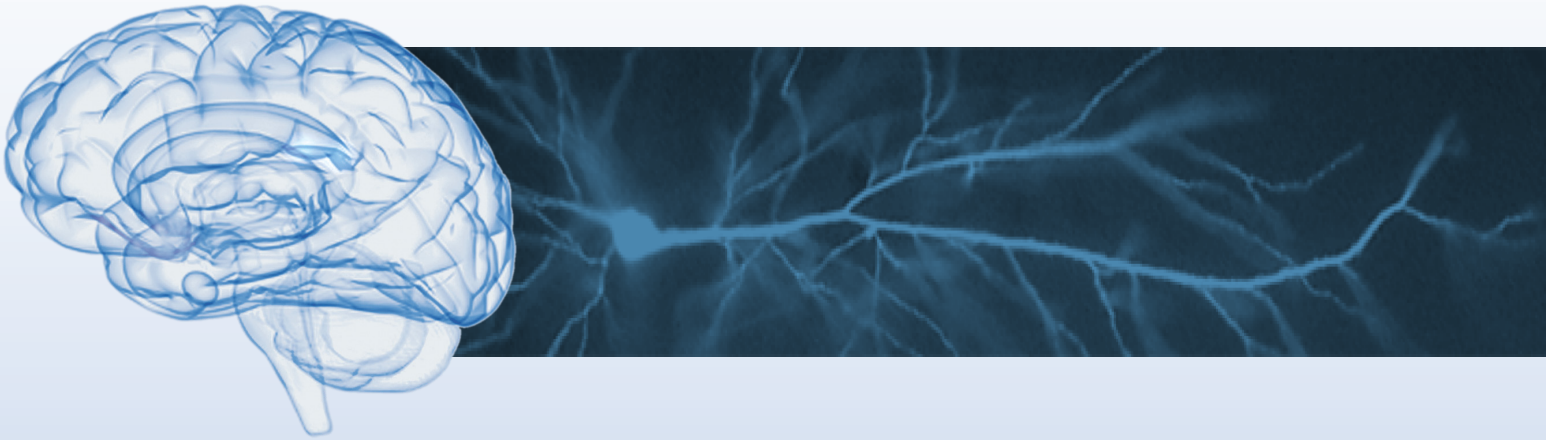

UT Austin Conference on Learning & Memory

Hosted by The Center for Learning and Memory
The University of Texas at Austin

April 15-16, 2011

Keynote Speaker
Susumu Tonegawa, MIT

Session Speakers
Kristen Harris
Kimberly Huber
Michael Mauk
Elizabeth Phelps
Alison Preston
Kimberly Raab-Graham
Michael Rugg
Alcino Silva
David Tank
Gina Turrigiano
Charles Wilson

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Conference Schedule

Friday, April 15th
Keynote Presentation and Cocktail Reception
AT&T Conference Center, Amphitheater 204
Sponsored by NeuroTexas Institute at St. David's Health Care

3:00-4:00	Conference Registration, Amphitheater 204 lobby
4:00-5:30	Susumu Tonegawa, MIT *"Neural circuit genetics of the hippocampal-entorhinal network"*
5:30-6:15	Cocktail Reception, AT&T Conference Center Interior Courtyard
7:00-9:30	Lake Austin Dinner Cruise

Saturday, April 16th
Conference sessions will be held in the Norman Hackerman Building (NHB)
Auditorium 1.720
Sponsored by Apple, Mind Science Foundation,
Neuralynx, NIH, Praxair, VWR International, Zeiss

8:00-8:50	Continental Breakfast, NHB 24th Street Patio Poster Session Open, NHB Lobby 1.400
8:50-9:00	Welcome Dan Johnston, Director, Center for Learning & Memory

Session 1
Molecular approaches to the study of learning & memory
Moderator: Richard Aldrich, UT Austin,
Department of Neurobiology

9:00-9:30	Alcino Silva, UCLA Department of Neurobiology *"Molecular and cellular mechanisms for memory allocation in neuronal networks"*
9:30-10:00	Kimberly Huber, UT Southwestern, Department of Neuroscience *"Regulation of synaptic function and number by Fragile X Mental Retardation Protein"*
10:00-10:30	Kimberly Raab-Graham, UT Austin, Department of Neurobiology *"Molecular mechanism of mTOR mediated suppression of Kv1.1 mRNA translation in dendrites"*
10:30-10:50	Break

