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Special thanks to Felecia Hester and Vicki Hixon (UAB) for their help in organizing this year's reception.

MBRF Poster Session Author and Title List

(* indicates poster presenter)

University of Miami:

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From the laboratory of Dr. Sari Izenwasser

Poster # 2) M. LENOIR*, J. LEDON, S. KHOKHAWALLA, A. RHODES, C. BOOTH, S. IZENWASSER. Nicotine conditioned place preference depends on sex and age.

From the laboratory of Dr. Miguel Perez-Pinzon

Poster # 3) K. R. DAVE*, A. P. RAVAL, R. A. DEFAZIO, H. W. LIN, C. DEZFULIAN, I. SAUL, S. K. BHATTACHARYA, M.A. PEREZ-PINZON. Identification of mitochondrial targets for protein kinase c delta following cardiac arrest.

Poster # 4) D. DELLA-MORTE, K. R. DAVE*, P. ABETE, F. RENGO, AND M. A. PEREZ-PINZON. The aged hippocampus exhibits lower levels of RACKI and RACKII, and increase GSK-3β levels.

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Poster # 5) N. HIRSCH, I. SAUL, K. R. DAVE, R. A. DEFAZIO, H. BRAMLETT, M. A. PEREZPINZON, A. P. RAVAL*. Cyclic pattern of 17β-estradiol pretreatment protects the hippocampal ca1 region against cerebral ischemia.

University of Florida:

From the laboratory of Dr. Tom Foster

Poster # 6) K. BODHINATHAN*, A. KUMAR, T. C. FOSTER. Influence of redox state on the afterhyperpolarization in CA1 pyramidal neurons: role for ryanodine receptor oxidation during aging.

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Poster # 8) A. KUMAR*, A. RANI, K. BODHINATHAN, AND T. C. FOSTER. Selective estrogen receptor agonists, PPT and DPN differentially regulate hippocampal synaptic transmission in estrogen receptor alpha and beta KO mice.

Poster # 9) W. -H. LEE* AND T. C. FOSTER. The role of SOD1 in brain aging.

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Poster # 10) B. K. ORMEROD, R. B. SPEISMAN*, A. KUMAR, T. C. FOSTER. Biomarkers predict successful versus unsuccessful aging in rats.

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Poster # 11) F. A. SIEBZEHNRUBL*, R. CORAS, E. PAULI, H. B. HUTTNER, M. NJUNTING, K. KOBOW, C. VILLMANN, E. HAHNEN, W. NEUHUBER, D. WEIGEL, M. BUCHFELDER, H. STEFAN, D. A. STEINDLER, I. BLUMCKE. Learning is related to the regenerative capacity of the human hippocampus.

University of Alabama at Birmingham:

From the laboratories of Drs. Rita Cowell, Linda Overstreet-Wadiche, and James Meador-Woodruff

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Poster # 35) M. R. PENNER*, T. L. ROTH, F. D. LUBIN, E. D. ROTH, L. T. HOANG, J. D. SWEATT, C. A. BARNES. DNA methylation of zif268 is not dynamically regulated within the aged hippocampus following spatial behavior.

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MBRF Sponsored Abstracts

at the

2009 Society for Neuroscience Conference

Executive Director: Dr. Ralph L. Sacco, M.D.
Scientific Director: Dr. Clinton Wright, M.D.

Pharmacological manipulations of NO signaling influence the acquisition and consolidation of fear learning

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Fear learning requires activation of the N-methyl-D-aspartate receptor (NMDAR) which leads to calcium influx and activation of neuronal nitric oxide synthase (nNOS). In the brain, nitric oxide (NO) produced by nNOS acts as a retrograde neuronal messenger that participates in synaptic plasticity including late phase long-term potentiation (LTP) and the formation of long-term memory (LTM). Yet, little is known about the role of NO signaling in classical Pavlovian conditioning. The fear conditioning paradigm is used to investigate the roles of various genes, neurotransmitters and substrates involved in learned fear associated with contextual and auditory cues. Recently we have shown that mice with a targeted deletion of the nNOS gene, nNOS knockout (KO) mice, exhibited a severe deficit in contextual fear learning (60%) and a relatively milder deficit in cued fear learning (15%), which implicated the nNOS gene in both hippocampus- and amygdala-mediated fear learning (Kelley, et al., 2009 Learn Mem, *in press*). To further define the role of NO signaling in fear conditioning, a pharmacological investigation was performed. The selective nNOS inhibitor S-methylthiocitrulline (SMTC; 20 & 50mg/kg; ip) was administered to WT mice and the NO donor molsidomine (10mg/kg; ip) was administered to nNOS KO mice 30min prior to fear conditioning. The acquisition and consolidation of fear learning were investigated in short-term and LTM tests. In WT mice the selective nNOS inhibitor significantly impaired LTM of contextual but not cued fear learning. In nNOS KO mice, treatment with the NO donor significantly improved the acquisition and LTM of both contextual and cued fear learning, which reached similar magnitudes observed in control WT counterparts. The results support the role of NO signaling in fear learning, and validate our previous studies with nNOS KO mice. Further, the results from WT mice suggest that inhibition of nNOS may suppress the development of context-dependent fear response. Understanding the requirement of NO signaling in fear conditioning could lead to the development of new treatments strategies for fear-related disorders such as posttraumatic stress disorder (PTSD).

Nicotine conditioned place preference depends on sex and age

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Department of Psychiatry and Behavioral Sciences, University of Miami Miller School of Medicine, Miami, FL 33136

Although women now constitute half of all smokers and many studies suggest that adult males and females may differ in factors that maintain tobacco smoking, there is little information about sex differences in nicotine reward. In addition to sex differences, there also are indications of age differences in nicotine reward. Limited studies suggest that adolescent male rats self-administer more nicotine than adults, suggesting that drug administration during adolescence leads to different behavioral effects than during adulthood. However, it is not known whether sex differences in nicotine reward exist during adolescence as well. In the present study, sensitivity to the conditioned reward of nicotine was evaluated in male and female adolescent and adult rats. The main hypothesis of this proposal was that females and males would respond differently to nicotine and that the differential responses would be age-specific. Adolescent (PND 34) and adult (PND 66) male and female rats were tested using a conditioned place preference (CPP) procedure. Nicotine CPP was begun with a pretest, followed by three days of training with nicotine (0.1, 0.2, 0.4, 0.6, 0.8 or 1 mg/kg) and saline. The day after the last conditioning session, a posttest was conducted and the time spent on each side was recorded for 30 min. Nicotine CPP in adolescent females was of greater magnitude than in adult females, suggesting that they are more sensitive to the conditioned rewarding effects of nicotine. In adolescents, nicotine CPP occurred in response to a lower dose of nicotine in males than females, suggesting than nicotine is more potent as a reward to males than females during adolescence. However, the dose-response curve for nicotine CPP was very steep in males, with only a single dose producing a significant reward. In contrast, for adolescent females, the curve was broader than males. Thus, it may be that it is more difficult to determine nicotine reward in males than females. In conclusion, these data support the idea that adolescence is a critical period of time where individuals are vulnerable to nicotine reward. In addition, sex plays an important role in determining nicotine reward during adolescence. These findings suggest that it will be necessary to take this into account in the development of sex- and age-specific strategies for improving smoking cessation treatments.

Support: NIDA and ORWH grants DA024584 and DA015119

Identification of mitochondrial targets for protein kinase c delta following cardiac arrest

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Mitochondrial dysfunction following cerebral ischemia results in cell death. Protein kinase c delta (δPKC) may be involved in mitochondrial dysfunction mediated apoptosis in hippocampal CA1 pyramidal neurons. During the initiation of apoptosis, δPKC translocates from cytosol to cellular organelles including mitochondria. We previously observed the release of cytochrome c from the mitochondria and the activation of δPKC following cerebral ischemia as proximate temporal events. We hypothesized that δPKC translocates to hippocampal mitochondria following global cerebral ischemia resulting in the phosphorylation of target proteins which contribute to mitochondrial dysfunction and sought to identify some of the mitochondrial substrates of δPKC. Eight minute of global ischemia was induced in a rat model of asphyxial cardiac arrest (CA) with control animals subjected to sham surgery without CA. One hour after resuscitation the hippocampi were rapidly isolated and δPKC protein levels quantified in the mitochondrial fraction by immunoblotting. Mitochondrial δPKC levels increased by 99±19% ($p<0.02$, $n=4$) when compared to sham animals. Hippocampal synaptosomes were treated with either the δPKC activator peptide ψδRACK or the carrier peptide Tat (control) (1 μM final concentration) for 15 min with subsequent mitochondrial isolation. Mitochondrial proteins were separated using 1D SDS-PAGE electrophoresis. Gels were stained for phosphoproteins using ProQ Diamond reagent followed by coomassie blue reagent and the ratio of phosphorylated proteins to total proteins was quantified by densitometry. Protein phosphorylation was significantly increased in three bands (MW ~45, 30 and 7 kDa) by 47 ($p<0.05$), 45 ($p<0.02$) and 199 % ($p<0.001$) as compared to control, respectively. Within the 45 and 30 kDa bands, 8 and 10 proteins were identified using liquid chromatography tandem mass spectrometry (LC MS/MS) analysis, respectively. Further studies are ongoing to determine which of these 18 proteins are phosphorylated in an unbiased manner. The identification of these specific targets will yield important information regarding the mechanism by which δPKC mediate ischemic mitochondria dysfunction.

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The aged hippocampus exhibits lower levels of RACKI and RACKII, and increase GSK-3β levels

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Several studies demonstrated that the aged rat brain is more susceptible to ischemia than young and middle-aged rat brains. εPKC is involved in neuroprotection against cerebral ischemia. Previously we showed lower levels of εPKC in particulate fractions of old rat hippocampus compared to those in young and middle-aged rats. Following activation of different PKCs, they translocate and bind to membrane anchoring proteins called receptors for activated C kinase (RACKs) and phosphorylate their protein targets. One such target is glycogen synthase kinase-3β (GSK-3β). GSK-3β is an apoptotic mediator that can be inactivated by εPKC-mediated phosphorylation. We hypothesized that the increase in vulnerability in the aged brain is, at least in part, due to lower phosphorylation of GSK-3β resulting in a subsequent activation of cell death pathways. To test our hypothesis, we first determined levels of RACK1, RACK2, total GSK-3β and phosphorylated GSK-3β (pGSK-3β) in hippocampus of young (n=4, 4 month-old), middle-age (n=4, 12 month-old), and old (n=4, 24 month-old) Fisher 344 rats by Western blot. Results were expressed as mean±SEM. Statistical significance was determined with an ANOVA test followed by a Bonferroni's post-hoc test. Both RACKI and RACKII levels were significantly lower in the aged animals by 41% (58.53±7.2, $p<0.05$) and 66% (34.14±8.2, $p<0.02$) when compared to young rats, respectively. When comparisons were made between middle-aged and aged animals, RACKI and RACKII levels were significantly lower in the latter group by 63% (121.95±3.7, $p<0.02$) and 43% (80.48±11.5, $p<0.05$), respectively. No significant differences in the RACKI and RACKII levels were observed between young and middle-aged groups. The levels of GSK-3β were significantly higher in aged animals by 145% (245±7.2, $p<0.01$) and 127% (118±9.7, $p<0.01$) compared to young and middle-aged rats, respectively. However, in aged rats, levels of pGSK-3β were significantly lower by 57% (42.0±5.3, $p<0.01$) and 69% (112±7.6, $p<0.01$) compared to young and middle-age rats, respectively. No significant changes were observed in the levels of GSK-3β and pGSK-3β among the young and middle-aged groups. We conjecture that the increased cerebral susceptibility after ischemic injury in aged rats may be mediated, at least in part, to lower phosphorylation of GSK-3β owing to the lower levels of εPKC in the particulate fraction of hippocampus from aged rats.

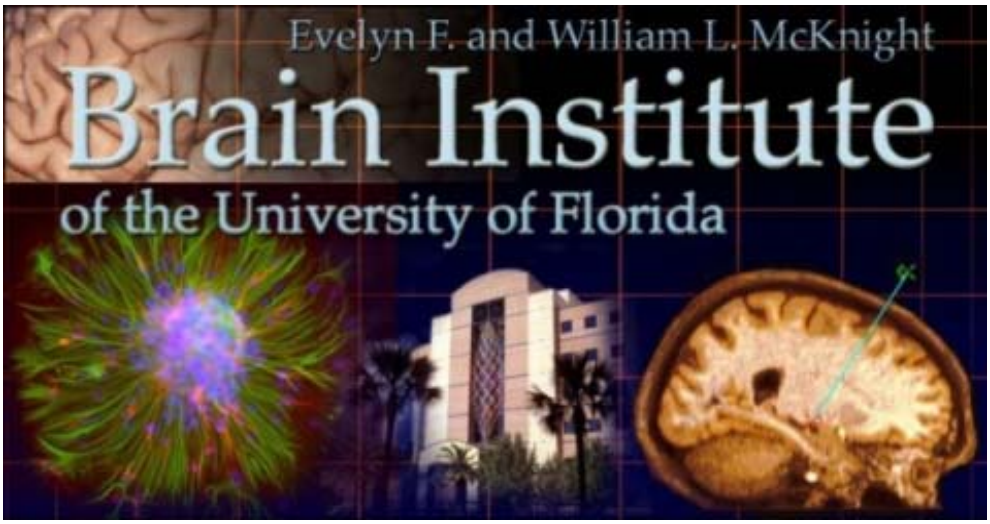
Cyclic pattern of 17β-estradiol pretreatment protects the hippocampal ca1 region against cerebral ischemia

N. HIRSCH[1], I. SAUL[1], K. R. DAVE[1], R. A. DEFAZIO[1], H. BRAMLETT[2], M. A. PEREZPINZON[1], A. P. RAVAL[1]

[1]Neurol., Univ. of Miami, Miami, FL; [2]Univ. of Miami, Neurological Surgery, FL

Failure of the Womens' Estrogen for Stroke Trial raised concern regarding the safety of chronic estrogen treatment in women. A single 17β-estradiol (E2) bolus 48 h prior to ischemia induces neuroprotection in the hippocampal CA1 region in rat model of global cerebral ischemia (Raval et al., Neuroscience; 2009). Based on this result and because E2 is released in a cycling manner, we hypothesized that cyclical E2 treatment provides neuroprotection against cerebral ischemia in the rat model. Normal cycling female rats were ovariectomized (OvX) and 7 or 30 days later an injection schedule of E2 (5 μg/Kg; i.p) or vehicle (oil) was started. Rats were injected with E2/oil at an interval of every 48 or 72h for 21 days. Forty eight or seventy two hours following the last E2 treatment rats were exposed to cerebral ischemia produced by 10min of bilateral carotid occlusion and systemic hypotension (50mmHg). Seven days after cerebral ischemia, rat brains were fixed for histopathological assessment. Hippocampal sections at the level of 3.8 mm posterior to bregma were examined for normal neurons in CA1 region. The number of normal neurons per slice in the CA1 hippocampal region in naïve rats was 1100±45 (mean ± SEM; n=4). The ischemic insult to OvX rats decreased the number of normal neurons to 18% of naïve (192±10, n=6, $p<0.05$; ANOVA test followed by a Bonferroni's post-hoc test). Intermittent estradiol-17β treatment to seven days OvX rats prior to cerebral ischemia increased the number of normal neurons to 52% (559±13, n=7, 48h interval) and 41% (446±14, n=7, 72h interval) compared to the naive group ($p<0.05$). Vehicle treatment did not show any significant difference in the number of normal neurons versus OvX groups. Female rats receiving sham-OvX showed no difference from naive in neuron loss after ischemia. Interestingly, intermittent E2treatment given to rats after thirty days of ovariectomy was not beneficial against ischemia. A cyclic pattern of E2 bolus treatment conferred protection against ischemia in seven days ovariectomized rats. This study emphasizes the need to investigate a cyclical E2 replacement regimen in post-menopausal women while avoiding the known side effects of chronic estradiol treatment.

Support: AHA-National center # 0730089N, James and Esther King Biomedical Research



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

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

Stress-induced changes in neural expression of the organic cation transporter: Insights from an animal model of depression

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[1]Neurosci., Univ. Florida, Gainesville, FL; [2]Psychology, Univ. of Florida, Gainesville, FL

Major Depression is one of the leading causes of disability, lost time from work, and diminished quality of life worldwide. Several lines of evidence indicate that stress-related processes invoke depressive symptoms in vulnerable individuals. The Wistar-Kyoto rat has been characterized as an animal model of depressive-like behavior and high stress responsiveness that may provide new insights into the neurobiological processes that underlie Major Depression. The organic cation transporter-3 (OCT3) is a low-affinity, corticosterone-sensitive monoamine transporter that has been associated with a variety of disorders including obsessive-compulsive disorder (OCD), substance abuse, and depression. Acute inhibition of the OCT3 by corticosterone causes a rapid increase in extracellular serotonin concentrations in parts of the brain that mediate behavioral responses to stress, which are often exaggerated in certain types of psychopathology such as mood disorders and depression. In this study, we quantified neural expression of the OCT3 in limbic regions of the brain by 1) in situ hybridization and 2) Western blot in both stressed and unstressed Wistar-Kyoto and Long-Evans rats. We postulate that altered endogenous and stress-induced expression of the OCT3 in the Wistar-Kyoto rat compared to Long-Evans may be related to its depressive phenotype and poor stress regulation. We are also characterizing the neurochemical and behavioral effects of decynium 22 (D-22), a specific OCT3 antagonist, in Wistar-Kyoto and Long-Evans rats. Previous studies have found an acute rise in extracellular serotonin in the dorsomedial hypothalamus following D-22 administration, while others have reported an antidepressant effect of D-22 in mice lacking the serotonin transporter (SERT). The results of these studies will indicate whether OCT3-mediated serotonin signaling is altered in the Wistar-Kyoto relative to Long-Evans, which may elucidate the neurobiological substrates underlying stress-related psychopathology.

Neurotensin plays a modulatory role in pharmacologically-induced self-injurious behavior

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Repetitive, self-injurious behaviors are frequently exhibited by individuals with neurodevelopmental and psychiatric disorders. These stereotyped behaviors can produce physical injury, and they include head-banging, self-punching, self-biting, and others. Pemoline, an indirect monoamine agonist, produces stereotyped self-biting in rats and we are using this model of self-injury to elucidate the neurobiological mechanisms that change in response to pemoline administration, and which underlie the expression of self-injury. Previously we found that changes in dopaminergic and glutamatergic neurotransmission are involved in initiation of pemoline-induced self-injury. We've also shown that circulating corticosterone is elevated in pemoline-treated rats and that chronic stress potentiates the self-injury. We are now focusing on modulators of dopamine, glutamate, and stress systems as potential mechanisms that underlie expression of self-injury, and as targets for pharmacotherapy. Neurotensin is a neuropeptide that increases dopamine and glutamate release in the striatum and stimulates the hypothalamic-pituitary-adrenal axis to increase circulating corticosterone levels. We have found that neuronal content of neurotensin is elevated in the striata of self-injurious pemoline-treated rats compared to the content in controls. Furthermore, a neurotensin agonist, PD 149163, significantly enhances pemoline-induced self-injury, whereas our preliminary data indicate that the neurotensin antagonist, SR 48692, reduces pemoline-induced self-injury. We are continuing to explore the exact neurobiological substrates that are responsible for the potentiating and attenuating effects of the neurotensin agonist and antagonist, respectively. A preliminary analysis reveals an important role of extracellular dopamine and serotonin in the striatum. Future analyses will focus on evaluation of postsynaptic events (e.g. phosphorylation of intracellular signaling molecules) that are important for the modulatory effects of neurotensin neurotransmission on the expression of pemoline-induced self-injury.

Influence of redox state on the afterhyperpolarization in CA1 pyramidal neurons: Role for ryanodine receptor oxidation during aging

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The excitability of the CA1 pyramidal neurons of the hippocampus is reduced in aged, memory impaired animals. The Ca2+-activated K+-mediated afterhyperpolarization (AHP) which has been previously reported to increase in the CA1 pyramidal neurons during aging, contributes in part to the decrease in neuronal excitability. In this study we investigated the role of redox state in mediating the increase in the AHP of the aged neurons. Dye-based detection of reactive oxygen species indicated a more oxidative state in the CA1 pyramidal neurons of aged (20-24 month old) F344 rats when compared to young (6-9 month old) rats. The AHP was recorded from the hippocampal CA1 pyramidal neurons of aged F344 rats using sharp microelectrode techniques. Application of the reducing agent dithiothreitol (DTT, 0.7 mM) significantly decreased the AHP to 47.68 ± 12.93%, ($p<0.05$) from the averaged baseline level (5.92 ± 1.43 mV). In order to investigate the mechanism for DTT effects, the ryanodine receptor (RyR) antagonist - ryanodine (20 μM) was applied before addition of DTT. Ryanodine reduced the AHP to 62.17 ± 3.34 % and blocked the effect of DTT. In order to eliminate the possibility that ryanodine-mediated blockade of DTT was due to floor effects as a result of a reduction in AHP by ryanodine, we increased the extracellular calcium to 4 mM. Under the supra physiological levels of calcium, a robust AHP could be recorded in the presence of ryanodine (7.38 ± 2.03 mV) and the reduction in the AHP by DTT was blocked. The results suggest that the decrease in neuronal excitability associated with age-related cognitive decline is linked to the redox state through RyR activity.

