The McKnight Brain Research Foundation Reception and Poster Session

JW Marriott Hotel
Ballroom Level Salon G
Monday November 17, 2008
6:30-8:30 pm

The McKnight Brain Research Foundation®

Dedicated to the Understanding and Alleviation of Age -Related Memory Loss

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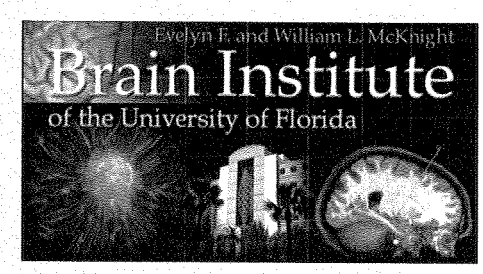
DEPARTMENT OF NEUROBIOLOGY



MILLER SCHOOL OF MEDICINE
UNIVERSITY OF MIAMI

Evelyn F. McKnight Brain Institute
The University of Arizona

The McKnight Brain Research Foundation (MBRF) Reception and Poster Session

In affiliation with the Society for Neuroscience

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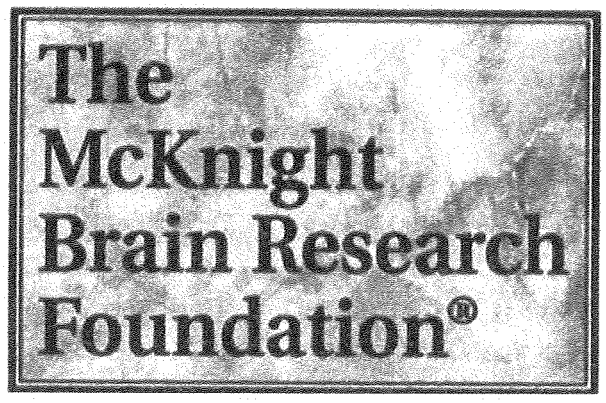

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Age-Related Memory Loss

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MBRF Poster Session Author and Title List

(* indicates poster presenter)

University of Arizona:

From the laboratory of Dr. Carol Barnes:

Poster # 1) M. R. Penner*, L.T. Hoang, T.L Roth, E.D. Roth, J.D. Sweatt, C.A. Barnes. "DNA methylation of Arc in the hippocampus of memory-impaired aged rats"

Poster # 2) K. Plange*, S.N. Burke, C.A. Barnes. "Control of response selection by reinforcer value in young and aged bonnet macaques"

From the laboratory of Dr. Gene Alexander:

Poster # 3 K. L. Bergfield *, K.D. Hanson, K. Chen, S.J. Teipel, H. Hampel, S.I. Rapoport, J. R. Moeller, G. E. Alexander. "Age-related regional MRI gray matter network pattern in healthy aging: A replication study"

Poster # 4) K. D. Hanson *, K. L. Bergfield, K. Chen, E. M. Reiman, M. A. Bernstein, J. Kornak, D. J. Harvey, N. W. Schuff, P. M. Thompson, M. W. Weiner, C. R. Jack, Jr, G. E. Alexander. "Twelve month MRI gray matter declines in Alzheimer's dementia evaluated by voxel-based morphometry with multivariate network analyses: Findings from the Alzheimer's Disease Neuroimaging Initiative"

From the laboratory of Drs. Lee Ryan and Betty Glisky:

Poster # 5) K. Walther*, B.B. Bendlin, E. Glisky, D.G. Walker, L-F. Lue, L. Ryan. "The relation between APOE gene dosage, diffusion weighted MRI and cognition in healthy older adults"

From the laboratory of Dr. Leyla De Toledo-Morrell:

Poster # 6) T.R. Stoub*, R.C. Shah, C.A. Barnes, L. deToledo-Morrell. "Parahippocampal white matter changes in healthy older individuals"

From the laboratory of Dr. Heather Bimonte-Nelson:

Poster # 7) J. Acosta*, L. Mayer, C.J. Smith, R. Audet, J. Castillo, L.M. Demers, C.K. Enders, H.A. Bimonte-Nelson. "Premarin enhances memory, prevents scopolamine-induced amnesia and increases cortical acetylcholine levels in middle-aged surgically menopausal rats"

University of Alabama at Birmingham:

From the laboratory of Dr. John Hablitz:

Poster # 8) G.D. Calfa*, J.J. Hablitz, L. Pozzo-Miller " Hippocampal hyperexcitability in Mecp2 null mice: a voltage-sensitive study"

From the laboratory of Dr. Linda Overstreet-Wadiche:

Poster # 9) C. Zhao*, S. Markwardt, L. Overstreet-Wadiche "Structural and physiological properties of newborn dentate granule cells after neonatal hypoxia-induced seizures"

Poster # 10) S.J. Markwardt*, L. Overstreet-Wadiche "Mechanisms of slow GABAergic signaling to adult-generated neurons"

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Poster # 13) L.C. Vedder*, L.L. McMahon "NR2B-containing NMDARs are responsible for the 17β estradiol-induced increase in novel object recognition"

University of Miami:

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Poster # 14) M. A. Perez-Pinzon*, A. P. Raval, K. R. Dave, R. A. Defazio. "Ischemic preconditioning-induced neuroprotection against cerebral ischemia in vitro requires BDNF"

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University of Florida:

From the laboratory of Dr. Tom Foster:

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Poster # 17) W.-H. Lee*, T. C. Foster "The role of SOD1 in brain aging"

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MBRF Sponsored Abstracts

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Estradiol-17β Priming activates cyclic-amp response element binding pathway and protects the brain against cerebral ischemia in rat

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[1]Dept Neurol (D4-5), Miami, FL

Failure of the Womens' Estrogen for Stroke Trial raised concern regarding the safety of chronic estrogen treatment in women. Estradiol-17b is released in a cyclic manner from ovaries and intermittent increased estradiol titer primes various tissues. Therefore, we hypothesized that (1) cyclic increases in endogenous 17b-estradiol confers neuroprotection against ischemia via activation of the cyclic-AMP response element binding protein (CREB); and (2) a single 17b-estradiol bolus provides protection against ischemia in the absence of endogenous estradiol. To test these hypotheses, rats were either sacrificed or subjected to cerebral ischemia at different stages of the estrous cycle. Ischemia was produced by 10min of bilateral carotid occlusion and systemic hypotension (50mmHg). Brains were examined for histopathology at 7 days of reperfusion. Result demonstrated that the higher serum levels of 17b-estradiol, during transition from diestrus to proestrus stage of estrous cycle, correlated with increased immunoreactivity of pCREB in hippocampus. Western blot analysis showed 152% (253 ± 14; n=4; $p<0.05$) increase in hippocampal pCREB level at proestrus stage as against diestrus. Interestingly, histopathology study demonstrated that ischemic insult to diestrus female decreased number of normal neurons in the CA1 hippocampal region by 82% (194 ± 34, $p<0.05$; n=5) as compared to the control rats (without ischemia; 1100 ± 68; n=5). Interestingly, the number of normal neurons was significantly increased by 13% (255 ± 24; n=5; $p<0.05$ against diestrus) and 20% (409 ± 75, n=5; $p<0.05$ against diestrus) when ischemia was induced during the proestrus and estrus stages, respectively. To further test the efficacy of a 17b-estradiol bolus, ovariectomized rats were treated with 17b-estradiol (5/10/50 μg/Kg) or vehicle-oil, and 48h later rats underwent ischemia. Depletion of 17b-estradiol levels at diestrus or after ovariectomy resulted in loss of this protective state. A single 17b-estradiol bolus treatment to ovariectomized rats 48h prior to ischemia significantly increased pCREB, Bcl-2 protein levels and number of normal neurons in CA1 region after ischemia. These results suggest that both endogenously cycling estrogens and an exogenous bolus 17b-estradiol protect hippocampus against ischemia via activation of CREB®Bcl-2 pathway in a manner similar to preconditioning.

Supported by: AHA-National center # 0730089N and the James and Esther King Biomedical Research Program, Florida Department of Health 07KN-10.

Ischemic Preconditioning-induced neuroprotection against cerebral ischemia in vitro requires BDNF

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Ischemic preconditioning (IPC) is a robust neuroprotective strategy against cerebral ischemia. A major emphasis in this field is elucidation of the mechanisms by which IPC affords protection against cerebral ischemia. Our previous studies demonstrated that (1) IPC requires activation of epsilon protein kinase C (ePKC), and activation of ePKC by itself could mediate preconditioning (pharmacological preconditioning; PPC); (2) IPC elevates brain-derived growth factor (BDNF) mRNA expression in hippocampal CA1, CA3, and dentate neurons; and (3) resveratrol (a phytoalexin) pretreatment mimics ischemic preconditioning (resveratrol preconditioning; RPC). Thus, in the current study using three different strategies to induce ischemic tolerance (viz. IPC, PPC and RPC) we investigated (1) whether BDNF protein level increases after preconditioning, and (2) whether inhibition of TrkB receptor diminishes hippocampal tolerance against ischemia. To test these hypotheses we used hippocampal organotypic slice cultures and an in vitro model of cerebral ischemia. To induce preconditioning the slices were exposed to either IPC (15 min of oxygen-glucose deprivation (OGD) or ePKC-agonist (15 min; 200 mM) or resveratrol treatment (1h; 100 mM). To test the hypothesis that BDNF protein levels increased after preconditioning, we collected slices for immunohistochemistry at 24 and 48h after all three types of preconditioning. Confocal images depicted increased BDNF protein immunoreactivity in the CA1 region of hippocampus after all three types of preconditioning. To further characterize the role of BDNF in preconditioning, we inhibited the TrkB receptor (K252a; 200nM) for 48 hours after preconditioning, then induced lethal ischemia. The quantification of neuronal death was carried out by using the propidium iodide (PI) staining. Our results demonstrated that inhibition of TrkB receptors resulted in loss of neuroprotection induced by each of the three types of preconditioning. The percent cell death in each group was: OGD (52 ± 2 %, n = 5; mean ± SD), IPC (18± 0.9%) (n = 4) vs IPC + TrkB inhibitor (50 ± 3%; n = 5; $p<0.05$), RPC (24 ± 8% ; n = 5) vs RPC + TrKB inhibitor (41% ± 6; n = 6; $p<0.05$) and PPC (25 ± 7%; n = 4) vs PPC + TrkB inhibitor (43 ± 7%; n = 5; $p<0.05$). We conclude that endogenous BDNF acting at TrkB receptors plays an important role in mediating neuroprotection conferred by all 3 preconditioning paradigms.

Supported by: NS34773, NS045676, and NS054147.

Activation of protein kinase c delta following oxygen glucose deprivation leads to release of cytochrome c from mitochondria

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Mitochondrial damage including release of pro-apoptotic factors such as cytochrome c from mitochondria following cerebral ischemia is a key event leading to cell death. Early activation of protein kinase c d (dPKC) following cerebral ischemia has been correlated with the release of cytochrome c from mitochondria. dPKC activation is considered to be involve in the release of cytochrome c. However, the mechanism by which activation of dPKC leads to cytochrome c release is not understood. The goal of the present study was to define whether activation of dPKC after cerebral ischemia resulted in release of cytochrome c. Using synaptosomes as a model system we tested if early activation of dPKC following cerebral ischemia induced cytochrome c release by decreasing phosphorylation of BAD (ser 136) via activation of protein phosphatases 2A (PP2A). To test this hypothesis we induced 60 min of oxygen glucose deprivation (OGD) (an in vitro model of ischemia) in synaptosomes. We observed that OGD resulted in increased levels of dPKC in mitochondria by 72% ($p<0.05$, $n = 4$ each). OGD in presence of dPKC inhibitor peptide (Tat- dV1-1) resulted in decrease in cytochrome c release and increased BAD phosphorylation by 71% ($n = 4$, $p<0.004$) and 312% ($n = 4$, $p<0.01$), respectively as compared to OGD in presence of or carrier peptide (Tat) group. Similarly, OGD in presence of PP2A inhibitor okadaic acid, but not in presence of protein phosphatases 1 inhibitor calyculin, also prevented OGD induced cytochrome c release and BAD dephosphorylation by 52% ($n = 4$, $p<0.05$) and 144% ($n=4$), respectively. These results suggest that early activation of dPKC following OGD initiates the release of cytochrome c from mitochondria by decreasing phosphorylation of BAD via activation of PP2A.

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Enhanced interneuron firing and network activity in CA1 region of the rat hippocampus after cardiac arrest

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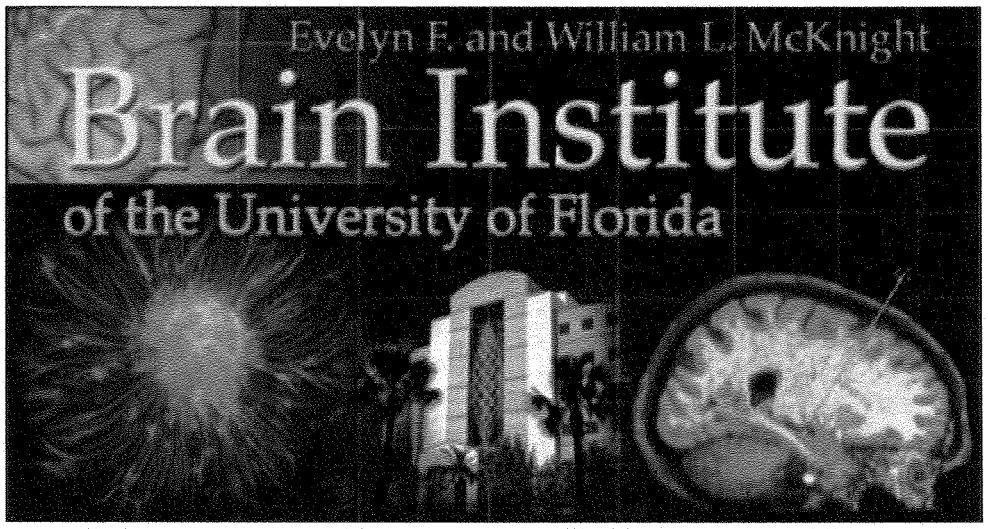
Cardiac arrest has devastating consequences for neuronal function and survival in the CA1 region of the hippocampus. After ischemic insult, CA1 pyramidal neurons exhibit decreased excitability and delayed cell death. The impact of cardiac arrest on interneurons has received little attention despite their critical role in maintaining the balance of excitation and inhibition in the hippocampus. We tested the hypothesis that interneuron function was impaired after cardiac arrest. Hippocampal brain slices were obtained from male Sprague-Dawley rats (250-300 g) 24 hours after cardiac arrest or sham surgery. First, we looked for deficits in action potential generation in interneurons. After cardiac arrest, whole-cell recordings revealed increased action potential firing upon depolarization (sham: 26.7 ± 10.5 Hz; cardiac arrest: 65.4 ± 13.4 Hz; $p<0.05$, n=4-8). Cardiac arrest significantly decreased the amplitude of action potentials (sham: 90.0 ± 6.4 mV; cardiac arrest: 67.8 ± 3.7 mV; $p<0.03$); however, other parameters including action potential threshold and width were not affected. A potential mechanism for enhanced action potential firing is a decrease in the efficacy of GABA synapses. Thus, we asked if GABA synapses were impaired by cardiac arrest. Whole-cell patch clamp recordings revealed prominent $GABA_A$ receptor mediated miniature postsynaptic currents (GABA mPSCs) in all stratum pyramidale interneurons. We found a doubling of the frequency of GABA mPSCs 24 hours after cardiac arrest (sham: 3.81 ± 0.46 Hz; cardiac arrest: 8.1 ± 1.2 Hz, $p<0.02$; n=4-6) with no change in amplitude. Given the persistence of action potentials and GABA synapses, we next asked if the network of interneurons in the CA1 region was altered by cardiac arrest. In the presence of 4-aminopyridine and excitatory amino acid receptor antagonists, interneurons generate network-driven depolarizations referred to as GABA waves. Spontaneous GABA waves occurred in all slices. The frequency of spontaneous GABA waves was significantly increased in brain slices obtained 24 hours after cardiac arrest (sham: 18.1 ± 3.1 Hz; cardiac arrest: 46.7 ± 7.3 Hz; $p<0.01$, n=6-9).

