY OF CINGULATE
PYRAMIDS FOLLOWING INTRA-AMYGDALOID INJECTION OF AMYLOID-b IN ADULT F344
RATS: A Quantitative Golgi Analysis**

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Abstract

A potential animal model of Alzheimer's disease (AD) was assessed, based on the neurotoxic effects of amyloid-b (25-35) stereotaxically injected directly into the amygdala. Previously, this was found (Sigurdsson et al, 1996) to result in progressive trans-synaptic cytoskeletal and astroglial reactions, including tau2 immunoreactivity distally in cingulate cortex and hippocampus. The present study reveals additional information regarding neurostructural damage of cingulate neurons in this model. Young-adult (3 months) F344 rats (N=8) were given a single intra-amygdala injection of Ab (25-35) (5nm/3µl) and killed 32 days post-operatively. Controls (N=7) were vehicle-injected. Formalin-fixed fronto-parietal tissue blocks were Golgi-stained. Layer V cingulate neurons (6/brain) were randomly selected from coded slides. Camera lucida drawings of the basilar tree were analyzed using Sholl analysis. Results revealed small, but consistent and statistically significant

dendritic atrophy in Ab injected rats (Wilcoxin test, $p < .05$). The findings lend additional validity to this animal model of AD and suggest that it may be useful in the evaluation of therapeutic strategies.

(Supported by NIH/SBIR Grant #1-R41-AG15740-01)

Presented at the Meeting of the Society for Neuroscience, Miami Beach, October, 1999

**HIPPOCAMPAL DISORGANIZATION AND DENDRITIC ATROPHY FOLLOWING ABNORMAL NEURONAL MIGRATION IN LIS1-DEFICIENT MICE:
a quantitative analysis of golgi-stained tissue.**

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Abstract

Human Type I lissencephaly is a severe neuronal migration disorder that results from mutation or deletion of the gene encoding the b subunit of platelet-activating factor acetylhydrolase (*Lis1*). Mice with a heterozygous deletion of *Lis1* show abnormal neuronal migration. In the hippocampus of *Lis1*^{+/-} mice both normally located, and a "stranded" subgroup of CA1 pyramidal neurons are found. We characterized dendritic parameters of both populations of CA1 neurons. Formalin-fixed coronal blocks from mutants (N=4) and wild type (WT) controls (N=7) were Golgi stained. From coded slides, normally located CA1 pyramids (in the str. pyramidale) and stranded CA1s were randomly selected for analysis. Camera lucida drawings were quantified. Dendritic analysis showed that normally located CA1s in both the KO mice and WT controls were not structurally different. However, stranded CA1s were significantly atrophic and had far more simplistic dendritic arbors. Nevertheless, spine density on these stranded CA1 neurons was normal. Such structural abnormalities may explain in part the reduced electrographic seizure threshold observed in *Lis1*^{+/-} mice. (Supported by NIH.)

Presented at the meeting of the Neurotrauma Society, Miami Beach, October, 1999

**NEOCORTICAL NEUROPROTECTION BY BONE MORPHOGENIC PROTEIN-7 FOLLOWING MIDDLE CEREBRAL ARTERY OCCLUSION IN THE RAT:
A Novel and Sensitive Histopathological Assessment using Golgi-Stained Tissue.**

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Abstract

Bone Morphogenic Protein-7 (BMP-7) a.k.a Osteogenic protein-1 (OP-1) has previously been shown to enhance recovery of function when administered 24 hours after unilateral middle cerebral artery occlusion (MCAO) in the rat. Using the microscopic appearance of Golgi-stained neuropil as a highly sensitive histopathological technique for assessing tissue damage, we now show that BMP-7 reduced the extent of tissue damage surrounding the infarct.

Adult S-D rats were subjected to permanent right MCAO. Approximately twenty-four and seventy-two hours after infarction the animals were administered BMP-7 (~0.4nmol) or vehicle by injection into the cisterna magna. All subjects were sacrificed 21 days post-MCAO. Brains were immersion-fixed in formalin and 3mm-thick coronal blocks within the infarcted region were removed and stained using the rapid Golgi method.

