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Granic, Ivica; Nyakas, Csaba; Kovacs, Gabor G.; Luiten, Paul G.M.; Eisel, Ulrich

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### **A $\beta$ -INDUCED NERVE GROWTH FACTOR TROPHIC DISCONNECTION IN ALZHEIMER'S DISEASE**

A. Claudio Cuello, Martin Bruno, Wanda Leon, *McGill University, Montreal, QC, Canada. Contact e-mail: claudio.cuello@mcgill.ca*

**Background:** The causative mechanisms for the atrophy of forebrain cholinergic neurons in Alzheimer's disease (AD) remain undefined. **Methods:** Western blot analysis of members of the NGF maturation/degradation cascade in the CNS and of key inflammatory markers in human brain samples of Alzheimer's patients and controls as well as in rodents after the application of oligomeric A beta 42 and treatment with minocycline. **Results:** We found evidence for a diminished conversion of the NGF precursor molecule to its mature form and for increased NGF-degradation in Alzheimer's brain samples, resulting in an up-regulation of proNGF levels. We further observed that soluble A $\beta$ -oligomers injections provoked a similar up-regulation of proNGF levels in the rat hippocampus. These experiments revealed an A $\beta$ -induced inflammatory microglia activation, increased inducible nitric oxide synthase (iNOS) which activated the NGF-degrading enzyme MMP-9 and facilitated the CNS accumulation of peroxynitrite-modified proNGF. These combined effects markedly reduced the level and biological activity of mature NGF and provoked the accumulation of proNGF. These changes were prevented with the application of minocycline. Accordingly, the administration of minocycline in a transgenic AD mouse model also corrected the altered levels of MMP-9, iNOS and microglial activation, preserving cognitive behavior and normalizing proNGF levels. **Conclusions:** These effects of A $\beta$  amyloid CNS burden on NGF metabolism would explain the well-known cholinergic atrophy found in AD and it offers a novel therapeutic target.

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### **FAMILIAL ALZHEIMER'S DISEASE PATHOLOGY DIFFERENTIALLY AFFECTS DISCRETE NEUROGENIC NICHES IN THE ADULT BRAIN**

Michael P. Demars, Orly Lazarov, *University of Illinois, Chicago, IL, USA. Contact e-mail: mdemar2@uic.edu*

**Background:** It is broadly accepted that new neurons and glia are generated in discrete areas in the adult mammalian brain throughout life. Increasing evidence suggests that some of these neurons integrate and function in local neural circuits. Neurogenesis in the adult brain has been postulated to play a role in learning and memory process' including episodic and spatial memory and following brain insult. Alterations in neurogenesis might be particularly significant in neurodegenerative diseases, such as Alzheimer's disease (AD). Patients affected with AD experience progressive loss of memory and cognitive decline. Neuropathologically, a progressive neuronal loss takes place in specific brain areas such as the hippocampus and cortex. Recent reports suggest that neurogenesis is altered in AD. However, information concerning the nature of alterations and the effect of Alzheimer's pathology on neurogenic processes has been insufficient and controversial. **Objective:** To explore the effect of familial Alzheimer's disease (FAD)-linked pathology on adult neurogenesis. **Methods:** The extent of neurogenesis was examined in the brains of FAD-linked transgenic mice with varying levels of pathology using immunohistochemical and biochemical analyses. **Results:** We show that neurogenesis is impaired in transgenic mice harboring FAD-linked APP<sup>swe</sup>/PS1 $\Delta$ E9 double mutation. Further, we demonstrate that FAD-linked pathology is differentially prominent in discrete neurogenic areas in the brains of these mice. **Conclusions:** This study suggests that neurogenesis is significantly altered by FAD-linked pathology. In addition, we postulate that neurogenic microenvironments are differentially affected in the AD brain. Taken together, these results may imply that FAD-linked alterations in neurogenesis could exacerbate the learning and memory deficits and neuronal vulnerability characteristic of the disease.