In contrast to vulnerable pyramidal cells, interneurons exhibit vigorous action potential firing, synaptic function, and network activity 24 hours after cardiac arrest. Robust interneuron activity likely tips the balance of excitation towards inhibition, contributing to synaptic dysfunction and impaired cognitive ability after cardiac arrest. Due to the activity-dependent release of pro-survival factors such as BDNF, enhanced inhibition after cardiac arrest may exacerbate delayed cell death in CA1 pyramidal cells.

Supported by: NS034773, NS045676, NS054147, and NS0820.



Director: Dr. Dennis A. Steindler, Ph.D.

Chair: Dr. Thomas C. Foster, Ph.D.

17β-estradiol induces rapid increase in hippocampal synaptic transmission in estrogen receptor beta WT and KO mice

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Estrogen has been implicated in the regulation of hippocampal physiology, including synaptic plasticity. Rapid modulation of hippocampal synaptic plasticity by estrogen has long been investigated in estrogen receptor alpha knock out (KO) and wild types (WT) littermates. The current study examined the effect of 17b estradiol (EB) on hippocampal synaptic function in slices obtained from estrogen receptor beta KO and WT female mice. All the mice (3 -5 months old) were ovarectimized and hippocampal slices were prepared 10-12 days following ovarectomy. Extracellular excitatory postsynaptic field potentials were recorded from CA3-CA1 synaptic contacts in an *in vitro* hippocampal slice preparation and effect of bath application of EB (100 pM) were examined. No genotype differences were observed in the baseline synaptic responses. Synaptic responses were significantly increased 30 min following EB application in slices obtained from WT, $F[(1,9) = 10.54\ p< 0.01]$ and KO $F[(1,10) = 7.36\ p< 0.02]$ littermates when compared to control (vehicle treated) group. The responsiveness of the hippocampus to estrogen appears to decrease in estrogen receptor beta KO mice and EB-induced potentiation of the synaptic responses were generally larger $[F(1,13) = 4.00\ p< 0.06]$ in WT hippocampi relative to KO hippocampi. Examination of paired-pulse facilitation ratios indicated that EB has no effect on facilitation in WT (101.51 ± 1.86, $n = 9$) and KO (101.16 ± 0.86, $n = 8$) littermates. Together with our previous work on ER alpha KO mice, the results suggest that both receptors are required to obtain the full extent of rapid EB effects on hippocampal synaptic transmission.

Supported by: MH59891, AG14979, and The McKnight Brain Research Foundation.

Impaired CaMKII function in the hippocampus contributes to age-related deficits in NMDA receptor function

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Aging is associated with impaired N-methyl D-aspartate (NMDA) receptor mediated synaptic plasticity. These studies examined whether oxidative stress contributed to a decrease in NMDA receptor function. NMDA receptor mediated field excitatory post synaptic potential (NMDAR-fEPSP) were recorded from the CA1 region in hippocampal slices from young (6-9 months) and aged (20-24 months) F344 rats. We confirmed that the NMDA receptor function was decreased in aged when compared to young animals ($p<0.0001$). The oxidizing agents, xanthine/xanthine oxidase (X/XO), decreased the NMDAR-fEPSP in young ($p<0.01$), but not aged animals. In contrast, the reducing agent dithiothreitol (DTT) increased the NMDAR-fEPSP to a greater extent in aged ($p<0.05$) relative to young animals. Application of X/XO and DTT did not affect the pre-synaptic fiber volley amplitude, suggesting that the effect of these redox agents on the NMDA receptor function was not due to changes in excitability and the number of axons activated. L-glutathione a relatively membrane impermeable reducing agent had no effect, suggesting that the site of oxidation is likely intracellular. No change was observed in the paired pulse ratio of the aged animals in the presence of DTT, suggesting a post synaptic site of action. Enhancement of the NMDAR-fEPSP by DTT was blocked by the general ser/thr kinase inhibitor H-7 and the CaMKII inhibitor KN-62. DTT effects on the NMDAR-fEPSP were not blocked by inhibition of PKC, calcineurin, or PP1. The results indicate that oxidative stress impairs CaMKII activity and the impaired CaMKII activity contributes to age-related deficits in NMDA receptor function in the hippocampal CA1 pyramidal neurons during aging. Current studies are examining the effect of redox agents on NMDA receptor dependent synaptic plasticity in area CA1 across aging.

Supported by: AG14979, The McKnight Brain Research Foundation, and University of Florida Graduate Alumni fellowship.

The role of SOD1 in brain aging

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During aging, oxidative stress homeostasis is lost resulting in either an over production of reactive oxygen species (ROS) or impairments in removing damaged molecules, resulting in an increase in markers of oxidative stress. The increased oxidative stress can influence synaptic plasticity and cognitive function. Superoxide dismutase 1 (SOD1), an enzyme located in the cytosol, plays an important role in balancing ROS by converting superoxides to the less reactive hydrogen peroxide. The objective of the present study is to determine if over expression of SOD1 in the hippocampus late in life can delay brain aging by reducing oxidative stress. cDNA encoding hSOD1 was cloned into a lentiviral transfer vector containing the human EF1a promoter and a green fluorescence protein (GFP) reporter. The integrity of the final lentiviral transfer vector, pTYF-EF1a-hSOD1-IRES-GFP, was verified by DNA sequencing. Lentivirus containing hSOD1 gene (LV-SOD1) was constructed with transducing vector (pTYF-EF1a-hSOD1-IRES-GFP), packaging vector (pNHP) and envelope vector (pHEF-VSVG). Long-term expression of hSOD1 *in vitro* was confirmed by observing GFP positive cells in the daughter human embryonic kidney 293FT cells infected with LV-SOD1. In addition, we have infected hippocampi with LV-SOD1 and LV-GFP in 4 month old and 19 month-old F344/Brown Norway F1 rats, and verified the hSOD1 over expression by Western blot. Long-term expression of hSOD1 *in vivo* was observed immunohistologically one month after gene delivery. An increase of activated microglia located around the injection site was not associated with hSOD1 expression throughout the hippocampus. In conclusion, we have successfully over expressed hSOD1 in the hippocampi of young and aged rats using lentivirus. Ongoing studies are examining the effects of 4 months of LV-SOD1 overexpression on behavior and oxidation products.

Supported by: MH59891, AG14979, and The McKnight Brain Research Foundation.

Cannabinoid receptor agonists potentiate calcium independent release of GABA in the dentate gyrus through a CB1 receptor independent mechanism

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A number of previous studies have shown that cannabinoids inhibit calcium-dependent exocytosis from excitatory or inhibitory terminals through activation of CB1 receptors. In the present study, we used electrophysiological techniques to demonstrate a novel effect of cannabinoids on calcium-independent release events which does not involve activation of CB1, CB2, or vanilloid type 1 receptors. Specifically, we found that exogenous application of either WIN 55,212-2 or anandamide (AEA, both cannabinoid agonists) increased the frequency, but not the amplitude, of ruthenium red enhanced GABAergic miniature IPSCs recorded from hilar mossy cells in the presence of NBQX, APV, and TTX. Neither AM-251 (a CB1 receptor antagonist), AM-630 (a CB2 antagonist), nor capsazepine (a vanillod receptor antagonist) blocked the effect of WIN55, 212-2, which was also preserved in $CB1^{-/-}$ mice. Importantly, we determined that this effect was not produced by ruthenium red in the absence of WIN55,212-2, nor was ruthenium red required to observe it, as a similar potentiation was produced by WIN55,212-2 in ~50% of cases when recording miniature IPSCs in normal ACSF. By sharp contrast, neither WIN55, 212-2 nor AEA could potentiate calcium-dependent events that were recorded in the presence of the AM-251, suggesting that this novel effect targets a different release process and/or vesicular pool than that which is normally subject to action potential dependent release and CB1 meditated inhibition. Likely consistent with that interpretation, we noted that preincubation of slices with BAPTA-AM failed to block the effects of WIN55, 212-2 on miniature IPSCs recorded in the presence of ruthenium red, and yet interestingly, did block the effect of WIN55, 212-2 on miniature IPSCs recorded in normal ACSF. Cumulatively, our results suggest that both endogenous and exogenous cannabinoid agonists are able to increase calcium-independent exocytosis in this system through activation of an as yet unidentified receptor subtype.

Supported by: NIDA Grant DA019576.

Modeling Alzheimer's tauopathy: Adeno-associated virus vector expressing mutated tau in the hippocampus of rats impairs spatial working memory

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The hippocampus is a brain structure that prominently exhibits the two anatomical hallmark features of Alzheimer's Disease (AD), amyloid plaques and neurofibrillary tangles, early in the disease process. Moreover, the hippocampus is an essential component of mnemonic processing and its degeneration results in significant memory impairment. In order to create an Alzheimer's model, we used gene transfer techniques and injected a recombinant adeno-associated viral vector with a mutated human tau gene (P301L) into the hippocampus of adult rats. Our goal was to determine whether localized expression of human mutated tau would produce behavioral and/or anatomical pathology reminiscent of AD. Spatial memory on a Y-maze was tested for six months post-surgery. When behavioral testing was completed, the brains were assessed for expression of human tau and evidence of tauopathy. Rats injected with the tau vector exhibited persistent memory impairments on the task beginning about 5 weeks after the injections. In contrast, rats injected with a green fluorescent protein control vector performed at criterion levels during that interval. Assessment of the brain tissue revealed labeled hyperphosphorylated tau and NFTs in the hippocampus, but not in untransduced brain regions. Thus, vector-induced tauopathy in the hippocampus significantly impaired mnemonic functioning in rats.

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Inducible neurogenesis from subventricular zone glia

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Spatially and temporally restricted glial cells within the mammalian CNS can generate neurons both transiently during development and persistently in adult germinal zones. We and others have studied such "neurogenic" glia in an in vitro model of inducible neurogenesis in which subventricular glial monolayers are conditioned by medium supplemented with serum, EGF, and bFGF. Upon withdrawal of these supplements the monolayer undergoes a dramatic burst of neurogenesis that can account for up to 30% of the total cellular population. Here we systematically examine the time course of this neurogenic period, and assess the role of each of the individual supplements in priming the glial monolayer for subsequent neuronal production. We show that supplement withdrawal leads first to a period of relative quiescence, with little change in total cell number due to either proliferation or death, followed by a sharp period of cellular increase during which new neurons are produced. Additionally we show a synergistic interaction between EGF and bFGF in suppression of this inducible neurogenesis. Finally, we present evidence that this model of neurogenesis may be epigenetically mediated by cellular retroelements, since reverse trancriptase antagonists perturb induction.
The model of inducible neurogenesis and the experimental manipulation of its environment allow us to monitor the key signals instructing fate determination. To answer how neuronal diversity is instructed in adult neurogenesis will give us understanding of stem and progenitor cell activity in the adult mammalian brain.

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Over-expression of GDNF in the nigrostriatal tract induces greater weight loss than hypothalamic over-expression in aged obese rats

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Intraventricular administration of glial cell line-derived neurotrophic factor (GDNF) in primate and human trials for treatment of Parkinson disease have revealed the potential for GDNF to induce weight loss. We have previously shown that bilateral hypothalamic over-expression of GDNF via recombinant adeno-associated virus (rAAV) results in significant failure to gain weight in young rats and weight loss in aged rats. However, in the previous study, we could not determine an underlying biological mechanism for the weight loss effect in hypothalamus. We hypothesized therefore that since the nigrostriatal tract passes through the lateral hypothalamus, increased activity mediated by nigrostriatal DA may have been responsible for the observed effect on body weight. In this study we compared bilateral injections of rAAV-GDNF in hypothalamus versus substantia nigra in aged Brown Norway X Fischer 344 rats. Nigrostriatal GDNF over-expression resulted in significantly greater weight loss than rats treated in hypothalamus. The nigral or hypothalamic GDNF-induced weight loss was unrelated to food intake or activity levels of the rats. Moreover, hypothalamic or striatal catecholamine levels did not account for the observed effects on body weight. In contrast, significant DA increases in nucleus accumbens was observed when GDNF was over-expressed either in hypothalamus or SN. However, GDNF-induced increases in accumbens DA levels were larger in the hypothalamic-treated group than in the SN treated group. Therefore, while increased accumbens DA may partially account for the observed weight loss, increased DA cannot completely account for the robust weight loss observed when GDNF is over-expressed in the nigrostriatal tract.

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Cell biology and Behavior of the Placozoan Trichoplax adherens: Identification of transmitters and cells with neuron-like properties

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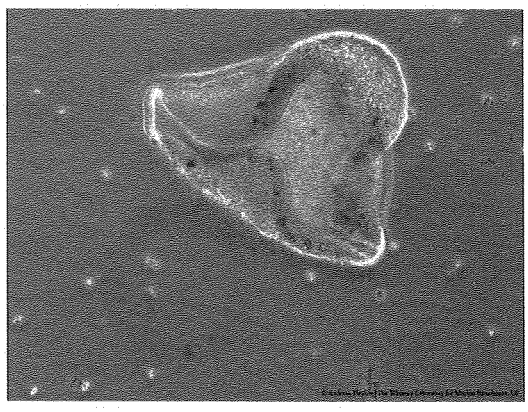
Trichoplax adhaerens is an enigmatic disk-like animal consisting of only four morphologically identifiable cell types arranged into 3 layers - surface, middle and lower. The animal lacks anterior-posterior polarity, but shows distinct dorsal-ventral surfaces through intriguing righting behavior, and the presence of gland cells with digestive function in the ventral epithelium. In the absence of sufficient morphological characters its phylogenetic placement has long been controversial and recent molecular data have not been able to resolve the issue. The recently released genome and obtained transcriptome information reveal several genes coding for transmitter synthesis enzymes and neuroendocrine-like signaling molecules. Thus the study of placozoans may provide insights into the early evolution of the nervous system. Using electron and light microscopy we characterized cell types by morphology, and we used histochemical markers for tubulin, actin and neuron-associated genes to identify cells with neuron-like properties. We also used cell cycle markers to identify regions of active cell division to identify the progenitors for each cell type. Finally, using capillary electrophoresis we have characterized the presence of putative transmitters and their metabolites in Trichoplax. Studies to assess the involvement of specific cells as well as putative transmitters in coordinating behaviors in the whole animal are in progress and will provide a better insight into how this basal animal functions.