Role of PHLPP1 in hippocampal neurons

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The PI3-Kinase/AKT pathway is a critical component of cellular survival mechanisms. Activation of AKT by growth factor mediated stimulation requires dual phosphorylation at Thr308 and Ser473, and regulation of these sites by kinases and phosphatases determines the level of AKT signaling within cells. Regulation of the Thr308 phosphorylation site has largely been elucidated; PDK-1 is the primary kinase responsible for phosphorylation of Thr308 while PP2A is the primary phosphatase responsible for dephosphorylation. Conversely, identification of the primary kinase and phosphatase responsible for regulation of the Ser473 site has only recently been described. mTOR/RICTOR is thought to be the primary kinase complex responsible for phosphorylation of AKT at Ser473, while the primary phosphatase is pleckstrin homology and leucine rich repeat protein phosphatase 1 (PHLPP1). PHLPP1 phosphatase exists as two distinct isoforms, PHLPP1α (~135KDa) and PHLPP1β (~190KDa). PHLPP1α is responsible for dephosphorylation of AKT at Ser473 and can strongly inhibit cell survival signaling. In addition, previous studies show BDNF mediated activation of calpain can degrade PHLPP1α in hippocampal neurons and serves to regulate total cellular PHLPP1α levels. However, whether PHLPP1β has the same function and regulation remains unknown. Recent experiments from our laboratory, using primary embryonic hippocampal neurons to study PHLPP1 signaling, observe a significant increase in PHLPP1β levels when treated with BDNF. Further, with western blot analysis, the beta isoform appears as a double band and suggests that the beta isoform is phosphorylated. Current work seeks to verify the identity of the double band using mass-spectrometry and determine what role calpains play in PHLPP1β regulation.

Selective estrogen receptor agonists, PPT and DPN differentially regulate hippocampal synaptic transmission in estrogen receptor alpha and beta KO mice

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Estrogen has been implicated in the regulation of hippocampal physiology, including synaptic plasticity. Our previous analyses suggest that both estrogen receptor (ER) subtypes, ERalpha and ERbeta, are required for rapid estradiol (EB) effects on hippocampal synaptic transmission. In the present study, we further sought to analyze role of ERalpha and ERbeta on hippocampal synaptic transmission by using selective agonist, PPT and DPN in slices obtained from ER alpha and ER beta KO female littermates. All the mice (3 -5 months old) were ovarectimized and hippocampal slices were prepared 10-12 days following ovarectomy. The effect of bath application of PPT (100 nM) and DPN (1 μM) on extracellular excitatory postsynaptic field potentials were examined at CA3-CA1 synaptic contacts using an *in vitro* hippocampal slice preparation. Synaptic responses were increased (115.80 ± 1.01 % of baseline, n = 3) 45 min following ER beta selective agonist, DPN application in slices obtained from ER alpha KO littermates; while application of PPT, an ER alpha selective agonist has no effect (100.87 ± 2.79 % of baseline, n = 5). Moreover, Synaptic responses were increased (117.17 ± 7.60 % of baseline, n = 11) 45 min following ER alpha selective agonist, PPT application in slices obtained from ER beta KO littermates; while application of DPN, an ER beta selective agonist has no effect (103.61 ± 1.39 % of baseline, n = 9) on synaptic responses. Taken together these data support the idea that both ERalpha and ERbeta contribute to rapid EB effects on hippocampal synaptic transmission.

The role of SOD1 in brain aging

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Our goal was to examine the hypothesis that impaired hippocampal function during aging is related to increased oxidative stress. We hypothesize that over expression SOD1 by viral vector in hippocampi of the aged rats can reduce cognitive deficits. The human SOD1 gene was delivered on dorsal hippocampus via lentivirus in young (3 months) and aged (19 months) F344/BN F1 male rats, and their performance in several behavior tasks were determined four months later. No effect of hippocampal over expression of SOD1 was found for sensory/motor learning in cue discrimination and object recognition memory. Examination of behavior on the spatial version of the Morris water maze indicated that over expression of SOD1 in hippocampi improved learning in aged rats, such that aged-SOD1 rats exhibited a reduction in the escape path length over the course of training ($p<0.05$). Further, aged-SOD1 rats exhibited an increased number of platform closing ($p<0.05$) relative to GFP controls during the acquisition probe trial. In contrast, no difference was observed for a retention probe trial delivered 24 hr after acquisition. For young rats, compared to aged matched GFP controls, over expression of SOD1 was associated with a tendency for impair retention of spatial discrimination ($p=0.12$ for the 24 hr platform crossings) and 1 hr retention of inhibitory avoidance training ($p=0.10$). The studies suggest that a reduction in reactive oxygen species may provide some benefit in aged animals. Therefore, current studies are attempting to improve on the treatment by examining the effects of 1 month of SOD1, SOD2 and catalase over expression on behavior and oxidation products.

GPCR dependent signaling systems are required for cannabinoid induced and CB1R independent potentiation of mIPSCs

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It has become clear that cannabinoid mediated activation of the CB1 receptor plays a prominent role in the regulation of synaptic transmission in the CNS. However, there is substantial evidence that cannabinoid ligands can also produce effects via other specific and non-specific mechanisms. Recently, our lab discovered a novel cannabinoid induced potentiation of miniature IPSCs observed in hilar mossy cells that is independent of CB1, CB2, and vanilloid type I receptors. This potentiation is limited to action potential independent exocytosis, and can be induced by exogenous application of both synthetic and natural cannabinoid receptor ligands. We used whole cell patch clamp recordings in an *in vitro* preparation of the rat dentate gyrus (18-25 days old) to determine whether cannabinoid induced potentiation of mIPSCs requires activation of a G-protein coupled receptor, and to further reveal the presynaptic signaling mechanisms involved. Although cannabinoid receptor ligands are generally small and highly lipophilic, several lines of evidence argue against a non-specific effect on mIPSC frequency. For example, consistent with prior data, we noted that bath application of 2-AG produced significantly less potentiation of mIPSC frequency than anandamide, even when delivered at 60X higher concentration (30 μM vs. 500 nM, respectively). Further, we found that both WIN55,212-2 and its analog, WIN55,212-3, can induce potentiation of mIPSCs even when bath applied at nM concentrations. While these experiments argue against a non-specific action, additional work suggests that GPCR dependent signaling is explicitly required. For example, we noted that bath application of WIN55,212-2 fails to enhance mIPSC frequency in slices preincubated for 1 hour in 20 μM suramin (a treatment which effectively uncouples G-proteins from their receptors). Although suramin is also a P2X receptor antagonist, another non-selective P2X receptor antagonist (PPADs, 20 μM) failed to block WIN55,212-2 mediated facilitation of mIPSCs. Further, we noted that 5 μM WIN55,212-2 fails to potentiate mIPSCs in the presence of the PKA inhibitor H-89 (10 μM). Because PKA is often activated downstream from adenylyl cyclase, we also tested the ability of forskolin, an adenylyl cyclase agonist, to induce potentiation of mIPSCs. Consistent with our previous result, we found application of 20 μM forskolin induces a robust increase in the frequency of mIPSCs. Cumulatively, our results to date imply that cannabinoid induced potentiation of mIPSCs in this system likely depends on GPCR mediated activation of both adenylyl cyclase and PKA.

Complex mechanisms govern multiple pulse depression of evoked IPSCs recorded from hilar mossy cells in the rat dentate gyrus: Distinct roles for both GABAA and GABAB receptors?

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Previous work from our lab has focused on identifying the mechanism through which bath application of carbachol (CCh) leads to inhibition of evoked IPSCs recorded from hilar mossy cells in the rat dentate gyrus. CCh-mediated inhibition of evoked IPSCs was found to be insensitive to CGP 52432 (a GABAB antagonist), to AM251 (a CB1 antagonist), and to AFDX-116 (a muscarinic M2 antagonist). In fact, several lines of evidence suggested that CCh-induced inhibition depends critically on an M1/M3 mediated increase in ambient GABA and subsequent activation of presynaptic or axonal GABAA receptors. Some more recent work has strengthened that hypothesis. For example, we now report that bath application of CCh sufficient to inhibit evoked IPSCs fails to block GABAB mediated IPSCs in the presence of picrotoxin (a GABAA receptor antagonist). Further, we find that sIPSC amplitude, as observed in CCh, is positively correlated with CCh-induced decreases in evoked IPSC amplitude. However, other recent findings have revealed further complexity in the system. For example, we now report that although CCh-mediated inhibition of evoked IPSCs is entirely insensitive to CGP, bath application of the GABA transport blocker NO-711 produces inhibition of evoked IPSCs that is entirely reversed by CGP. Consistent with that observation, bath application of baclofen (a GABAB agonist) profoundly inhibits CCh-sensitive evoked IPSCs, apparently through a presynaptic mechanism. Subsequent work has examined the relationship between CCh-sensitive and GABAB-sensitive mechanisms in multiple pulse depression of minimally evoked IPSCs. Intriguingly, robust MPD produced by a 10 Hz train is effectively eliminated by the combination of CCh and CGP. Further, separate experiments clearly indicate a role for GABAB dependent inhibition early in the pulse train, while CCh-sensitive (presumably GABAA dependent) depression is more prominent at later time points. Current work is testing a model in which GABAB receptors located on the presynaptic terminal function as conventional autoreceptors and produce feedback inhibition under light to moderate load, while ionotropic GABAA receptors located at slightly more distant axonal/preterminal positions function as an analog gate capable of scaling the action potential before it enters the terminal under conditions where the synaptic load is high and/or ambient GABA concentrations are more elevated. One as yet untested prediction of this model is that axonal/preterminal GABAA receptors present in this system should be less sensitive to desensitization than presynaptic GABAB receptors.

Group I mGluR mediated and endocannabinoid dependent LTD in the hilar region of the rat dentate gyrus

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We report that bath application of the group I mGluR agonist (RS)-3,5-dihydroxyphenylglycine (DHPG) causes acute inhibition of evoked IPSCs recorded from hilar mossy cells, and that significant long term depression (LTD) of synaptic transmission remains following washout of DHPG. Subsequent experiments using minimal stimulation techniques revealed that expression of both acute and long term effects of DHPG are restricted to a subset of GABAergic afferents that are also sensitive to depolarization induced suppression of inhibition (DSI). Experiments with a selective CB1 antagonist and with transgenic animals lacking CB1 receptors indicate that all effects of DHPG, like DSI, depend on activation of CB1 receptors. Further work with selective mGluR antagonists suggests a direct involvement of mGluR1 receptors. Interestingly, we also report that induction of LTD under our experimental conditions does not require direct somatic depolarization via the patch pipette, and does not appear to depend critically on the level of activity in incoming GABAergic afferents. In fact, in sharp contrast, we noted that the level of activity in glutamatergic afferents was strongly, and inversely, correlated with the effects of DHPG. Further, bath application of a low nM concentration of tetrodotoxin was found to selectively inhibit excitatory inputs to hilar mossy cells, and to substantially enhance both the acute and long term effects of DHPG. This result suggests that synaptic activity from TTX sensitive glutamatergic afferents to hilar mossy cells is likely to be a strong endogenous regulator of mGluR dependent and endocannabinoid mediated inhibition in this system.

Adeno-associated viral vector delivered somatostatin as a neuroprotective agent for temporal lobe epilepsy

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Background: Gene therapy provides a promising, less invasive alternative treatment for epilepsy. Preliminary results in our lab show an antiepileptogenic and neuroprotective effect of AAV delivered somatostatin (SST) in electrically kindled rats. However, the putative mechanism(s) remain elusive. Our study will elucidate these mechanisms using in vitro slice electrophysiology, while also addressing co-morbidities associated with temporal lobe epilepsy. This will be done by testing learning and memory using the Morris Water Maze.

Methods: The efficacy of gene delivered SST was assessed in electrically kindled adult male Sprague-Dawley rats by monitoring behavior and EEG dynamics in three groups of rats. Group 1 comprised of rats with bilateral bipolar stainless steel electrodes implanted in the amygdala for electrical stimulation. The kindling paradigm continued until the rats were fully kindled (i.e. attained 3 consecutive grade 5 seizures on the Racine scale). Group 2 consisted of identically prepared animals with the addition of injections of 8-10 ul AAV5-SST into the dentate gyrus and CA1 of the hippocampi bilaterally prior to electrode implantation. Group 3 was composed of sham-injected rats with AAV5 expressing GFP instead of SST. All animals were then perfused, and brains dissected out for histology to detect the presence of SST and GFP as well as any micro- and astro-gliosis. Another set of rats was injected with AAV5-SST and trained in the Morris Water Maze to assess cognitive impairment. These rats were also used for electrophysiological experiments to record the frequency of sIPSCs from granule cells to get a first estimate of the mechanism of SST action.

Results: Group 1 rats (n=7) were fully kindled at 21 +/- 3 stimulations with 1270 seconds spent in afterdischarges, which are a hallmark of seizure activity as detected by EEG. 6/8 group 2 animals did not achieve a fully kindled state, while the remaining 2 reached this endpoint at 26 +/- 3 stimulations and visual histological analysis revealed a higher number of SST positive cells as compared to group 1. Animals in group 3 (n=5) were fully kindled after 20 +/- 3 stimulations and 1256 seconds spent in afterdischarges. Preliminary analysis of cognitive impairment assessed using the water maze showed SST injected rats performing better 24 hrs post training when compared to uninjected controls.

Conclusion: Our results suggest that AAV delivered somatostatin in the dentate gyrus and CA1 hippocampal regions modulates seizure threshold, protects against the development of generalized seizures, and may lead to effective alternative treatments for refractory epilepsy with minimal side effects.

Peripheral expression of gelsolin and behavioral effects in a mouse model of Alzheimer's disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disease. It is the leading cause of dementia and is the 7th leading cause of death in the United States. A pathological hallmark of Alzheimer's disease is amyloid plaques, which are mostly composed of the 40-42 amino acid long peptide amyloid β (Aβ). The action of γ and β secretases on the transmembrane amyloid precursor protein results in the production of Aβ, whose fibrillar form is prone to abnormal folding and deposition to form amyloid plaques. The widely supported amyloid cascade hypothesis attributes a causative role to these plaques in Alzheimer's disease. Thus, they have been considered targets for therapeutic intervention. Plasma gelsolin is an 89kD actin-binding protein that has been shown to bind to and disassemble preformed Aβ fibers and to prevent the fibrillization of Aβ. Our group has shown that peripheral delivery of plasmid DNA coding for plasma gelsolin reduces the amyloid burden and total guanidine-extracted amyloid concentration in two different APP/PS1 lines of transgenic mice. The effects of peripheral gelsolin expression on memory related behavior have yet to be described. Adeno-associated virus serotype-8 vector coding for plasma gelsolin was delivered via the tail vein to APPSWE/PS1δE9 mice (APP + GEL). APP+GEL mice and aged-matched non-treated APP/PS1 and wild type controls were tested in a five day radial arm water maze (RAWM) to test spatial learning and memory. Performance on the maze was measured by latency to find a platform that was hidden at the end of one arm of the maze over repeated trials when starting from a randomly selected start arm, and the number of errors (entries into an arm where the platform was not located). On probe trials the wild type mice showed significant improvements on both measures, by the third day of testing and, the APP/PS1 control mice showed improvement by the fifth day, indicating that each of these groups eventually learned the task. However, the gelsolin mice did not show improvement by the end of the testing. These data support the hypothesis that amyloid plaque formation may be protective and serve to prevent memory loss and support the finding that soluble Aβ levels correlate more strongly with dementia severity. The dissolution of these plaques as a result of increased gelsolin may increase levels of soluble Aβ oligomers. Recent evidence suggests that these oligomers are more toxic to nerve cells than insoluble aggregates because oligomers attack nerve cell synapses. Thus, reduction of insoluble Aβ aggregates due to peripheral gelsolin expression may result in impaired memory.

Hippocampus-specific overexpression of inhibitor 1 of protein phosphatase 2A via adeno-associated virus serotype-5 reduces alternation behavior in wild-type and APPSWE/PS1δE9 transgenic mice

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Age-related inhibition of protein phosphatase 2A (PP2A) may play a key role in the development of tauopathy in Alzheimer's disease (AD). PP2A is a primary dephosphorylator of tau and also regulates many tau kinases. It was hypothesized that overexpression of I1PP2A (inhibitor 1 of protein phosphatase 2A), an endogenous inhibitor of PP2A, would introduce tau pathology to the B-amyloid pathology in transgenic APP mice, and that this would accelerate the cognitive decline observed in this AD model. Using a recombinant adeno-associated virus serotype 5 (rAAV-5) vector, we transduced I1PP2A or GFP (as a control) via bilateral hippocampal injections in 3-4 month old double-transgenic APPSWE/PS1δE9 (APP) and wild-type (WT) mice (Treatment Groups: APP+I1PP2A, APP+GFP, WT+I1PP2A, & WT+GFP). Animals were tested in a Y-maze for memory-related alternation behavior one and two months after injections. One month following injections, 3 animals from each group were examined histologically for amyloid (via 6E10 antibody), hyperphoshorylated tau/paired helical filaments (AT8 antibody), and for thioflavin S labeling. Spontaneous alternation was significantly reduced one month after gene delivery in WT+I1PP2A, APP+I1PP2A, and also APP+GFP. At two months, spontaneous alternation by the APP+GFP mice recovered to similar levels as the WT+GFP, while both groups receiving I1PP2A continued to show deficits, with APP+I1PP2A showing the least amount of spontaneous alternation. After one month, no differences were detected among the groups for 6E10 staining. There was an apparent increase in AT8 immunoreactivity in the APP+I1PP2A and WT+I1PP2A groups. APP+GFP and APP+I1PP2A mice showed only limited thioflavin S labeling at this age and expression duration, but some intracellular thioflavin S staining was detected in the APP+I1PP2A group. The behavioral impairment indicates that hippocampus-specific inhibition of neuronal PP2A activity via I1PP2A upregulation accelerates or complements neuropathology associated with B-amyloid overexpression. Active PP2A suppression may be a key mechanism involved in neurofibrillary and intracellular transport pathology in AD, and an important therapeutic target.

Characterizing neuronal viability within the trisynaptic pathway in rat hippocampal slices

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The hippocampus is widely studied for 'secondary damage' after mechanical brain injuries. To develop a model for studying mechanical brain injury, cell death and degeneration in the trisynaptic circuit from the entohinal cortex to the dentate gyrus (DG), DG to CA3, and CA3 to CA1 were characterized in the hippocampus of an *in vitro* brain slice preparation. Brains excised from five Sprague Dawley rats (250g female) were cut into 500 μm coronal slices and incubated in a temperature-controlled (35 - 36°C) perfusion chamber. Neurobasal-A medium was used as an artificial cerebrospinal fluid and 95% O_2/5% CO_2 was continuously supplied. Tissue slices were taken from the chamber after 1, 2, 4, 6, 8, and 10 hours and fixed in 4% buffered formaldehyde solution. Slices were frozen-sectioned at 50 μm, mounted, and stained with Fluoro-Jade C (FJC) and DAPI. Optimization of FJC staining procedures (particularly permanganate concentration and time) resulted in consistent and sensitive detection of degenerating neurons. Digital epifluorescence microscopy images were used to estimate neuronal degeneration as a function of incubation time. Image segmentation threshold values were found by statistical analysis of intensity differences between degenerating neurons and background. The number of degenerating neurons was estimated by the total number of pixels of FJC staining divided by the average number of pixels of individual neurons. The fraction of degenerating neurons was calculated by dividing the estimated number of FJC-positive neurons by the number of DAPI-labeled neurons in the same field. FJC revealed significant degeneration in hippocampus slices over 10 hours. Neurodegeneration was not observed in freshly fixed slices but was detected in dentate granule cells 1 hour after tissue slicing. Degeneration in CA3 and CA1 was detected in 3 to 4 hours after tissue slicing. After 6 hours, 30 to 40% of granule cells were labeled in DG but less than 10% of pyramidal neurons were FJC-positive in CA1 and CA3. Neuronal injury during slice preparation may mediate early excitotoxic cell death in the DG, and a subsequent release of glutamate from granule cells may induce delayed pyramidal cell death. This slice model will be useful for future analysis of mechanical injury effects on brain tissue.

Early damage in the parahippocampal gyrus, after status epilepticus, as a structural signature for the development of spontaneous seizures - A longitudinal study using enhanced MRI

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Rationale: Damage to the hippocampus and parahippocampal gyrus is observed in both patients and animal models of temporal lobe epilepsy (TLE) but it remains unclear whether this is the cause or consequence of spontaneous seizures. We used diffusion tensor imaging (DTI) and T2 relaxation times in a rat model of TLE to identify and evaluate structural changes in and around the hippocampus. **Methods:** Adult rats (n=23) were stereotactically implanted with electrodes in the right ventral hippocampus and electrically stimulated to induce status epilepticus (SE). Seizures were assessed behaviorally and longitudinal *in vivo* MRI scans were obtained pre- and post-implantation and at 1, 3, 5, 7, 10, 20, 40 and 60 days post-SE at 11.1 Tesla (T). Evans blue was injected prior to perfusion to assess breakdown of the blood brain barrier (BBB). Excised brains were imaged at 17.6T and histology performed for days 1 (n=3), 3 (n=3), 10 (n=3), 20 (n=3) and 60 (n=11) post-SE. Average diffusivity (AD), fractional anisotropy (FA) and T2 were analyzed in regions of interest in the limbic system. Histological images were co-registered with MRI using thin plate spline warping. **Results:** 8/11 rats developed spontaneous seizures (SS rats) post-SE and 3/11 (NS rats) did not. An increase in AD was observed *in vivo* in the ipsilateral dentate gyrus (DG) and CA3 subregions of the hippocampus 1 day post-SE, which correlated with Evans blue distribution suggesting BBB breakdown. In SS rats, a decrease in AD and increase in T2 was observed in the parahippocampal regions, the ipsilateral CA1 and subiculum suggestive of seizure-linked cytotoxic edema. A similar reduction in AD was observed in NS rats in the hippocampi at day 3 but no change was observed in the parahippocampal gyrus. No further structural changes were observed in these rats. An increase in AD and T2 in the parahippocampal gyrus at day 3, seen in SS rats only, corresponded to vasogenic edema resulting from cell loss (seen with Fluoro Jade C stain) and astroglial activation (GFAP immunolabeling). An increase in FA in the DG region was observed during and after the latent period which corresponded to mossy fiber sprouting (Timm's stain). Fiber tracking showed SS rats had lower fimbria/fornix volume compared to rats at day 1 post-SE or control rats. **Conclusions:** Only rats with parahippocampal damage during the first 72 hours post-SE developed spontaneous seizures, suggesting that these regions play a role during SE and subsequent epileptogenesis. DTI, not T2-weighted imaging, was useful in differentiating cytoxic and vasogenic edema during the acute phase. Co-registration of histology to MR images allowed for accurate correlation between the two modalities.