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### **THE ROLE OF PRESENILIN-1 IN ADULT NEUROGENESIS: IMPLICATIONS FOR ALZHEIMER'S DISEASE**

Archana Gadadhar<sup>1</sup>, Robert Marr<sup>2</sup>, Orly Lazarov<sup>1</sup>, *<sup>1</sup>University of Illinois, Chicago, IL, USA; <sup>2</sup>The Rosalind Franklin University, North Chicago, IL, USA. Contact e-mail: agadad1@uic.edu*

**Background:** Neurogenesis in the adult brain is thought to play a role in learning and memory, adaptation to novel environments, disease and injury. Modulation of neurogenesis may provide a compensatory mechanism for brain damage caused by neurodegenerative disorders, such as Alzheimer's disease (AD). Patients affected with AD experience memory loss and cognitive impairments caused by a progressive and massive neuronal loss in specific brain areas such as the hippocampus and cortex. The familial forms of the disease (FAD) are caused by mutations in amyloid precursor protein (APP) presenilin-1 (PS1) and presenilin-2 (PS2). PS1 and PS2 are thought to play a major role in the gamma-secretase complex that cleaves APP. In addition, this enzymatic complex regulates or cleaves critical signaling molecules of cell proliferation and fate determination, such as notch-1, L-1 and members of the tyrosine kinase family. Intriguingly, increasing evidence suggests that neurogenesis is altered in the AD brain. However, the mechanism underlying these alterations is largely unknown. **Objective:** To establish a role for PS1 in regulation of adult neurogenesis. **Methods:** We developed a siRNA-expressing lentiviral vector to effectively knock down expression of PS1 in the adult mouse brain. Lentiviral vectors offer stable and long-term gene knockdown, which is ideal for studying short and long term effects of the silenced gene. **Results:** We show that stereotaxic injection of these lentiviral vectors into the dentate gyrus (DG) and subventricular zone (SVZ) of adult mice knocks down PS1 expression in neural stem cells in a spatially- and temporally-specific manner. We further show that specific reduction of PS1 in neurogenic areas alters neurogenesis in the adult brain. **Conclusions:** This study suggests that PS1 plays a major role in adult neurogenesis and that FAD-linked mutations may directly affect adult neurogenesis.

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### **TNF EXPRESSING CELLS IN THE SUBEPENDYMAL ZONE OF THE THIRD VENTRICLE OF AGED MOUSE AND HUMAN BRAIN WITH ALZHEIMER'S DISEASE PATHOLOGY**

Ivica Granic<sup>1</sup>, Csaba Nyakas<sup>1,2</sup>, Gabor G. Kovacs<sup>3</sup>, Paul G. M. Luiten<sup>1</sup>, Ulrich L. M. Eisel<sup>1</sup>, *<sup>1</sup>University of Groningen, Haren, Netherlands; <sup>2</sup>Neurophysiological Research Laboratory of Hungarian Academy of Sciences, Semmelweis University, Budapest, Hungary; <sup>3</sup>National Institute of Psychiatry and Neurology, Budapest, Hungary. Contact e-mail: I.Granic@rug.nl*

**Background:** Pro-inflammatory cytokines including Tumor Necrosis Factor (TNF) are upregulated in many neurodegenerative diseases such as Alzheimer's disease (AD), but also in stroke or Multiple Sclerosis. Main sources for TNF in the brain are activated microglial cells, but it was also reported that astrocytes or neurons are able to express TNF. **Methods:** We have investigated by immunohistochemistry the distribution of TNF in the brains of transgenic APPS<sub>L</sub>/PS1mut mice. These animals develop after several months various aspects of the neuropathological hallmarks known of AD patients, in particular the precipitation of amyloid plaques in the forebrain region. **Results:** Interestingly, in addition to TNF positive glial cells in the direct vicinity of the amyloid deposits, we noticed cells highly positive for TNF immunoreactivity of unknown origin located in a region surrounding the third ventricle and in the subventricular zone. None of these cell could be detected in non-transgenic brains. Co-stainings with marker molecules for glial, astrocytic and neuronal cells failed to be positive. However, markers specific for proliferating cells and various neuronal stem cell markers including nestin and Ki-67 were found to be colocalized with TNF. To assess whether these cells are AD related, we investigated postmortem tissue samples from AD patients and age-matched

non-dementing control individuals. In fact we could verify the presence of TNF positive cells in the surroundings of the third ventricle only in the AD brains. **Conclusions:** In conclusion we could verify the presence of TNF positive progenitor cells in the surrounding of the third ventricle in our AD model. Ongoing research aims at identifying the functional role of those stem cells in Alzheimer's disease and AD animal models.