Session 2
Systems approaches to the study of learning & memory
Moderator: Ila Fiete, UT Austin, Department of Neurobiology

10:50-11:20	David Tank, Princeton University, Neuroscience Institute *"Neural circuit dynamics in mice navigating in virtual reality"*
11:20-11:50	Charles Wilson, UT San Antonio, Department of Biology *"Prospects for the striatum as a locus for storing memories of value and cost"*
11:50-12:20	Michael Mauk, UT Austin, Department of Neurobiology *"Cortical persistent activity and trace eyelid conditioning: a working memory hypothesis"*
12:20-2:00	Buffet lunch, NHB 24th Street Patio Poster session open and poster competition judging, NHB lobby 1.400

Session 3
Learning & Memory in humans
Moderator: Russell Poldrack, UT Austin,
Director, UT Imaging Research Center

2:00-2:30	Elizabeth Phelps, NYU, Department of Psychology *"Changing Fear"*
2:30-3:00	Michael Rugg, UT Dallas, Center for Vital Longevity *"Brain circuits supporting recollection: fMRI studies"*
3:00-3:30	Alison Preston, UT Austin, Department of Psychology *"Reactivation of prior experience during learning promotes the formation of relational memory networks"*
3:30-3:50	Break

Session 4
Synaptic approaches to the study of learning & memory
Moderator: Helmut Koester, UT Austin, Department of Neurobiology

3:50-4:20	Gina Turrigiano, Brandeis University, Department of Biology *"The self-tuning neuron: homeostatic synaptic plasticity"*

4:20-4:50	Kristen Harris, UT Austin, Department of Neurobiology *"Balancing synaptic weight during long-term potentiation"*

Poster Speakers

4:50-5:05	Miriam Meister, UT Austin, Graduate Student *"Dissociation of persistent memory activity and decision signals in parietal cortex of awake monkey"* Poster Abstract [4]
5:05-5:20	Loredana Stoica, Baylor College of Medicine, Graduate Student "*Selective pharmacogenetic inhibition of mTORc1 blocks long-term synaptic plasticity, memory consolidation and reconsolidation"* Poster Abstract [23]

Closing

5:20-5:30	Wrap-up and announcement of poster competition winners Dan Johnston, Director, Center for Learning & Memory
5:30-6:15	Cocktails in NHB lobby 1.400 Poster session open, NHB lobby 1.400
6:15-7:15	Buffet dinner, NHB 24th Street Patio

CENTER *for* LEARNING *and* MEMORY
The University of Texas at Austin

Poster Abstracts

[1] A role for medial prefrontal cortex in delay eyelid conditioning

Brenda D. Houck and Michael D. Mauk
Center for Learning and Memory, The University of Texas at Austin

Savings is the phenomenon of relearning at a faster rate than original learning. Delay eyelid conditioning engages the cerebellum in such a way as to reveal the properties of cerebellar learning and displays both same-CS savings (where a single CS is trained, extinguished and retrained) and different-CS savings (after training and extinction to one CS, retraining to a different CS is faster). We have recently shown that when contributions from medial prefrontal cortex (mPFC) are prevented, either with lesions or by using stimulation of mossy fibers as the CSs, different-CS savings is eliminated whereas same-CS savings is intact. Along with results showing that trace eyelid conditioning requires input to the cerebellum from this same region of mPFC, these results suggest mPFC can influence delay eyelid conditioning. We hypothesize that during delay eyelid conditioning the cerebellum learns in response to both tone-driven and mPFC-driven inputs, and that during relearning to a new CS, faster learning is supported through the mPFC-driven input which is reestablished quickly, enabling the cerebellar same-CS savings mechanism. This hypothesis predicts that delay eyelid responses will be vulnerable to mPFC lesions during initial learning to the new CS. To test this prediction we pharmacologically silenced the mPFC by infusing a GABA agonist during initial acquisition to one CS and during relearning to a new CS. These data support the hypothesis that different-CS savings requires interactions between mechanisms in mPFC and in cerebellum.