Neocortex from the infarcted hemisphere was categorized as: (1) missing; (2) heavily damaged (severely necrotic with no Golgi-impregnated neurons); (3) moderate-to-mildly damaged (with well-impregnated neurons and a grainy neuropil background); and, (4) undamaged (impregnated neurons with a clear neuropil background). From coded slides, neocortical areas ascribed to the different categories were quantified.

BMP-7 treated rats had less tissue missing ($p=0.0012$) and more lightly-to-moderately damaged ($p=0.0098$) and undamaged tissue ($p=0.001$) than control rats. Since Golgi-stained neurons in the moderately-to-lightly damaged penumbral region had less dendritic material ($p=0.002$) this population of neurons may be characterized by injured cells which were rescued from subsequent neuronal death by BMP-7.

(Funded by Creative BioMolecules, Inc.)

Presented at the IBC Conference on Alzheimer's Disease, Boston, MA 1999

AGING PDAPP TRANSGENIC MICE WITH A-beta DEPOSITION SHOW DIFFERENTIAL PATTERNS DENDRITIC ATROPHY OF HIPPOCAMPAL CA1 PYRAMIDS & DENTATE GRANULE CELLS.

A quantitative Golgi study of an animal model of Alzheimer's disease

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Abstract

PD-APP transgenic (tg) mice overexpress human amyloid precursor protein (hAPP) V717F, develop A-beta plaques, and mirror other Alzheimer-like pathology. Here we further characterize neurostructural changes in the hippocampus of these mice. Coronal blocks of formalin-fixed tissue encompassing the hippocampal formation from aged (26 months) PD-APP mice and age-matched controls were stained using the Rapid Golgi method. CA1 pyramids (n=66) and granule cells (n=66) in the dentate gyrus were randomly selected from coded slides. Camera lucida drawings were made of their dendritic arbors and analyzed using the Sholl Method. The basilar tree of the CA1 pyramids from the PD-APP mice showed significant atrophic changes including loss of dendritic material and simplification of branching. By contrast, the dendritic arbor of the granule cells showed atrophic changes only in the outer 2/3rds of the molecular layer, e.g., that dendritic region

receiving input from the entorhinal cortex, an area severely affected in AD. These findings support the validity of this animal model of AD and provide an anatomical substrate for the cognitive impairment described in these mice. (Supported by Elan Pharmaceuticals)

Presented at The American Association of Veterinary Anatomists
July, 1999

**ANALYSIS OF SELECTED GOLGI-STAINED BRAIN REGIONS OF TOTTERING, LEANER
AND COMPOUND HETEROZYGOUS, TOTTERING/LEANER MICE.**

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77843 NeuroStructural Research Laboratories, 2109 West Fifth Ave., Columbus, OH 43212.*

Abstract

Tottering (tg/tg), leaner (tgla/tgla), and compound heterozygous tottering/leaner (tg/tgla), mutant mice exhibit, to different degrees: ataxia, petit-mal-like epilepsy and an intermittent, myoclonus-like movement disorder. The tottering (tg) and leaner (tgla) mutations occur in the alpha1A calcium channel subunit which is highly expressed by many CNS neurons. This suggests a correlation between calcium channel dysfunction and occurrence of the neurological disorders exhibited by these mice, but the pathogenesis is far from clear. We initiated a morphologic study of the CNS to gain information concerning the influence these mutations have on CNS neurons. We have carried out a quantitative morphometric analysis of tg/tgla cerebellar Purkinje cells, as 90+ % of calcium channels expressed by these cells contain alpha1A subunits. We also examined the cerebral cortex to study neurons in another brain region. Brains from adult mutant and control mice were prepared using the Golgi-Cox method. Decreased branching area and spine density were observed for tg/tgla cerebellar Purkinje cells. However, tg/tgla cerebral layer II/III pyramidal neurons showed significantly larger dendritic arbors compared to controls, but no significant difference in spine density was observed. Cerebral pyramidal neurons may undergo compensatory neuroplastic changes, possibly in response to impaired cerebellar circuitry. Supported by a NIH grant (K08NS01681) to L.C.A.