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### IMPAIRED HIPPOCAMPAL ADULT NEUROGENESIS IN AN ANIMAL MODEL OF ALZHEIMER'S DISEASE

**Brian E. Halabisky**<sup>1,2</sup>, Binggui Sun<sup>1,2</sup>, Yungui Zhou<sup>1</sup>, Sarah Mueller-Steiner<sup>1,2</sup>, Xin Wang<sup>1</sup>, Giuqiu Yu<sup>1</sup>, Lennart Mucke<sup>1,2</sup>, Li Gan<sup>1,2</sup>, <sup>1</sup>J. David Gladstone Institutes, San Francisco, CA, USA; <sup>2</sup>Department of Neurology, University of California, San Francisco, San Francisco, CA, USA. Contact e-mail: [bhalabisky@gladstone.ucsf.edu](mailto:bhalabisky@gladstone.ucsf.edu)

**Background:** The hippocampal formation is severely affected by Alzheimer's disease (AD). It contains the dentate gyrus (DG), which is among the few brain regions in adult rodents and humans in which new neurons are continually born and functionally integrated into the neural network. How  $\text{A}\beta$  and AD affect the newborn neurons remains to be fully elucidated. Our objective was to assess the effect of amyloid- $\beta$  ( $\text{A}\beta$ ) on neurogenesis, determine underlying mechanisms, and develop strategies to counteract relevant pathogenic processes. **Methods:** We used human amyloid precursor protein (hAPP) transgenic mice from line J20 and nontransgenic controls at 3 weeks, 2-3 months or 6-7 months of age. Newborn neurons in the subgranular zone of their DG were labeled by stereotaxic injection of a GFP-expressing retroviral vector that only labels cycling progenitor cells. Neuronal dendrites and spines were quantitated by computer-assisted analysis of confocal microscopic images and electrophysiological responses by patch-clamp recordings. **Results:** At 2-3 weeks after the birth of adult-born neurons, when their response to GABAergic stimulation is still excitatory, adult-born granule cells in hAPP mice had more dendritic spines, received more GABAergic input, and showed stronger excitatory synaptic transmission than adult-born granule cells in controls. New granule cells in hAPP mice also exhibited an accelerated switch from a depolarizing to a hyperpolarizing chloride reversal potential. Thus, adult-born neurons in hAPP mice initially appear to undergo an abnormally accelerated development, which may relate to the excessive GABAergic sprouting in the dentate gyrus of hAPP mice (*Neuron* 55:697). By 4 weeks after the birth of adult-born neurons, when their response to GABAergic stimulation had switched from excitatory to inhibitory, adult-born granule cells in hAPP mice showed shorter dendrites, fewer dendritic spines, and poorer functional integration into the DG circuitry than controls. Importantly, nicotine treatment restored the normal dendritic and functional development of adult-born neurons in hAPP mice. **Conclusions:** hAPP/ $\text{A}\beta$  first accelerates and then impairs the development of adult-born granule cells, possibly through increases in GABAergic input. Nicotine treatment blocks this process, possibly by improving the balance between excitatory and inhibitory activities. Supported by Whittier Foundation.

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### REELIN-APP-INTEGRIN INTERACTIONS PROMOTE NEURITE OUTGROWTH IN HIPPOCAMPAL NEURONS

**Hyang-Sook Hoe**<sup>1</sup>, Kea Joo Lee<sup>1</sup>, Ji Yeon Lee<sup>2</sup>, Rosalind S. E. Carney<sup>3</sup>, Alexandra Makarova<sup>4</sup>, Lina Chakrabarti<sup>3</sup>, Ji-Yun Lee<sup>1</sup>, Brain W. Howell<sup>5</sup>, Bradley T. Hyman<sup>4</sup>, Daniel T. S. Pak<sup>1</sup>, Guojun Bu<sup>2</sup>, G. William Rebeck<sup>1</sup>, <sup>1</sup>Georgetown University, Washington, DC, USA; <sup>2</sup>Washington University, St. Louis, MO, USA; <sup>3</sup>Children's Research Institute, Washington, DC, USA; <sup>4</sup>Massachusetts General Hospital, Charlestown, MA, USA; <sup>5</sup>NIH, Bethesda, MD, USA. Contact e-mail: [hh69@georgetown.edu](mailto:hh69@georgetown.edu)