In vivo and in vitro AZT perturbs adult and perinatal neural stem/progenitor cells in mouse

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Zidovudine (3'-azido-3'-deoxythymidine; AZT) is a nucleoside reverse transcriptase inhibitor that has been used in the treatment and prevention of human immunodeficiency virus-1 (HIV-1) infection alone or in combination with other antiviral agents. In addition, AZT monotherapy has been used in pregnancy to reduce vertical transmission of HIV-1 from mother to infant since 1994. Although AZT's effect is proven in controlling viral infection in adult patients and in reducing vertical viral transmission, several studies showed adverse effects of AZT such as bone morrow suppression, cardiomyopathy, hepatotoxicity, neuropathy and mitochondrial damage. Despite the lack of active transport across blood brain barrier, AZT is shown to accumulate in high levels in cerebrospinal fluid with limited penetration into the parenchyma via passive diffusion. Due to the anatomical proximity of the neurogenic niches -Subventricular Zone (SVZ) and Dentate Gyrus (DG)- to the ventricular surfaces, we suggest that passive diffusion from CSF may be sufficient to expose neural stem and progenitor cells to deleterious levels of AZT. Here we systematically examine the effect of AZT on postnatal and adult neurogenesis in vivo and in vitro, and show that AZT substantially decreases the neurogenic potential of neural stem and progenitor cells. AZT blocks neuronal differentiation and proliferation in a model of inducible neurogenesis from astrocyte monolayers. In addition to adult neurogenesis, AZT affects postnatal neurogenic potential following in utero exposure. Investigation of the neurotoxic effects of AZT suggests the need to develop additional interventional strategies to decrease risk-benefit ratio of AZT treatment in HIV-1 therapy.

Regional and temporal functional heterogeneity among microglia: Population expansion and support of neurogenesis

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The subependymal zone (SEZ) represents one of the two regions of persistent neurogenesis in the adult mammalian brain, with new neurons being generated throughout life. Recent data utilizing a model of inducible neurogenesis (IN) indicates that microglia (MG), the resident immune cell of the brain, play an important role in the regulation of SEZ neuronal production: MG deficient cultures are incapable of IN, but IN can be restored by exposure to MG-conditioned media. As MG in the aged brain display a dystrophic morphology concurrent with diminished function, it is possible that replacement of these aged MG with a younger, more robust population could spare or partially ameliorate the diminished neurogenesis observed in the aged brain. We have shown that SEZ MG are capable of massive *in vitro* expansion, while MG derived from cortex (CTX) display a limited capacity for expansion, suggesting that the SEZ contains a unique MG progenitor cell type. Here we ask: 1) is the capacity for massive expansion intrinsic to SEZ MG, or does the expansion capacity result from microenvironment interactions with the SEZ niche; and 2) do CTX MG and SEZ MG differ in their ability to modulate IN. By combining SEZ and CTX tissue to generate heterospatial cultures, we found that exposure to the SEZ environment does not enhance CTX MG expansion. Conversely, exposure to the CTX environment inhibits SEZ MG expansion. Furthermore, addition of donor SEZ MG to either CTX- or SEZ-derived cultures suppresses the expansion of host MG, while the addition of donor CTX MG enhances the over-all MG yield. These data demonstrate that SEZ MG possess intrinsic, spatially restricted characteristics that cannot be conferred upon CTX. It is possible that SEZ and CTX MG represent unique and functionally distinct populations Introduction of expanded SEZ-derived MG to post-neurogenic SEZ cultures does not restore lost IN, yet does result in an increase in visible neuroblasts both before and after IN in neurogenic cultures, indicating that a critical period exists for the introduction of MG after which IN cannot be restored. We found that the repeated supplementation of expanded SEZ MG to neurogenic SEZ cultures allowed for sustained IN, provided that the overall MG proportions remained constrained within a fairly narrow value. It is possible that this proportion is critical for proper neurogenic function not only *in vitro* but also *in vivo*, and that deviations from this proportion may be an underlying factor in age-related neurogenic decline.

Thymidine analog-mediated anti-proliferation in brain cancer

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Thymidine analogs (TA) incorporate into newly synthesized DNA during S phase and are used ubiquitously for studies such as cell birthdating and assessment of proliferative index. Conventionally, it has been thought that TA incorporate relatively benignly into DNA, in the absence of secondary stressors such as irradiation. However, we and others have shown that incorporation of the TA bromodeoxyuridine (BrdU) and ethynyldeoxyuridine (EdU) cause a profound and progressive slowing of the cell cycle in all mammalian cells. While chemically similar, BrdU and EdU appear to exert distinct effects on treated cells. We show here that a single, low-dose pulse of BrdU or EdU leads to a progressive reduction in the proliferation rate of normal and transformed cells *in vitro*. In comparison, the anti-proliferative effect of EdU is more marked than that of BrdU. This is perhaps due to the fact that while BrdU apparently exerts this effect in the absence of overt cytotoxicity, EdU treatment is characterized by an initial period of massive DNA damage. These effects are shown in both transformed cell lines as well as in primary human glioblastoma multiforme (GBM)-derived neurospheres. This data suggests the ability of TA to, over time, severely impair cancer stem cell proliferation. Enticingly, in vitro EdU administration is effective in both temazolamide (TMZ)-susceptible and TMZ-resistant human GBM neurosphere lines, highlighting the promise of TA as a highly effective therapeutic approach that compliments current standard therapy. Finally, we show that in vivo EdU administration substantially reduces tumor bulk in a mouse model of human primary GBM. Taken together, these results support the continued investigation of TA such as BrdU and EdU as chemotherapeutic agents in the treatment of primary and recurrent brain tumors.

Top-down control of visual spatial attention: An fMRI study

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How attention modulates baseline brain activity in advance of sensory stimulation is a question of theoretical and practical interest. FMRI studies have identified bilateral frontal eye fields (FEFs) and inferior parietal sulcus/superior parietal lobe (IPS/SPL) as the essential components of a top-down attentional control network. How these components interact with one another and how attention modulates the strength of these interactions remain not well understood. To address these questions, a visual spatial attention experiment was performed in which subjects were cued on a trial-by-trial basis to covertly attend a predefined location either in the up left or the up right visual field while maintaining central fixation. Following either a long (10 seconds) or a short (2 seconds) time delay after the cue, an imperative stimulus was presented in one of the two locations. Key responses were required after stimulus discrimination. Event-related fMRI data were acquired using a Philip 3T scanner. For trials with the long delay period, GLM analysis was applied to the data sampled during the delay period to identify the activated cortices, which are presumed to correspond to the brain network involved in top-down attentional control. Functional connectivity measures such as cross correlation and Granger causality were then applied to study the interaction between these cortices and the influence they exert on the visual cortex.

Tight long-term dynamic doxycycline responsive nigrostriatal GDNF using a single rAAV vector

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Glial cell line-derived neurotrophic factor (GDNF) gene transfer is being developed as a treatment for Parkinson's Disease (PD). Due to the potential for side-effects, external transgene regulation should enhance this strategy's safety profile. Here, we demonstrate dynamic control during long-term expression of GDNF using a rAAV based bi-cistronic tetracycline-off construct. rAAV mediated over-expression of GDNF in the nigrostriatal tract has been shown to lead to loss of body-weight in rodents. In this study we utilized this phenomenon in order to study the in vivo activity of our regulated GDNF vector. Animals were bilaterally injected in the substantia nigra with rAAV expressing GDNF either under the control of the tet regulated expression cassette or a constitutive CBA promoter. Animals receiving rAAV-CBA-GDNF displayed an immediate loss of body-weight following viral injections as did animals in the rAAV-tet GDNF group receiving normal rat food (ON). Over a period of 6 months the animals in the two rAAV-tet-GDNF groups were studied in a cross-over fashion where the food was switched between normal and 3 g/kg doxycycline (dox, OFF) every 6 weeks. At the apex of each food cycle the ON animals displayed a significantly lower body-weight than that off the OFF group and a rAAV-GFP injected control, whereas the OFF group returned to body-weights indistinguishable from that of the GFP-control. GDNF levels in OFF rats were undetectable either via ELISA or histology. Striatal GDNF levels in the ON group were roughly nine-fold lower than that of the CBA-GDNF group. An additional study was designed to evaluate the whether there was a dynamic dox dose-response for striatal GDNF levels. Using the same injection paradigm as in the previous study five groups of animals were placed on dox doses ranging from 0 to 500 mg/kg food. Surprisingly, all dox doses-blocked weight loss induced in ON animals (normal food). A clear dox dose-response of striatal GDNF levels was apparent with total shut off occurring at 500 mg/kg diet. Measurements of corresponding serum levels of dox levels showed that serum levels below 0.8 g/ml were required for GDNF expression shut-down whereas 5 g/ml is the level required for antibiotic activity. Finally, since the striatum has been a preferred target in clinical trials aimed at reducing neural degeneration in PD, we also wanted to investigate whether direct injections of rAAV-GDNF to the striatum would lead to any measurable weight-loss. However, even after achieving striatal levels of GDNF similar to those resulting in significant weight-loss in the nigral injection paradigm, no reduction in body-weight due to intrastriatal injections was observed.

Epigenetic bases of neuronal diversity and plasticity in Aplysia californica: Toward single neuron epigenome

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What are the genomic bases of unique neuronal phenotypes? Epigenetics refers to inheritable modifications in phenotypes that do not involve changing the underlying DNA sequences. These mechanisms include DNA methylation of the cytosine residues in CpG dinucleotides, covalent and non-covalent modifications to the histone proteins, chromatin remodeling by the exchange of histone variants, chromatin dynamics involving the switching of active and silent chromatin, non-coding RNA including siRNA, and regulation of transcription. First, epigenetic processes can support self-perpetuating long-term molecular and structural changes in neurons and therefore, contribute to learning and memory mechanisms. Second, epigenetic modifications can be responsible for the generation of enormous diversity in neuronal phenotypes. To test these hypotheses we identified and cloned 13 canonical histones and their variants expressed in the CNS of Aplysia californica including a unique molluscan H3.4 as well as the H2Macro only described in vertebrates. We also identified 15 major histone modifying enzymes as well as more than 50 other components involved in static and dynamic chromatin remodeling. Astonishingly, using 454/SOLiD sequencing from single neurons and in situ hybridization, we show that the histones, histone modifying enzymes and many other chromatin associated proteins revealed a high level of differential expression: nearly all central neurons in Aplysia have their own unique expression profiles. This mapping study supports the idea that neuron-specific epigenetic modifications are involved in the generation of enormous neuronal diversity. Next, we investigated DNA methylation, hallmark of gene regulation; we identified and cloned an Aplysia DNA methyltransferase (DNMT). We show that as with the histone localization the AcDnmt1 also shows high levels of differential expression with enormous cell-specificity. For the first time we demonstrated biochemically the presence and dynamics of DNA methylation in single identified neurons suggesting that this process is involved in both establishing of unique neuronal identity and long-term plasticity. Moreover, we have demonstrated that two facilatory transmitters (serotonin and nitric oxide) associated to long-term forms of memory in Aplysia induced active DNA demethylation. Combined with the mapping studies, these experiments clearly point toward the central role of epigenetic processes in mechanisms of neuronal diversity, plasticity and disease. This work also opens unprecedented opportunity to study the epigenome of individual neurons at a resolution difficult to achieve elsewhere.

On the independent origins of neurons and complex brains: Insights from genomic and comparative analysis of basal animal lineages

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Here, I will summarize results of initial genomic analysis of five Ctenophore species (Pleurobrachia, Beroe, Mnemiopsis, Bolinopsis, and Dryodora) Glass and Calcium Sponges (Porifera), Placozoa (Trichoplax) and several Cnidaria species representing one of the earliest lineages of the animal kingdom as well as deep transcriptome profiling of neurons from representatives of 10 animal phyla including basal bilaterians, Xenoturbella, selected Lophotrochozoa (molluscs, annelids, phoronids) and Ecdysozoans (selected arthropods and nematodes). As a result of this comparative analysis and a novel animal phylogeny, I will discuss and reevaluate two classical scenarios for the origin of neuronal organization and memory mechanisms in animals: Monophyly vs Polygenesis. The Polyphyletic Origin (i.e. independent origin of neurons and complex brains in different lineages) is a more favorable scenario that we implemented to analyze three fundamental problems: (i) the logic of gene regulation at the scale of the entire genome in different neurons, (ii) the independent origin, parallel evolution and maintenance of the enormous diversity of neuronal cell lineages (homologous neurons) and (iii) comparative data related to trans-differentiation and neurogenesis. Injury-associated mechanisms leading to secretion of signal peptides (and related molecules) can be considered as evolutionary predecessors of inter-neuronal signaling and the major factors in the appearance of neurons in the first place. Emerging data dealing with genome-scale expression profiling at the level of single identified neurons provide additional support for this hypothesis. The evolutionary logic has been also applied to explain complex transcriptome responses following plasticity tests. Consequently, models for parallel evolution of neurons and plasticity mechanisms has been developed. Such evolutionary models will be discussed in terms of transcriptional “noise” at the genomic scale (as random sources of variability) and principles of “neuroeconomics” with feedback loops (as selection criteria in the self-organization of specific gene-regulatory circuits). In addition, growing neurogenomic information from diverse cell/neuronal lineages provides unique resources for novel strategies in Neuronal Classification and NeuroSystematics.

Synaptic transcriptome: Identification and characterization of kinesin-transported mRNAs from the central nervous system of Aplysia

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Specific mRNAs are transported from the cell body to synapses where their translation can modify communication of pre-existing synapses and induce formation of new synaptic connections. This synaptically localized pool of mRNAs consists of both translationally active and dormant mRNAs and may contribute to the molecular basis of synaptic autonomy during memory formation. Little is known about the identity of mRNAs that are actively transported, how this transport is regulated and when and how these dormant mRNAs are utilized. To identify mRNAs that are actively transported from the cell body to synapses, we focused on mRNAs that are associated with kinesin, a motor protein that transports gene products from the cell body to synapses. We have identified ~ 600 mRNAs in the kinesin complex using Aplysia microarrays, developed specifically from the neuronal EST collection, and by deep sequencing of an mRNA cargo library prepared from Kinesin-RNA protein complex isolated from the central nervous system (CNS) of Aplysia. These mRNAs encode neuropeptides, vesicle proteins, kinases, ion channels as well as those involved in protein synthesis, degradation and remodeling of cytoskeleton. We are now investigating (1) how the stoichiometry of this mRNA population are modified and (2) when these mRNAs are utilized, in response to 5HT, a modulatory neurotransmitter involved in learning and memory storage in Aplysia. By focusing on association of 10 candidate cargos to polysomes isolated from CNS, we find that application 5HT, a modulatory transmitter released during learning, induce differential loading of RNA cargos (Plexin, Spectrin, PKC, and Synaptotagmin) to polysomes, suggesting that the mRNA pool at the neuronal processes may be differentially utilized during synapse formation. Alpha-synuclein silencing via AAV mediated RNAi delivery causes nigrostriatal degeneration.

Alpha-synuclein silencing via AAV mediated RNAi delivery causes nigrostriatal degeneration

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Clinical and experimental data has shown that over expression and/or mutations of alpha-synuclein (α-syn) can evoke Parkinson-like neurodegeneration in humans and animals suggesting that down regulation of α-syn can provide therapeutic modalities for synucleinopathies. In our study we designed and selected active small interfering RNAs (siRNAs) to cleave the rat α-syn messenger RNA. Two active siRNAs and three control siRNAs were embedded in a small hairpin RNA (shRNA) and cloned in Adeno-associated virus (AAV) vectors under control of the H1 promoter and containing a GFP reporter. AAV5 expressing either active or irrelevant siRNAs were injected into the rat substantia nigra pars compacta (SNc). At 4, 8 and 12 weeks post-injection, animals were examined by amphetamine rotation test and sacrificed. Nigral injection of AAV-siRNA targeting α-syn resulted in a reduction of TH-positive cells and striatal dopamine and led to behavioral deficit at all time points. The level of neurodegeneration evoked by different siRNAs correlated with their ability to down regulate α-syn protein in tissue culture and in vivo. Use of different AAV titers demonstrated nigrostriatal degeneration in a dose-dependent manner. No cell loss was observed in the nearby ventral tegmental area in spite of a reduction in α-syn. CONCLUSIONS: Our data revealed that silencing of the rat α-syn gene by vector-based RNA interference in neurons of SNc leads to a rapid neurodegeneration of nigrostriatal dopaminergic system in adult rats. This result confirms the important role of α-syn for normal function of dopamine neurons and suggests that both up and down regulation α-syn expression can lead to nigrostriatal dysfunction.

Dynamic regulation of the Schwann cell microRNAome is essential for proper myelination

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The process of myelination in the peripheral nervous systems requires the coordinated expression and repression of a wide array of genes. Schwann cells, the myelinating cells of the peripheral nervous system, differentiate upon axonal contact, which leads to the up-regulation of myelin associated genes. MicroRNAs (miRNAs), small endogenously derived regulatory RNA molecules, have been demonstrated to repress the expression of genes by binding in a reverse complementary manner to the 3'-untranslated region of targeted genes. Previous work from our laboratory has shown at least one Schwann cell myelin gene, peripheral myelin protein 22, is subjected to regulation by miR-29a. This current work is focused on elucidating the role of Schwann cell miRNAs during the process of myelination. Utilizing lentiviral shRNA vectors, we are able to achieve over 90% transduction efficiency of Schwann cells when evaluated using an encoded GFP marker. Employing shRNA lentiviral particles, we inhibited Dicer expression by greater then 70% in primary rat Schwann cells, thus reducing their ability to produce mature miRNAs. By selectively reducing Dicer expression in the Schwann cells, we are able to focus our investigation exclusively on the glial miRNA contribution to the myelination process. When compared to cells transduced with a control shRNA, the Dicer shRNA cells had a 50% increase in proliferation as measured by BrdU assay, a characteristic of non-differentiated Schwann cells. In addition, the Dicer shRNA cells when seeded onto cultured dorsal root ganglion neurons did not form compact myelin as measured by myelin basic protein immunoreactivity and western blot. Finally, forced expression via lentiviral vectors of a miRNA, miR-29a, previously demonstrated to be down-regulated in differentiated Schwann cells, also impaired the myelination capacity of the cells *in vitro*. These data suggest that dynamic regulation of the Schwann cells 'miRNAome' is essential for myelination and implicates the miRNA pathway in both development and differentiation of Schwann cells.

Lipopolysaccharide induced activation of circulating inflammatory molecules exert a negative influence on hippocampal progenitor cell differentiation in the adult brain

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Although the function of adult neurogenesis has not been elucidated completely, a strong correlation is emerging between performance on spatial memory tasks and optimal levels of neurogenesis. Previous reports have shown that neuroinflammation, induced by lipopolysaccharide (LPS) ablates adult hippocampal neurogenesis and results in a latent memory impairment in the Morris Water Maze (Ormerod BK., 2005, Soc Neurosci Abstr 141.7). Both deficits can be ameliorated with non-steroidal anti-inflammatory drug (NSAID) treatment; however, chronic NSAID treatment is associated with adverse side effects. Here we attempt to more specifically identify components of the neuroinflammatory response that are malicious to neuronal differentiation by quantifying cytokine levels in the blood and brain of LPS-treated (0, 5, 7.5 and 10 mg/kg) adult female C57Bl/6 mice using a multiplex ELISA strategy that permits the detection of up to 32 analytes simultaneously. The mice were injected with bromodeoxyuridine (BrdU; 50mg/kg; 1 to 4 days) so that hippocampal neurogenesis could be evaluated. As expected, LPS treatment (all doses versus 0mg/kg) immediately (within 5h) increased all pro-inflammatory cytokines and chemokines, with the most robust elevations observed in TNF-α, IL-6, IL-1β, IFN-γ, IL-17 and MCP-1 levels in the blood (p's < 0.01) and hippocampus (p's < 0.01). By 96h, most analytes had returned to baseline levels. However, levels of IL-12 (p40), KC and LIX significantly overshot baseline levels and, were significantly lower in LPS- versus vehicle-treated mice 96h after treatment. Interestingly circulating levels of IL-12(p40), KC and LIX correlated negatively with new neuron (BrdU/DCX+ve) number (p's < 0.03). Our data suggest that these factors may modulate the pathways responsible for regulating cell fate decisions among hippocampal progenitor cells.