Published in Brain Research, 1998

**DENDRITIC ALTERATIONS IN CORTICAL PYRAMIDAL CELLS
IN THE SPARCE FUR MOUSE**

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Abstract

Ornithine carbamoyltransferase deficiency, an X-linked trait, leads to toxic hyperammonemia in *sparce fur* (*spf/Y*) mice. Quantitative analysis of the basilar dendritic tree of layer V pyramidal cell in frontoparietal cortex stained by the Golgi Kopsch method revealed a significant decrease in both the complexity of the dendritic arbor and in dendritic terminal spine density (60%) in *spf/Y* mice compared with controls. Such

reductions may contribute to behavioral dysfunction observed in *spy/Y* mice.

Presented at the Annual Meeting of the Neurotrauma Society, Los Angeles, November, 1998

QUANTITATIVE ASSESSMENT OF CORTICAL DENDRITIC ALTERATIONS FOLLOWING TRAUMATIC BRAIN INJURY IN THE RAT: A GOLGI-IMPREGNATION STUDY.

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Abstract

Traumatic brain injury (TBI) was produced by a controlled pneumatic impactor striking the entire right sensorimotor cortex of the anesthetized rat. Neurologic testing reveals initial deficits with a biphasic pattern of functional behavioral recovery. Golgi-impregnation staining which permits detailed microscopic assessment of degenerative and regenerative dendritic changes was used to quantitatively evaluate the basilar trees in superficial (layers II-IV) and deep (layers V-VI) pyramidal cell populations in the impact zone ("damaged region") and adjacent cortical ("undamaged") region. Animals were sacrificed at 6 days (n=3), 3 months (n=3), and 4.5 months (n=1) after the TBI. There were 5 sham controls. A total of 219 neurons were evaluated. Slides were coded and assessments made blind with respect to the post-trauma times. Parameters quantified included Sholl analyses, measurement of total dendritic lengths, and changes in branch segments. The most salient findings were: Six days following TBI, neurons in both damaged and undamaged cortex revealed widespread dendritic atrophy. Deep pyramidal neurons appeared to be more affected than superficial pyramids. Indices of damage included loss of terminal branches, reduction in dendritic length, and significant reductions in Sholl dendritic arbor profiles. At 3 months post-injury, most dendritic parameters still were suppressed in both damaged and undamaged regions. However, there was an increase in terminal tip segments, possibly predicting new neuronal growth. By 4.5 months we find preliminary but compelling evidence of neuroplasticity in both damaged and undamaged cortex, the undamaged region showing the more robust effect. Golgi-staining thus appears to be a valuable tool to help ascertain the extent of neuronal damage and dendritic plasticity and could be useful in evaluating the efficacy of neuroprotective agents. (Supported by U.S. Army contract DAMD-17-93-C-3008)

Presented at the Annual Meeting of the Society for Neuroscience, Los Angeles, November, 1998

COMPENSATORY CORTICAL NEUROPLASTICITY IN THE COMPOUND, HETEROZYGOUS, TOTTERER-LEANER MUTANT MOUSE.