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Neuronal transcriptome of the crab Cancer borealis: Brain, stomatogastric ganglion and identified neurons

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Despite the many advantages of the crab Cancer borealis as a model organism for cellular and system neuroscience, a major limitation has been the lack of genomic information. We have sequenced ~400,000 ESTs/cDNAs representing >50,000 putative unique gene products including splice forms and non-coding RNAs, which likely correspond to ~50-60% of the total number of protein-coding genes expressed in the nervous system. Specifically, we have constructed three groups of cDNA libraries amenable for both standard EST collection/cloning applications and high-throughout pyrosequencing. These groups consist of: (i) the entire CNS of Cancer, (ii) the stomatogastric ganglion (STG), and (iii) individual neurons representing the pyloric central pattern generator. As a result, we have annotated >6,000 protein coding genes expressed in the Cancer CNS and STG. A selected list of genes identified in this study represents major groups of transcripts relevant for control of neural excitability, synaptic functions, receptors and adhesion molecules, novel neuropeptides, etc. There are also >50 neuronal genes that are orthologues of defined markers for various neurodegenerative diseases. We have also identified candidates for regulatory molecules known to be involved in Ca-mediated signal transduction pathways linking electrical activity to gene expression as well as developmental genes. In addition, we have developed a reliable in situ hybridization protocol for mapping studies. To this end, full-length cDNA sequences were obtained for three neuropeptide prohormones (Orcokinin, Red Pigment Concentrating Hormone, and Pigment Dispersing Hormone) that were not previously cloned from Cancer but that are important modulators of the rhythmic activity generated in the STG. We have mapped their expression in whole-mount preparations of the entire brain as well as in the STG and associated ganglia. Each of the three neuropeptides has its own unique expression profile pattern and most neuropeptide releasing neurons are located outside of the STG, supporting their role as mediators of descending modulatory control. Finally, using electrophysiological tests combined with double-labeling, we were able to unambiguously identify two orcokinin-containing neurons in the STG as lateral posterior gastric (LPG) neurons. In summary, the developed molecular resources and the ability to map gene expression should allow detailed study of the genomics of identified cells and circuits and provide a critical bridge between genes, circuits and behavior in Cancer and related decapod species.

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Nearly complete genomic profiling of individual identified neurons: SOLiD approach

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What makes a neuron a neuron? What are the genomic bases of unique neuronal phenotypes? How different is the transcriptional profile of one neuron from another? Here, we attempt to identify and quantify nearly all RNA species present in a given neuron. Therefore, we have provided the first unbiased view of the operation of an entire genome from a single characterized neuron. First, we developed protocols for digital expression profiling of identified *Aplysia* neurons representing interneuron, sensory and motor neuronal classes. The generated single-neuron cDNA libraries accommodate the emPCR for two complementary massive parallel sequencing technologies (stating from pyrosequencing to the-sequencing-by-ligation -SOLiD) and allow assembly of the shorter sequence reads. In summary, >8,900,000,000 bases from just three identified neurons were obtained (~80 million sequences from each neuron). It is estimated that such coverage represents >99% of all RNA species in a single neuron. Using absolute real-time PCR we demonstrated that our method is fully quantitative with the dynamic range covering the entire neuronal transcriptome (from the rarest transcripts with only a few copies per cell to the most abundant RNAs with many thousands of copies). Quantitative Real-time PCR and *in situ hybridization* further validated this method of digital profiling. As a result of our initial analysis we propose that >50% of a genome is expressed in a single neuron with a significant fraction being non-coding RNA including antisense RNAs. Many TxFrags, a fragment of a transcript defined as a genomic region, are also found to be specific to neuron subtypes, strongly suggesting a role in the generation of neuronal identity. Furthermore, the numerous classes of non-coding RNAs revealed here likely represent both enormous complexity and crucial contributions of epigenetic RNA signaling mechanisms in the regulation of multiple neural functions, including establishing and maintaining diverse neuronal phenotypes in neural circuits.

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Toward evolutionary dynamic of neuronal transcriptomes: Insights from simpler nervous systems of gastropod mollusks

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For more than 60 years, Gastropod molluscs have served as powerful model organisms for cellular and system neuroscience. Their numerically simpler nervous systems with giant identified neurons provide unprecedented opportunities to study the principles of organization of neural circuits as well as learning and memory mechanisms. However, a major limitation has been the lack of genomic information. As the first step in this direction, we have sequenced >500,000 ESTs/cDNAs from five key model species (Pleurobranchaea californica, Clione limacina, Tritonia diomedea, Melibe leonina, Lymnaea stagnalis). These sequences were assembled and cross-annotated using the extensive transcriptome and genomic information from Aplysia californica. This comparative approach allowed identification of both evolutionary conserved neuronal genes and numerous novel genes including neuropeptides, prohormones and other predicted secretory products. Using bioinformatics tools and web-based applications, we were also able to create a suite of peptides likely to be processed from these gene products. It is estimated that there are >150,000 putative unique gene products (with non-coding RNAs) present in our comparative neurogenomic database, which likely correspond to >50-60% of the total number of genes expressed in the nervous systems of these molluscs. Specifically, for each species, we have constructed cDNA libraries amenable for both standard EST collection/cloning applications and high-throughout pyrosequencing. A selected list of genes identified in this study represent major groups of transcripts relevant for control of neural excitability, synaptic functions and plasticity, receptors, adhesion molecules, developmental genes, homologs of genes involved in neurological disorders, etc. We have also developed a reliable in situ hybridization protocol for mapping studies and used relevant molecular markers to identify homologous neurons and neural circuits across these species. In summary, the developed molecular resources and the ability to map gene expression should allow detailed study of the genomics of identified cells and provide a critical bridge between genes, circuits and behavior in the broad evolutionary context.

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Alpha-synuclein expression suppresses phospholipase D2 toxicity and neurodegeneration in rat substantia nigra

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The phospholipase D 2 (PLD2) is a membrane-bound enzyme that hydrolyzes phospholipids and is involved in vesicular transport and membrane signaling in the brain. PLD2 was shown to be inhibited by a-synuclein (a-syn) in vitro. In present study we provide genetic evidence that the role of a-syn is to inhibit PLD2 *in vivo* as well. Specifically, a series of recombinant Adeno-associated viral (rAAV) vectors carrying human a-syn and/or rat PLD2 cDNAs or expressing small interfering RNA targeting rat PLD2 and a-syn mRNAs were packaged into AAV5 serotype capsids. All possible combinations of these vectors were injected into rat Substantia Nigra pars compacta (SNc) to determine their effect on dopamine (DA) neurons. Over expression of PLD2 in rat SNc caused severe neurodegeneration of DA neurons, loss of striatal DA and behavioral deficits. Coexpression of wild type a-syn suppressed PLD2 neurodegeneration, while an a-syn mutant defective for inhibition of PLD2 *in vitro* failed to inhibit PLD toxicity *in vivo*. Further, both upregulation and knock down of PLD2 was toxic to DA neurons, suggesting that a-syn pathology is at least partially due to dysregulation of PLD2. Finally, increased expression of PLD2 and knockdown of a syn produced the same amphetamine induced rotational asymmetry, and knockdown of PLD2 and increased a syn expression produced the opposite amphetamine induced rotational behavior. Since over expression of a-syn in humans causes early onset of Parkinson's Disease, this suggests that PLD2 and a-syn are essential for DA homeostasis and implies that the primary initiating defect in Parkinson's Disease may be dysregulation of PLD2.

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Monocyte-neprilysin gene therapy in alzheimer's disease transgenic mice

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The recruitment of peripheral blood monocytes into the brain during the development of amyloid pathology is poorly understood. Previous work by other groups as well as our own has shown that bone marrow derived monocytes have the ability to migrate to the brain and associate with amyloid deposits in the AD transgenic mouse model. Studies implicating the homing of monocytes to regions of CNS damage have led to the idea that these cells could be used to deliver therapeutic genes to the brain in Alzheimer's disease. Recent in vitro and in vivo studies demonstrate that the up-regulation of Ab-degrading enzymes can significantly reduce the accumulation of the Ab peptide. Identification of a method to selectively up-regulate brain neprilysin activity may provide a new therapeutic potential.

To study the effects of neprilysin(NEP)-transfected monocytes on Ab, we utilized bone marrow harvested from C57BL/6-Tg mice that express GFP under the control of the human ubiqutin C promoter. Cells were mixed with microbeads conjugated to CD11b+ antibody in order to separate monocytic population from entire bone marrow cells using a magnetic field. Fifteeen month old APP mice were injected with 200,000 transfected monoctyes in both the right cortex and right hippocampus. Monocytes were transfected with one of two plasmids. The first was a NEP-S (secreted)-HA plasmid in which the membrane binding domain was replaced with a signal peptide triggering secretion sequence and appended with an HA tag. The second group received the NEP-M(mutant)-HA plasmid which lacks any enzyme activity and serves as a control for the exogenous protein. Mice were sacrificed one week after the injection. The sections were stained for Ab, Congo and HA. There were significant reductions in both HPC and CX for Ab and Congo in the NEP-S mice compared to NEP-M mice.

To demonstrate that peripheral monocytes can enter the brain and deliver neprilysin to Ab plaques, 10 month old APP+PS1 transgenic mice received 5 x 10^6 GFP+ CD11b+ monocytic cells injected into the jugular via a microvascular port. The numbers of monocytes entering the brain was virtually nonexistent in nontransgenic mice, butincreased considerably in APP transgenic mice. These monocytes concentrated near the amyloid paque deposits. Manipulating monocytes to express an amyloid-degrading enzyme such as neprilysin offers a powerful novel therapeutic tool for the treatment of Alzheimer's disease.

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AAV- mediated over expression of glucose regulated protein 78 (GRP78) ameliorates alpha-synuclein (α-syn) neurotoxicity in the rat model of Parkinson Disease

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Parkinson Disease is characterized by protein misfolding and the formation of inclusions called Lewy bodies. The presence of misfolded proteins leads to endoplasmic reticulum (ER) stress. The ER stress response serves to protect cells against the toxic build-up of misfolded proteins. GRP78 is a major ER-stress regulated protein and up-regulation has been shown to be important in protecting cells from challenge with cytotoxic agents. In our study we over expressed GRP78 in rat Substantia Nigra via AAV mediated transfer to test whether this ER chaperone is able to slow down the onset of a-syn induced neurodegeneration. The data suggests a link between the GRP78 mediated unfolded protein response (UPR) and a-syn. Briefly, immuno-precipitation studies in cell lysates from HEK293 cells transfected with human wild type (wt) a-syn, S129A and S129D a-syn mutants revealed GRP78 interaction with all three forms. Western blot analysis showed a significant increase of GRP78 expression only in S129D expressing cells. Meanwhile, CHOP protein, an ER-stress elevated marker, was up-regulated in all three a-syn forms, but to a significantly lesser extent in S129D expressing cells compared to S129A. Additionally, we found that nigral over expression of GRP78 in rats reduced the toxic effect of S129A, increasing striatal dopamine and eliminating the behavior deficit in the amphetamine rotation test at 8 weeks after AAV gene transfer. Further, our data shows that phoshorylation at Ser-129 is a critical step in normal a-syn degradation. It implies that non-phosphorylated a-syn induces ER-stress (increased CHOP expression), but, apparently does not activate UPR survival pathways (no changes in GRP78 expression). At the same time, over-expression of exogenous GRP78 diminishes S129A toxicity in rat SNc. Our data suggests, that the ER stress chaperone, GRP78, can play a neuroprotective role in a-syn induced Parkinson-like neurodegeneration.

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Post-transcriptional regulation of peripheral myelin protein 22 by miR-29a

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Peripheral myelin protein 22 (PMP22) is a dose-sensitive, disease-associated protein primarily expressed in myelinating Schwann cells. Either deletion or duplication of the PMP22 locus can result in hereditary peripheral neuropathy, suggesting a requirement for precise regulation of this gene. Previous studies demonstrate that PMP22 is post-transcriptionally regulated and the 3'UTR of the gene exerts a negative effect on translation. The restricted expression of the PMP22 protein in comparison to its ubiquitously detected mRNA supports a role for post-transcriptional regulation. MicroRNAs (miRNA) are small regulatory molecules that function at a post-transcriptional level by targeting the 3'UTR in a reverse complementary manner. In these experiments, we used cultured primary rat Schwann cells to demonstrate that not only do alterations in the miRNA biogenesis pathway affect PMP22 levels, but endogenous PMP22 is subjected to miRNA regulation. GW body formation and Dicer (an RNase III endonuclease) expression are co-regulated with the differentiation state of Schwann cells, both demonstrating increased levels in growing cells, as compared to differentiated cells. Furthermore, the levels of Dicer inversely correlate with PMP22, while Dicer knock-down via siRNA leads to elevated PMP22. These results support a role for miRNA pathway in regulating PMP22 protein expression. We used microarrays to characterize the miRNA profile of actively growing and differentiated Schwann cells, and to identify differentially regulated miRNAs. The microarray results, in conjunction with miRNA bioinformatics programs, identified several candidate PMP22-targeting miRNAs. Using *in vitro* assays, we demonstrate that a differentially regulated miRNA, miR-29a, can bind to and repress PMP22 reporter expression through a specific miRNA seed binding region. Immunoprecipitation of Ago2, a RNA binding component of the RNA-induced silencing complex, reveals that endogenous PMP22 RNA binds Ago2, and over-expression of miR-29a enhances this association. Finally, over-expression of miR-29a reduces steady-state PMP22 levels, while inhibition of endogenous miR-29a relieves the miRNA-mediated repression of the gene. These data reveal another level of regulation of myelin gene expression and identify PMP22 as a target of miRNAs, which may have implications in development and disease.