**Background:** Amyloid precursor protein (APP) processing to  $\text{A}\beta$  is an important event in the pathogenesis of Alzheimer's disease, but the normal physiological function of APP is not well understood. Understanding APP

function, trafficking, and processing in neurons may provide valuable information in generating interventions against AD pathogenesis and its accompanying memory loss. **Objective:** Our previous studies have shown that APP processing and  $\text{A}\beta$  production are regulated by the extracellular matrix protein Reelin. In the present study, we further examined whether Reelin is a ligand for APP and whether the interaction between APP, Reelin and integrins affects dendritic neurite outgrowth. **Methods:** We used co-IP's, immunostaining, and FLIM assay to determine whether Reelin and APP interact. Neurons were infected with GFP or APP, treated with control or Reelin, then neurite lengths were assessed using immunostaining. **Results:** We co-transfected COS7 cells with Reelin and APP constructs, and found that Reelin 3-6 domain interacted with the APP E1 domain. In addition, Reelin treatment increased cell surface levels of APP and decreased endocytosis of APP in hippocampal neurons, resulting in decreased  $\text{A}\beta$  production. Because of the known role of Reelin and integrins in regulating neuronal development, we examined whether the interaction between Reelin and APP, or integrins and APP, affected neurite outgrowth in primary hippocampal neurons. We found that APP shRNA prevented Reelin's effect on neurite outgrowth. In addition, we found that  $\alpha 3\text{b1}$  integrin antibody also prevented Reelin-induced neurite outgrowth. Our preliminary data show that APP promotes neurite outgrowth; thus, we tested whether APP or APP combined with Reelin could affect the cytoskeleton when expressed in heterologous cells. We found that transfected cells with control vector showed a regular pattern of fine actin stress fibers. However, we found that APP-transfected cells showed dispersed clusters and APP combined with Reelin dramatically reorganized F-actin, forming a dispersed cluster and smaller well-defined star-like clusters. **Conclusions:** These data strongly suggest that Reelin is a novel ligand for APP that affects APP trafficking and processing. Furthermore, a Reelin-APP-integrin interaction is critical for regulation of neurite outgrowth and neuronal development.

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### LOCALIZATION OF PHOSPHORYLATED TAU PROTEIN IN IMMATURE NEURONS OF ADULT HIPPOCAMPUS

**XiaoPing Hong**<sup>1</sup>, Qing Tian<sup>2</sup>, YingHua Liu<sup>2</sup>, Wei Wei<sup>2</sup>, Jian Zhi Wang<sup>2</sup>, <sup>1</sup>Institute of Neuroscience, Tongji Medical College, HuaZhong University of Science and Technology, Wuhan, China; <sup>2</sup>Tongji Medical College, HuaZhong University of Science and Technology, Wuhan, China. Contact e-mail: [xphong100@126.com](mailto:xphong100@126.com)

**Background:** Tau is a neuronal microtubule-associated protein and its functions are modulated by site-specific phosphorylation. Expression of phosphorylated tau decreases with the development of brain, and high levels of tau phosphorylation correlate with the presence of dynamic microtubule during periods of high plasticity in the developing brain. **Methods:** Rat brains of different ages (newborn, one week, one month, and three months old) were used to study the distribution of phosphorylated and unphosphorylated tau in the dentate gyrus of hippocampus during development. Double immunofluorescence staining and confocal laser microscope were used to investigate the relationship between different species of tau protein and immature neurons. To examine the influence of tau phosphorylation on the survival of newborn cells, stereotaxic technique was used for administrating wortmannin into rat lateral ventricle. **Results:** Both the phosphorylated and unphosphorylated tau immunoreactivity decrease significantly during the postnatal development, while phosphorylated tau still remains highly expressed in a few cells residing the subgranular zone of adult hippocampus. And immature neurons, identified by double immunohistochemistry for doublecortin or neuroD, are also positive for phosphorylated tau protein. In addition, up regulation of tau phosphorylation (via pharmacological inhibition of phosphoinositol-3 kinase by wortmannin) increases the number of immature neurons and the survival of BrdU-positive cells, but has no effects on proliferation. **Conclusions:** These findings indicate that tau phosphorylation play a role in adult neurogenesis, possibly by regulating the survival of newborn neurons.