Biomarkers predict successful versus unsuccessful aging in rats

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Chronological age does not predict cognitive success across senescence, which can vary from "successful" with minimum impairment to "unsuccessful" with significant impairment despite no identifiable pathology. We have shown previously that senescent rats can be characterized as memory unimpaired (MU) and memory impaired (MI) using the spatial water maze and inhibitory avoidance tasks, which exhibit a good degree of concordance in their sensitivity to age-related memory impairments. Interestingly, senescent rats that are categorized as MI begin to show impaired cognition in middle age. We take advantage of this observation to investigate whether either hypothalamic-pituitary-adrenal axis or inflammatory biomarkers for "unsuccessful aging" emerge in middle-aged rats. To test this hypothesis young (8 mo), middle aged (14 mo), and aged (20 mo) male Fischer 344 rats were trained and tested on the spatial water maze task and then in an inhibitory avoidance task, where some animals received a mild foot shock during training and some acted as no shock controls. Based upon their performance in these tasks, the rats were described as memory unimpaired (MU) or memory impaired (MI) and then sacrificed so that blood and hippocampal/cortical tissue samples could be collected. Analytes in blood serum and protein harvested from brain tissue samples were quantified using a multiplex ELISA strategy that permits the detection of up to 23 pro- and anti-inflammatory chemokines/cytokines or 3 stress hormones simultaneously. Our preliminary results show that circulating levels of the homeostatic cytokine eotaxin and proinflammatory cytokine RANTES tended to increase with age ($p=0.086$ and 0.078 respectively). Interestingly,circulating levels of eotaxin, RANTES, leptin, Interleukin-4, and growth related oncogene (GRO-KC) were lower in animals that received shock during inhibitory avoidance training. Within the cortex and hippocampus, multiple pro-inflammatory cytokines including interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β), and interestingly interleukin-17 (IL-17), interleukin- 4 (IL-4), leptin and monocyte chemoattractant protein-1 (MCP-1) initially increased with age but then decreased in old age. Corticosterone levels increased across age, but the age x shock interaction was significant in that shock-induced increases in hippocampal (and perhaps serum) corticosterone levels decreased with age ($p < 0.05$). Our preliminary data have revealed interesting candidate biomarkers of aging that we are currently validating against performance in behavioral tasks.

Fetal and adult neural networks plated long-term on microelectrode arrays exhibit disparate activity patterns: Implications for neural progenitor cell addition

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Neural progenitor cells (NPCs) hold great potential for repairing the diseased or damaged brain, yet our knowledge about the extent that NPC-generated neurons integrate functionally into compromised adult neural networks is lackluster. We have begun characterizing the effects of transplanted NPCs on the activity of neural networks using a microelectrode array (MEA) approach that allows us to measure firing rate and spike form in 1-5 neurons located at each of the 60 electrodes and network bursting across the array. To this end, we have typically added NPCs to neural networks derived from dissociated embryonic day 18 (E18) cortical cultures, which exhibit spontaneous action potentials within a few days of plating and network bursting within one week. These well characterized cultures exhibit an "immature" modulated bursting pattern with intermittent periods of quiescence by 20-30 days and a "mature" steady bursting pattern at 35-40 days, which remains stable for the life of the culture (> 90 days). We previously demonstrated adding NPCs to a mature E18 cortical tissue-derived neural network reduced network activity (see Soc Neurosci Abs 738.8, 2008). However, primary cultures derived from the developing fetal brain may exhibit properties that are different from cultures derived from the adult brain that we are attempting to model with our MEA system. Therefore, we characterized primary cortical cultures prepared from dissociated E18 or 4-week old rat brain immunohistochemically and electrophysiologically. Similar numbers of cells in both cultures expressed neuron (β-tubulin; ~26.4±5% in adult and ~31.5±6% in fetal), glial (GFAP; ~13.7±2% in adult and 20.3±2% in fetal), and oligodentrocyte (NG2; 2.37±1% in adult and 19.4±4% in fetal). In mature cultures derived from E18 cortex, we found that NPCs, relative to NPC-conditioned media or no cell addition, reverted to an electrophysiologically "immature" state approximately 14 days after NPC addition ($p<0.05$). Our preliminary data suggest that neural networks derived from adult cortex develop more slowly than networks derived from fetal cortex, and we are currently examining the effect of NPC addition to these adult-generated networks. Our results suggest that an appropriate model may be required to accurately predict the outcome of stem cell transplant studies.

Functional somatic nicotinic receptors of the median and dorsal raphe nuclei

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Projections from neurons of the median raphe (MR) and dorsal raphe (DR) form the majority of the ascending 5-HT projections to the forebrain. Cells of the MR and DR are involved in the generation of oscillations in the hippocampus whereby the activation or inhibition of MR cells can produce long trains of desynchronization or theta rhythm, respectively, and DR cell firing is correlated with hippocampal theta rhythm *in vivo*. While immunohistochemical studies have identified nicotinic receptors (nAChRs) in both the MR and DR, there is a paucity of information available concerning functional nicotinic responses in these neurons. We used whole-cell patch clamp methods with brainstem slices from p15-22 rats and measured responses to focal somatic application of 1mM ACh (pipette concentration) in the presence of atropine. Four types of nAChR response profiles were identified in neurons of the MR nucleus: 1) cells with fast inward currents completely blocked by 50nM MLA, 2) cells with inward currents completely blocked by 50nM MLA and 1μM DHβE, 3) cells with inward currents partially blocked by MLA and DHβE containing a residual current that was blocked by mecamylamine, and 4) cells that were not responsive to ACh. In contrast, only two types of nAChR response profiles were observed in neurons of the DR nucleus: cells with fast inward currents blocked by 50nM MLA, and cells not responsive to ACh. The results indicate putative functional α7 subtype nAChRs in both DR and MR neurons and functional α4β2 in MR neurons which may also express a third functional nAChR subtype. The functional expression of nAChRs in both MR and DR serotonergic neurons implicates a potential role for nicotinic compounds in treating psychoneurological conditions such as schizophrenia, anorexia, and depression. Further, procognitive effects of nicotine-like drugs may occur from activation of neurons in the brainstem as well as traditionally studied target regions such as the cortex and hippocampus.

The pharmacological modulation of acetylcholine-evoked responses of nicotinic receptors in the lateral geniculate nucleus

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We used whole-cell patch-clamp methods with transverse brain slices from p16-25 rats to measure responses of LGN neurons to focal somatic application of acetylcholine (ACh) in the presence of atropine. In animals older than postnatal day 20 we found that neurons contain a relatively restricted/pure population of putative α4β2* nAChRs, insensitive to 50nM α-conotoxin MII, with approximately 95% of the ACh-evoked response blocked by 1μM dihydro-beta-erythroidine. ACh-evoked responses were reduced by bath applications of the weak α4β2 partial agonist cytisine in both a time and concentration dependent manner. With 10nM cytisine in the bath for 7 minutes, ACh-evoked peak responses were reduced to 18.8 ± 1.7% of their initial amplitude. We also investigated the modulatory effects of TC-2559, an agonist we confirmed in oocyte expression studies to be a selective activator of the putative high sensitivity (HS) conformation of α4β2 receptors. Using double barrel pipettes with ACh and TC-2559 (pipette concentrations of 1mM and 100μM, respectively), no measurable responses to somatic application of TC-2559 could be detected, and the magnitude of peak responses to ACh were less than half of those recorded in single barrel experiments where only ACh was present. Responses to ACh with peak magnitudes closer to control values (single barrel pipettes) could be produced by reducing the pipette concentration of TC-2559 to 30μM. However, a steady reduction in the magnitude of ACh response peaks (to ~50%) was observed over 8 min when ACh and TC-2559 were alternately applied, and there were no measurable responses to the applications of TC-2559. Bath application of 1 μM TC-2559 also produced a 63% reduction in ACh-evoked peak responses. Although it has been proposed that chronic nicotine treatments should increase the relative abundance of HS α4β2 receptors, our preliminary results indicated there was no significant increase in TC-2559 responses in animals treated for 11-12 days with nicotine at 3 mg/kg/day via osmotic minipumps. In conclusion, our results show that ACh-evoked responses of α4β2* receptors in the rat brain are potently inhibited by low concentrations of weak partial agonists. Moreover, the chronic nicotine treatments used did not appear to increase the expression of TC-2559 activated (HS α4β2) receptors. TC-2559 was generously supplied by Targacept, Inc.

Investigation of a nicotinic acetylcholine receptor with only one putatively functional agonist binding site at the single channel level

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Early single-channel data from muscle-type nicotinic acetylcholine receptors (nAChR) revealed populations of short and long duration open events. The short-lived events occurred primarily at low acetylcholine (ACh) concentrations, but longer duration openings became prevalent at higher ACh concentrations. The short and long duration events were hypothesized to arise from singly- and doubly- liganded receptors, respectively. However, other evidence suggested both agonist binding sites must be occupied by agonist for the channel to open. The question regarding what degree of agonist occupancy is required for effective nAChR activation has remained important, and open. Muscle-type nAChR contain two structurally distinct agonist binding sites; this property allows for selective perturbation of one of the two binding sites whilst the other is undisturbed. A solvent-exposed cysteine residue was introduced in the delta subunit (δL121C), which contributes a complementary face to one of the two agonist binding sites. Initial examination of this mutant receptor expressed in *Xenopus* oocytes showed similar ACh response profiles to those seen in wild-type receptors until the mutant receptor is treated with the sulfhydryl reagent, 2-aminoethyl methanethiosulfonate (MTSEA), which covalently binds the accessible cysteine residue that was introduced in the binding site. Responses of the mutant receptors containing a single MTSEA-sensitive binding site were greatly reduced at low agonist concentrations, but responses to high agonist concentrations (≥1 mM) showed little effect. Receptors containing two MTSEA-sensitive binding sites showed nearly complete loss of sensitivity to ACh. BOSC23 cells were transiently transfected with mouse muscle cDNA clones encoding either the wild-type or δL121C mutant receptor, and cell-attached patch-clamp recordings were obtained from cells that were either untreated or treated with MTSEA (2mM for 120 s). Following MTSEA treatment of mutant receptors containing a single MTSEA-sensitive binding site, the distributions of apparent open event dwell-times contained greater proportions of short events, defined as less than 1.29 ms in duration, than were observed in untreated cells at both low (1 μM) and high (30 μM) concentrations of ACh. Additionally, the upper limit of P_{open} was reduced in the presence of low ACh concentration, but remained unchanged at a high ACh concentration following treatment with MTSEA. Future experiments will include outside-out patches because this configuration will allow the same patch to be studied at various concentrations of agonist prior to and after MTSEA treatment.

Purification of immature neurons from neural stem cell progeny

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Neural stem cells (NSCs) represent a promising renewable source of cells that overcoming many of the ethical and supply issues related to transplantation. The ability to generate large quantities of cells from a small amount of starting tissue and the capacity for multi-lineage differentiation makes NSCs an attractive choice of donor material. However, several problems exist. First, following transplantation the vast majority of the cells die and second, most of the progeny differentiate into astrocytes. The development of methods to obtain an enriched population of neurons, together with the discovery of molecules that enhance their survival *in vivo*, would overcome some of the present hurdles that impede clinical application of this technology. To address these issues we have developed a new method for the generation and enrichment of neurons from a mixed population of NSC progeny and used this enriched population as a source of cells to screen for neuronal survival factors. Cultured neurospheres were plated at high density in a growth factor, serum-containing medium resulting in the production of a monolayer of astrocytes and a smaller population of precursors that would proliferate and give rise to colonies of neuronal cells (beta III-tubulin +). Using flow cytometry we exploited the distinct morphology characteristics of these two populations, allowing us to isolate an enriched population (75%) of immature neurons based solely on forward and side scatter properties. Further enrichment was obtained using a positive sorting strategy that produced a near pure population of immature neurons (97%). We also noted that the *bona fide* stem cell frequency in the neuronal cell population was reduced by two orders of magnitude (0.03% to 0.0005%). Using the NSC derived purified population of neurons as a source of immature neurons; we screened a number of growth factors and discovered that BMP4 demonstrated a strong survival effect on the cells *in vitro*. This effect was maintained following transplantation into the adult striatum where we observed a 2-fold increase in the survival of the implanted cells, a 3-fold increase in NeuN expression, an absence of proliferation and differentiation of the donor cells into glia. Our results illustrate that NSC derived immature neuronal cells can be purified to near homogeneity using the outlined strategy and will not undergo uncontrolled proliferation and glial differentiation following transplantation. We have also identified BMP4 as a survival factor for immature neurons both *in vitro* and *in vivo*. Application of this technology to human neural stem cell progeny may benefit clinical application.

Slow cycling-stem cell attribute does not impart therapy resistance in glioblastoma multiforme brain tumor

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The cogency of the cancer stem cell hypothesis is based on the notion that some cancer cells have somatic stem cell characteristics that are responsible for tumor initiation and that it is these stem cell features that impart resistance to therapy. Although compelling, this model has not been tested for many solid tissue cancers. As infrequent or slow cycling is a universal feature of adult stem cells, and a characteristic that provides protection to conventional cancer treatments, here we ask whether human Glioblastoma multiforma (hGBM) brain tumors contain a tumor-initiating slow cycling population and whether this population is resistant to radiation. In support of the cancer stem cell hypothesis we find a population of slow cycling hGBM that are able to initiate tumor formation, however, counter to the cancer stem cell hypothesis, this putative stem cell population is not resistant to radiation treatment.

Human progenitor cells differentiate into multiple neuronal phenotypes in rat brain

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Objectives:
Adult human neural progenitor cells (AHNPs) have been identified in brain specimens from elective surgery performed for the treatment of epilepsy (Walton et al. 2006). These cells can be expanded, manipulated in vitro and transplanted into host rat or mouse brains. These cells could potentially be used to treat variety of neurological diseases by replenishing populations of neurons lost due to injury or degenerative processes. We investigated the survival and development of AHNPs transplanted into immature rat brains.
Methods:
AHNPs were obtained from hippocampal specimens from patients with intractable epilepsy. AHNPs were expanded for multiple passages in vitro before being transduced with a lentiviral vector encoding recombinant human GFP. GFP-positive AHNPs were FACS sorted and expanded until transplantation. For in vivo differentiation studies, 50,000 cells were grafted into the lateral ventricle of postnatal day 1 rat pups (N=10) using a Hamilton syringe. Rats were sacrificed for histology at postnatal days 14-35 (P14-35). Rat brains were studied with fluorescence microscopy and immunohistochemistry using a confocal microscope.

Results:
AHNPs spread throughout both hemispheres of the brain and developed into mature-appearing neurons. They were seen more frequently in the hippocampus and less frequently in the neocortex. By P35, neurons with both pyramidal and non-pyramidal morphologies could be seen in all subfields of the hippocampus and dentate gyrus. They were also seen throughout all layers of the neocortex. The morphology of the AHNP-derived neurons was appropriate to the location of the cells. GFP-labeled puncta that appeared to be pre-synaptic terminals could be seen surrounding the somata of adjacent host neurons; this raises the possibility of functional communication between transplanted and host neurons. Some neurons co-expressed pavalbumin and others co-expressed somatostatin. This suggests that some neurons differentiated into specific subtypes of inhibitory interneurons.

Conclusions:
AHNPs are capable of dispersing, migrating and surviving when transplanted into immature rat brain. They can also develop morphological characteristics of mature neurons of various sub-types that appear appropriate for the host structures in which they reside. This provides

encouraging preliminary data regarding the potential of these cells for neurorestorative therapy in humans.

Learning is related to the regenerative capacity of the human hippocampus

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The brain maintains its capacity to generate new neurons throughout life. Several animal studies support neurogenesis in the hippocampus as a cellular substrate for learning. Using hippocampal resection tissue obtained from patients with focal seizures, we show that neurogenesis is decreased in humans with compromised learning. Using a novel expansion and differentiation paradigm, hippocampal progenitor cells failed to differentiate into neurons in vitro when obtained from patients with learning impairment and granule cell loss in the hippocampus. Memory scores were assessed using intracarotid amobarbital injections (WADA test). Both neuronal differentiation in vitro and granule cell loss in vivo predicted memory scores in patients. We observed loss of BDNF and cdk5 expression in patients with learning impairment. Neurons were successfully differentiated in vitro when obtained from patients with normal memory acquisition, suggesting that the regenerative capacity of the hippocampus is required for encoding new memories in the human brain.

Expansion, differentiation and transplantation of adult human neural progenitor cells from Parkinson's patients

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Parkinson's disease (PD) is one of the most prevalent movement disorders among the elderly, characterized by motor as well as non-motor (mood, behavioral and cognitive symptoms) and caused by loss of dopaminergic neurons in the substantia nigra and progressive neurodegeneration. Novel therapeutic avenues that are being explored for regenerative medicine in PD have included cell replacement therapy and gene therapy. Here, we present a novel source for autologous cell replacement in PD - adult human neural progenitor cells (AHNPs). We derived AHNP cultures from the subventricular zone, hippocampus, cortex and substantia nigra of PD patient autopsy specimens (n=4, PMI < 24 hours) as well as from intra-operatively obtained deep-brain stimulation (DBS) electrodes (n=3). New AHNP lines were isolated and cultured according to our previously published protocol (Walton et al. 2006). Patient-derived AHNP lines could be expanded to yield large numbers of progeny. Differentiation paradigms in vitro resulted in neuronal and glial phenotypes. Some cells expressed tyrosine hydroxylase in addition to neuronal markers, indicating a dopaminergic lineage. Furthermore, we transplanted AHNPs into the striatum of adult mice. A total of 50,000 cells were used per graft. AHNPs revealed unprecedented functional engraftment into host circuitry in vivo. Planned future studies will include motor and behavioral outcomes testing in rodent models of PD. These data show the possible future applicability of autologous and/or endogenous stem cell therapy for PD patients.

Insights into tumor invasion using a novel human glioblastoma cell line with lessons learned from regeneration biology

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With an average life expectancy of only 14.6 months post diagnosis, glioblastoma multiforme (GBM, WHO grade IV anaplastic astrocytoma) is both the most prevalent and perhaps, the most destructive of all adult human brain cancers. Notwithstanding its aggressive mitotic index, the root cause of this poor clinical prognosis is arguably the highly invasive character of the malignancy. While testing for the presence of multipotent, self-renewing "cancer stem cells," we challenged an array of primary human brain cancers with a novel monolayer culture system and the neurosphere assay. A new GBM cell line was established from a rare pediatric glioma, with an unsurpassed invasive phenotype. Here, we present a comparative *in vitro* and *in vivo* characterization of this new cell line against the well-characterized U-87MG human glioma cell line focused specifically on tumor infiltration within the brain. Migratory pathways followed by these GBM-initiating cells are not random; rather, highly patterned pathways are delineated. When this glioma cell line is stereotaxically transplanted into the brain of immune-compromised NOD/Scid mice, the resulting glioma circumscribes blood vessels, invades within the sub-pial space, and demonstrates a strong preference for migration along myelinated fiber tracts such as the corpus callosum. These cells are fully capable of migrating as individual "pioneer" tumor cells, however, they are more commonly observed moving collectively in parallel bands of cells, precisely following the geometry of the white matter pathway. This parallel orientation of tumor invasion strikingly resembles parallel, intra-fascicular axonal regeneration within white matter. Thus, we hypothesize that many of the selfsame "inhibitory" or instructive guidance factors that influence regenerating axons within myelinated fiber tracts, also influence the movement of an invading glioma. It is also possible that invading GBM cells may be guided by additional and/or alternative substrates interleaved between myelinated fibers. With that in mind, we have extended our investigation to include tumor invasion from the standpoint of the extracellular matrix (ECM). Within the brain, at the invasion front of these long parallel bands of cells, the expression of the ECM molecule chondroitin sulfate proteoglycan (CSPG) is decreased below background levels. This suggests that tumor invasion requires mechanisms to overcome both myelin as well as matrix-mediated inhibition. Thus, capitalizing on the infiltrative prowess of this novel cellular model, we have begun to investigate the basics of GBM invasion, guided in part, by lessons learned from regeneration biology.

Development of cilia in the mouse neocortex

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The development and function of neuronal cilia in neurons is poorly understood. Our group and others previously showed that in the hippocampus, cilia on neural precursors appear to respond to secreted factors such as sonic hedgehog, the signaling of which in turn regulates neuronal proliferation. In neocortex, a few signaling molecules and receptors are enriched in neuronal cilia such as adenylyl cyclase III (ACIII). We have studied the development of cilia in neocortical neurons using immunostaining and electron microscopy, and here we describe the dynamics of the appearance of these cellular protrusions both in projection neurons and in inhibitory interneurons during early postnatal ages. The relationship between neuron polarization and cilia orientation in the different cortical layers is also examined in mature animals.