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Differential inhibition of nicotinic responses in the ventral tegmental area by nAChR subtype-selective antagonists

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Examples of nicotinic acetylcholine receptor (nAChR) subtype-selective antagonists come from a family of novel *N,N'*-alkane-diyl-*bis*-3-picolinium (bAPi) compounds. The C_{10} analog of bPiDDB, bPiDI is a potent antagonist of nicotine-evoked dopamine (DA) release in striatal slices. We characterized the effects of bPiDI on the ACh-evoked responses of ventral tegmental area (VTA) neurons. The VTA has a heterogeneous nAChR expression, and nAChRs in the VTA are thought to play important roles in the stimulation of DA release in the nucleus accumbens (NAcc) produced by systemic administration of nicotine. We performed whole-cell recordings in rat brain slices of the VTA to evaluate the inhibitory action of bPiDI on ACh-evoked responses and to compare that activity to the effects of the selective antagonists, methyllycaconitine (MLA), dihydro-ß-erythroidine (DHßE), and a-conotoxin MII. bPiDI was also tested on hippocampal interneurons which predominantly expressed a7 nAChRs. We observed a dose-dependent differential effect of bPiDI on neurons that exhibited a slow component to their ACh-evoked responses. When 1 μM bPiDI was bath-applied there was an increase in ACh-evoked net charge responses of neurons that had a predominant slow inward current response, while peak currents were generally unaffected. However, treatment of cells exhibiting slow ACh-evoked responses with 10 μM bPiDI mostly resulted in reduction of both peak currents and net charge. Following bPiDI application, some of the neurons showed residual current that was further blocked in most of the cases by DHßE. Interestingly, cells with fast transient responses, putatively a7-mediated, were largely insensitive to 1 μM bPiDI, and the increase in net charge of the slow responses was not sensitive to glutamate receptor antagonists. Consistent with this finding, ACh-evoked responses from hippocampal interneurons were effectively reduced by 10 μM but not 1 μM bPiDI. The novel antagonist bPiDI shows a selective concentration-dependent modulation of nicotinic receptor function in the VTA. Further evaluation of such novel nAChR antagonists that inhibit nicotine-evoked DA release could aid in the development of smoking cessation therapies.

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Identification of multiple pharmacophores for the selective activation of nicotinic alpha7-type acetylcholine receptors

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The activation of heteromeric and homomeric nAChR was studied in Xenopus oocytes in order to identify key structures of putative agonist molecules associated with the selective activation of homomeric alpha7 receptors. We observed that selectivity between alpha7 and alpha4beta2 was more readily obtained than selectivity between alpha7 and alpha3beta4. Based on structural comparisons of previously characterized selective and nonselective agonists, we hypothesized that there existed at least three chemical motifs which, when present in molecules containing an appropriate cationic center, could be associated with the selective activation of alpha7 receptors. We identify three distinct structural motifs, based on prototypical drugs: the choline motif, the tropane motif, and the benzylidene motif. The choline motif involves the location of an oxygen-containing polar group such as a hydroxyl or carbonyl separated by two carbons from the charged nitrogen. The tropane motif provides alpha7-selectivity based on the presence of multiple hydrophobic groups positioned away from the cationic center at specific orientations. We show that this motif can convert the nonselective agonists quinuclidine and ethyltrimethyl-ammonium to the alpha7-selective analogs methyl-quinuclidine and diethyldimethyl-ammonium, respectively. We have previously shown that the benzylidene group of GTS-21 converts anabaseine into an alpha7-selective agonist. The benzylidene motif was also applied to quinuclidine to generate another distinct family of alpha7-selective agonists. Our results provide insight for the further development of nicotinic therapeutics and will be useful to direct future experiments with protein structure-based modeling and site-directed mutagenesis.

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The activation and inhibition of neuronal nicotinic acetylcholine receptors (nAChR) subtypes by novel cytisine analogs

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Recent studies using the nicotinic partial agonist cytisine and the nicotinic antagonist mecamylamine have suggested that inhibition of neuronal nAChRs can have antidepressant-like effects in mouse behavioral models. More recently, it has been shown that the S(+)isomer of mecamylamine can antagonize low sensitivity (LS) a4(3)b2(2) nicotinic acetylcholine receptors while activating high sensitivity (HS) a4(2)b2(3) nAChRs. This raises the possibility that the combination of activation and inhibition of different nAChR subtypes may be necessary for antidepressant-like activity. In order to clarify the nAChR subtypes involved in the antidepressant-like properties of cytisine, we have generated a number of substituted cytisine analogues. In the current study, we have tested these cytisine analogues to determine their activities at rat a4b2, a3b4 and a7 nAChRs, as well as high and low sensitivity forms of human a4b2 nAChRs, expressed in *Xenopus* oocytes. We compare the activity of these new analogs to that of cytisine. We find that while cytisine has ten-fold greater efficacy on LS a4b2 receptors (I_{max} 60% that of ACh) compared to the HS receptors, the behaviorally active cytisine analog CE-140 (Mineur et al., this meeting) is a relatively weak partial agonist for both the HS and LS receptors (efficacy 8% and 3%, respectively) with similar potencies for both forms of a4b2. Unlike cytisine, which is an efficacious agonist of a3b4 and a7 receptors, CE-140 produced very little activation (less than or equal to 5% ACh maximum) of a3b4 or a7 receptors at concentrations less than or equal to 100 μM. As expected, the co-application of the partial agonist CE-140 with the full agonist ACh to cells expressing rat a4b2 receptors produced decreased responses compared to those produced by ACh alone, dependent on CE-140 concentration and limited by the intrinsic activity of CE-140. An alternative cytisine analog, CE-145 was more efficacious than CE-140 for all the receptor subtypes tested and had with no significant differences in its activity for HS and LS forms of human a4b2 receptors. These results demonstrate the great potential for tuning the pharmacological profile of cytisine analogs for improved use either as antidepressants or smoking cessation agents with fewer side effects.

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A stem cell/gene therapy approach for treatment of spinocerebellar ataxia

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Spinocerebellar ataxia type 1 (SCA 1) belongs to a family of polyglutamine repeat disorders where mutant ataxin 1 is believed to cause protein misfolding and impaired protein clearance that eventually leads to severe Purkinje cell loss and progressive incoordination of movements. It has been shown that Purkinje cells are targets of bone marrow cell fusion, and we hypothesized that this somatic stem cell fusion could be exploited for a novel therapeutic strategy for SCA 1 using bone marrow derived cells (BMDCs) that are ex vivo genetically modified through the use of recombinant, self-complementary adeno-associated virus serotype 7 (scAAV7) engineered to carry genes that have been reported to suppress SCA 1 symptoms: DnaJb4, Lissencephaly-1, Pcbp3, and Gstt2. Virally transduced whole BMDCs or Sca 1+, c-kit+, Lin- hematopoietic stem cells (SKL HSCs), originally isolated from young male GFP mice, were used as donor cells for transplantation into the retro-orbital sinus of lethally irradiated, young female Sca 1 mice. Animals survived from 16 to 40 weeks and their brains analyzed, including immunophenotypic and confocal microcopic analysis. Purkinje neurons represented the cell population with the largest numbers of labeled cells, being immunopositive for GFP and calbindin, and they were observed starting at 24 weeks post transplantation. Serial confocal images confirmed the occurrence of cell fusion, including the presence of two nuclei within GFP+ Purkinje neurons.This study represents a proof of principle that focused cell fusion combined with viral vector directed gene therapy offers a novel therapeutic approach for diseases like SCA1, given that efficiency of fusion events and successful expression of neuroprotective factors contribute to enhanced survival of at-risk cells and positive behavioral outcomes. Thus, gene therapy combined with targeted somatic cell fusion of autologously transplanted, adult stem/progenitor cells might be efficacious for at-risk neuronal protection in a variety of neurodegenerative diseases.

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Investigation of Parallel and Anti-parallel Configurations of Syntaxin 1A and Synaptobrevin 2 Interaction

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Syntaxin 1A and Synaptobrevin 2 (also known as vesicle-associated membrane protein 2) along with SNAP25 belong to the SNARE (soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptors) family of proteins. They are involved in the exocytosis of synaptic vesicles. It has been shown that both parallel (N-termini of two proteins are aligned at same end) and anti-parallel (C-terminal of one protein and N-terminal of the other protein are aligned at same end) configurations are present, while details of the interactions between these proteins in these configurations have not yet been defined. We used an Atomic Force Microscope (AFM) in force spectroscopy mode to investigate the mechanical interactions between syntaxin 1A and synaptobrevin 2 at single molecule level. Various terminal configurations of proteins (N-N, N-C, C-N and C-C) were studied by attaching the recombinant proteins via their histidine 6 tags to nickel-coated AFM tips and glass coverslips. The syntaxin-synaptobrevin intermolecular interaction forces, extensions, spontaneous lifetimes and interaction energies were obtained. The measured interaction extensions difference (up to 6 nm) is related to alignment of different terminal configurations. Their activation energy (ΔH) difference is as large as 5 k_BT, and implies that parallel configurations might be energetically favorable to tether/dock vesicles at the plasma membrane. These findings provide additional insight on the characteristics of the Sx1A-Sb2 binary complex and they aid better understanding of the possible role for various configurations of the complex in exocytosis.

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The mitochondria modulate Ca^{2+}-dependent glutamate release from rat cortical astrocytes

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Vesicular glutamate release from astrocytes depends on the mobilization of free Ca^{2+} from the endoplasmic reticulum (ER), and from the extracellular space to elevate cytosolic Ca^{2+} (Ca^{2+}cyt). While the mitochondria in neurons, and other secretory cells, have been shown to sequester free Ca^{2+} and have been implicated in the modulation of Ca^{2+}-dependent transmitter release, the role of the mitochondria in Ca^{2+}-dependent glutamate release from astrocytes is not known. A pharmacological approach was taken to manipulate Ca^{2+} accumulation in the mitochondria and thereby affect Ca^{2+}cyt of solitary astrocytes in response to mechanical stimuli. The Ca^{2+}cyt response and the release of glutamate were measured optically in parallel experiments using a fluorescent Ca^{2+} indicator and an enzyme-linked assay, respectively. It was observed that inhibiting mitochondrial Ca^{2+} accumulation is correlated to increased Ca^{2+} cyt and glutamate release, while enhancing mitochondrial Ca^{2+} accumulation is correlated to decreased Ca^{2+} cyt and glutamate release. These observations suggest that the mitochondria modulate Ca^{2+}cyt dynamics in astrocytes along with the ER and plasma membrane ion channels, and play a role in Ca2+-dependent glutamate release from astrocytes.

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Micropit: a new culturing system to characterize solitary astrocytes and small networks of these glial cells

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Astrocytes play an important role in cell-cell signaling in the mammalian CNS and have been implicated to play a role in synaptic neurotransmission in physiological and pathophysiological conditions. The property of astrocytes to couple via gap-junction coupling and to undergo paracrine signaling by releasing gliotransmitters, makes characterization of these cells more difficult in vitro and even more so in vivio. To minimize the complexity of the system introduced by intercellular signaling in astrocytes, we developed a cell culturing method of purified rat visual cortical astrocytes in micropatterned cell-adhesion substrate that are spatially well-defined. Our micropatterning approach involves the use of a microfabricated polydimethylsiloxane (PDMS) mold to generate arrays of well-defined circular cell adhesive surfaces, which we called micropits, by micropatterning agarose on glass coverslip pre-coated with a cell-adhesion substrate polyethyleneimine (PEI). The micropits are 75 mm in diameter and 50 mm in height, and they are positioned 300 μm apart from each other from center to center. The size of the micropit limits the number of cells that can be cultured within the micropit while the inter-micropit distance reduces the paracrine signaling amongst cells in different micropits. The same coverslip besides the array of micorpits also contained two segments of non-pit regions for standard astrocyte cell culturing. We showed that solitary astrocytes or small networks of these cells cultured in micropits were viable and exhibit expected characteristics for this cell type in terms of Ca2+ dynamics and astrocytic markers expression; these characteristics were similar to those of cells cultured in non-pit regions in the same coverslip. However, the intracellular Ca2+ oscillations in solitary micropit astrocytes were less complex when compared to non-pit astrocytes with intact paracrine signaling. The power spectral analysis of these intracellular Ca2+ oscillations revealed that solitary astrocytes in micropits can exhibit a single dominant oscillation frequency compared to multiple oscillation frequency peaks observed in non-pit astrocytes which maybe indicative of paracrine signaling and/or gap-junction coupling. Additionally, solitary cells in micropatterned regions can undergo Ca2+-dependent glutamate release and ATP-mediated astrocyte-to-microglia signaling, so this culturing method can be used to investigate glial-glial or glial-neuronal interactions in a spatially well-defined microenvironment.

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Hippocampal hyperexcitability in Mecp2 null mice: a voltage-sensitive study

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Some of the most important clinical manifestations in Rett syndrome (RTT) are partial and generalized convulsive or silent (i.e. absence) seizures, with concomitant EEG impairments. Experiments in mouse models of RTT have shown an imbalance of neuronal networks in favor of neuronal inhibition, suggesting that the absence or loss-of-function mutations in *Mecp2* specifically impair excitatory synapse number and/or function. To directly estimate the excitation/inhibition balance in a seizure prone region of the brain, we performed voltage-dye imaging in hippocampal slices from symptomatic *Mecp2* null mice (5-6 weeks). Acute hippocampal slices (300μm-thick) were stained with RH-414 and imaged with an array of fast photodiodes. The spread of membrane depolarization in CA1 *stratum radiatum* evoked by a single afferent stimulation (100μsec, 30μA) is significantly larger in *Mecp2* null mice compared to wildtype littermates. In addition, bath application of the K^+ channel blocker 4-AP caused a much larger, longer lasting and further spreading depolarization in *Mecp2* null mice than in wildtype animals. To test whether this hyperexcitability is due to enhanced transmitter release from excitatory presynaptic terminals, we performed multiphoton imaging of the recycling dye FM1-43. Despite the pronounced hyperexcitability of *Mecp2* null slices, and the reports of impaired release from excitatory synapses in cultured *Mecp2* null neurons, the rate of FM1-43 destaining from the total recycling pool of vesicles is similar in CA1 *stratum radiatum* of *Mecp2* null and wildtype slices. Current studies are addressing the possibilities of differential effects on the total vs. the readily releasable pool, which can be assessed by using sucrose-evoked FM dye loading (Tyler et al. J Physiol 2006), and the release capability of inhibitory synapses, which can be identified by their location and pharmacological properties (Mathew et al. J Neurosci 2008).

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Activity-dependent release of BDNF from mossy fibers evokes a membrane current in CA3 pyramidal neurons that requires TRPC3 channels

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The interest in BDNF as an activity-dependent modulator of synapse structure and function in the CNS has intensified in recent years. Several lines of evidence have implicated endogenous BDNF/TrkB receptor signaling in the consolidation of synaptic plasticity in area CA1 and during hippocampal-dependent learning. We have shown that afferent stimulation evokes a TRPC-like membrane current in CA1 pyramidal neurons, reminiscent of currents evoked by local application of recombinant BDNF (Amaral & LP-M, J Neurosci 2007). To follow up these studies in a region where presynaptic fibers exhibit significant endogenous BDNF immunostaining, we performed simultaneous whole-cell recordings and Ca2+ imaging in CA3 pyramidal neurons while stimulating the mossy fiber (MF) pathway. Afferent stimulation consisted of brief theta-burst stimuli (5 bursts at 5Hz, each with 4 pulses at 100Hz) and was performed in the presence of a cocktail of ionotropic and metabotropic GluR and GABAR antagonists (MF origin confirmed with DCG-IV). Under these conditions, MF stimulation reliably evoked slow inward currents reminiscent, but significantly larger than TRPC3-dependent currents recorded in CA1 neurons. MF-evoked responses in CA3 neurons were completely blocked by the extracellular BDNF scavenger TrkB-IgG, confirming that they represent activity-dependent BDNF release. In addition, MF-evoked inward currents were accompanied by transient elevations of intracellular Ca2+ concentration, similar to the Ca2+ signals evoked by recombinant BDNF in CA1 neurons (Amaral & LP-M, J Neurohysiol 2007). Lastly, the requirement of TRPC3 channels was confirmed by biolistic gene transfer of a specific TRPC3 shRNA construct (and eYFP for identification). CA3 pyramidal neurons expressing TRPC3 shRNA showed significantly smaller MF-evoked responses (50-65%) compared to non-transfected neighboring cells in the same slice, or neurons transfected with either a random shRNA construct or eYFP alone. Taken together, these observations demonstrate that presynaptic release of BDNF from mossy fiber terminals evokes a depolarizing membrane current and Ca2+ elevation in CA3 pyramidal neurons mediated by TRPC3 channels. It remains to be tested whether this BDNF/TrkB/TRPC3 signaling pathway participates in synaptic plasticity at the MF-CA3 synapse.