[2] Identifying the forebrain circuits necessary for trace eyelid conditioning

Jennifer J Siegel*, Frank Riusech*, Maria Vicky Moya, Brian Kalmbach, Nikolai Dembrow, Raymond A. Chitwood, Daniel Johnston and Michael D. Mauk *Denotes equal contribution
Center for Learning and Memory, The University of Texas at Austin

The association of two stimuli that do not overlap in time poses a special problem for neural circuits. While the cerebellum can support learning conditioned responses (CRs) when a tone conditioned stimulus (CS) and an unconditioned stimulus (US, e.g. eye stimulation) overlap in time (delay conditioning), it requires forebrain input via the pons when stimuli are separated by a temporal gap (trace eyelid conditioning, TEC). Persistent activity in the prefrontal cortex (PFC) appears to be particularly well suited for this purpose. We are defining a PFC-to-pons-to-cerebellum circuit in the rat that is involved in the association during TEC. Anterograde tracers infused into the mPFC formed discrete plexuses of dense axon terminals in the rostral pons. Reversibly inactivating the rostromedial pons selectively abolished the forebrain-dependent trace CRs. By combining the reversible inactivation of muscimol with retrograde tracers labeled layer V neurons in PFC. Single-units recorded from the caudal mPFC during trace conditioning displayed significant activity increases in response to the tone that persisted to overlap with the US, in addition to phasic stimuli responses. Importantly, persistent activity was observed during training before the expression of learned responses. Finally, we are using somatic and dendritic patch clamp recordings *in vitro* to test whether after TEC training there are changes in the physiological properties of pons-projecting neurons in areas of PFC involved in TEC.

[3] **Heterogeneity in mPFC layer V neurons depending on long-range projection targets**

Nikolai Dembrow*, Raymond A Chitwood*, Brian Kalmbach*, Jennifer J Siegel, Frank Riusech, Maria Vicky Moya, Michael D Mauk and Daniel Johnston *Denotes equal contribution
Center for Learning and Memory, The University of Texas at Austin

Persistent activity in the PFC during the interval between stimulus and response is hypothesized to constitute the neural basis of working memory. To elucidate the mechanisms of this persistent activity we are identifying the intrinsic properties of pyramidal neurons in layer V. Interestingly, the dynamic and integrative properties of layer V neurons that project to the pons (CPn) at the soma are distinct from those that project to the contralateral cortex (commissural, COM). CPn neurons act as band-pass filters and will resonate between 2 and 6 Hz, whereas COM neurons act as low-pass filters, resonating below 2 Hz. Differences in the intrinsic properties extend to the dendrites of these neurons. Using dual recordings, we find that neurons that are non-resonant at their soma are similarly non-resonant in their dendrites. In contrast, neurons that are resonant at their soma display higher resonance frequency in their distal apical dendrites (>500 μm). The distinct subthreshold properties of CPn and COM neurons can be accounted for in part by differences in the hyperpolarization-activated cyclic nucleotide gated cation h-current. We further find that cholinergic, dopaminergic and adrenergic modulation alter CPn and COM neurons' subthreshold and suprathreshold properties differently. Importantly, CPn neurons have a higher propensity than COM neurons to fire persistently in the cholinergic agonist carbachol. Furthermore, we find that resonant neurons display synaptically-induced intrinsic plasticity whereas non-resonant neurons do not. Together, these data indicate that the two categories of projection neurons may serve separate functions in PFC during working memory-related behavior such as trace eyelid conditioning.