TMS and locomotor therapies improved motor and cognitive disabilities of an experimental autoimmune encephalomyelitis (EAE) rat model for multiple sclerosis (MS)

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Symptoms and disabilities of MS appear across a broad spectrum, including motor (spasticity, weakness, and balance disorder) and cognitive impairments. Our recent EAE rat model has revealed significant neurophysiological, locomotor, balance and cognitive changes which have collectively demonstrated the feasibility of reproducing significant features of human spasticity and other disabilities in this model. Thus this animal model became amenable to applications of rigorous outcome measures and potential treatment approaches that can have direct translational potential. We used two rehabilitative approaches using a custom made bicycle locomotor training and Transcranial magnetic stimulation (TMS) to expand the evidence base for new rehabilitative interventions to document the extent to which these can safely reduce symptoms of MS-disabilities, and how these interventions influence the underlying neurobiology. By week-5 post-inoculation (pi, MBP in CFA), the EAE rats revealed significant velocity-dependent ankle extensor spasticity which contained both dynamic (higher velocities) and tonic component (at low velocities) and increased over time (to pi week 17). Moreover, TMS motor-evoked potentials (tcMMEPs) amplitudes increased in soleus (SOL) and tibialis anterior (TA) and forelimb flexor (FF) at pi week 3 (in acute stage of the disease) and drastically decreased or absent (~ 50%) especially in SOL & TA and remained increased in FF from pi week 5 through 17. Interestingly, there was a recovery in TA tcMMEPs amplitudes at pi week 17 which was not observed in SOL. In addition, these animals showed significant motor weakness in the forelimbs, a cognitive deficit for serial learning in MWM, and a significant reduction in balance tested on a rotorod. Our data indicate that EAE animals treated using TMS (25 single magnetic pulses with graded stimulus intensities from 1.2 - 2.8 Tesla (30% to 70% of Max) or cycle training (two 20 min. sessions for 5 weeks) revealed significantly decreased spasticity, increased forelimb grip strength, improved scores for serial learning, and increased balance performance. We propose that a significant portion of these disabilities are companion disorders correlated with decreased noradrenergic (NE) function in neural regions that are critical to these functions. We hypothesize that the therapeutic treatments using locomotor training or TMS significantly improved MS symptoms through an upragulation of NE function in selected spinal, brainstem, and cortical regions. These data support the exploration of these treatment modalities for delaying and reducing the severity of MS symptoms.

Director: Dr. David J. Sweatt, Ph.D.

Multi-system deficiencies in parvalbumin in mice lacking the transcriptional coactivator PGC-1α

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The transcriptional coactivator peroxisome proliferator activated receptor γ coactivator 1α (PGC-1α) is a master regulator of metabolism in numerous tissues, including heart, muscle, and liver, and it has been assumed that PGC-1α plays a similar role in the brain. We have previously shown that PGC-1α becomes localized to GABAergic neurons within the first month of postnatal life in the rodent, but little is known about its transcriptional targets in this neuronal population. Using immunohistochemistry, we observed a striking reduction in the calcium binding protein parvalbumin (PV), but not other GABAergic markers, throughout the forebrain in PGC-1α null and heterozygous mice as early as postnatal day (P) 14. Regional Q-RT-PCR confirmed this finding, as PGC-1α null and heterozygous animals had 9- and 4-fold reductions in PV mRNA levels, respectively, in the hippocampus compared to wild-type littermates at P30 (n=7/group). Interestingly, mice lacking PGC-1α did not have significant changes in the expression of glutamic acid decarboxylase 67 or other proteins expressed by interneurons at the mRNA or protein level. Electrophysiological analysis in the dentate gyrus revealed that, although spontaneous GABA release was not significantly impaired, PGC-1α null mice exhibited paired-pulse facilitation rather than depression and had increased variability in inhibitory postsynaptic currents. Behaviorally, PGC-1α null animals were hyperactive with drastic motor impairments at P30 but lacked deficits in sensorimotor gating (n=15/group). These functional and behavioral abnormalities occur before the previously described signs of neurodegeneration or decreases in metabolic gene expression. As PV is expressed outside of neural tissue, we also measured PV mRNA expression in peripheral tissues and found significant reductions in eye and heart of PGC-1α null animals. The global PV loss in PGC-1α deficient animals indicates that PGC-1α may be a direct regulator of PV. In support of this hypothesis, PGC-1α overexpression in neuroblastoma cells robustly induced PV expression by 14-fold. To our knowledge, this is the first evidence for a direct transcriptional regulator of PV. In light of the dysregulation of PV in neurological and psychiatric disorders, PGC-1α holds promise as a novel therapeutic target in such illnesses.

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Learning-related changes in unconditioned response diminution in the prefrontal cortex during Pavlovian fear conditioning

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During Pavlovian fear conditioning, a conditioned stimulus (CS) is paired with an aversive unconditioned stimulus (UCS). The expression of a conditioned response (CR) is typically taken as evidence that the CS-UCS association has been learned (Kamin, 1968; Rescorla, 1968; Wagner et al., 1968), while the unconditioned response (UCR) is considered an automatic, unlearned reaction to the aversive UCS. However, learning-related changes in the UCR have been observed in prior conditioning research. Several Pavlovian conditioning studies have previously demonstrated reductions in UCR magnitude to paired CS-UCS presentations compared to a UCS presented alone (Baxter, 1966; Orlebeke & Van Doornen, 1977). This effect is often referred to as UCR diminution. In addition, UCR diminution has been demonstrated within a number of brain regions including dorsolateral prefrontal cortex (dlPFC), suggesting that top-down mechanisms may partly mediate this learning-related process (Dunsmoor et al., 2008). The present study further investigated the role of the prefrontal cortex in UCR diminution. Healthy volunteers participated in a fear conditioning study in which skin conductance response (SCR) and UCS expectancy ratings were measured concurrently with functional magnetic resonance imaging (fMRI) using a 3T Siemens Allegra scanner. Volunteers were exposed to a two tone discrimination procedure in which one tone (CS+) predicted a loud (100dB) white noise UCS, and the second tone (CS-) was presented alone during the acquisition phase of the study. During a test phase, participants received UCS presentations that coterminated with the CS+ and CS-, as well as UCS presentions alone. UCRs within the dlPFC were reduced (UCR diminution) when the UCS followed the CS+ compared to when it followed the CS-. These findings demonstrate UCR diminution within the dlPFC, and suggest that learning-related changes within this brain region modulate UCR production during Pavlovian fear conditioning.

Histone methylation is dynamically regulated in the entorhinal cortex during consolidation of long-term memory

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Epigenetic mechanisms play an important role in the storage of long-term memories. In previous work, we observed that fear conditioning triggered changes in histone H3 acetylation and phosphorylation marks in the hippocampus at actively transcribing gene promoters (Lubin and Sweatt *Neuron* 2007, Lubin et al. *J. Neurosci.* 2008). Whether post-translational modifications of histone-tails occur in other brain regions in response to learning is not currently known. The entorhinal cortex (EC) is the principal brain structure that regulates and modulates the information being presented and processed by the hippocampus. Here we report investigations into histone methylation changes during memory consolidation; H3K9 dimethylation; a mark of transcriptional repression and H3K4 trimethylation; a mark of transcriptional activation in the EC with contextual fear conditioning (CFC). One hour after fear conditioning we observed no significant changes in H3K4 trimethylation when compared to the context alone or naive animals. Interestingly, we observed a significant increase in H3K4 trimethylation in animals exposed to context alone compared to naïve animals. We found a significant increase in H3K9 dimethylation 1 h after fear conditioning ($p < 0.05$) suggesting an increase in transcriptional repression of genes in the EC during memory consolidation. These results demonstrate differential regulation of histone methylation marks in EC in response to novel context learning versus associative contextual fear conditioning. In correlation with histone methylation, we found changes in the transcription of memory related genes, such as *mGluR1*, *bdnf, Dnmt 3b,* and *MeCP2*. In addition, our results show that specifically inhibiting histone H3K9 dimethylation (transcriptional repression) prior to CFC enhances long-term memory formation. Furthermore, pilot studies suggest that changes in histone methylation are linked to regulation of DNA methylation of genes in area CA1 of hippocampus following CFC. Current studies will determine whether this coupling of histone methylation to DNA methylation is maintained in the EC in response to CFC. Together, our findings provide additional insight into the regulation of epigenetic mechanisms within the EC (one of the foremost regions of the brain affected in Alzheimer's disease and Schizophrenia) that may contribute to abnormal regulation of gene expression in neurological disorders.

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Dynamic NR2B-chromatin structure regulation triggered by status epilepticus

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Alterations in gene expression for NR2B (an NMDA receptor subunit) is associated with the development of epilepsy. However, the molecular mechanisms regulating expression of the NR2B gene have not been explored in epilepsy. Recent work has implicated epigenetic mechanisms in the regulation of the NR2B gene in the adult CNS (Lee et al. 2008). We investigated whether altered epigenetic mechanisms contribute to abnormal regulation of genes such as NR2B following seizures. Epigenetics involves the active marking of DNA by methyl groups and post-translational modifications of histone such as acetylation, phosphorylation or methylation. In the studies presented here, we first determined the gene expression pattern of the NR2B gene in hippocampus of kainate-treated animals. We report that NR2B mRNA levels are increased in area CA1 and dentate gyrus region of the hippocampus 1 h following kainate-induced prolonged seizure activity or status epilepticus (SE: $p < 0.05$). No changes in NR2B mRNA levels were observed in area CA3 of hippocampus during SE. These results suggest altered NR2B gene expression in specific regions of the hippocampus during prolonged seizure activity. In conjunction with NR2B gene expression changes, the DNA methylation status of the NR2B promoter was dynamically regulated in hippocampus of animals experiencing kainate-induced prolonged seizures. These results suggest that in the hippocampus DNA methylation correlates to aberrant regulation of the NR2B gene during seizure activity. Finally, we assessed the effect of histone deacetylase (HDAC) inhibition on cognitive deficits associated with epilepsy. In chronic epilepsy, we found that administration of a non-selective Class I HDAC inhibitor (NaB; 1.2g/kg, IP) to kainate-treated animals for seven consecutive days prior to fear conditioning significantly improved long-term memory retention assessed by freezing behavior ($p < 0.001$). Thus, these results suggest that manipulation of chromatin remodeling through HDAC inhibition rescues at least one of the severe phenotypes associated with epilepsy; deficits in memory formation. Together our preliminary findings implicate altered epigenetic regulation of gene expression changes in epilepsy.

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Estradiol-induced NR2B and ERK dependence of LTP at TA synapses in hippocampus

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Elevated circulating estradiol (E2) in mammals is associated with enhanced hippocampal dependent memory. In examining the mechanisms which mediate this improved learning we have previously shown that in 7-9 week old ovariectomized (OVX) female rats that E2 replacement increases the magnitude of long-term potentiation (LTP), increases current mediated by NR2B containing NMDA receptors (NMDARs), and increases CA1 pyramidal cell apical dendritic spine density. Whether these E2 induced enhancements in synaptic morphology and function are specific for CA3-CA1 synapses or are conserved mechanisms that occur at other synapses in hippocampus when E2 levels are elevated is not clear. Because of the role entorhinal cortex plays in facilitating learning and memory we tested whether E2 would similarly increase NR2B current, LTP magnitude and spine density at entorhinal cortex-CA1 synapses (TA). We show E2 increases NR2B containing receptor current (E: 36.8$\pm$%; V:19.0$\pm$% contribution of NR2B receptors; $p<0.04$). However, the magnitude of LTP at TA synapses was not significantly different between vehicle and E2 treated animals (E: 150$\pm$7%; V: 151$\pm$12%; $p>0.05$). Interestingly, when NR2B containing receptors were blocked (0.5μM RO25-6981), LTP magnitude in slices from E2 treated animals was significantly decreased (E: 150$\pm$7%; E2+RO: 128$\pm$5%; $p<0.04$) with no change observed in slices from vehicle treated animals (V: 151$\pm$12%; V+RO: 148$\pm$7%; $p>0.05$) indicating an E2 mediated role for NR2B containing receptors. We next tested a role of ERK activation in mediating LTP at TA synapses. Blockade of ERK activation (20μM U0126) did not significantly alter the magnitude of LTP in slices from vehicle treated animals(V: 146$\pm$9%; V+U0126: 154$\pm$14%; $p>0.05$). However, similar to NR2B containing receptor inhibition, blocking ERK activation in slices from E2 treated animals significantly decreased LTP magnitude(E: 144$\pm$9%; E+U0126: 118$\pm$6%; $p<0.04$). These data suggest an E2-induced selective role for NR2B and ERK in mediating LTP at TA synapses. Future studies will examine whether E2 similarly increases dendritic spine density at TA synapses as occurs at CA3-CA1 synapses.

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O-glcnacylation is a dynamic modulator of synaptic transmission at ca3-ca1 synapses

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Diabetes affects approximately 20.8 million people in the US. Many diabetics experience hippocampal dependent memory loss, although the mechanisms are unclear. Under basal conditions, glucose is processed through the glycolytic pathway; however, 2-4% of circulating glucose is metabolized through the hexosamine biosynthetic pathway, where it is modified to produce an O-linked N-acetylglucosamine (O-GlcNAc) moiety that can be added to serine/threonine residues. When circulating glucose is in excess, as in diabetes, flux through the HBP is increased, leading to increased O-GlcNAcylation, which likely contributes to pathological consequences of hyperglycemia. In brain, hippocampal neurons have the highest expression of O-GlcNAc transferase (OGT) and O-GlcNAcase, the enzymes responsible for adding and removing, O-GlcNAc to serine/threonine residues. Because synaptic function is tightly controlled by serine/threonine phosphorylation, and the same residues can undergo O-GlcNAc modification, O-GlcNAcylation is likely as important in modulating synaptic function and plasticity as phosphorylation. Furthermore, pathologically elevated O-GlcNAcylation could play a role in the cognitive deficits associated with diabetes. In extracellular dendritic field recordings from hippocampal CA3-CA1 synapses, we find that pharmacologically increasing O-GlcNAcylation significantly depresses basal transmission, which interferes with subsequent induction of LTP. However, preventing O-GlcNAcylation using an OGT inhibitor causes synaptic potentiation. These data demonstrate that O-GlcNAcylation is tonically active and contributes to the level of basal transmission. Further these data show that chronically elevated O-GlcNAcylation limits the ability of synapses to undergo LTP, consistent with a mechanism that could account for cognitive deficits in diabetic animal models where O-GlcNAcylation is increased. Therefore, further elucidating the mechanisms by which O-GlcNAcylation modulates synaptic transmission could be vital to understanding the relationship between diabetes and deficits in hippocampal function.

Estrogen induced modification of NR2B subunits enhances learning in ovariectomized rats

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Cognition varies in women over the menstrual cycle, such that a higher level of 17β-estradiol (E2) is associated with stronger working memory. In rodents, proestrous levels of E2 are correlated with increased acquisition and working memory. The mechanisms underlying E2's affects on learning and memory are not fully understood, but likely involve enhanced synaptic function in hippocampus, a brain region required for learning and memory. Proestrous levels of circulating E2, in cycling and ovariectomized (OVX) rats treated with E2, increases dendritic spine density, NMDA transmission and long term potentiation (LTP)at CA3-CA1 synapses. We have shown previously that E2 increases NMDA transmission mediated specifically by NR2B containing receptors. Importantly, this increase in NR2B transmission is causal to the E2-induced increase in LTP magnitude. It is not known whether the increase in NR2B current is a consequence of an increase in expression of NR2B subunits, an increase in phosphorylation or both. However, E2 has been shown to increase NR2B subunit mRNA in hippocampus, suggesting that protein levels may be increased. We hypothesize that E2 treatment in OVX rats causes an up regulation of the NR2B subunit expression and phosphorylation which increases NR2B current, permitting the E2-induced enhanced hippocampal dependent learning and memory. The current study uses electrophysiology and synaptosomal western blot analysis in area CA1 of E2 or vehicle treated adult OVX rats to determine whether there is an increase in expression and phosphorylation of NR2B subunits. Ongoing studies are investigating a direct link between the E2-induced up regulation of NR2B current and increased learning.

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Analysis of downstream signaling proteins of the NMDA receptor in schizophrenia

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Converging evidence implicates NMDA receptor dysfunction in schizophrenia. Chronic blockade of NMDA receptors with PCP alters the expression levels of NMDA receptors and changes the stoichiometry of NMDA receptor subunits. PCP increases psychotic symptoms in patients with schizophrenia, treatment with NMDA receptor modulators such as glycine or D-serine reduces negative symptoms, and several postmortem studies have found altered expression of NMDA receptor subunits and binding sites in schizophrenia. Recent work from our lab has shown that SynGAP, a RasGTPase that directly binds to PSD95 and MUPP1 (a multi-PDZ domain containing scaffolding protein), localizes to the PSD and is decreased in subjects with schizophrenia off antipsychotic medication. SynGAP is a modulator of p38 MAPK and ERK function, and directly regulates GluR1 subunit AMPA receptor recycling. A decrease in SynGAP suggests there may be altered downstream signaling in patients with schizophrenia. We have begun Western blot analysis on proteins that lie downstream of SynGAP in order to determine if and how its reduction affects downstream targets in subjects with schizophrenia. We will present data on the abundance of ERK, pERK, p38 MAPK and phoshpo-p38 MAPK in the anterior cingulate cortex and the dorsolateral prefrontal cortex in subjects with schizophrenia and a comparison group. These data will be a significant contribution to the literature on ionotropic glutamate receptor signaling in schizophrenia and may support the overarching hypothesis of decreased NMDA receptor function in this illness.

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AMPA interacting proteins in endosomes in schizophrenia

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Accumulating evidence suggests that glutamate receptor dysfunction in schizophrenia is not a problem of too much or too little receptor expression, but instead a problem of receptor trafficking. Trafficking of receptors is controlled, in part, by proteins that interact with the AMPA receptor subunits. Protein interaction between AMPA receptors and SAP97, GRIP1, or PICK1 may lead to stabilization of a receptor at the synapse or internalization of the receptor in endosomes. Turnover of receptors via endosomes is a critical event for regulation of neuronal transmission at the synapse. We postulate that alterations in endosome content may underlie neuropathological alterations in schizophrenia. We hypothesize that there is an increase in AMPA receptor interacting proteins in early endosomes in schizophrenia, suggesting decreased stabilization of AMPA receptors at the synapse in this illness. The aim of this study is to isolate the early endosomes from postmortem human brain tissue and using a modified subcellular fractionation technique to probe for alterations in endosome content and AMPA receptor interacting protein expression. Tissue homogenates were pre-cleared of non-specific binding via incubation with magnetic beads. Using this pre-cleared tissue homogenate, we targeted endosomes for immunoisolation using a magnetic bead-antibody complex (specific binding) or using magnetic beads only (negative control). Captured material was removed from the beads and analyzed by Western blot analysis. The early endosome marker, Early Endosome Antigen 1 (EEA1) was detected in the pre-clear pellet (non-specific binding) and in the immunoisolation (specific binding) lanes, but not in the negative control lane. Data on expression of EEA1 and AMPA receptor interacting proteins in the early endosome fraction for subjects with schizophrenia and a comparison group will be presented. In schizophrenia compared to control, we found no significant difference in the expression of SAP97 ($p=0.82$) or GRIP1 ($p=0.37$) in total homogenate in anterior cingulate cortex. Overall protein expression of SAP97, GRIP1, and PICK1 in anterior cingulate cortex and dorsolateral prefrontal cortex will be presented. In summary, we have developed a modified immunoisolation protocol to isolate early endosomes from postmortem tissue. This technique will permit us to test the hypothesis that there is an alteration in trafficking of AMPA glutamate receptor subunits in the endosomal compartment in schizophrenia.

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Actin-myosin dynamics contribute to the consolidation of amygdala-dependent fear memories

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Plastic changes in neural circuits are believed to encode information necessary for memory formation. Synaptic potentiation is associated with changes in dendritic spine morphology and these structural changes likely both precede and mediate later functional alterations to brain circuits. Polymerization of actin filaments, the primary cytoskeletal element found in dendritic spines, is thought to promote structural plasticity and play a crucial role in the formation of fear memories. However, the molecular mechanisms that regulate filament dynamics are poorly understood. Myosin II, a hexameric protein complex essential for regulating actin dynamics in a variety of cell types, is highly expressed in dendritic spines of pyramidal neurons. Therefore, we hypothesized that myosin II is a critical regulator of actin dynamics during fear memory formation in the lateral amygdala. By infusing a variety of small molecule inhibitors directly into the lateral amygdala (LA), we were able to investigate the role of actin-myosin dynamics in cued fear conditioning. Here we show that pre-training infusions of a myosin II ATPase inhibitor into the LA blocks the formation, but not expression, of long term memory formation. Furthermore, infusion of an actin depolymerizing agent prior to fear conditioning results in a similar blockade of long-term fear memory. Interestingly, when infusion of the myosin II ATPase inhibitor precedes the actin polymerizing agent, the memory blockade is not additive. This suggests that myosin dynamics precede actin polymerization during long-term memory formation. Additionally, pre-training infusions of the myosin II ATPase inhibitor disrupt long-term memory formation while leaving short term memory intact, indicating that actin-myosin interactions mediate an early consolidation mechanism. Taken together, these results support the hypothesis that myosin-driven reorganization of the actin cytoskeleton is one of the earliest steps in fear memory consolidation.

Actin-myosin dynamics regulate an early consolidation stage of hippocampal LTP and memory

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Memory consolidation involves multiple stages, the first of which must begin within seconds of learning and then progress rapidly over the following several minutes. While substantial evidence indicates that later memory phases involve protein synthesis, almost nothing is known about the earliest cellular events that underlie this process. By investigating long-term potentiation and associative learning, we discovered a Myosin II-dependent mechanism for rapid reorganization of the actin cytoskeleton in dendritic spines that is necessary for synaptic plasticity and learning. Inhibiting Myosin II prevented Theta Burst Stimulation (TBS)-induced actin polymerization in spines of CA1 pyramidal neurons. This inhibitor also blocked LTP stabilization with identical kinetics to inhibitors of actin polymerization, suggesting that this molecular motor participates in the processes that drive actin filament assembly at synapses in response to coincident afferent stimulation. Interestingly, agents that promote actin polymerization prevented the effect of myosin II inhibitors on synaptic plasticity, further indicating that Myosin II participates in regulated changes to actin dynamics. Importantly, neither agent affected baseline synaptic transmission. Based on these findings, we then investigated the role of Myosin II in memory consolidation. An inhibitor of Myosin II activity blocked long-term memory (LTM) formation in the hippocampus without affecting short term memory or retrieval. Further, this inhibitor has no effect on memory performance when given immediately after training. Finally, actin polymerizing agents delivered to the hippocampus prevented the amnestic effect of the myosin II inhibitor. Taken together, these data indicate that an actin-myosin II system of force generation has been co-opted in the nervous system to generate plasticity at dendritic spines, and this process may support the storage of information at synapses that leads specifically to LTM formation. Because structural remodeling of synapses is thought to allow neural circuits to quickly imprint recently acquired information, our data may describe some of the earliest events that lead to LTM consolidation in the hippocampus.

