TNF Expressing Cells in the Subependymal Zone of the Third Ventricle of Aged Mouse and Human Brain with Alzheimer's Disease Pathology
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A precursor molecule to its mature form and for increased NGF-degradation cholinergic neurons in Alzheimer’s disease (AD) remain undefined.

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 Aβ-oligomers injections provoked a similar up-regulation of proNGF levels in the rat hippocampus. These experiments revealed an Aβ-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 Aβ amyloid CNS burden on NGF metabolism would explain the well-known cholinergic atrophy found in AD and it offers a novel therapeutic target.

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 processes 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 in neurogenesis is insufficient and controversial. 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 stereotoxic 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.

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 brain of transgenic APPswe/PS1ΔE9 mouse. 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 cells 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 these stem cells in Alzheimer's disease and AD animal models.

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**IMPAIRED HIPPOCAMPAL ADULT LOCALIZATION OF PHOSPHORYLATED TAU PROTEIN IN IMMATURE NEURONS OF ADULT HIPPOCAMPUS**

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**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 Aβ and AD affect the newborn neurons remains to be fully elucidated. Our objective was to assess the effect of amyloid-β (Aβ) 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 120 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 stereotactic 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 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/Aβ 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**

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**Background:** Amyloid precursor protein (APP) processing to Aβ 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 Aβ 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 Aβ 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 a3b1 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**

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**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, stereotactic 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 phosphoinositide-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.