[4] **Dissociation of persistent memory activity and decision signals in parietal cortex of awake monkey**

Miriam L.R. Meister[1] and Alexander C. Huk[2]
[1]Institute for Neuroscience, [2]Neurobiology & [2]Center for Perceptual Systems, The University of Texas at Austin

Cells in the lateral intraparietal area (LIP) with persistent memory activity before an instructed eye movement have long been exclusively sampled out of the whole population for research on the electrophysiology of decision-making. This is done because it is thought that persistent memory activity indicates that a cell will also show the memory signals of integrating relevant sensory evidence over time in order to reach a decision of whether to next look at the cellular response field (RF) (Newsome & Shadlen, 1996). However, the relationship between this persistent activity and decision signals has never actually been tested. We therefore performed an experiment to see if decision signals existed even in cells without persistent memory activity. Data was collected from two rhesus macaque monkeys and two hemispheres during an oft-used decision-making task (discriminating direction of motion in a noisy visual stimulus like TV snow). The monkey indicated his decision at the end of each trial with an eye movement to either the cellular RF or a location diametrically opposite. We took data from cells that ran the gamut from very strong to totally absent memory activity during the instructed saccade task. Our results show that persistent memory activity is actually not a predictor of whether a cell will show decision signals during decision-making.

[5] Recurrent seizures suppress dendritic growth of developing hippocampal pyramidal cells

Masataka Nishimura[1], Xue Gu[1] and John W. Swann[1,2]
[1]The Cain Foundation Laboratories, The Jan and Dan Duncan Neurological Research Institute, Departments of Pediatrics and [2]Neuroscience, Baylor College of Medicine, Houston, TX

Impaired learning and memory are common in epilepsy syndromes of childhood. Clinical investigations suggest that the developing brain may be particularly vulnerable to the effects of intractable seizure disorders. The earlier the onset of an epilepsy the larger the effects seem to be on both brain anatomy and cognition. Experiments reported here explore these ideas by examining the effects of seizures in infant mice on learning and memory and on the growth of CA1 hippocampal pyramidal cell dendrites.

Fifteen brief seizures were induced by flurothyl between postnatal days 7 and 11 in mice that express green fluorescent protein (GFP) in hippocampal pyramidal cells. One to 20 days later, dendritic arbors were reconstructed to measure growth. Spatial learning and memory were assessed in a water maze.

Recurrent seizures produced marked deficits in learning and memory. Analysis of pyramidal cells showed that after the cessation of seizures dendritic arbors dramatically slowed their growth while neurons in their littermate controls continued to add new dendritic branches and lengthen existing branches. When experiments were performed in older mice, seizures had no measureable effects on either dendrite arbor complexity or spatial learning and memory. The recurring seizures of intractable childhood epilepsy are likely to contribute to learning and memory deficits associated with these syndromes by suppressing dendrite growth.

[6] Previous ethanol experience enhances synaptic plasticity of NMDA receptors in the ventral tegmental area

Brian E. Bernier, Leslie R. Whitaker and Hitoshi Morikawa
Waggoner Center for Alcohol and Addiction Research, Section of Neurobiology and Institute for Neuroscience, The University of Texas at Austin

Addiction is thought to arise, in part, from a maladaptive learning process in which enduring memories of drug experiences are formed. However, alcohol (ethanol) generally interferes with synaptic plasticity mechanisms and thus impairs various types of learning and memory. Therefore, it remains unclear how powerful memories associated with alcohol experience are formed during the development of alcoholism. Here, using brainslice electrophysiology in mice, we show that repeated *in vivo* ethanol exposure (2 g/kg, i.p., three times daily for 7 days) causes increased susceptibility to the induction of long-term potentiation (LTP) of NMDA receptor (NMDAR)-mediated transmission in mesolimbic dopamine neurons, a form of synaptic plasticity that may drive the learning of stimuli associated with rewards, including drugs of abuse. Enhancement of NMDAR plasticity results from an increase in the potency of inositol 1,4,5-trisphosphate (IP_3) in producing facilitation of action potential-evoked Ca^{2+} signals, which is critical for LTP induction. This increase in IP_3 effect occurs through a protein kinase A (PKA)-dependent mechanism. Additionally, we found that ethanol-treated mice display enhanced place conditioning induced by the psychostimulant cocaine. These data suggest that repeated ethanol experience may promote the formation of drug-associated memories by enhancing synaptic plasticity of NMDARs in dopamine neurons.