Low expression of the NMDAR-associated signaling protein, SynGAP1, as a model of abnormal neocortical circuit development

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Intellectual disabilities, autism and certain psychiatric illnesses are believed to arise from abnormal development of brain circuits. The refinement of connections in sensory cortices is dependent upon NMDA receptor (NMDAR) activation. As such, these channels and associated signaling pathways are attractive candidates to control processes that underlie the wiring of neocortical circuits during critical periods of brain development. However, it is still an open question how association areas of the cortex connect to each other during childhood. SynGAP, a synaptically enriched protein with both RasGAP and RapGAP activity, is downstream of NMDARs and regulates synaptic structure and function. In addition, this protein has been implicated as a cause of non-syndromic mental retardation and is reduced in persons with schizophrenia. Therefore, we broadly hypothesize that this protein is critical for functional connectivity between brain regions during development, and, as a consequence, the emergence of normal behaviors during childhood. To begin to test this hypothesis, we subjected SynGAP heterozygous mutant mice to a battery of tests designed to uncover behavioral abnormalities that model autism and schizophrenia. The results from this extensive behavioral testing demonstrated an array of abnormalities. The behavioral endophenotype of the SynGAP mutant was strikingly similar to that of mice with reduced function or expression of NMDARs. Some of these abnormities included reduced pre-pulse inhibition, enhanced startle responses, reduced socialization, novelty-induced hyperactivity and severely deficient spatial working memory. In addition, these mice were less responsive to psychomotor effects of NMDAR antagonists, further suggesting that some of these abnormal behaviors are related to NMDAR hypofunction. Functional analysis of prefrontal cortex in brain slices demonstrated that circuits in this area have abnormal spread of neuronal activity between layer 5/6 and layer 2, indicating these cortical micro-circuits are disorganized. Interestingly, many of these abnormal behaviors were present as early as 3-4 weeks of age, suggesting that SynGAP is necessary for the development of circuits that control fundamental behaviors. Our immediate future studies are aimed at understanding how the brain adapts during development to abnormal levels of SynGAP proteins by comparing structural and functional measures of circuits before, during and after the critical period of development.

MyH7B, a muscle-type Myosin II heavy chain, regulates synaptic function and dendritic spine morphology in hippocampal neurons

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The actin cytoskeleton is crucial for maintaining dendritic spines in a plastic state. Plasticity of dendritic spines is believed to underlie experience-dependent modifications of neural circuits that support learning and memory. Precise regulation of dendritic spine shape and volume is linked to the polymerization state of actin filaments and is believed to occur through precise moment-to-moment changes in the turnover of actin filaments at excitatory synapses. Proteins that interact with actin filaments are therefore likely candidates to regulate the complex processes that support constant actin turnover at synapses. Recently, we have cloned and characterized a novel class II Myosin heavy chain**,** MyH7B, which is expressed in rat brain tissue and regulates synaptic function in hippocampal neurons. We show that Myh7B is present in 80-90% of dendritic spines, in dendritic shafts and in cell soma. In dendritic spines, it localized predominantly either in the neck or in the head, areas where it only partially colocalizes with actin. Despite very low expression levels of this Myosin II heavy chain in neuronal tissue, compromising its function had profound effects on dendritic spine structure and excitatory synaptic strength without altering the total number of spines. Neurons where Myh7B had been knocked down presented large, irregularly shaped and bifurcated spine structures. Further analysis revealed that the effect on spine morphology is distinct from that other Myosin II isoforms. Interestingly, disruption of actin dynamics caused by reduced MyH7B expression occluded the effects of actin depolymerizing agents on AMPAR-mediated mEPSCs. Taken together, our data provide evidence that distinct Myosin II isoforms differentially regulate dendritic spine structure in hippocampal neurons, and these protein complexes may regulate the interplay between actin filaments and AMPA receptor function in neurons. Future experiments will be focused in addressing the effects of myh7B in spine morphology and synaptic transmission.

Histone deacetylase inhibitors reverse contextual memory deficits in a mouse model of Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized clinically by progressive cognitive impairments that eventually lead to dementia and death. The earliest symptoms of AD present as a pure deficit in memory retrieval. Therefore, drug treatments that intervene in the early stages of AD by rescuing memory deficits could be promising therapies to slow, or even reverse the progression of the disease. In this study, we tested the hypothesis that histone deacelylase Inhibitors (HDACi's) can rescue certain cognitive deficits in a mouse model of AD. APPswe/PS1deltaE9 mice demonstrated profound contextual memory impairments beginning at six months of age. Chronic HDACi injections did not alter contextual memory formation in normal mice, but had profound effects in the mutants. Both sodium valproate and sodium butyrate completely restored contextual memory in these mutant mice. Further behavioral testing demonstrated that the newly consolidated memories in HDACi-treated transgenic mice were stable over a two-week period. At six-months of age, APPswe/PS1deltaE9 mutants had reduced histone acetylation, which was reversed by five weeks of repeated HDACi injections. Together, these data indicate that histone acetylation is abnormal in a mouse model of AD and that restoring HDAC levels in these transgenic mice may contribute to increased cognitive performance. Our results suggest that HDACi's may be a promising therapeutic intervention for the cognitive deficits associated with AD.

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Lasting cortical DNA methylation following hippocampus-dependent learning

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How the brain structurally and functionally preserves memories in the face of constant molecular turnover is a long-standing mystery of cognition. Because a behavioral memory's lifetime represents multiple molecular lifetimes, this suggests the necessity for a self-perpetuating signal. One candidate is DNA methylation, which maintains cellular memory throughout developmental cycles of differentiation and division. Here we report evidence that the adult nervous system has co-opted this epigenetic modification to subserve the maintenance of behavioral memory throughout cycles of protein turnover. Previously, we demonstrated that hippocampal DNA methylation is critical for memory formation. However, consistent with the hippocampus' limited temporal role in maintaining memory, methylation returned to basal levels within one day of learning. We now report that a single associative learning experience induced persistent, gene-specific changes in DNA methylation with corresponding transcriptional alterations in the prefrontal cortex, a brain region thought to support lasting, remote memories after system consolidation. These changes include hypermethylation and transcriptional repression of the memory suppressor gene, calcineurin. Importantly, this hypermethylation and transcriptional repression is dependent on associative learning events occurring in the hippocampus and persisted for at least 30 days, well into the time that the cortex has incorporated the memory into its network. The rapid methylation changes observed in the hippocampus versus sustained methylation in the prefrontal cortex suggests that DNA methylation is a potential mechanism participating in system consolidation and may serve as a marker of the memory trace. In addition, these results demonstrate that learning-induced DNA methylation is both impervious to protein turnover in the adult cortex and capable of influencing the transcription of memory-associated genes for long periods. Thus, we propose that the adult brain utilizes DNA methylation as a self-perpetuating signal to preserve memories in the face of molecular turnover.

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Protease-activated receptor-1 (PAR1) function modulates hippocampal synaptic plasticity

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Protease-activated receptor-1 (PAR1) is a G-protein coupled receptor (GPCR) that is activated by proteolytic cleavage of its amino terminus by serine proteases. While previous work has shown that inhibiting PAR1 activation is neuroprotective in models of ischemia, traumatic injury, and neurotoxicity, surprisingly little is known about PAR1's contribution to normal brain function. In the CNS, PAR1 is expressed in the amygdala and the hippocampus, which are two brain regions critical for memory formation. Within the hippocampus, PAR1 is expressed predominantly in astrocytes. Mounting evidence indicates that activation of certain Gαq-coupled GPCRs on astrocytes results in the release of various neurotransmitters, a process which can modulate synaptic activity. In particular, activation of PAR1 leads to glutamate release from astrocytes and potentiation of NMDA receptor responses in CA1 pyramidal cells. We have previously demonstrated that PAR1 activation is upstream of extracellular-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) activation in area CA1 of the hippocampus and that PAR1 knockout mice show impairments in passive avoidance and cued fear-conditioning tasks, suggesting an important and specific role for PAR1 in memory formation. In our present work, we demonstrate that PAR1 knockout mice show decreased levels of long-term potentiation at Schaffer collateral-CA1 synapses, while having normal baseline synaptic transmission at these same inputs. These data suggest that normal PAR1 function is important for glial-neuronal interactions subserving learning and memory. Current experiments are directed towards further investigating the roles of PAR1 in synaptic function.

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The role of DNA methylation in spatial learning and memory

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Recent research continues to establish links between epigenetic mechanisms and learning and memory. One such mechanism, DNA methylation, involves covalent bonding of methyl groups to cytosines in cytosine-guanine dinucleotide rich regions of a gene. Our goal was to explore the role of DNA methylation in spatial learning and memory, using both biochemical and neurophysiological approaches. The first experiments were aimed at examining hippocampal DNA methylation modifications following a spatial behavior. Using methylation specific real-time PCR and direct bisulfite sequencing PCR, we quantified DNA methylation patterns of the *bdnf* (brain-derived neurotrophic factor) gene, a gene often evoked in cell signaling cascades in learning and memory, in male rats that had explored familiar (control) and novel environments. Relative to controls, significant differences in *bdnf* DNA methylation were observed across hippocampal subregions (CA1, CA3, and dentate gyrus). Results provide support for a potential role of DNA methylation in spatial learning and memory, but detailed mechanisms and relationships remain unclear. One possibility is that methylation acts as an epigenetic mechanism for encoding aspects of a novel environment /experience/event. The second set of experiments were aimed at assessing the potential role of DNA methylation in regulating neurophysiological properties of hippocampal pyramidal neurons during a spatial task. Hippocampal pyramidal neurons (i.e., place cells) exhibit increased firing rates in specific spatial locations (i.e. place fields). In a novel environment, these spatial representations are encoded or generated during the initial exploration and then may be stable for several months. While pharmacologically manipulating DNA methylation levels, we simultaneously recorded place cells in CA1 and CA3 as rats ran laps around a closed loop track. We then examined spatial correlations of place cell firing rate maps generated during sequential exposures to familiar and novel environments. Stability of place fields significantly varied across drug treatments. In summary, both biochemical and physiological data provide support for an underlying role of DNA methylation in spatial learning and memory.

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Epigenetic modification of the *bdnf* gene in the amygdala and hippocampus by early-life experiences

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The molecular mechanisms by which early-life experiences, including child abuse and neglect, are able to induce robust alterations in mental health remain unclear. Epigenetic mechanisms, or the chemical markings of the DNA and the surrounding histone proteins that regulate gene activity in the CNS, are an attractive molecular hypothesis for the enduring effects of early experiences on neural substrates of mental health. Using a candidate gene approach, we have previously shown in the rat that caregiver abuse and neglect disrupts epigenetic regulation of the *brain-derived neurotrophic factor* (*bdnf)* gene in the prefrontal cortex, and that this epigenetic dysregulation persists through the lifespan and across at least one generation (Roth et al., 2009, Biol. Psychiatry 65:760-769). With this study, we begin to further characterize the effects of early-life experiences on epigenetic regulation of the *bdnf* gene, broadening our focus to the amygdala and hippocampus. During the first postnatal week, rat neonates were exposed for 30 min daily to either a stressed, abusive caregiver or a non-stressed, positive caregiver. Littermates that remained in the home cage served as baseline controls. Results indicate that these early postnatal experiences produced lasting changes in *bdnf* DNA methylation and gene expression patterns in the amygdala and hippocampus. In summary, data continue to implicate epigenetic modification of the *bdnf* gene in the neural and behavioral outcomes of early-life experiences.

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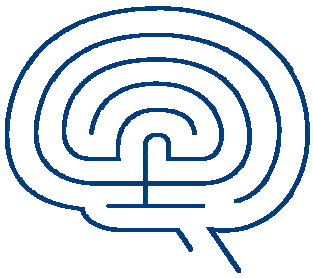

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Multivariate regional network pattern of MRI gray matter preceding conversion to dementia in amnestic mild cognitive impairment

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Previous studies in Alzheimer's disease (AD) patients have demonstrated brain volume reductions on magnetic resonance imaging (MRI), with temporal lobe structures preferentially affected compared to healthy aging. Amnestic mild cognitive impairment (aMCI) is viewed as a transitional stage between healthy cognitive aging and AD, where individuals with aMCI develop dementia at a higher rate than the general elderly population. We sought to identify the characteristic regional pattern of gray matter atrophy associated with aMCI in those individuals known to subsequently convert to dementia. We used voxel-based morphometry (VBM) with multivariate network analysis to identify the regional pattern of gray matter associated with aMCI in individuals who converted to dementia in the 3 years after their baseline assessment compared to healthy controls. Analyses included 80 aMCI converters (mean age=75.6±7.5; M/F=48/32, mean years to conversion=1.41±0.61, range=0.4-3.1 years) selected from a cohort of 375 participants with aMCI with follow up ranging from 6-months up to 3-years as part of the Alzheimer's Disease Neuroimaging Initiative (www.loni.ucla.edu/ADNI). A group of 159 healthy controls (mean age=75.2±4.9; M/F=96/63) were matched to the aMCI converters in age, gender, and years of education. Using volumetric T1 MPRAGE MRIs obtained at baseline, VBM processing was performed with statistical parametric mapping (SPM5) to produce smoothed gray matter volume maps. Multivariate Scaled Subprofile Model (SSM; Moeller et al., 1987; Alexander and Moeller, 1994) analysis was performed to identify a regional pattern of gray matter reductions that distinguished the aMCI converters from healthy controls. SSM analysis of the aMCI and control groups combined identified a linear combination of four component patterns that best distinguished the aMCI converters from healthy controls (R^2=0.37, $p<0.000001$) with each significantly contributing to the model. This combined pattern was characterized mainly by bilateral reductions in the medial and lateral temporal lobes including in the hippocampus, with small areas of relative preservation in bilateral mid and anterior cingulate

and in the vicinity of the basal ganglia. The results indicate a regionally distributed pattern of MRI gray matter atrophy that precedes the conversion to dementia in individuals with aMCI and includes reductions in brain regions that are known to be affected early in AD. Investigating network patterns of MRI brain atrophy in those destined to develop dementia may aid efforts toward identifying individuals with aMCI at greatest risk for conversion to AD within the first few years of follow up.

Network analysis of MRI gray matter in amnestic mild cognitive impairment:relation to rates of cognitive decline and conversion to dementia

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Amnestic mild cognitive impairment (aMCI) is thought to be a transitional stage between normal aging and the dementia of Alzheimer's disease (AD). We used voxel-based morphometry (VBM) with multivariate network analysis to determine if regional patterns of gray matter from magnetic resonance images (MRI) could predict subsequent declines in cognitive performance and conversion to AD in individuals with aMCI. Analyses included 100 AD patients (mean age=75.7±7.2; M/F=50/50), 100 individuals with aMCI (mean age=74.9±7.1; M/F=50/50) and 100 healthy controls (HC; mean age=75.5±4.9; M/F=50/50) from the Alzheimer's Disease Neuroimaging Initiative (www.loni.ucla.edu/ADNI). The groups did not differ in age, years of education, or gender and the aMCI group included participants who did not convert to dementia during the first 12 months of follow up. Baseline volumetric T1 MPRAGE MRIs were processed using VBM with statistical parametric mapping (SPM5). Neuropsychological performance was assessed at baseline and after a 12-month follow-up and 85 participants in the aMCI group were followed additionally over an average of 21.8±2.9 months post-baseline. Using multivariate network analysis with the scaled subprofile model (SSM; Moeller et al., 1987; Alexander and Moeller, 1994) for all 300 participants combined produced a regional pattern of gray matter reductions associated with group membership (R^2=0.34, p□0.000001), reflecting the continuum of clinical severity from HC to aMCI to AD. This pattern showed gray matter reductions in bilateral mid and inferior temporal cortices, including in hippocampal and parahippocampal regions, with areas of relative preservation in the anterior and mid cingulate and bilateral superior, mid, and inferior frontal cortices. After we controlled for baseline levels of cognitive performance, greater group-related pattern expression was associated with greater 12-month declines in attention (p<0.00002) and language (p<0.025) functions, but not in memory (p=ns) in the aMCI group. The association with declines in language was reduced to non-significance after controlling for attentional decline (p=ns). Further in the aMCI participants with extended follow up, higher network pattern scores were associated with conversion to dementia (p<0.009). The

findings suggest that individual differences in network patterns of gray matter atrophy may assist in predicting subsequent cognitive decline in aMCI, where attentional decline may precede conversion to dementia. MRI with voxel-based network analyses may aid efforts toward identifying those individuals who progress to dementia and may benefit from early treatment.

Regional reduction of cortical thickness in cognitively normal late middle aged adults with APOE ε4

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In previous studies, we have demonstrated reductions in cerebral metabolism in cognitively normal late middle-aged adults with the apolipoprotein E (APOE) ε4 allele compared to ε4 non-carriers, involving brain regions typically associated with the effects of Alzheimer's disease (AD) as well as healthy aging (Reiman et al., 1996, 2005). We now investigate differences between APOE ε4 carriers and non-carriers in regional measures of cortical thickness assessed with magnetic resonance imaging (MRI) in a group of cognitively normal late-middle aged adults. All participants had a reported family history of dementia, had no cognitive difficulties or complaints, and included 19 individuals with two copies (mean age=57.0±4.9; M/F=6/13), 26 with one copy (mean age=57.9±4.1; M/F=8/18), and 35 with no copies [non-carriers (NC); mean age=57.3±5.3; M/F=10/25] of the ε4 allele. The groups did not differ in age, gender, or years of education. Volumetric T1 MRI scans were processed using Freesurfer software (Fischl et al., 1999; Dale et al., 1999) to measure cortical thickness in 35 regions of interest (ROI) from each hemisphere. Repeated-measures analyses with group as the between-subject and hemisphere as a repeated-measures factor indicated bilateral main effects for group ($p<0.05$, uncorrected for multiple omnibus tests) with less cortical thickness in the APOE □4 carriers combined (n=45) compared to non-carriers (n=35) in anterior cingulate, fusiform, medial orbitofrontal, and superior temporal regions ($0.007<p<0.022$). There were no significant group by hemisphere interactions. APOE ε4 gene dose (the number of ε4 alleles in a person's APOE genotype) was associated with corresponding reductions in cortical thickness in left fusiform, bilateral superior temporal, right inferior parietal, and right medial orbitofrontal regions ($0.017<p<0.05$). In the APOE ε4 carriers, poorer memory performance was associated with less cortical thickness in bilateral superior temporal and right caudal anterior cingulate ($0.001<p<0.05$). The results suggest that cognitively normal late-middle-aged people with the major genetic risk factor for AD have decreased cortical thickness in brain regions known to be affected in AD and in healthy aging. MRI measures of cortical thickness may be helpful in detecting the earliest effects of AD on the brain. If these effects are found to progress over time, this method may assist efforts in evaluating prevention therapies for AD prior to the onset of cognitive impairment.

Distributed regional pattern of gray matter volume in Alzheimer's disease: A comparison with the effects of healthy aging

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Alzheimer's disease (AD) is characterized by brain volume reductions on magnetic resonance imaging (MRI) with temporal and parietal regions most consistently and severely affected early in the clinical course. Healthy aging preferentially affects frontal regions, but reductions in other brain areas have been observed. We investigated the relative effects of AD and healthy aging on MRI regional brain volume using voxel-based morphometry (VBM) with multivariate network analysis to first identify a regional pattern of MRI gray matter reductions in AD patients compared to healthy controls, and to subsequently test the ability of this pattern to distinguish the groups after we statistically controlled for aging effects using an age-related network pattern of MRI gray matter from an independent sample of healthy adults, 22 to 84 years of age. Ten otherwise healthy patients with possible or probable AD (mean age=65±9, M/F=6/4, mean Mini-Mental State Exam (MMSE)=16.1±6.3) and 15 age-matched healthy controls (mean age = 63±8, M/F=10/5, mean MMSE=29.5±0.7) were studied using volumetric T1 MRI scans with statistical parametric mapping (SPM5) VBM and customized tissue priors to produce gray matter volume maps. Multivariate network analysis with the Scaled Subprofile Model (SSM) identified a linear combination of two component patterns with higher expression in the AD patients than controls (R^2=0.74, p<0.000001). The combined pattern showed gray matter reductions in bilateral medial and lateral temporal and parietal areas, with relative preservation in cerebellum and anterior/mid cingulate regions. We then forward applied an age-associated network pattern of MRI gray matter to the AD and healthy control groups, which was derived from 29 healthy adults, 22 to 84 years of age using the same SPM5 SSM methods. The age-related pattern showed mainly bilateral reductions in medial and dorsolateral prefrontal, perisylvian, and precuneus regions, with relative preservation in thalamus. In a multiple regression model, expression of this age-related pattern was higher in the AD patients than controls (R^2=0.24, p=0.013). When subject scores for the AD-related pattern was added to the model, the additional 50% of the variance in distinguishing the groups was explained (p<0.000002) and the subject scores for the age-associated pattern were reduced to non-significance. These results suggest that, despite prominent differences in the regional patterns of gray matter reductions, healthy aging and AD share some regional features of gray matter atrophy that may reflect underlying, developing disease in the context of healthy aging or exaggerated aging effects in AD dementia.