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HDAC activity is required for BDNF to increase dendritic spine density and quantal neurotransmitter release onto CA1 pyramidal neurons

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BDNF is a potent modulator of synaptic structure and function, exerting its actions across a widespread time window. Considering that long-tem actions of BDNF involve gene transcription and its well-described role in synaptic plasticity and learning and memory processes, and the recent observations of epigenetic changes for those same events, we hypothesized that chromatin remodeling was required for the synaptic effects of BDNF. Since chromatin remodeling by histone acetylation is one of the epigenetic mechanisms responsible for transcriptional regulation, we tested whether inhibition of histone deacetylases (HDAC) affected BDNF actions. Hippocampal slice cultures from postnatal rat hippocampus were exposed to hrBDNF (250ng/mL; 48hs) in presence and absence of trichostatin A (TSA, 1.65μM). As previously shown (Tyler and Pozzo-Miller 2001), long-term BDNF exposure increased the frequency of AMPAR-mediated mEPSCs in CA1 pyramidal neurons, without affecting their amplitude or individual kinetics. Consistent with a requirement of histone modifications, this effect was significantly reduced by co-incubation with TSA. However, HDAC inhibition per se had no consequences on mEPSC frequency, amplitude or kinetics. Similar observations were obtained when recording mEPSCs from CA1 neurons in acute hippocampal slices exposed to TSA for 15min. Considering that the increased mEPSC frequency caused by BDNF resulted from both enhanced transmitter release and a higher density of excitatory spine synapses (Tyler and Pozzo-Miller 2001), we next quantified dendritic spine density as a surrogate for excitatory synapses. As previously shown, BDNF increased spine density in CA1 pyramidal neurons, an effect completely prevented by co-incubation with TSA. Consistent with the observations on quantal transmitter release, TSA by itself did not affect spine density. Taken together, these results suggest that the transcriptional program initiated by long-term BDNF exposure requires chromatin remodeling such as histone acetylation. Future studies will address whether enhanced transmitter release is a consequence of structural remodeling of release sites, and potential involvement of other epigenetic mechanisms, such as DNA methylation.

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Persisting changes in DNA methylation with learning

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DNA methylation is a covalent chemical modification of DNA catalyzed by DNA methyltransferases. DNA methylation is associated with transcriptional silencing and has been studied extensively as a lifelong molecular information storage mechanism put in place during development. Recently, we have begun investigating the hypothesis that the adult central nervous system utilizes this covalent modification of DNA to aid in the formation and storage of long-term memories. Accumulating evidence indicates that hippocampal methylation is a critical participant in the transcriptional regulation of memory-associated genes during consolidation. But interestingly, within the hippocampus, the alterations in methylation produced by learning return to baseline within 24 hours after training, at least in the subset of genes examined to date. To investigate the possibility that the CNS employs methylation as a more permanent storage mechanism, methylation was examined in the medial prefrontal cortex (mPFC) or basolateral amygdala (BLA) 7 days after training for contextual and cued fear conditioning. There were no differences between trained animals and their control counterparts (context only or shock only) in the promoters of the immediate early gene zif or the negative regulator of memory, calcineurin, in the mPFC 7 days after contextual fear conditioning. However, the promoter region of reelin was hypermethylated in animals that had received associative training a week earlier. Interestingly, this effect was NMDA receptor-dependent, as treatment with MK-801 at the time of training prevented the reelin hypermethylation. In contrast, both reelin and calcineurin were hypomethylated in the BLA one week after cued fear conditioning relative to controls, while zif was hypermethylated. Taken together, these results demonstrate that a single trial of fear conditioning is sufficient to produce lasting, gene-specific changes in DNA methylation. In addition, the rapid methylation changes observed in the hippocampus versus sustained methylation in the cortex are consistent with the notion that associative memories may shift from the hippocampus where they are initially consolidated to a more permanent storage site in the cortex.

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Regulation of MeCP2 and MBD1 in learning and memory

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Ninety-five percent of females with Rett Syndrome have mutations in the methyl-CpG-binding domain protein MeCP2. Mutations in both MeCP2 and the related protein MBD1 cause learning deficits in mice. However, while these proteins are known to be essential for development, their role in normal learning and memory has not yet been investigated. Therefore, we are examining the regulation of these proteins in response to a classical model of learning, fear conditioning. C57/bl6 mice were trained for contextual fear conditioning, a hippocampus-dependent learning task. Following training, hippocampal area CA1 was removed for biochemistry. Sprague-Dawley rats received cued fear conditioning, an amygdala-dependent learning task, after which the basolateral amygdala was removed. Tissue was removed at three time points during the memory consolidation phase, thirty minutes to two hours following training, and RNA or protein was extracted. *Mecp2* and *mbd1* mRNA levels were assessed using real time PCR, and protein levels were examined using Western blotting. Associative training did not significantly alter the transcriptional regulation of *mecp2* or *mbd1* at the time points examined. MeCP2 and MBD1 can also be regulated through protein modifications. MeCP2 is inactivated through phosphorylation by CDKL5, and PIAS1 and PIAS3 inactivate MBD1 through sumoylation. Changes in levels of the inactive forms of both proteins, phospho-MeCP2 and sumo-MBD1, are currently being examined, as are CDKL5 and PIAS proteins 1 and 3. These studies help elucidate the role of MeCP2 and MBD1 in normal learning and memory.

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The molecular persistence of memory: Histone methylation, memory formation, and the molecular markers of extinction

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Memories may last a lifetime, yet the underlying molecular mechanisms of memory formation are transient. It is well established that 1) protein synthesis is required for memory formation and 2) that protein half-lives are measured in hours. In the face of rapid protein turnover, how can a memory persist beyond the lifetimes of its constitutive molecular building blocks? Clearly, there must be a persisting molecular signal - some way to retain information beyond the lifespan of the individual proteins associated with a given memory trace. Such a persisting signal might recapitulate the state of a synapse by inducing consistent patterns of protein expression, allowing for replacement of degraded proteins with new proteins of the same type and function.
Our laboratory has identified alterations in histone H3 acetylation and phosphorylation in memory formation. Both of these modifications may serve to regulate the way in which genes are transcribed, thereby affecting the resultant proteins necessary for -- or involved in -- the process of learning and memory. Recently, we have identified a role for another form of histone-tail modification, histone H3 methylation. We hypothesize that histone H3 methylation is a candidate molecular mechanism for a long-term protein recapitulation of memory storage. Histone H3 trimethylation at Lys 4 (H3K4me3) is associated with transcriptional activation while Lys 9 dimethylation (H3K9me2) is associated with transcriptional silencing. Preliminary studies suggest that histone dimethylation modifications persist for at least 5 days following a behavioral learning paradigm, such as contextual fear conditioning. Furthermore, we found that the specific pattern of H3K4me3 and H3K9me2 in areas CA1, CA3, and DG of hippocampus is unique to the learning experience of the animal. We observed distinct patterns of histone methylation following contextual fear conditioning, context pre-exposure, and extinction. Overall, these results are of particular interest in that histone Lys methylation, until quite recently, was believed to be irreversible. Furthermore, previous studies of histone methylation have largely been limited to the role that these modifications play in gene regulation during development. Thus, our results indicate that histone H3 methylation is dynamically regulated in the adult CNS during different stages of memory formation.

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The role of DNA methylation in maintaining stable hippocampal place fields

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The rat hippocampus plays a critical role in spatial navigation. Hippocampal pyramidal neurons (i.e., place cells) exhibit increased firing rates in specific spatial locations (i.e. place fields). In a given environment, these spatial representations may be stable for several months. Although much work has been done characterizing many aspects of place cell properties, very little is known about the molecular mechanisms maintaining these spatial representations. Presumably, maintaining a long term stable spatial representation requires gene expression. Indeed, some studies have demonstrated that hippocampal gene expression and protein synthesis may be induced by spatial exploration, and long term stability of place fields can be abolished by blocking protein synthesis. Recent studies also suggest that memory formation and gene expression may be regulated by epigenetic mechanisms, such as DNA methylation. In the present study we investigated the potential role of DNA methylation in maintaining stable place field representations. While pharmacologically manipulating DNA methylation levels, we simultaneously recorded place cells in CA1 and CA3 as male rats ran along a closed loop track. To assess the stability of place fields in control and drug-treated conditions, we compared spatial correlations of place cell firing rate maps generated during sequential exposures to familiar and novel environments. Our results suggest a role of DNA methylation in maintaining spatial representations and provide further insight into potential epigenetic regulation of spatial learning and memory.

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Early-life adversity and its impact on DNA Methylation patterns in the amygdala

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Neural mechanisms responsible for the enduring effects of childhood maltreatment on mental health remain undefined. On a molecular level, one such mechanism may be aberrant programming of DNA methylation, an epigenetic mechanism that represses gene expression. Indeed, aberrant DNA methylation continues to be highlighted for its role in the etiology and expression of several mental disorders. In this study, we addressed whether early-life adversity in the form of caregiver abuse and neglect disrupts DNA methylation in the adolescent and adult amygdala. To model abuse and neglect, rat neonates were exposed to a stressed caregiver 30 min daily during the first postnatal week. Littermate controls were exposed to either a non-stressed caregiver or remained in the home cage. Results indicate that the quality of early postnatal experiences profoundly influences DNA methylation patterns and gene expression in the developing and adult amygdala. Such alterations may provide a framework for enduring effects of early stressors on mental health.

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Structural and physiological properties of newborn dentate granule cells after neonatal hypoxia-induced seizures

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Hypoxia is a major cause of neonatal seizures that are often unresponsive to conventional medications and can lead to lifelong neurocognitive deficits, mental retardation and epilepsy. Seizure activity adversely affects the dentate gyrus, a region where significant neurogenesis occurs during the postnatal period. Although seizures in adults can cause abnormal migration and development of seizure-induced newborn dentate granule cells (DGCs), the effects of experimental seizures on developing DGCs in neonates is not known. In this study we used global hypoxia to induce brief seizures at P10 in proopiomelanocortin (POMC)-EGFP mice that provide a transgenic marker for newborn DGCs. We examined the morphology and synaptic properties of newborn DGCs one week after seizures. We used dendrite-tracing software to measure the total dendritic length and intersections with reconstructed confocal stacks, and we used stereological methods to calculate the total number of newborn DGCs. For assessment of physiological properties, we used whole cell recording in acute brain slices. One week after hypoxia-induced seizures, the total number of newborn DGCs was significantly decreased compared with controls (10799 ± 595 in hypoxia mice versus 21399 ± 1182 in controls, $P<0.05$). There was a significant increase in total dendrite length (166.5 ± 6.0μm in hypoxia mice versus 120.9±4.5μm in controls, $P<0.05$), as well as an increase in dendrite length and intersections at successive radii in newborn DGCs from hypoxia treated animals. These morphological changes were accompanied by an increase in spontaneous inhibitory postsynaptic currents (0.027 ± 0.009 Hz of hypoxia mice, 0.005 ± 0.001 Hz of controls, $P<0.05$) in newborn DGCs, with no changes in amplitude. In addition, we also studied the effects of kainate acid induced seizures on neurogenesis in (POMC)-EGFP mice at P10. Surprisingly, prolonged seizures induced by kainate acid produced similar alterations in newborn DGC number and morphology. These results indicate that even brief neonatal hypoxia-induced seizures alter structural and physiological properties of newborn DGCs, potentially contributing to abnormal development and long term changes in dentate gyrus function.

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Mechanisms of slow GABAergic signaling to adult-generated neurons

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Granule cells in the dentate gyrus are continually produced throughout life. Adult generated dentate granule cells initially receive depolarizing GABAergic synaptic input that is important for activity dependent synaptic integration into the hippocampal network. GABAergic postsynaptic currents (PSCs) in newborn granule cells have slow rise and decay phases, possibly reflecting the postsynaptic receptor subunit composition (Overstreet Wadiche et al., 2005). However, an alternative possibility is that slow GABAergic PSCs result from unusually slow and low GABA concentration transients. To test whether the GABA transient contributes to the slow rise and decay of evoked $GABA_A$-mediated postsynaptic currents (PSCs) in newborn granule cells, we recorded evoked PSCs in newborn granule cells identified by POMC-GFP expression that are at an early developmental stage with exclusively GABAergic synaptic input. In newborn granule cells, PSCs had slow kinetics with a 20-80% rise time of 6.26 ± 0.38 ms (n = 9). The amplitude of PSCs in newborn cells were dramatically enhanced by the GAT1 blocker NO711 (185.9 ± 11.8% of control; n = 8), whereas fast (1.2 ± 0.15ms) IPSCs in neighboring mature cells were less affected (88.8 ± 4.8% of control; n = 4). Addition of the $GABA_B$ antagonist CGP55845 (2 μM) increased the amplitude of IPSCs to 129.8 ± 9.4% of control ($p < 0.05$; n = 6) and the paired pulse ratio from 0.51 ± 0.04 to 0.63 ± 0.02 ($p < 0.01$; n = 6), suggesting both tonic and stimulus-induced activation of presynaptic $GABA_B$ receptors. Importantly, the low-affinity $GABA_A$ antagonist TPMPA (200 μM), reduced the amplitude of PSCs in newborn cells to 52.6 ± 5.2% of control, while reducing fast IPSCs in mature cells to only 69.1 ± 3% of control ($p < 0.01$; n = 6 and n = 5 respectively). However, the high-affinity $GABA_A$ antagonist (SR 95531, 100 nM) reduced PSC amplitude similarly in both newborn (50.8 ± 3.6% of control; n = 6) and mature granule cells (53.3 ± 5.6% of control; n = 5; $p > 0.5$). These data suggest that the GABA transient contributes to slow synaptic responses in newborn granule cells. Slow GABAergic signaling may promote trophic functions of GABA during neuronal maturation.