[7] IP3-induced activation of Ca^{2+}-sensitive K^+ channels in striatal medium spiny neurons

Mike A. Clements and Hitoshi Morikawa
Waggoner Center for Alcohol and Addiction Research, The University of Texas at Austi

Inositol (1,4,5)-trisphosphate receptors (IP_3Rs) are intracellular Ca^{2+} release channels that play a key role in controlling neuronal excitability and plasticity. Production of IP_3 is caused by synaptic inputs activating metabotropic neurotransmitter receptors, such as metabotropic glutamate or acetylcholine receptors. The resulting increase in intracellular Ca^{2+} levels influences a plethora of neuronal processes by activating Ca^{2+}-sensitive enzymes and ion channels. Previous studies have shown that Ca^{2+}-sensitive SK and BK K^+ channels are activated by spike-evoked Ca^{2+} influx in medium spiny neurons (MSNs), the sole output neurons of the striatum, thereby shaping their firing pattern. However, it is not clear how SK and BK channels are coupled to IP_3-induced Ca^{2+} release in these neurons. To address this issue, we performed flash photolysis of caged $IP_3$to trigger IP_3R-mediated Ca^{2+} release in MSNs using mouse striatal slices. Photolytic application of IP_3 produced a transient suppression of firing evoked by depolarizing current injections in current clamp. In voltage clamp (HP = -57 mV), both SK and BK channels were found to contribute to IP_3-induced outward currents. The kinetics and IP_3 concentration dependence, together with differential sensitivity to fast and slow Ca^{2+} chelators, have allowed us to predict the arrangement of SK and BK channels with respect to IP_3Rs. These responses may play a role in neurotransmitter modulation of MSN firing.

[8] Social isolation during adolescence enhances a dopamine neuron learning mechanism

Leslie R. Whitaker, Mickael Degoulet and Hitoshi Morikawa
Waggoner Center for Alcohol and Addiction Research, Section of Neurobiology, The University of Texas at Austin

Social isolation is an environmental manipulation that heightens addiction risk and enhances pathological reward learning. Following a period of social isolation, clinical studies have demonstrated increased compulsive drug use and seeking, and animal studies have shown enhanced drug self-administration and reward learning. These effects are most pronounced when the isolation occurs during adolescence. Dopamine (DA) neurons of the VTA are critical to reward learning and motivated behavior. Synaptic plasticity at DA neurons is thought to be important for the development of drug addiction. Our lab has recently described LTP of NMDA receptors in DA neurons that is dependent on burst-induced Ca^{2+} signaling. This type of plasticity requires the amplification of burst-induced Ca^{2+} signals by preceding activation of mGluRs, leading to the generation of inositol trisphosphate (IP_3). This plasticity is enhanced following *in vivo* amphetamine or alcohol exposure, and may play a role in pathological reward learning. Using *in vitro* brain slice electrophysiology in conjunction with UV flash photolysis of caged IP_3, we show enhanced IP_3-mediated intracellular Ca^{2+} signaling in animals isolated for a period of 3-4 weeks during the adolescent period. We also show that social isolation occludes the effect of *in vivo* amphetamine administration on mGluR/IP_3-mediated Ca^{2+} signaling. Further, we show data indicating that social isolation enhances amphetamine conditioned place preference (CPP). Our results provide a putative neural mechanism that may contribute to the heightened addiction risk associated with exposure to prolonged social isolation.

[9] Modeling of small peptide fragments as inhibitors of the FGF14:Nav channel complex formation

Svetla Stoilova-McPhie[2], Alexander Shavkunov[1], Tetyana Buzhdygan[1,3], Anesh Prasai[1], Fernanda Laezza[1]
[1]Dept. of Pharmacology and Toxicology, [1]Neuroscience and Cell Biology, [1]Neuroscience Graduate Program, University of Texas Medical Branch, Galveston, TX

The pore-forming alpha subunit of the voltage-gated Na^+ (Nav) channels provides the basis for electrical excitability in the brain. Dysfunction of specific Nav channels is linked to a plethora of excitability-driven human disorders that mostly lack effective therapeutic options. Current medications targeting Nav channels are designed toward highly conserved channel domains and, as such, lack specificity. Here, we have explored the Nav channelosome as a source of less conserved protein-protein interaction interfaces toward the design of safe and specific drugs against Nav channelopathies.