Age effects on neuronal activity in the perirhinal cortex

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Numerous studies have shown that normal object recognition requires an intact perirhinal cortex. The fact that this cognitive skill is altered by the aging process, suggests that the functional integrity of this structure may be altered across the lifespan. The current experiment investigated whether age-related deficits in stimulus recognition could be attributed to alterations in the underlying activity patterns observed in single perirhinal cortical neurons. Multiple single neurons in the perirhinal cortex were recorded simultaneously with 'hyperdrives' as 6 young (9 months old) and 6 aged (25-27 months old) rats traversed a circular track for a food reward. During some behavioral conditions the track was empty except for two food dishes in which reward was placed. During other conditions the track 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. During all behavioral conditions it was observed that, in both young and aged rats, a proportion of perirhinal cortical neurons exhibit increased activity at the locations of the food dishes ('food dish fields'). Additionally, when objects were on the track, a proportion of perirhinal cortical neurons in young and old rats showed increased firing at the location of objects ('object fields'). Importantly, the proportions of perirhinal cortical neurons showing food dish fields or object fields was significantly less in the aged compared to the young rats. Conversely, the proportions of perirhinal cortical neurons showing no activity during track running was significantly higher in the aged relative to the young rats. The changes observed here in perirhinal cortical neuron activity patterns could contribute to the behavioral deficits that have been observed in old rodents and humans in object recognition tasks.

Arc transcriptional responses are modulated by degree of context familiarity

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The immediate-early gene Arc (activity-regulated cytoskeletal gene) plays a critical role in the maintenance of long-term potentiation and long-term memory function. Arc is also regulated in hippocampal CA1 and CA3 neurons in a context-specific manner that closely parallels 'place cell' firing. Recent work demonstrates that a single lap around a track (i.e., a single pass through a place field) is sufficient to initiate Arc transcription within CA3 place cell ensembles, but not in CA1, where additional laps are required to engage the complete ensemble (Miyashita et al., 2009). Although a significantly smaller ensemble of neurons are activated, granule cells in the dentate gyrus (DG) also show place selectivity, but it is not known how these conditions affect neural ensemble development within this hippocampal subregion. The current study investigated the development of DG and CA1 place cell ensembles in rats that were trained to run a circular track. Using fluorescence in situ hybridization (FISH) and RT-PCR, we were able to determine the number of cells that expressed Arc, as well as the amount of Arc transcribed following circular track trajectory in the CA1 and DG regions. On the day of testing, one group of rats ran either 2 laps or 10 laps around the same track they were trained on, while a second group were taken to an unfamiliar testing room and ran either 2 laps or 10 laps on a novel track. We determined that rats trained and tested on the same track and within a familiar room activated similar sized ensembles of neurons that expressed Arc in CA1 and the DG regardless of the number of transversals around the track (2 or 10 laps). Similarly, using RT-PCR, we observed that comparable levels of Arc mRNA were measured in either CA1 or the DG following 2 or 10 laps around the track. When these results were compared to those obtained from rats that ran 2 or 10 laps in an unfamiliar environment, however, we observed that rats tested in a novel room had significantly less Arc mRNA within both CA1 and the DG. These results suggest that while the Arc transcriptional response is reliably activated in ensembles of 'place cells' of CA1 and the DG following an experience in a familiar environment, additional experience is required for this response to be optimized within both of these brain regions as rats learn about the new space.

Maximal electro-convulsive shock induced c-fos mRNA expression is reduced in the hippocampus of aged rats

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Rapid and transient increase in immediate-early genes (IEGs) have been reported following electroconvulsive shock (Cole et al., 1990). Transcription of these genes regulates a cascade of subsequent genomic responses leading to long lasting changes in neuronal activity, morphology and receptor redistribution (Morgan and Curran, 1989). Previous studies have shown both an increase in *c-fos* mRNA following LTP (using high frequency stimulation) in fascia dentata of aged rats and a decrease in c-fos protein following seizure in aged mice (D'Costa et al., 1991). To ascertain whether a brief electroconvulsive shock can elicit a differential response in aged animals we used fluorescence *in situ* hybridization and high-resolution confocal microscopy to obtain a measure of the numbers of cells that express *c-fos* mRNA and RT-PCR to measure the mRNA levels of *c-fos.* These data were obtained for the fascia dentata, and CA hippocampal sub-regions at 5, 30 and 60 min following maximal electroconvulsive shock treatment (MECS) in young and old rats. With one exception (pyramidal cells; 60 min following seizure), there were equivalent numbers of *c-fos* expressing cells following seizure treatment in young and old rats. There was no age difference revealed by RT-PCR analysis following resting behaviors in either pyramidal or granule cells. Aged animals, however, exhibited a two-fold decline in *c-fos* mRNA levels as measured by RT-PCR at 5, 30 and 60 min intervals in the granule cells and at 30 and 60 min intervals in the CA pyramidal cells. While the consequence of reduced *c-fos* mRNA per cell in older animals, following seizure, remains unknown at present, it is possible that it is a compensatory or neuro-protective response. The beneficial or detrimental consequence of this age-related change remains to be investigated.

Pre-activation of hippocampal CA1 activity patterns is reduced in old rats

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The hippocampus may play a role in the coordination of memory consolidation processes by reactivating sequences of cell activity (Wilson and McNaughton, 1994). Repetitive track running results in recurring pyramidal cell activity patterns, and cell pair correlations of firing between neurons during behavioral episodes are preserved in subsequent quiet waking and slow wave sleep. It is unknown, however, whether multiple episodes of similar behavioral experiences within the same day interact with the reactivation process. Five young (9-12mo) and 4 old (26-28mo) Fischer 344 rats ran 35-40 laps for food reward on a circular track, and rested in a pot for 30-min before and after track running, while hippocampal area CA1 pyramidal cell activity was recorded. Rats ran two sessions per day (2-hr inter-session-interval) for 31 days in an environment that was not experienced prior to the first day of recording. For both the morning and afternoon recording period, the electrophysiological expression of the reactivation process was calculated using the explained variance method (Kudrimoti et al., 1999). When cell pair correlations from all rats over all 31 days were considered together, no differences in reactivation were observed between the first and second sessions within days. Additionally, as reported previously, we confirm that no differences are observed in explained variance in the rest period following the first session of the day between age groups (Gerrard et al., 2001). Greater "pre-activation", however, was observed during rest before the second session of the day than before the first session of each day. To determine whether this effect changes as the environment goes from being novel to familiar, we examined correlations of firing patterns during days 1-3 versus days 11-13 of exposure to the environment. During days 1-3, pre-activation prior to the second session was significantly less in the old compared to the young rats. This reduction in pre-activation in old rats was not apparent by days 11-13. These data suggest that 1) behavioral experience can affect activity pattern reactivation processes in CA1 for up to 2 hours in both young and old rats; and 2) that this pre-activation effect is attenuated in old compared to young rats for the initial days in which the rats are learning about a novel environment. This reduction in pre-activation in the old rats could reflect defective encoding processes in these spatial memory-impaired animals.

Characterization of behaviorally-induced Arc expression in ventral tegmental neurons during aging

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Dopaminergic neurons of the ventral tegmental area (VTA) have been shown to be highly correlated with reward. As rewards are thought to be central for predicting the outcome of future events which can guide behavior, possible changes in the reward system during aging might lead to impairments in cognitive or behavioral flexibility. We previously reported that the immediate-early gene (IEG) Arc is expressed in the VTA of young and aged male rats after exposure to a sexually receptive female rat, a behavior known to activate the VTA (Hoang et al., 2008). Although video-scored behavioral responses were similar across age groups, the proportion of Arc-expressing VTA neurons of aged animals was significantly attenuated in the rostral versus caudal regions of the VTA. IEG expression is thought to be dynamically regulated by specific forms of patterned synaptic activity that underlie information storage and plasticity. These results support the hypothesis that VTA plasticity is diminished over the lifespan. Because of the heterogeneity of cells in this region, it was important to further characterize those VTA cells that express Arc in the rostral versus caudal regions. We used florescence in situ hybridization for tyrosine hydroxylase (TH) mRNA, a dopamine biomarker, and Arc mRNA to determine the distribution of neurons that co-express these markers in the VTA of young and aged rats. Behaviorally-induced TH mRNA expression was significantly attenuated in the rostral VTA of aged animals. Additionally, we found that Arc mRNA and TH mRNA co-localized only in the caudal region of the VTA, and that this co-localization is also reduced in aged animals. These results suggest that behaviorally-induced Arc expression in the VTA varies not only by cell type or location along the rostro-caudal axis of the VTA, but also during normal aging. Specifically, although TH+ neurons in the rostral VTA were activated for sexual behavior in young and aged animals, these neurons did not express Arc, and were not engaging the same molecular cascades known to be important for plasticity mechanisms in other brain regions such as the hippocampus. Conversely, TH+ neurons activated by sexual behavior in the caudal VTA largely expressed Arc, indicating that they may well undergo similar plasticity processes. Given that dopaminergic afferents arising from different regions of the VTA project to different areas of the brain, these data may have important implications for the processing of reward and event salience. Furthermore, given that dopamine biomarkers are known to generally decline during normal aging, these data may provide insights into the specificity with which the VTA is altered over the lifespan.

Neuron population activity in the rat anterior cingulate cortex tracks the advancement through a trial during a decision-making task

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Cognitive functions proposed for the anterior cingulate cortex span a range that includes action-outcome learning, effortful behavior, attention, working memory, and memory retrieval. Lesion studies have confirmed the diversity of anterior cingulate involvement in behavior, and these have been complimented by a wide range of results that have correlated task and behavioral factors with single-neuron activity. In the present experiment we examined the relative contribution of several task and behavioral factors to network activity by examining their correlation with the principle components of neuron population activity (i.e., hidden, uncorrelated variables that account for sequentially descending degrees of variance in the population). Neurophysiological recordings were collected from the anterior cingulate of five young adult rats during a three-choice, cued-place decision task. A task trial began when a rat entered a central platform and was presented with two cues (a ringing sound and a blinking light), localized to independently random arms. If the rat followed the correct cue it was presented with liquid food reward; alternatively the rat was presented with an error sound for an incorrect choice. The rewarded cue was experimentally reversed approximately every seven days. Single neurons from the anterior cingulate cortex were found that exhibited increased firing rates to a range of variables, notable examples including outcome and trial-completion-time. For population analyses, neuron firing rates were converted into z-scores and binned at 120ms. Using only the initial principle components (roughly accounting for 10% of the variance in population activity), it was possible to reconstruct where the rat was within the sequence of a given trial; i.e., returning to the central platform, initiating a trajectory to the feeder zone, or experience the outcome. Individual component values often correlated with task-relevant spatial variables (whether the rat was in the feeder or cue zones, but not which feeder zone), and velocity (in many instances differentiating the journeys toward and returning from the feeder zones). In many recording sessions, there was a rapid change in population state as the rat left a feeder zone to initiate a new trial, which progressively shifted in the opposite direction as the trial was completed. This method provides a relatively assumption-free starting point for decoding anterior cingulate activity in a decision task. It will be valuable to use these population-based factors to further dissect the contribution that anterior cingulate networks make to attention, decisions, and outcome learning.

Statistical analysis of microcolumn structure in the rodent neocortex

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Physiological data dating back to Mountcastle (1957) and Hubel and Wiesel (1963) demonstrated a columnar organization of function in neocortex. Observations from cytoarchitectonic studies also reveals that cortical neurons are arranged vertically in microcolumns. Until recently, this aspect of cortical structure has not been quantified. Advances in imaging technology, combined with techniques from statistical physics and automated neuronal detection, enable large-scale quantitative analyses of such structural properties of neocortex in Nissl-stained tissue. Specifically, non-random spatial relationships among neurons can be quantified by a two-dimensional probability map (or density map function) that describes the probability of finding a neuron at a given distance from an arbitrary origin. This density map gives information about the strength, width and spacing of microcolumns. These properties are of interest in making quantitative comparisons of cortical structure between normal and treatment conditions as well as across species. The utility of these measures has been demonstrated in a recent study of area 46 of monkey prefrontal cortex where the strength of microcolumns was shown to decrease with age, a change correlated with cognitive impairment (Cruz et al., 2004). We have now adapted these methods to quantify columnar organization in rat cortex using digitized images captured with the DMetrix DX-40 scanning array microscope. This instrument contains 80 miniaturized 20X objectives and can image 40 slides per hour, a hundredfold increase in rate of image acquisition compared to other commercial solutions. This increase in digitization speed, when coupled with computing systems able to store and automatically analyze terabytes of image data, removes the restriction of focusing on a limited cortical region such as area 46 and allows assessment of the entire rat neocortex. Initial results demonstrate 85% accuracy of the automatic neuron recognition algorium applied to the DMetrix images which is comparable to semi-automated recognition in area 46. Applying density map analysis to Nissl-stained sections of adult Fischer-344 rat somatosensory cortex revealed a microcolumnar strength of 1.10, exceeding a value of 1.00 which indicates a non-columnar, uniform distribution. These data provide evidence for an identifiable, statistical tendency among neurons to be organized into microcolumns in rat neocortex. Extension of these methods to compare the cortex of young and old rats will allow determination of whether rat cortex shows age-related changes in microcolumns and if there is region selective vulnerability.

Hippocampal sequence compression increases with increasing velocity

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Theta phase precession is the systematic shifting of hippocampal neuronal spikes relative to the hippocampal theta rhythm as the rat passes through the neuron's spatial receptive field. Because this phenomenon occurs across the entire population of principal neurons, the temporal sequence in which CA1 pyramidal cell place fields are encountered is replicated within individual theta cycles, in highly compressed form (Skaggs et al., 1996). The preservation of the temporal order results in a sweeping forward of the network activity, such that the hippocampal representation transitions from the location the rat presently occupies to locations ahead of the animal (Tsodyks et al., 1996). Extrapolations of this concept suggest that the amount of sequence compression should increase as rats travel at higher velocities due to an increased "look ahead" at high velocities (necessary for maintaining place field size). Prior investigations, however, have observed that the amount of compression remains constant across different velocities (Geisler et al., 2006). Because these results were in apparent conflict with extrapolations of the Tsodyks et al. (1996) schematic, we revisited this issue in more detail. Using data from recording locations that are more anterior than are the traditional implants (where changes in sequence compression will be the easiest to observe), we examined the relationship between cross correllogram lag and the distance between the place field centers as a function of velocity. When the data were collapsed across the population using a method that allows velocity to be represented in a continuous manner, a significant increase in sequence compression was found to occur with increases in velocity. This increase in sequence compression may be a mechanism through which the prediction of upcoming locations is optimized. An extrapolated 'sketch' of the Tsodyks et al. (1996) model, incorporating these observations, will also be presented.

CA1 pyramidal cell activity characteristics are modulated by 3-dimensional objects

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The perirhinal and lateral entorhinal cortices send projections to the distal portion of the CA1 subfield of the hippocampus (Canning and Leung, 1997; Liu and Bilkey, 1997; Naber et al., 1999; Naber et al., 1999; Burwell, 2000; Witter et al., 2000). Although these medial temporal lobe structures are a major source of input to the hippocampus, relatively little is known regarding the contributions of the perirhinal cortex and lateral entorhinal cortex to the activity patterns observed in hippocampal cells. It has been shown previously that 3-dimensional objects lead to selective increases in the firing rates of both perirhinal and lateral entorhinal cortical neurons (Burke et al., 2008; Deshmukh and Knierim, 2008). The current experiments investigated the degree to which 3-dimensional objects affect place field size and activity in the middle portion of the dorsoventral axis of the CA1 hippocampal subfield. The activity of CA1 pyramidal cells was monitored as rats traversed a circular track. In some conditions the track contained no objects while in other conditions 3-dimensional objects were placed on the track to increase the likelihood of lateral entorhinal and perirhinal involvement. In the middle CA1 subfield, three factors differentiated the objects-on-track conditions from the no-object conditions: more pyramidal cells expressed place fields, the size of the fields decreased, and adding or removing objects from the environment lead to global remapping in CA1. Because overall running velocities were similar between conditions with objects and without objects, it is unlikely that the effects of objects on CA1 activity patterns reflect differences in running speed. Additionally, a small but significant portion of place fields remapped under conditions in which the object locations were shuffled, which suggests that at least some of the CA1 neurons were selective for a particular object in a particular location. Together, these data suggest that 3-dimensional objects can influence the activity characteristics in the middle portion of the CA1 hippocampal subfield, perhaps through inputs to the hippocampus from the perirhinal and lateral entorhinal cortex.

The effects of distraction and interruption forms of interference on delayed-nonmatching to sample task performance

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Memory performance is vulnerable to distracting stimuli, particularly, if the distracter requires attention and the human participants are older. The current experiment adapted a task that has been used with humans to measure the effects of external interference factors on memory performance in nonhuman primates. The task was modified such that it could be used in a Wisconsin General Testing Apparatus. Monkeys learned to perform a trial-unique delayed-nonmatching to sample task with a 30 sec delay period. All animals were required to reach a criterion performance of 90% over 5 consecutive days of testing. After animals reached this criterion, they participated in 5 days of testing in the "distraction" condition, in which irrelevant stimuli are presented and should be ignored during the delay period. During this condition the monkey was presented with a sample object that she was able to displace to obtain a food reward. A wooden guillotine door was closed for 10 sec, and then was raised, afterwhich the monkey was presented with an irrelevant distracter that was behind a Plexiglas screen, so that the monkey could not touch the object. The wooden guillotine door was then lowered again for another 10 sec. Finally, the wooden door was raised and the monkey was presented with the sample object and a novel object. A correct trial occurred if the monkey displaced the novel object and obtained the food reward. After the distractor condition was complete, the monkeys participated in 5 days of testing in the "interruption" condition, in which a stimulus was presented that did require attention, but was not relevant to the final choice. The procedure for the interruption condition was similar to that of the distraction condition, except that the Plexiglas screen was not used and an irrelevant object was baited with a food reward that the monkey could retrieve by displacing the object. Overall, performance on the delayed-nonmatching to sample task was not affected by the distraction condition, in which the interferring stimulus was irrelevant to obtaining food reward. In contrast, monkeys exhibited significantly more errors during the interruption condition, where the object, if attended, and displaced, could result in reward. This suggests that, like humans, monkeys show disrupted memory performance in conditions in which interferring variables require attention.

DNA methylation of zif268 is not dynamically regulated within the aged hippocampus following spatial behavior

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The neurobiological underpinnings of age-related memory deficits include aberrant changes in gene transcription that affect the ability of the aged brain to be "plastic". Memory and synaptic plasticity processes are associated with transcription of immediate-early genes (IEGs), such as Arc (activity-regulated cytoskeletal gene) and zif268. Blocking the expression of these genes in adult animals prevents the consolidation of memory, and decreased IEG expression is observed as a result of the normal aging process. The molecular mechanisms underlying these changes in gene transcription are not currently known, but recent work points to DNA methylation as a potential novel mechanism. Epigenetic changes involving the covalent chemical modification of DNA by DNA methyltransferases, typically results in transcriptional silencing and loss of gene function, although transcriptional activation is also a possible effect. Regardless of whether silencing or activation of transcription results from this modification, DNA methylation can play a key role in dynamically regulating gene transcription in the adult CNS. In previous work we have observed significant age-associated changes in the DNA methylation status of the Arc gene (Penner et al., 2008) within the hippocampus (CA1 and the dentate gyrus). Here, we report that like Arc, the DNA methylation status of the zif268 gene can be dynamically regulated by spatial behavior within the adult hippocampus. In area CA1 there is a significant reduction in methylation of the zif268 gene, whereas in the dentate gyrus, adult rats show a significant increase in methylation of zif268. In contrast to the adult animal, the DNA methylation status of the zif268 gene is not dynamically regulated following spatial behavior in aged rats. The absence of a dynamic change in the methylation status of zif268 within the aged hippocampus may contribute to the reduced levels of zif268 mRNA that have been previously observed. Moreover, because zif268 has been shown to play a key role in the maintenance of long-term memory, these changes are likely contribute to the memory deficits observed in aged animals.

Age differences in performance of appetitive instrumental tasks

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Emotions differentially influence memory for learned items in old versus young adults. Specifically, older adults tend to remember items with a positive value more accurately than neutral or negative ones, whereas younger adults do not tend to remember positive items better than neutral or negative ones (e.g., Mather and Carstensen, 2005; Trends Cogn Sci.). To investigate further the influence of emotions on learning, young (9-12 mo) and aged (24-27 mo) Fischer 344 rats were paired while performing a series of appetitive instrumental learning tasks. Rats were initially trained to press two different levers, one associated with maltodextrin and the other with sucrose. The performance levels of young rats were matched to levels in old rats, leading to a similar learning curve during training. In this training, the probability of obtaining a reward was gradually decreased to reach a random ratio (RR) probability of 20% at the end of 11 days. The effect of the change in the incentive value of the reward was then assessed in a reinforcer devaluation task using selective satiation. Both groups were sensitive to the devaluation of the outcome, lever pressing more for the non-devalued outcome. No differences were detected between age groups. Conversely, old rats were found to be generally more sensitive to the degradation of the action-outcome contingency as shown by a selective decrease in pressing to the degraded lever-outcome pair. In this contingency degradation task, one of the lever-outcome associations was degraded by also delivering rewards in the absence of actions (RR of 5%). Finally, old rats tended to extinguish lever pressing faster than did young rats in a subsequent extinction test involving the lever that was not previously degraded. These data suggest that older rats are more sensitive to changes in reward contingencies and to the absence of reward following their actions. While the data do not necessarily suggest that old rats learn or adapt faster, it is possible that that they are more prone to refrain from producing actions when they are not needed or when the reward is not readily available. Other experiments that have examined young and old rat behavior using extinction tests of aversive stimuli, have shown greater resistance to extinction in aged rats (e.g., Bevilaqua et al., 2008; Topic et al., 2008). However, these experiments were conducted under more stressful situations, which may explain the apparent differences between our studies. It will be interesting to extend the present experiment to different instrumental and Pavlovian situations to determine the degree to which the effects of aging on extinction learning can be generalized.