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NR2B-containing NMDARs are responsible for the 17β estradiol-induced increase in novel object recognition

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Hippocampal dependent learning, including novel object recognition, is enhanced by proestrus levels of circulating 17[[Unsupported Character - ]]b estradiol (E2) (Frye et al., 2007; Walf et al., 2006). In cycling female rats, LTP at CA3-CA1 synapses is increased during proestrus compared to diestrus. Previously, we have shown that proestrus E2 levels in ovariectomized (OVX) rats increases synaptic NMDA current mediated by receptors containing NR2B subunits. Furthermore, this increase in NR2B current is required for the E2 induced heightened LTP magnitude. Given the role of LTP in hippocampal dependent learning, this finding suggests that the increase in NR2B mediated current causes the heightened learning at proestrus. This idea is supported by published findings that increased NR2B subunit expression is correlated with enhanced learning (Tang et al., 1999; Xu et al., 2005).
The goal of this study was to test the hypothesis that the E2 induced increase in novel object recognition requires NMDA receptors containing NR2B subunits. To test this, estrogen or vehicle treated OVX rats were injected with the NR2B antagonist RO25-6981 (5mg/kg) or saline thirty minutes prior to training. We find that E2 increases novel object recognition, confirming previous reports. In support of our hypothesis, we found that animals treated with RO25-6981 spent significantly less time investigating the novel object versus the familiar object when compared to animals treated with E2 alone ($p<0.05$). Importantly, no significant differences were found in time spent with the novel object in vehicle treated OVX rats with or without RO25-6981. Our findings show that the NR2B subunit is essential for E2 induced heightened novel object recognition. Moreover, the shared requirement of NR2B subunits for the E2 induced heightened LTP and learning suggests a direct link between the increased LTP and heighten learning in vivo.

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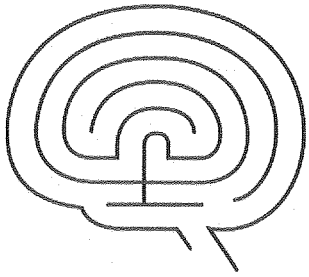

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DNA methylation of Arc in the hippocampus of memory-impaired aged rats.

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Several recent studies suggest that gene transcription necessary for long-term memory formation can be dynamically regulated by epigenetic mechanisms, including DNA methylation (Miller and Sweatt, 2007). DNA methylation involves the covalent chemical modification of DNA by DNA methyltransfereases (DNMTs), which act by adding a methyl group to the 5' position of cytosine residues. This activity typically results in transcriptional silencing and the loss of gene function. In the memory-impaired aged rat, the mRNA levels of several memory-promoting immediate-early genes (IEGs) are attenuated in the hippocampus after spatial behavior. One such gene to show age-related and sub-region specific reductions in the hippocampus is *Arc* (activity-regulated cytoskeletal gene). In order to determine if epigenetic mechanisms modulate this attenuation of *Arc* in the hippocampus of memory-impaired aged rats, we investigated the activity of several DNMTs (DNMT1, 3a and 3b) as well as the DNA methylation status of *Arc* under resting conditions and following spatial behavior within area CA1 and the dentate gyrus. Our results demonstrate significant age-related differences in basal levels of methylated *Arc* in both area CA1 and the dentate gyrus, with aged animals having more methylated *Arc* compared to adult rats. Following spatial behavior, no significant dynamic DNA methylation occurs within area CA1 following exploration of a novel environment in aged rats, even though dynamic DNA methylation *does* occur in the adult CA1 following the same spatial behavior. In the dentate gyrus, dynamic DNA methylation of *Arc* is observed in both adult and aged rats following spatial behavior, and a significant age difference in methylated *Arc* is observed. These results are the first to demonstrate that sub-region specific age-associated changes in the DNA methylation status of *Arc* occurs. Because *Arc* is necessary for the maintenance of long-term memory, and is reduced in the aged brain, an understanding of how *Arc* transcription may be regulated via epigenetic mechanisms may lead to effective treatment strategies aimed at ameliorating the cognitive dysfunction associated with normal aging.

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Automatic 3-dimensional joint montage synthesis from arrays of confocal images and neuronal layer identification by associative image analysis.

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Mapping behaviorally relevant neural circuits depends on knowing not just how many neurons are activated but also which neurons in which layers of brain tissue are engaged by a specific event. Quantitative neuroanatomy historically has been constrained by the time and labor involved in extracting measurements from histological sections. Methods such as stereology can estimate morphological parameters, but only sample fractions of the tissue of interest. In order to study patterns of activation at spatial scales that exceed the field of view of the microscope, while retaining sub-cellular resolution, we developed an automated 3D registration algorithm that jointly aligns images with sub-pixel accuracy. The joint registration overcomes the problem of error accumulation that limits pair-wise image registration methods, and provides a basis for large-scale montage synthesis. The same coordinate transformations can also be used to montage results of automated 3-D segmentation of cell nuclei and cell classification. Montage synthesis is completed in less than a day on a standard computer. The algorithm was tested on tissue areas covered by over 60 confocal image stacks, each measuring about 0.1 mm^2 in area, enough to encompass the entire entorhinal cortex in a section of rat brain. The tissue was dual-stained for cell nuclei (Topro) and a neuronal marker (NeuN). For each of the 60 stacks, the stained nuclei in the Topro channel were segmented automatically using an improved algorithm that is much more scalable compared to our prior work. Segmentation masks were used as a spatial basis to quantify the amount of NeuN marker associated with each nucleus. The NeuN-positive nuclei are designated as neurons. A rich set of intrinsic and associative measurements are computed for each cell. The montage synthesis algorithm merges measurements for nuclei that are in the overlapping regions between adjacent confocal stacks to generate a composite table of quantitative variables that are machine readable. Analysis of this table can be used to investigate a range of relationships between individual components of brain tissue, using both structural and functional markers. The efficiency of these automated methods increases the amount of tissue that can be sampled in a reasonable time frame and represents the next step towards whole brain imaging. In the future we plan to implement our catFISH analyses of behaviorally-activated neural circuits over montages of entire cortical regions, which should enable the investigation of not only the numbers of activated neurons but also the spatial distributions of activated cells over wide regions of brain.

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Cerebral blood volume magnetic resonance imaging reveals localized correlates of age-associated cognitive decline in rhesus monkeys.

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Studies among neurologically healthy humans suggest dramatic age-associated decline in medial temporal lobe- and prefrontal cortex-dependent cognitive function, including memory and executive abilities. Results from these investigations remain somewhat inconclusive, however, because of the possibility that participants with incipient Alzheimer's disease (AD) were included, even in well-screened samples of non-demented adults. In the current study, we used high resolution MRI to derive cerebral blood volume (CBV) maps in 10 adult rhesus monkeys (*Macaca mulatta)* (mean $\pm$ SD age = 19.79 $\pm$ 8.47 yrs, range = 9.50 - 30.92 yrs), who, like all non-human primates, do not develop AD. We examined metabolic correlates of age and performance on a spatiotemporal memory test, the delay response (DR) task, and a recognition memory test, the delayed non-matching-to-sample (DNMS) task. Using a 1.5T head-dedicated MRI scanner, whole brain 3D T1-weighted images were acquired before and 4 minutes after i.v. administration of the contrast agent gadolinium. Regional, individual subject, CBV maps were derived by subtracting the pre-contrast image from the post-contrast image and dividing each voxel by an estimate of 100% blood volume taken from voxel intensities in the superior sagittal sinus. Images were smoothed with a 4 mm kernel and spatially normalized to the same stereotactic space defined by a customized MRI template. CBV values greater than 10% were masked out. Univariate voxelwise statistical parametric mapping was used to examine correlates of CBV. Older monkeys performed worse across increasing delays on the DNMS task ($r = -0.885$, $p = 0.002$), but not on the DR task ($r = -0.128$, $p = 0.735$). Correlations between age and CBV were observed bilaterally in prefrontal cortex, primarily in dorsolateral regions (height threshold $F = 5.32$, $p < 0.05$). CBV in prefrontal regions, also predominantly in dorsolateral regions, was correlated with performance on the DNMS task (height threshold $F = 5.32$, $p < 0.05$) but not on the DR task. On the other hand, there was a strong correlation between CBV bilaterally in the anterior hippocampus and performance on the DR task (height threshold $F = 11.26$, $p < 0.01$), but not on the DNMS task. We previously demonstrated an age-associated correlation between CBV in the dentate gyrus and performance on the DNMS task (Small et al., 2004). The lack of association in the current analysis is likely due to the diminished spatial resolution inherent in voxelwise analyses compared to ROI analyses. Findings from this study highlight the vulnerability of the prefrontal cortex and its potential role in normal age-associated cognitive decline.

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The effect of aging and novelty on the single-unit activity of perirhinal cortical neurons.

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Object recognition memory requires the perirhinal cortex and it is believed that this process is mediated by a reduction in the response of perirhinal cortical neurons when a stimulus is repeated (e.g., Fahy et al., 1993; Zhu et al., 1995). This 'response reduction' could provide a familiarity signal used to judge the prior occurrence of a stimulus. During the normal aging process humans, monkeys and rats all show a decline in object recognition. The extent to which this impairment is related to age-associated functional alterations in the perirhinal cortex, however, is unknown. The current experiment investigated whether age-related deficits in recognition memory could be attributed to alterations in response reduction properties of perirhinal cells in aged animals. Multiple single neurons in the perirhinal cortex were recorded simultaneously with 'hyperdrives' as 6 young (9 months old) and 5 aged (25-27 months old) rats traversed a circular track that contained 8 objects evenly spaced around the track. During the first epoch of behavior, all 8 objects were novel. After completing 20 laps (10 clockwise and 10 counterclockwise), rats were allowed to rest for either 20 minutes or 2 hours. After this delay period, the rat returned to the track to run another 20 laps. During this second behavioral epoch, the track contained 6 familiar objects from the first behavioral epoch and 2 novel objects. The extent to which the firing rate decreased within a behavioral epoch was measured in both the young and the aged rats. In both age groups, approximately one-third of perirhinal principal neurons selectively increased their firing rate in relation to specific objects. There was no evidence in either age group, however, that these neurons decreased their firing rate as the objects became familiar within a single epoch of track running. These data are inconsistent with previous reports of response reduction that recorded perirhinal cortical neurons in stationary rats (Zhu et al., 1995; Zhu & Brown, 1995). Among the reasons for this discrepancy is the more active exploration of objects employed in the current investigation. This suggests that, for a rat, there may be a fundamental difference between viewing an object in a fixed location and actively exploring it while moving through space.

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Reduced Arc transcription in CA1 pyramidal cells of aged, memory-impaired rats.

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The formation and maintenance of memories relies on rapid and sustainable synaptic modification, and these processes require new gene expression. Numerous studies have reported age-associated changes in gene expression, including changes in *Arc* (activity regulated cytoplasmic gene), an immediate-early gene necessary for reliable memory consolidation. We have observed that the proportion of pyramidal neurons that transcribe *Arc* are similar between adult and aged rats in area CA1 after exploration of a novel environment, but using RT-PCR, we have shown that aged rats actually have less *Arc* mRNA relative to adult rats. Because the same number of cells transcribe *Arc* following spatial behavior in CA1, this reduction in *Arc*, as measured by RT-PCR, may be the result of some or all aged pyramidal neurons transcribing less. Within ~2 minutes of stimulation, *Arc* can be detected at the genomic site of transcription using FISH (Guzowski et al., 1999), and these intranuclear foci are detectable up to 15 minutes following the initiation of transcription (Vazdarjanova et al., 2002). In order to address the question of whether aged neurons make less *Arc* than adult neurons, we measured the average and integrated fluorescent intensity of *Arc* intranuclear foci to determine if the amount of primary transcript detected per Arc^+ CA1 neuron is different between adult and aged rats. The results of this analysis indicate that the average intensity and integrated fluorescent intensity from aged rats are significantly lower than adult rats, suggesting an age-related decrement in the amount of primary transcript after 5 min of spatial exploration. A reduction in the amount of *Arc* transcript per cell could interfere with the ability of the aged neuron to respond effectively to relevant stimuli, and contribute to age-associated memory deficits.

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Sparse behavior-induced expression of the immediate-early gene Arc in the rat ventral hippocampus.

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Spatial experience induces the IEG *Arc* in CA1 neuronal ensembles in a context-specific manner (Guzowski et al., 1999), similar to that observed in electro-physiological recordings of hippocampal place cells. Spatial learning is impaired by selective dorsal excitotoxic lesions of the hippocampus while such effects are not observed following ventral hippocampal lesions (Moser et al., 1995; Bannerman et al., 1999). In addition, fewer cells exhibit place fields in the ventral hippocampus and the place fields are larger than those observed in dorsal regions (Jung et al., 1994; Maurer et al., 2006). We report here that the expression of Arc in dorsal and ventral CA1 is consistent with the electrophysiologically observed differences in spatial scaling along the dorso-ventral axis. Rats ran in boxes of two different sizes (60 cm square vs. 120 cm square) with a 20 min rest period. In dorsal CA1, about 45% of pyramidal cells expressed Arc (combined foci + cytoplasmic) whereas in the ventral hippocampus only 20% were arc positive. Thus, these data provide further correspondence between electrophysiological and IEG expression methods for assessing proportions of active cells in a given brain region.

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Expression of the immediate-early gene Arc in ventral tegmental neurons during aging.

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Accumulating evidence suggests that neurons of the ventral tegmental area (VTA) of the midbrain are highly correlated with reward (e.g., Schoenbaum et al., 2002; Balfour et al., 2004). Rewards are thought to play an important role in making predictions about the outcome of future events which can guide behavior. Possible changes of the reward system during aging might lead to impairments in cognitive or behavioral flexibility. Recently, anatomical methods that monitor the expression of neural-activity-dependent, immediate-early genes (IEGs) have been developed that can map the distribution of neurons activated during specific behaviors (Guzowski et al., 2000). IEG expression is thought to be dynamically regulated by specific forms of patterned synaptic activity that are believed to underlie information storage. To determine whether the IEG *Arc* is expressed in the VTA of young and old rats, we exposed two young and two aged male rats to a sexually receptive female, a manipulation known to activate the VTA. There was no significant difference in the behavioral responses of the young and the aged animals. Namely, both young and aged animals had similar amounts of physical contact with the female rat. Utilizing *in situ* hybridization for *Arc* mRNA as well as the catFISH cellular imaging technique, we confirm *Arc* expression in VTA neurons of young and aged animals. Under caged control and maximum electro-convulsive shock conditions, similar proportions of VTA neurons in both young and aged animals express *Arc*. However, after the exposure to a sexually receptive female rat, aged animals exhibited significantly smaller proportions of *Arc*-expressing neurons in the VTA compared to young animals. These results demonstrate age-related changes in VTA neural activity which may affect the functionality and efficacy of the VTA and its projection sites. These alterations may also contribute to the deficits in learning observed in aged animals.

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Aged rats show intact learning in a cross-modal switching task.