Of the intracellular fibroblast growth factors (FGF11-14), FGF14 is the most potent and specific regulator of the Nav channels. Through a monomeric interaction with the intracellular C-terminal tail of Nav channels, FGF14 regulates gating and trafficking of neuronal Nav channels. We have aligned the FGF14 model with the crystal structure of the FGF13 to define the FGF14:Nav channel interface. Three model-based peptide fragments, Fpep1, Epep1 and Ppep1, were selected as potential candidate disrupters of the FGF14:Nav channel complex. The efficacy of the synthesized peptides in reducing the FGF14:Nav channel complex formation was evaluated using the split-luciferase complementation assay and co-immunoprecipitation. Our results indicate that Fpep1 inhibits significantly the association of FGF14 with the Nav channel. Molecular modeling revealed that Fpep1 overlays with amino acid residues critical for FGF14 binding to Nav channel. Further docking analysis will be employed to optimize potency and selectivity of this fragment as a platform for probe development and drug discovery against neuronal Nav channels.

[10] Real-time detection and functional regulation of the FGF14:Nav channel complex

Shavkunov AS, Panova-Elektronova NI, Veselenak R, Bourne N, Laezza F.
Dept. of Pharmacology and Toxicology, Neuroscience Graduate Program, Summer Undergraduate Research Program, Assay Development Service Division (ADSD), Galveston National Laboratory, University of Texas Medical Branch, Galveston, TX

The brain relies on the integrity of the pore-forming alpha subunit of the voltage-gated Na^+ (Nav) channels as key substrate of excitability. Dysfunction of these channels is linked to a plethora of brain disorders still lacking effective therapeutic options. In pursuit of new functional proteomic and drug discovery goals, we have explored the Nav channelosome as a new source for prospective therapeutics against Nav channelopathies. We have previously demonstrated that fibroblast growth factor 14 (FGF14) is a biologically relevant component of the Nav channelosome. Through an interaction with the intracellular C-terminal tail of Nav channels, FGF14 acts as a unique chaperone-like molecule controlling gating properties and subcellular localization of native Nav channels.

Here, we apply the bioluminescence-based luciferase complementation assay (LCA) to detect the FGF14:Nav1.6 channel C-tail complex formation real-time in living cells. To discover new intracellular pathways targeting the FGF14:Nav channel complex, we screened a library of 385 kinases inhibitors seeking compounds that could robustly and specifically regulate the FGF14:Nav channel complex formation. We have identified 28 compounds which had a significant effect on the FGF14:Nav1.6 channel complex formation, leading to a range of 4-fold decrease to 5-fold increase in luminescence intensity. Our findings reveal a potential kinase-regulated intracellular network at the basis of neuronal excitability that could serve as a platform for future therapeutic interventions against Nav channel-driven brain diseases.

[11] CK2-dependent regulation of the FGF14:Nav channel complex

Buzhdygan TP[1,2], Shavkunov SA[1], Panova-Elektronova NI[1], Sohail M[3], Laezza F[1].
[1]Dept. of Pharmacology and Toxicology, [2]Neuroscience Graduate Program, [3]Summer Undergraduate Research Program, University of Texas Medical Branch, Galveston, TX

The integrity of neuronal excitability relies on functioning of the voltage-gated Na^+ (Nav 1.1-1.9) channels. Recent evidence indicates that the intracellular fibroblast growth factor 14 (FGF14) binds to and modulates the activity of Nav channels through a direct interaction with the channel C-tail. However, the dynamic regulation of this protein complex remains unknown. In this study, we tested the hypothesis that selective kinase inhibitors would interfere with the FGF14:Nav channel complex. Using the split luciferase complementation assay, we show that functional coupling between FGF14 and the Nav1.6 channel is prevented by application of the casein kinase 2 (CK2) inhibitor 4,5,6,7-tetrabromobenzotriazole (TBB). Furthermore, we demonstrate that pretreatment with TBB decreases the co-immunoprecipitation of FGF14 and full length Nav1.2 channels in HEK293 cells and that FGF14 is directly phosphorylated by CK2. In support of the physiological relevance of this finding, we show that exposure of hippocampal neurons to TBB causes a striking loss of FGF14 from the axonal initial segment, causing a reversal of the protein polarity, while Nav channel subcellular distribution remained intact. In conclusion, we propose a model in which inhibition of CK2 activity might result in a rapid loss of Nav channel function and excitability through an FGF14-mediated mechanism.