Hippocampal CA1 place representations stabilize as young and old rats gain experience in a novel environment

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Place cells in hippocampal area CA1 exhibit increased firing rates in a particular region of an environment (i.e., place fields). These place fields form an environment-specific representation ('map') that remains consistent between exploration episodes. However, when exploring a familiar environment, aged rats sometimes exhibit global "remapping" such that most place field representations are mismatched between consecutive sessions (Barnes et al., 1997; Nature, 388: 272-275). We also know that place fields form during the first minutes an animal explores a novel environment and require NMDAR activity for long-term stability. These findings led us to ask whether gaining experience in an environment is associated with increased stability of place fields, and whether there are age differences in remapping as an environment transitions from novel to familiar. In a novel environment, 5 young (9-12 mo) and 4 old (26-28 mo) Fischer 344 rats ran 35-40 laps on a circular track twice daily (2-hr inter-session-interval), during which activity of ensembles of single cells in hippocampal area CA1 was recorded. Firing rates were determined for each cell over all track locations, and spatial correlations were calculated to quantify the stability of place fields between the sessions of each day. While the majority of correlations between the two recording sessions within a day were strong, about 25% of cells in both age groups showed weak spatial correlations on the first day the rats experienced the novel environment. The proportion of cells with low correlations between sessions on a single day significantly decreased during days 1 to 13 from 23 to 6% in young rats and 24 to 11% in old rats. Interestingly, global remapping (defined as low spatial correlation in at least 70% of cells within a session) was not observed until day 14 in either age group. In summary, place field representations of a novel environment stabilize in both young and old rats during two weeks of daily exposure to the environment. Increased stabilization includes both fewer cells expressing low spatial correlations, and more cells expressing high spatial correlations between sessions. The lack of global remapping until day 14 suggests that while initial representations of an environment are stabilizing, the formation of new ones may be inhibited.

Electrophysiological responses of rostral versus caudal ventral tegmental neurons

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Accumulating evidence suggests that neurons of the ventral tegmental area (VTA) of the midbrain are highly correlated with the salience of events. In particular, VTA neurons have been shown to be especially sensitive to unexpected rewards. Several studies have shown that the activity of putative dopamine neurons from the VTA modulate their firing rates in response to reward magnitude (e.g., Schoenbaum et al., 2002; Tober et al., 2005). Little is known, however, about the response of VTA neurons across its rostro-caudal axis. Different regions of the VTA have different anatomical projection sites, with the rostral VTA sending heavy projections to the prefrontal cortex and the caudal VTA projecting more strongly to limbic areas such as the nucleus accumbens. High density recording methods were used that allowed ensembles of cells to be recorded from the rostral (-5.00 to -5.50mm Bregma) and caudal (-6.00 to -6.30mm Bregma) VTA simultaneously. Recordings were obtained from three adult male rats as they ran for food reward. The magnitudes of the rewards were varied as a function of trial and recording session. Specifically, the magnitudes of reward were unexpectedly changed within a recording session and varied from session to session. Overall we confirm reports of the sensitivity of VTA neurons to reward and to reward magnitude. Furthermore, we observed that two main 'classes' of VTA neurons were responsive to reward delivery: 1) neurons that significantly increased their firing rates to reward; and 2) neurons that displayed significantly decreased firing rates to the onset of reward delivery. Within these two categories of VTA neurons, cells tended to cluster into those that either had gradual or that had steep changes in firing rates following reward delivery. While the broad categories of response inhibition versus excitation to reward delivery were present in both the rostral and caudal VTA, the rostral VTA showed a significantly higher proportion of those cells with steep onset and offset characteristics. These results suggest that the VTA as a whole exhibits complex and heterogenous responses to reward delivery that may have important implications for processing in the upstream structures that receive differential VTA output.

Altered pattern separation in the aged fascia dentata correlates with memory deficits

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Principal cells across the hippocampal formation display robust high-frequency activity when an animal is in a specific spatial location. These place cells are thought to collectively encode the context of an episode, with each structure making a unique contribution to maintaining the balance between the formation of many unique, unambiguous representations despite similar input (pattern separation) against the ability to retrieve memories from altered or incomplete cues (pattern completion). Within this interconnected network, the fascia dentata (FD) is thought to mediate pattern separation through the orthogonalization of incoming activity from the entorhinal cortex. While it is known that the amount of activity in the aged FD is dramatically reduced, it remains unknown whether this change is accompanied by alterations in the pattern of activity during memory formation. This question was addressed using zif268, an immediate-early gene which is critical for enduring memory and durable changes in synaptic transmission and which reliably labels cells expressing place fields. Following an initial exploration session in a novel environment, young (12 months) and aged (24 months) F344 rats returned to their home cage for 25 minutes, and then either returned to the same environment (A/A), or visited a visually distinct environment in a different location (A/B). A caged control (CC) group remained undisturbed in their home cages. Results indicate that in the aged FD, granule cells are far less reliably activated by repeated exposures to the same environment. Moreover, this decreased reliability correlates with spatial memory deficits in the Morris swim task. This shift in the pattern of activity during spatial processing may explain why place fields in areas downstream of FD are less stable over trials in aged rats. Moreover, this deficit in the ability to reliably represent a spatial context in which an event occurred could contribute to the general decline in episodic memory observed during progressive age. Arc expression throughout the hippocampus:

Contrasting spatial and non-spatial navigation with an identical trajectory

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The immediate-early gene (IEG) Arc is activated in a behavior-dependent manner and is required for enduring synaptic plasticity. In the hippocampus, the proportion of neurons expressing Arc is comparable to the results of single unit recordings. Compartmental analysis of temporal activity using in situ hybridization (catFISH) timestamps the cellular activity of cells by localizing Arc transcription to either the nucleus or the cytoplasm. Thus, the animal can perform two behavioral sessions separated by 25 minutes and cells activated in the first, second, or both sessions can be identified. Place and response learning are mediated by the dorsal hippocampus and dorsal striatum, respectively (Packard & McGaugh, 1996). In the current study, rats were trained to switch between place and response strategies. On test day, rats were trained on the place task, returned to their home cage, then trained on either the place or response task. Both place and response trials required the rat to perform the same trajectory, only the cognitive strategy used was changed. We examined the expression of Arc in different sub-regions throughout the hippocampus, as well as the dorsal and ventral striatum. A large proportion of CA1 cells had overlapping activity during the place/place task in the dorsal hippocampus. A significantly proportion of dorsal hippocampus CA1 cells showed different activation dependent on whether the animal completed a spatial or motor response trajectory. This dissociation was not seen in the ventral CA1 and striatum. These data suggest that within the hippocampus sub-regions different in their sensitivity to task demands. Identifying this “division of labor” will facilitate our understanding of hippocampal function.

Preserved IEG reactivation in the aged fascia dentate

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Abundant evidence suggests that the hippocampus facilitates the consolidation of episodic memories. One strongly supportive observation is that patterns of hippocampal ensemble activity expressed during behaviour persist during subsequent "off-line" resting states [i.e., quiet waking states, slow wave sleep (e.g, Wilson & McNaughton, 1994)]. Recently, the catFISH method has been applied to studying this phenomenon (Marrone et al, 2008). It was found that a subpopulation of cells transcribing immediate-early genes (IEGs) during exploration expresses IEGs again during a subsequent period of rest. Thus, reactivation of spatial activity-induced ensembles also reactivates IEG expression. Moreover, both tetrode recordings and IEG expression indicate that reactivation is compromised in CA1 in aged animals. The next critical step is to understand how gene mis-regulation may occur in other regions that may contribute to age-related memory decline. Towards this goal, we examined zif268 expression in the fascia dentata (FD), a region known to show adramatic decline in activity with progressive age. Young (12 months) and aged (24 months) F344 rats were exposed to an episode of spatial exploration alongside a rest episode. In one group (pre-rest), rest occurred prior to exploration, while in a second group (post-rest), the rest episode occurred following exploration (n = 6 rats/age/group). During rest, both young and aged rats recapitulate zif268 expression patterns induced during previous exploration. Moreover, this recapitulation during rest occurred in the same number of cells, despite a dramatic reduction in the number of cells transcribing zif268 during spatial exploration. These data are consistent with the hypothesis that zif268 transcription during behaviour is driven largely by perforant path afferents, which are dramatically reduced in the aged hippocampus, while transcription during reactivation in the FD is the result of back projections from CA3, which may be preserved in the aged hippocampus.

IEG reactivation across multiple regions: Relation to coding sparsity

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Following wakeful, attentive behaviour, the patterns of ensemble activity in the hippocampal formation are recapitulated during subsequent "off-line" resting states (e.g., Wilson & McNaughton, 1994). Consistent with this observation, the patterns in the transcription of immediate-early genes (IEGs), such as Arc, are also recapitulated (Marrone et al, 2008), suggesting that IEGs contribute to "off-line" consolidation. Specifically, it was found that a subpopulation (~25%) of cells transcribing IEGs during exploration expresses IEGs again during a subsequent period of rest. However, since the discovery of experience-dependent neuronal reactivation, this replay of ensemble activity patterns has been found in several brain regions and across many species, indicating a very general biological phenomenon. If IEG reactivation reflects a correlate of this ensemble replay, then the same generality should be observed for IEG expression. This hypothesis was tested by examining Arc expression in several regions of the hippocampal formation and surrounding cortices in a group of F344 rats exposed to an episode of spatial exploration alongside a rest episode. In one group (post-rest), the rest episode occurred following exploration, while in a second control group (post-rest), rest occurred prior to exploration (n = 6 rats/group). In post-rest animals, all brain regions examined showed recapitulation of Arc expression patterns induced during previous exploration in a fraction of cells. Moreover, this recapitulation was absent in pre-rest control animals. Furthermore, it is clear that IEG reactivation, while detectable in multiple brain regions, occurs in a small fraction of the cells engaged during previous waking behaviour. Examining Arc reactivation in regions known to have differing degrees of coding sparsity will permit investigation of what quantitative relationship, if any, exists between the sparsity of the neuronal code expressed during behavior and its subsequent recapitulation at rest.

D1 receptor activation is not required for place cell-related Arc expression

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Several lines of evidence suggest the involvement of D1 receptors in certain forms of learning and memory as well as in hippocampal synaptic plasticity. In fact, activation of D1 receptors has been shown to be necessary for enduring synaptic plasticity in the CA1 region of the hippocampus. Similarly, the activity regulated immediate early gene Arc is involved in enduring plasticity and memory consolidation. It is well established that following spatial exploration, hippocampal neurons express Arc in an environment-specific manner such that they can reliably label cells that express place fields. Taking advantage of in situ hybridization for Arc mRNA and catFISH cellular imaging technique, we investigated whether dopamine D1 receptor activity is necessary for normal place cell-related Arc expression in the CA1 region of the hippocampus. F344 rats were habituated to handling and injection (saline) prior to the behavioural test. On test day, rats exposed to an open-field environment (A) for 5 min and immediately after the exploration received either a single injection of a D1 receptor antagonist, (SCH23390, 1 mg/kg, subcutaneously) or vehicle (saline). The rats were placed back in their home cages and 25 min later were exposed to either the same open field (A/A condition) or to a visually distinct open field located in a different environment (A/B condition). Immediately following the second exploration session, rats were sacrificed. Control groups consisted of a negative control group of undisturbed rats sacrificed immediately after being removed from their home cages and a positive control group of rats receiving maximal electroconvulsive shock. Preliminary data revealed no drastic change in Arc induction following spatial exploration, although it remains possible that subtle changes have occurred.

Spatial context sensitivity partially explains differences in episodic encoding between deep and superficial neocortical layers

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When a rat performs the same behavior in two different rooms, the cells in the superficial layers of neocortex show patterns of immediate-early gene (IEG) expression that indicate that the two experiences are differentiated. The deep layers of neocortex, however, do not respond differently to the two experiences (Burke et al., 2005). A possible reason for this layer-dependent difference in episodic encoding is that superficial layers are sensitive to spatial context, but deep layers are not. To test this possibility, we examined whether the layer-dependent difference in IEG expression disappears when a rat performs the same behavior in the same room. As in Burke et al. (2005) rats were trained to traverse a rectangular track, repeatedly, for food reward. The rats were exposed to two, five-min epochs of track running, separated by a 20-min period in the home cage. One group of rats was exposed to the same room twice (AA) and the other group was exposed to two different rooms (AB). Both conditions used the same food reward and the same behavior (turning direction). Patterns of immediate-early gene expression in activated cells were examined with the cellular compartment analysis of temporal activity by fluorescence in situ hybridization (catFISH) method. As in the Burke et al. (2005) study, the superficial and deep layers of the posterior parietal and gustatory cortices were examined. Consistent with the hypothesis that the superficial neocortical layers encode spatial context, greater overlap was observed in the superficial layers of the AA group as compared to the superficial layers of the AB group. Contrary to expectations, however, the amount of overlap between activated neural populations in the AA group was lower in superficial neocortical layers as compared to deep layers. Neither the percentages of activated cells nor the running speed differed between the 1st and 2nd epoch in AA and AB group. These results suggest that while sensitivity to spatial context can partially explain why the superficial layers differentially encode two behavioral epochs in two different rooms, other factors are also likely to play a role. The variables that may contribute to this difference include order of the experience, the animals' internal state, and intrinsic network properties of the superficial layers. How these factors contribute to the difference in episodic encoding between the superficial and deep neocortical layers remains an open question.

NK3 receptor activation in the median preoptic nucleus reduces core temperature in the rat

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One of the most dramatic symptoms of menopause is the hot flush, a proposed disorder of hypothalamic thermoregulation. Hot flushes are caused by ovarian hormone withdrawal and are effectively treated by estrogen replacement. The mechanism of menopausal flushes remains an enigma. We have previously shown that human menopause is associated with increased NKB gene expression in the hypothalamic infundibular/arcuate nucleus. We hypothesize that, in addition to its role in reproduction, these estrogen-responsive NKB neurons may participate in the regulation of body temperature and contribute to the onset of menopausal flushes. We have shown that arcuate NKB neurons project to the MnPO, a site implicated in the integration of estrogen with the thermoregulatory axis (Dacks and Rance, Soc.Neurosci.Abstr. 2008). In the present study, we investigated whether pharmacological activation of NK3 receptors (the preferential receptor for NKB) in the MnPO affects core temperature and thermoregulatory mechanisms. The MnPO of freely-moving, estradiol-treated, ovariectomized rats was infused (over 3 minutes) with a selective NK3 receptor agonist (senktide, 90 pmol) or vehicle (300 nl aCSF). Core temperature was measured by telemetry and tail skin temperature was measured using a tethered thermocouple. Senktide microinfusion elicited a robust drop in core temperature of approximately 1 oC with the maximal decrease observed approximately 20 minutes post-injection. These results demonstrate that activation of NK3 receptors in the MnPO alters body temperature and provides the first evidence that NKB signaling affects thermoregulation.

An interconnected network of neurokinin B neurons in the arcuate nucleus of the rat

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A recent study in humans showed that neurokinin B (NKB) and the NK3 receptor are essential in the regulation of reproduction (Topaloglu et al., *Nature Genetics*, 2008). NKB neurons in the infundibular/arcuate nucleus are likely to be key elements in this regulatory circuitry. These NKB neurons coexpress dynorphin, kisspeptin and ERα and exhibit marked changes in neuronal morphology and gene expression in postmenopausal women (Rance*, Peptides,* 2009). In the arcuate nucleus of the rat, there is a homologous population of NKB neurons that project to NK3 receptor-expressing GnRH terminals in the median eminence. Interaction among these arcuate neurons is suggested by the dense plexus of NKB/dynorphin fibers in close apposition to NKB/dynorphin neurons and coexpression of the NK3 receptor. We hypothesize that this population of arcuate NKB neurons has extensive interconnections that would provide an infrastructure for synchronization. In the present study, we determined if NKB fibers in the arcuate nucleus originate from arcuate cell bodies. The anterograde tracer biotinylated dextran amine (BDA, 10,000 MW) was iontophoretically injected into the rat arcuate nucleus. Dual-label immunofluorescence was then used to identify NKB-ir axons that were anterograde-labeled with BDA (NKB/BDA). NKB/BDA fibers were identified throughout the rostral-caudal extent of the arcuate nucleus and in the external zone of the median eminence. Interestingly, commissural NKB/BDA fibers were also identified in the internal zone of median eminence projecting to the arcuate nucleus contralateral to the site of injection. In addition, close apposition was observed between NKB/BDA axons and single-labeled NKB somata within the arcuate nucleus. These results provide strong evidence that arcuate NKB neurons project to other NKB neurons within the arcuate nucleus. Combined with previous studies (Krajewski et al., *J Comp Neurol* 2005, *Soc Neurosci Abstr* 2008), we describe an interconnected network of arcuate NKB-ir neurons that are sex-steroid responsive and project to GnRH terminals in the median eminence. The importance of the NK3 receptor in this network is underscored by its presence on both arcuate NKB neurons and GnRH terminals. This circuitry provides a mechanism for arcuate NKB neurons to be bilaterally synchronized to influence pulsatile secretion of GnRH.

Effect of Actinomycin D on the sustained transcription of Arc in the rat hippocampal granule cells

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Memory formation requires the induction of synaptic plasticity in the cells engaged in information processing. Some of these plastic events are modulated by immediate-early gene expression induced during activation of neural ensembles following behavioral experience. Particularly relevant is the expression of the immediate-early gene Arc that is known to be related to persistent forms of synaptic plasticity. In the hippocampus dentate gyrus (DG) it has been observed that Arc mRNA expression following a single 5 min exploration occurs in ~2% of the granule cell population (Chawla et al., 2005) that lasts for more than 8 hrs (Ramirez-Amaya et al., 2005; Chawla et al., 2006; 2007). We have observed that more than 50% of the DG granule cells that contain cytoplasmic Arc mRNA > 30 min after exploration also express intronic Arc in the nuclei, representing recent transcription without further stimulation and suggested that DG neurons activated by exploration show sustained Arc transcription for several hours. In order to demonstrate that this is actually a sustained transcription mechanism we administered the transcription inhibitor Actinomycin D intraventricularly (IV) in rats immediately after spatial exploration and compared them with animals receiving IV vehicle administration. Different group of animals (n=3 or 4) were sacrificed at 30, 120 and 480 minutes. The results show that Actinomycin D administration as compared to vehicle notably reduced the amount of recently transcribed Arc 30 minutes after exploration and also reduced the amount of total activated cells at the 120 and 480 min time points. Since the same IV Actinomycin D administration produced a reduction in Arc transcription that lasts ~2hrs, the current observation suggests that the long lasting presence of Arc observed in DG granule cells after spatial exploration represents a sustained transcription in granule cells that were activated during the behavioral experience.

Diffusion measures of white matter integrity in older females related to Body Mass Index

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Increased body fat has been associated with alterations of the brain structure. In a recent study we used voxel based morphometry to examine the relationship between Body Mass Index (BMI) and structural differences in gray and white matter volumes in a sample of 95 community-dwelling older females (ages 52 – 92; BMI range 18.5 – 45). Higher BMI was associated with regions of decreased gray matter volumes as well as several regions of increased volumes of white matter in frontal, temporal, and parietal regions. Similar increases in white matter volumes have been observed in a study of obese young adults (Haltia et al., 2006) and the effect was partially reversed by dieting, suggesting that increased body weight may enhance the density of the myelin sheath. However, it is not known whether these increases in volume result in functional changes to the diffusivity of white matter. To address this question, diffusion-weighted magnetic resonance imaging was used to assess the relation between BMI and diffusion in the same sample of older females. Images were acquired using an echo planar sequence (GE ASSET), with 58 axial sections, 2.6 mm no gap, covering the entire brain (TE/TR = 70.4/13000 ms, matrix 96x96, FOV = 25cm). Diffusion was measured in 25 directions (2 averages, B0 = 1000s/mm^2). Measures of fractional anisotropy (FA) and apparent diffusion coefficients (ADC) were extracted from the same regions of white matter determined by the VBM analysis to show increases in volume related to BMI: right orbital, inferior, and superior frontal; left and right temporal stem; right cingulum and posterior cingulum; right superior temporal; and left parietal. After controlling for age, BMI was negatively correlated with FA in the right superior temporal white matter ($r = -.46$, $p < .001$), left temporal stem ($r = -.32$, $p < .01$), and right cingulum ($r = -.24$, $p < .05$). These correlations were against the expectation that BMI is associated with increased FA considering the notion that higher volumes of white matter might reflect better myelinisation. However, in one region we observed a negative correlation between BMI and ADC of the inferior frontal white matter ($r = -.21$, $p < .05$) supporting our expectation of better white matter integrity with increased BMI. The results suggest that increased white matter volumes seen in VBM analysis are not strongly associated with changes in white matter diffusivity. Further investigation is needed to identify the underlying mechanism of white matter volume changes associated with increasing BMI.