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Aging in humans is associated with a decline in the ability to inhibit task-irrelevant information, as well as declines in the ability to switch to a new set of behaviors when the current set ceases to be rewarding (e.g., Braver et al., 2001). Previous work has shown that rats also undergo age-related impairments in a task which requires the animals to shift between responding to olfactory and somatosensory cues (Barense et al., 2002; see Birrell and Brown, 2000). The present study examined whether an age-related impairment would also be found in an auditory-visual switching task. Rats were trained to follow visual stimuli (a blinking light) and, in separate sessions, auditory stimuli (a localized ringing sound), to receive reward at the end of arms on a three-armed platform. When the rats were trained to optimal performance, they were introduced to the experimental task in which both cues were presented simultaneously, but only one rewarded. After approximately seven sessions (one week), the rewarded stimulus was changed, requiring the rats to make a corresponding change in the stimulus that they responded to. Aged rats tended to perform worse than did young adult rats while responding to auditory cues, independent of whether the visual distracter cue was present; however, performance and learning rates in the visual condition were the same for both age groups. The aged rats were clearly not more sensitive to the distracting cue than were young adult rats in either condition; if anything, younger animals were more likely to inappropriately follow the distracter auditory cue during the visual condition. Unlike the olfactory-somatosensory switching paradigm, in which learning takes place within several trials, learning and switching in the current task took place across hundreds of trials and many sessions. Also, error rates in both age groups did not appear to be affected by the success or failure of the immediately preceding trials. Thus, "switching" in the present task may result from a slow relearning of specific stimulus-response associations. The neural systems required for this learning may be relatively preserved with age, in contrast to those mechanisms required to switch between quickly-learned, stimulus-outcome associations.

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Moving through "fictive space": Hippocampal expression of the immedate early gene Arc during running on a fixed wheel occurs equivalent to proportions of cell exploring an environment.

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The immediate-early gene *Arc* is critical for the maintenance of long-term potentiation and memory consolidation, and is induced in hippocampal pyramidal neurons by awake, attentive behavior. *Arc* mRNA first appears in the nucleus at 2 min and moves completely to the cytoplasm 20 min later when the same environment is explored twice, separated by 20 min, *Arc* is found in nucleur and cytoplasmic compartments of the same populations of neurons, consistant with electrophysiological data on maintenance of place fields between sessions. The relationship between *Arc* expression and neuronal firing is not always static, as massed exposure to the same environment lowers the numbers of neurons expressing *Arc* despite unchanged firing properties (Guzowski et al., 2006). The present study aimed to characterize *Arc* activation in CA1 neuorns as a result of running constantly in a fixed location. Our expectations were: 1) fewer place cells would be active in the fixed space of the wheel compared to running in larger spaces and 2) running for 45 min might decouple *Arc* expression from neuronal firing. There were four groups: positive controls with *Arc* induced by maximal electroconvulsive shock (MECS); negative controls sacrificed immediately after removal from their home cages (CC); and two groups that were trained to run on an exercise wheel in 16 lap increments for food reward. On the experiment day, one group ran for 5 min, the other for 45 min. *Arc* was detected with fluorescent *in situ* hybridization and positive cells were quantified in CA1. Both running groups showed *Arc* expression that was intermediate to the MECS and CC rats. Surprisingly, both running groups had total percentages of *Arc*-positive cells around 40-45%, comparable to proportions for rats that explored an environment of 3600 cm^2 (Guzowski et al., 1999) and shows that there is no reduction in *Arc* positive cell numbers as is seen with massed exposure. The results also indicate that the activity of rats running for reward on an exercise wheel is similar to a rat freely moving through multiple place fields in an environment, which is contrary to what would be expected for a rat remaining in a fixed location. Contrary to our predictions, it seems that the path integrator of the medial entorhinal cortex (McNaughton et al., 2006) overrides the "space clamp" of wheel running and prevents potential transcriptional decoupling that would occur if the hippocampus was treating the wheel as a fixed, limited space. This agrees with a prior report tha neuronal assembly activity during wheel running can be similiar to activity generated on a linear track (Pastalkova and Buzsaki, SfN 2007, poster 742.17).

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Temporal compression in precessing neuronal ensembles increases with running speed.

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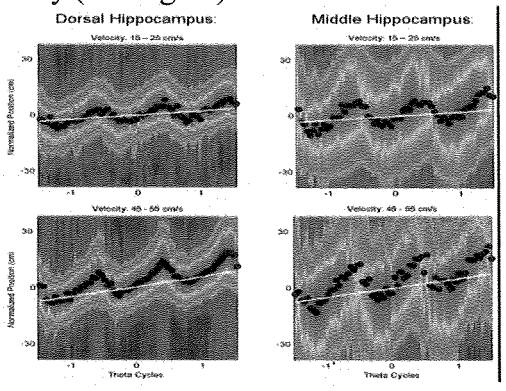
Theta phase precession occurs across the entire population of principal cells, supporting the hypothesis that the temporal order of cells during a single pass through the fields is temporally compressed within multiple theta cycles. Derivatives of the Tsodyks schematic (Tsodyks et al., 1996) suggest that this compression is a sweeping forward of the network activity which 'looks-ahead' to upcoming locations. According to the Tsodyks model (but see Navratilova et al., this session) at the beginning of a theta cycle, hippocampal neurons are driven by external input, activating a network state that represents the rat's current position in the environment. The asymmetric connectivity of these neurons then causes the network to sweep forward through a sequence of states corresponding to the upcoming sequence of place field centers. In order to preserve place field size with increases in running speed, this hippocampal 'look-ahead' must increase with velocity; otherwise apparent place field size decreases. A method was developed to asses the 'look-ahead' in populations of CA1 pyramidal neurons as rats traverse one dimensional tracks. Population vector based reconstruction utilizing a correlation of a spatial population vector matrix (N neurons x P position bins) with a short timescale temporal matrix (N neuron by T temporal bins) captured the 'look-ahead' as predicted by Tsodyks et al. (1996; See Figure; color density reflects average correlation value of theta time bin versus position bin, black dots are the normalized position of highest correlation for each theta bin, and white line is the rats average normalized position by theta time). The slope of the 'look-ahead' increases with running speed, suggesting that place field size does not decrease with running speed. Additionally, the application of this method to populations of middle hippocampal CA1 neurons revealed an increase in temporal compression associated with the larger spatial metric, compared to the dorsal region, at the same velocity (see Figure).



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Activation of immediate-early gene transcription in hippocampus and neocortex during REM sleep.

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The hippocampus is thought to play a critical role in encoding episodic information rapidly by linking distributed cortical circuits. These initially weak connections are proposed to be strengthened during off-line processing (e.g., sleep), with the hippocampus driving the reactivation of experience-specific activity patterns to allow gradual plastic change in neocortical networks. This study sought to: i) identify cell networks activated during sleep following novel open-field exploration and ii) determine whether activity/plasticity is induced in a similar network of cells during REM sleep as those activated during experience. EEG electrode implanted rats were exposed to an open-field environment for 5 min, placed in a recording chamber, and EEG was monitored for indication of sleep, specifically for REM and slow wave sleep. Rats that slept and met criteria for REM were then gently awakened and kept awake for 25 min. After rats were exposed to the same environment (A/REM/A) or to a different environment (A/REM/B) for 5 min. A separate group of rats were exposed to the environment, allowed to sleep, awakened from sleep without entering REM, and returned to the same environment 25 min later (A/non-REM/A). Rats from these groups were sacrificed after the second session, and brains were processed for *Arc/H1a* catFISH. In this design, *Arc* nuclear signal detects neurons activated during the second session and *H1a* nuclear signal detects neurons activated during the preceding sleep period. A control group was exposed to the environment as other groups, kept awake for 1 hr in the recording chamber, and then sacrificed for *Arc/H1a* catFISH. This group (A/awake) served to obtain basal levels of IEG expression by controlling for handling procedures associated with environmental exposure and EEG recording. Preliminary analyses show that sleep induced *H1a* in a significantly higher percentage of hippocampal CA1 neurons (~10-14%) compared to the A/awake controls (~7%). Moreover, a slight increase in *H1a* activation was observed in the REM groups (~13%) compared to non-REM group (~10%). In the A/REM/A group, ~50% of neurons active during REM were also active in the second session, which was significantly higher than the A/REM/B group (~35%). The parietal cortex overlying the hippocampus showed a similar trend, with the highest induction of IEGs observed during REM sleep (~10%) compared to awake (~3%) or non-REM (~7%). These data show that even a brief period of REM (~90-120 sec in a 5 min period) can drive IEG expression in ~7% of hippocampal and neocortical neurons, suggesting a specific engagement of plasticity mechanisms during sleep.

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The effect of age and context on object recognition.

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Normal aging causes a decline in both spatial and object memory. This is considered to be a result of physiological changes in the hippocampus and perirhinal cortex, respectively. In the current investigation, 17 young (9 months) and 17 aged (24 months) rats were tested using the spatial version of Morris swim task, which is dependent on the hippocampus, and the spontaneous object recognition task (SOR), which requires the perirhinal cortex. Consistent with previous experiments, for the swim task, after four days of training, aged rats took longer to find the hidden escape platform compared to young rats. On the SOR task, young rats showed an exploratory preference for the novel object up to delays of 24 hr, whereas the aged rats only showed exploratory preference after a 2 min delay. These results suggest that aged rats were impaired at remembering previously explored objects at longer delays. When the swim task results were compared to SOR performance it was observed, in the aged rats, that there was no significant correlation between an animal's ability to recall the location of a hidden escape platform and the recognition score. This supports the idea that the aging process affects different regions of the brain independently. In young rats, there was a significant correlation between recognition memory scores and swim task performance at both the 2 min and 24 hr delays. For the 2 min delay, poorer performance on the swim task correlated with better performance on the recognition task. In contrast, for the 24 hr delay, lower performance on the swim task correlated with lower preference for novel objects. This suggests that at the 2 min delay, young rats may use a single cue based strategy to discriminate the novel object from the familiar object. Such a strategy may not be effective for the swim task in which many cues are needed to determine the location of the hidden escape platform. In the young rats that show stronger recognition memory, the familiar object may become bound to the original environment and a configural representation of the environment promotes the recognition. To test this hypothesis, a third experiment was performed with 15 young and 15 aged rats in which the environmental context was changed between the sample and the test phase of the SOR task for both a 2 min and a 24 hr delay. For the 2 min delay, context had no affect on SOR for both age groups. For the 24 hr delay, context affected the ability of young rats to distinguish between novel and familiar objects. These findings suggest that the maintenance of object representations over time becomes tied to the environmental context that the objects were viewed in and that these representations become impaired in advanced age.

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Control of response selection by reinforcer value in young and aged bonnet macaques.

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The orbital prefrontal cortex is particularly vulnerable to the process of normal aging. Consistent with this observation, significant age-related impairments are observed in behavioral tasks that require this area for good performance. Specifically, reversal learning is disrupted in advanced age, with older subjects showing an increased tendency for perseveration. It is unknown, however, if other behaviors that require both the orbital prefrontal cortex and amygdala are also disrupted by advanced age. The current experiment used a task that depends on the integrity of the connection between the orbital prefrontal cortex and amygdala, namely a reinforcer devaluation task (e.g., Baxter et al., 2000). The degree to which response selection can be guided by reward value in both aged (20-27 years old) and young (9-10 years old) bonnet macaque monkeys was tested using a Wisconsin General Testing Apparatus. Initially, the bonnet macaques were trained on a Visual Discrimination task in which the animal had to learn to select the rewarded object from an object pair. Forty object pairs where used and one object in the pair was always rewarded and one was always not rewarded (i.e., unbaited). Twenty of the forty rewarded objects were rewarded with food 1 and twenty with food 2. After each animal reached criterion performance (at least 90% accuracy over five consecutive days), they participated in the selective satiation procedure. For the selective satiation tests, the monkey was first allowed to consume as much of food 1 or food 2 in their home cage until eating ceased. After satiation, the monkey then performed an object discrimination test with twenty object pairs in which only the rewarded objects from the initial object discrimination learning were presented to the monkey. For this test, the objects that had always been rewarded with food 1 were now paired with the objects that had always been rewarded with food 2 and the monkey was allowed to select only one of the rewarded objects. Following selective satiation, young monkeys chose the objects associated with the unsatiated food more than they had prior to the satiation procedure. In contrast, even when satiated, the aged monkeys continued to choose the objects associated with both food items. Because the object preference of the aged monkeys did not differ as a function of reinforcer value, this suggests that the aged monkeys did not learn to associate the specific objects with the related food item. These results suggest that in addition to age-related functional alterations in the orbital prefrontal cortex, aged monkeys may also show a decline in the functional connectivity between the amygdala and orbital prefrontal cortex.

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A spatial twist on classical eyeblink conditioning

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The hippocampus contributes to spatial learning and memory in rats, and also undergoes changes in physiology and function during aging. Here, we assessed the effect of age on a spatial version of a classical eyeblink conditioning task (Kawahara et al., Society for Neuroscience Abstract, 2003). Young (12 month old) and aged (25 month old) male rats received eyelid EMG electrode and electrical stimulation wire implants, and were trained to shuttle on a circular track for food reward. Rats received blink-inducing electrical stimulation with 50% probability on their crossings of two specific locations on the circular track. EMG recordings were analyzed to determine the time and location of eyelid activity. For each training session, mean and SD of activity were calculated for a control zone on the circular track in which no blink stimuli were delivered. Data from zones of the track analyzed for blink behavior were normalized by subtracting this control mean, and were expressed as z-scores to control for individual variability in background EMG activity. This method of analysis ensures that any conditioned responses identified are, in fact, location-specific. Further, considering all trials in all sessions, blink activity increased over the last 500 msec of the approach to the stimulus locations in young and aged rats, demonstrating spatial precision in the expression of this conditioned response. EMG activity 2SD above the control mean was considered to be above threshold, and percentages of activity above threshold were calculated in 100 msec intervals for the 1000 msec before the two stimulus locations. Overall, young and aged rats both showed significantly increased threshold EMG activity in the 100 msec preceding blink stimulus locations when the first session was compared to the last session of training. However, both between groups and within groups, rats expressed significant variability in blinking activity above threshold when all sessions were considered. Many individual rats also varied widely in the expression of the conditioned response between sessions. Although these data indicate that both groups learned the locations of the stimuli, there was a wide range of individual performance in accuracy and consistency of expression of the conditioned response, and in the rate of learning. Further electrophysiological studies are necessary to determine the neurobiological underpinnings of these individual differences in expression of spatial memory and temporal accuracy of the conditioned response.

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Parahippocampal white matter changes in healthy older individuals.

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Recent work from our laboratory has demonstrated decreased parahippocampal white matter volume in the region of the perforant path not only in patients with very mild Alzheimer's disease (AD), but also in people who are at risk of developing AD compared to healthy older controls (Stoub et al., PNAS 2006;103:10041-10045). Since this region is pathologically involved very early in the disease process, such alterations could degrade information flow into the hippocampus and contribute to the declarative memory deficit observed early in AD. It is unclear, however, how early such changes take place in older healthy people.
Previous electrophysiological and morphological studies comparing young and old rats have demonstrated a decrease in synapses in the middle molecular layer of the hippocampal dentate gyrus, as well as a decrease in the presynaptic potential in old rats. These findings are indicative of loss of axons from the perforant path to the dentate gyrus. It is, therefore, important to examine if the parahippocampal white matter region that includes the perforant path is also affected in humans as a function of age.
To examine this question we included 51 healthy older individuals (mean age=77, range 65-89) and 41 young participants (mean age=27, range 20-36) in the present investigation. T1 weighted high resolution MRI scans were acquired using a 3-D SPGR pulse sequence. Parahippocampal white matter volume was calculated using voxel based morphometry and statistical parametric mapping (SPM2). Comparisons between healthy older and young participants showed a significant difference in parahippocampal white matter volume ($p=0.003$). These findings suggest that parahippocampal white matter volume loss is present not only as a function of pathological aging, but also as a result of the normal aging process.