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Abstract

Compound heterozygous, tottering/leaner mice (tg/tg^{la}) exhibit ataxia, intermittent myoclonus and absence seizures. These mice carry one allele each of two autosomal recessive mutations: tottering (tg) and leaner (tg^{la}). Golgi impregnation studies of the cerebellum showed Purkinje cells (PC) from adult tg/tg^{la} mice have reduced branching area and fewer dendritic spines (Abbott et al, *Soc. Neurosci. Abst.*, 1998). Broad interaction between cortex and cerebellum with respect to motor and memory functions infers that reduced cerebellar circuitry in these mutant mice may influence cortical circuitry. Blocks of fronto-parietal cortex from tg/tg^{la} (N=5) and control (+/+) (N=4) mice were stained using the Golgi-Cox method. Sections were cut at 120um. From coded slides, five layer II/III pyramidal neurons were randomly selected from each subject. Camera lucida drawings were made of the basilar tree of each neuron. Sholl analysis showed that the dendritic arbors from tg/tg^{la} mutant mice were significantly larger than controls (Wilcoxin test, $p=0.001$). There was no significant difference in the spine density along 30um terminal tip segments between tg/tg^{la} mutants and controls. Overall, the increased amount of dendritic branching in the mutant suggests that cortical synaptic circuitry is enhanced in this group. Previously, it was found that Lurcher mutants (with postnatal loss of PCs) showed enhanced dendritic arbor of cortical pyramids (Goss et al, *Soc. Neurosci. Abst.* 22:1132, 1996). The tg/tg^{la} mutant mouse reveals an apparently comparable compensatory neuroplastic response of cortical neurons -- possibly to subserve functions associated with impaired cerebellar circuitry. [Supported by a NIH grant (K08NS01681) to L.C.A.]

Published in the Journal of Neurochemistry, **69**, 1997

ENHANCED HEMICHOLINIUM BINDING AND ATTENUATED DENDRITE BRANCHING IN COGNITIVELY IMPAIRED ACETYLCHOLINESTERASE-TRANSGENIC MICE

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Abstract

In a search for behavioral, neuroanatomical, and metabolic characteristics of Alzheimer's disease that may result from cholinergic malfunction, we used transgenic mice overexpressing acetylcholinesterase (AChE) mRNA and active enzyme in brain neurons. Mapping by in situ hybridization revealed that transgenic and host AChE mRNAs were distributed similarly. In a Morris water maze working memory paradigm, adult transgenic mice did not display the characteristic improvement found control mice either between or within test days and spent less time than control mice in the platform zone. In 5-week-old transgenic mice, the basilar dendritic trees of layer V pyramidal neurons from the frontoparietal cortex were essentially as developed as in age-matched controls. However, branching totally ceased after this age, whereas in control adults it continued up to at least 7 months. Therefore, dendritic arbors became smaller in adult transgenic mice than those of controls. Furthermore, the average number of spines was significantly lower

on dendritic branches of 7-month-old but not 5 week-old transgenics compared to controls. Binding of tritiated hemicholinium-3, a blocker of the high affinity uptake characteristic of active cholinergic terminals, was over two-fold enhanced in the brain of transgenic mice. In contrast, no differences were observed in the mRNA and ligand binding levels of several different subtypes of nicotinic and muscarinic acetylcholine receptors. These findings suggest that three different hallmarks associated with Alzheimer's disease namely, progressive cognitive failure, cessation of dendrite branching and spine formation, and enhanced high-affinity choline uptake are outcomes of cholinergic malfunction.

Presented at the Annual Meeting of the Society for Neuroscience, New Orleans, October, 1997

ALTERED NEURONAL MORPHOLOGY AND NEURONAL CELL DENSITY IN HIV INFECTED PRIMATES

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R. F. Mervis, NeuroStructural Research Laboratories, Columbus, Ohio

Abstract

Identification of altered neuronal morphology has been reported in altered postmortem CNS tissue from patients with AIDS. In experimental models it has been found that HIV peptides can also cause alteration in the neuronal morphology in rodents. Such alteration in neuronal morphology can have important implications for understanding both the neurologic and cognitive symptoms. In the present study, Golgi impregnated tissue from SIV infected primates was analyzed. Changes in neuronal morphology were identified which include: atrophic neurons, neurons with decreased spines and, in some instances, varicosities. In addition, special Nissl sections (75um thick) were prepared for analysis with a computer-assisted optical dissector. Regional neuronal counts, adjacent to multi-nucleated giant cells did not demonstrate neuronal loss. But areas in which gliosis can be identified (with or without multi-nucleated giant cells) presented with neuronal cell loss. Studies are underway to characterize the spectrum of morphologic changes. In addition, our work with cell counts is continuing. Further data may reveal a pattern associated with neuronal cell loss.

(Supported by NIH grants NS31857, RR00168, NS30769, NS35732)

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