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A dissociation of the influence of objects on the firing characteristics of perirhinal and hippocampal neurons.

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Since the seminal observation that lesions of the medial temporal lobe (MTL) produced a retrograde amnesia with profound anterograde amnesia, it has been understood that the MTL is necessary for memory formation. Lesion and electrophysiological studies, however, show that the MTL does not act as a unitary structure, but rather shows functional specificity. In fact, while lesions of the hippocampus (HPC) in rats lead to deficits in spatial memory, lesions of the neighboring perirhinal cortex (PRC) lead to deficits in object recognition. Additionally, the activity of neurons in these regions also shows a functional distinction. When a rat is placed in an environment, principal neurons of the rat HPC show an increase in the firing rate in specific areas, also referred to as the neuron's place field (O'Keefe and Dostrovsky, 1971). In contrast, the PRC shows very little place-specific firing, and it is hypothesized that the PRC represents information about objects for both perceptual and mnemonic functions (Murray et al., 2007). This experiment was designed to determine differences in the role that objects play in the assembly of cognitive maps in the HPC and PRC. Male rats were implanted with a hyperdrive, which was used to record the activity of single neurons in the PRC and CA1 subregion of the HPC. The rats ran two maze epochs on a circular track, separated by a 20-minute sleep session. The first epoch consisted of familiar objects in previously experienced positions, while in the second epoch, the objects' positions were reconfigured. It was observed that the CA1 place fields of principal neurons showed similar firing patterns between the two epochs. Conversely during epoch 1, the spike timing of the perirhinal cells correlated with object position, such that perirhinal cells demonstrated "object fields." These object fields were affected by the reconfiguration of the objects. Between the two maze sessions, the firing rate of the second epoch was significantly different from the first. The data suggest that visual signals received by the PRC can be used to construct "object fields," and lend support to the perceptual mnemonic theory of PRC function.

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Premarin enhances memory, prevents scopolamine-induced amnesia and increases cortical acetylcholine levels in middle-aged surgically menopausal rats

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Conjugated equine estrogen (CEE; trade name Premarin) is the most commonly prescribed estrogen therapy, and is the estrogen used in the Women's Health Initiative study. While in vitro studies suggest that CEE is neuroprotective, no study has directly evaluated CEE's effect on cognition and neurochemistry in an animal model. The current experiment tested whether CEE impacted: I) reference memory, working memory and long-term retention; and, II) the cholinergic system via the two approaches of pharmacological challenge during memory testing, and determination of acetylcholine and acetylcholinesterase levels in hippocampus (CA1/2), frontal cortex, anterior cingulate and entorhinal cortex after testing. Middle-aged ovariectomized (Ovx) rats were given injections of either Oil (vehicle), CEE-Low, CEE-Medium or CEE-High treatment. Relative to the Oil group, the CEE-High group showed less overnight forgetting for reference memory and enhanced platform localization during the probe trial, which was correlated with increased hippocampal acetylcholine levels. All three CEE groups exhibited enhanced working memory performance, and CEE dose-dependently protected against scopolamine-induced amnesia with, remarkably, every rat receiving the highest CEE dose maintaining zero errors after scopolamine challenge. CEE also increased acetylcholine levels in the frontal cortex and anterior cingulate. This is the first study to assess the cognitive effects of CEE in an animal model. The findings suggest that CEE can provide cognitive benefits on reference and working memory, possibly through cholinergic mechanisms.

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Tonic premarin enhances memory retention, increases cortical NGF and BDNF and alters hippocampal gene expression in middle-aged surgically menopausal rats

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Premarin (Wyeth Pharmaceuticals) is the most commonly prescribed hormone therapy (HT) to women in the United States. We have found that cyclic Premarin treatment improves working memory and prevents scopolamine-induced amnesia in middle aged ovariectomized (Ovx) rats (Acosta et al., in review). The current study examined the cognitive effects of Premarin in middle-aged Ovx rats, with Premarin administered tonically, a more similar method to how women receive HT. We administered either vehicle treatment or one of three Premarin doses (low, medium or high) to Ovx rats. Rats were tested on a battery of maze tasks tapping working and reference memory function. While no Premarin dose affected learning on the working or reference memory tasks, on two different tasks high dose Premarin aided memory retention. After testing, BDNF and NGF proteins were evaluated in the frontal cortex, cingulate gyrus, entorhinal cortex, and dorsal and ventral hippocampus, and gene expression was evaluated in the dorsal hippocampus. All Premarin doses increased BDNF and the two highest doses increased NGF in the cingulate gyrus. Expression profiling analysis of vehicle, low and high Premarin dosed rats was performed on Affymetrix Rat Genome 230 2.0 arrays, which probes for 28,000+ rat genes. Comparison of vehicle and low dose treated rats led to the identification of widespread significant ($P<0.05$, fold>=1.5) transcriptomic changes of nearly 3000 genes. Metacore Genego pathway analysis of these genes indicated that affected processes include cellular organization and biogenesis, macromolecule/protein localization, metabolism, and transport. Comparison of vehicle and high dose treated rats led to identification of 649 genes. Of these genes, those demonstrating the greatest expression changes include Homer1 (homer homolog 1 (Drosophila); +1.73 fold, P=3.95E-03; baseline=vehicle treated) and Scd (stearoyl-Coenzyme A desaturase 1; -1.54 fold, P=1.81E-02). Pathway analysis of these 649 significantly-altered genes indicated that affected processes include protein transport, translation, microtubule-based processes, protein localization, metabolism, and behavioral fear and defense responses. Taken together, these findings suggest that in middle-aged Ovx rats tonic Premarin treatment yields beneficial effects when memory is under challenge, increases growth factors in the cingulate gyrus and alters dorsal hippocampus gene expression.

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Twelve month MRI gray matter declines in Alzheimer's dementia evaluated by voxel-based morphometry with multivariate network analyses: Findings from the Alzheimer's Disease Neuroimaging Initiative

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Previous studies in Alzheimer's disease (AD) patients have demonstrated brain volume reductions on magnetic resonance imaging (MRI), with medial temporal structures preferentially affected compared to healthy aging. We used voxel-based morphometry (VBM) with multivariate network analysis to investigate twelve-month declines in the regionally distributed patterns of gray matter atrophy in AD patients and healthy controls from the Alzheimer's Disease Neuroimaging Initiative (ADNI; www.loni.ucla.edu/ADNI). Analyses included 56 AD (mean age = 76.0±7.7; M/F = 32/24) patients and 100 healthy controls (mean age = 76.0±5.4; M/F = 59/41), who did not differ in age or gender. Using volumetric T1 MPRAGE MRIs obtained at baseline and after a twelve-month follow up visit, longitudinal VBM processing was performed with statistical parametric mapping (SPM5) to produce smoothed gray matter maps. To identify a regional pattern of gray matter reductions that distinguished the groups and to test for a group x time interaction in pattern expression over the twelve-month interval, multivariate Scaled Subprofile Model (SSM) analysis was performed. SSM analysis of the two groups combined identified a linear combination of component patterns that best distinguished the AD patients from controls ($R^2 = 0.48$, $p \leq 0.0000001$) with each significantly contributing to the model. This combined pattern was characterized mainly by bilateral medial and lateral temporal, perisylvian, and parietal reductions with relative preservation in visual cortical regions. Using the SSM network subject scores derived from each subject's baseline and twelve month follow up scan, a group x time interaction ($p \leq 0.01$) was observed with the AD patients showing greater pattern expression from baseline to 12 months compared to the controls. After controlling for

baseline Mini Mental State Exam (MMSE) scores, greater longitudinal increases in pattern expression in the AD patients were associated with greater declines in MMSE performance over the twelve month period ($p \leq 0.003$) and this association was not observed in the controls. Together, the findings indicate a regionally distributed MRI gray matter pattern associated with AD that shows a progression of gray matter reductions over 12 months which is not observed in healthy elderly and which is associated with the rate of cognitive decline in the AD patients. SSM network analysis with MRI longitudinal VBM may aid in tracking the progression of AD and potentially assist in evaluating treatments and prevention therapies.

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Age-related regional MRI gray matter network pattern in healthy aging: A replication study

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Healthy aging is associated with gray matter reductions on magnetic resonance imaging (MRI), preferentially affecting the prefrontal cortex. We previously used a multivariate model of regional covariance, the Scaled Subprofile Model (SSM), with voxel-based morphometry (VBM) to identify a network pattern of MRI gray matter reductions associated with aging in 26 healthy adults, 22 to 77 years of age (Alexander et al., *NeuroReport*, 2006) which included areas of reduced gray matter mainly in bilateral prefrontal, perisylvian, and lateral and medial temporal regions. In the current study, we sought to evaluate the reproducibility of the network pattern of MRI gray matter reductions associated with aging in an independent sample of 29 healthy adults, 22 to 84 years of age (mean age = 47±18 yr, 11M/18F, apolipoprotein (APOE) ε4 carriers/non-carriers = 9/20) and to evaluate the potential influence of APOE ε4 on the observed age-related regional gray matter pattern in this cohort. Statistical parametric mapping (SPM5) with VBM and customized tissue priors were used to spatially normalize and segment the T1 volumetric MRI scans into smoothed gray matter maps. Multivariate SSM analysis was performed to identify the regional network pattern of MRI gray matter associated with age in this sample. The SSM identified a single network pattern (first component) that was highly associated with age ($R^2 = 0.64$, $p < 0.000001$) and was mainly characterized by reductions in bilateral medial and dorsolateral prefrontal, perisylvian, and precuneus regions with relative preservation in bilateral thalamus. In this sample, presence of the APOE ε4 allele was not associated with the subject expression of this age-related gray matter pattern ($p = 0.81$). That the major areas of gray matter reduction in the age-related pattern were consistent with our previous study suggests common regions of age-related gray matter reductions in prefrontal and selected temporal regions, whereas the reductions in other brain areas may be due to individual cohort differences in the context of aging. The lack of an association of the age-related pattern with APOE carrier status

suggests that the regional reductions in gray matter associated with aging in this sample cannot be attributed to an increased risk for Alzheimer's disease due to the APOE ε4 allele. Multivariate SSM analysis can assist in the detection and tracking of structural brain changes due to aging and has the potential to aid the evaluation of putative anti-aging therapies.

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Regional network of MRI gray matter reductions associated with aging in non-demented adults with Down Syndrome

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Healthy aging has been associated with a regionally distributed network pattern of gray matter reductions on magnetic resonance imaging (MRI) that preferentially involves frontal and selected temporal brain regions (Alexander et al., 2006, 2008). Down syndrome (DS) provides a model of abnormal aging in which there is increased beta amyloid deposition and risk for Alzheimer's dementia from the triplication of the APP gene on chromosome 21. To identify the age-related network pattern of MRI gray matter reductions in non-demented adults with DS, we used a multivariate model of regional covariance, the Scaled Subprofile Model (SSM; Moeller et al., 1987), with voxel-based morphometry (VBM). T1-weighted volumetric MRI scans from 26 DS adults (mean age = 39.9 ± 9.3 years; M/F = 12/14) ranging in age from 25 to 61 years and clinically screened to exclude dementia (previously reported in Teipel et al., 2004) were included. Statistical parametric mapping (SPM5) VBM was used to transform the MRI scans to a standard brain atlas using customized tissue priors to segment them into smoothed gray matter volume maps. SSM analysis was performed on the MRI VBM gray matter maps to determine the regional network associated with age in the DS group. Greater subject expression of the first SSM component pattern was correlated with increasing age ($R^2 = 0.36$, $p \leq 0.001$) in the DS subjects and this association remained significant after we controlled for gender, total intracranial volume, and general intellectual ability on the Peabody Picture Vocabulary Test (PPVT-R). The age-related pattern was characterized mainly by reductions in bilateral parietal, precuneus, posterior cingulate, perisylvian, and lateral temporal regions with relative preservation in bilateral thalamic, and cerebellar regions. After we controlled for PPVT-R, higher expression of the age related pattern was associated with poorer performance on tests of delayed memory for hidden objects ($p < 0.01$), visuospatial construction ($p = 0.03$), and object naming ($p = 0.03$). These findings indicate that aging in DS is characterized by a regionally

distributed pattern of gray matter reductions in brain regions that have been associated with both aspects of healthy aging and to a greater extent the progressive effects of Alzheimer's disease and its associated cognitive decline. Multivariate SSM network analysis with MRI VBM may help to distinguish the effects of healthy from pathological aging; and may potentially assist in the evaluation of interventions for age-related cognitive decline.

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The relation between APOE gene dosage, diffusion weighted MRI and cognition in healthy older adults

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Background: The apolipoprotein E e4-allele is the most important known genetic risk factor for Alzheimer´s disease (AD). The risk for developing AD increases and the age at onset decreases with increasing number of the APOE e4 allele. Higher gene dosage has also been associated with faster cognitive decline and greater volume loss in preclinical older adults. The following study used diffusion weighted MRI (DWMRI) to examine the relationship between APOE e4 dosage, white matter integrity, and cognitive functioning in healthy older adults. We hypothesized that higher gene dose may be related to greater changes in diffusion measures as people age.
Methods: Participants included 85 community-dwelling controls (ages 52 - 92) with no APOE e4 allele, 28 heterozygote and 6 homozygote carriers. All participants underwent extensive neuropsychological testing and were assessed as cognitively normal. DW EPI scans (ASSET) were carried out on a GE 3T MRI scanner acquiring 2.6 mm sections covering the whole brain. Fractional anisotropy (FA), apparent diffusion coefficients (ADC), and radial diffusivity (RD), were determined for the frontal white matter, centrum semiovale, parietal white matter, temporal stem white matter, and the genu and splenium of the corpus callosum. Results: ANOVAs comparing age group (age 70yrs) and gene dose (controls, heterozygote, homozygote) revealed significant main effects for age for ADC and RD for all regions. Importantly, age group interacted with gene dose for ADC in the frontal and parietal white matter, the centrum semiovale, and temporal stem, and for RD in the frontal white matter and temporal stem. Post hoc comparisons showed significant gene dose effects only for older adults ages 70 and older, but not for those younger than age 70. Homozygotes showed higher ADC and RD values in the frontal and parietal white matter and centrum semiovale compared to both controls and heterozygotes, while ADC and RD in the temporal stem increased linearly with higher gene dose across the three groups. In addition, executive and memory functioning were highly correlated with ADC and RD in the frontal and parietal white matter, the centrum semiovale, and temporal stem in both genetic risk group but not the controls.
Conclusion: Higher gene dose of the APOE e4 allele was associated with a faster decline of white matter integrity at the age of 70 years in areas early affected by AD and these changes were predictive of cognitive changes. Diffusion weighted MRI might be a sensitive method to detect early pathological changes before the onset of AD.

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