[12] Aging-induced changes in mouse neuromuscular junctions are explained by degeneration and regeneration of muscle fiber segments at the synapse

Yue Li and Wesley Thompson
MCDB Section, School of Biological Sciences, The University of Texas at Austin

The vertebrate neuromuscular junction (NMJ), the synapse near the center of each skeletal muscle fiber, is formed by the terminal branches of a motor neuron apposed to accumulations of acetylcholine receptors (AChR) in the muscle fiber membrane. Early in postnatal development each NMJ achieves a unique "pretzel" shape given by the pattern of these terminal branches and AChR. In mice this structure is remarkably stable throughout most of life, except for intercalary growth (and shrinkage) as the muscle fiber changes in size. However, this structure appears to become unstable during the events of aging. Previous studies suggest a gradual loss of synaptic area as the AChR fragment into small islands and the nerve terminal develops varicosities. Here we used repeated vital and static imaging to show that NMJs in aging mice undergo sudden, profound changes in their morphology over the course of a few days, but that accumulate in the muscle over time. These changes occur as the segment of the muscle fiber underneath the synapse degenerates, is phagocytosed, and a new muscle fiber segment regenerates in its place. Similar changes can be induced by deliberate muscle fiber injury, suggesting injury occurs during the events of aging. Most of the disruption of morphology occurs as the terminal branches that persist above the degenerated muscle fiber segment grow into contact with the regenerating segment. Thus, the morphological changes that affect aging NMJs are caused by events that lead to the fiber's loss and subsequent replacement. Alterations in the course of aging of these synapses, e.g. those resulting from caloric restriction and exercise, likely act by altering the susceptibility of muscle fibers to damage.

[13] Schwann cell mediated remodeling of the mouse neuromuscular synapse

Matt Lee, Yue Li, Michelle Mikesh and Wesley Thompson
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At the adult vertebrate neuromuscular junction (NMJ) acetylcholine receptors (AChRs) are aggregated in the postsynaptic muscle fiber and precisely apposed by presynaptic nerve terminal branches that, in turn, are capped by non-myelinating terminal Schwann cells (tSCs). This structure is highly stable throughout most of life. However, NMJs in transgenic mice whose motor axons overexpress a membrane-associated form of neuregulin1-type III (NRG1-III), a ligand that binds a receptor tyrosine kinase present in SCs, are unstable and dynamic. In these mice, all three components of NMJs are dramatically altered. tSCs are increased in number (probably as a consequence of mitogenic activity of the NRG). The SCs also extend processes absent from control NMJs. AChR aggregates are found, not in smooth and continuous gutters, but in small islands with matching presynaptic nerve terminal boutons/varicosities connected by thin neurites. A subset of these AChR-rich islands lack presynaptic inputs and, from their dimmed appearance and subsequent fate, are in the process of elimination. Vital imaging suggests that morphological changes to the nerve terminal precede those to AChR aggregates and that terminal branches are continuously being modified. Electron and light microscopic examination strongly suggests tSCs actively destroy portions of the presynaptic nerve terminal. Moreover, near the onset of NRG expression in neonatal mice, SC processes actively intrude between the nerve terminal and muscle fiber. The postnatal elimination of polyneuronal innervation is accelerated in these mice. Moreover, such SC intrusion appears to be a feature of control, nontransgenic mice during the period of synapse elimination. Our results suggest that tSCs can actively remodel synaptic morphology/connection at vertebrate NMJs and that this activity can be induced by NRG signaling from the motor axon to the SC.

[14] Optimal tuning curve widths for grid cells

Yongseok Yoo and Ila Fiete
Center for Learning and Memory, The University of Texas at Austin

We analyze the grid cell code (GC) for animal location to determine its dependence on tuning curve width. Each grid cell fires as a function of location with periodic response that tiles the explored space. One grid network (all cells with a single spatial response period) encodes location only as a phase relative to its period. Further, different grid cell populations have distinct response periods. The GC therefore consists of a family of multi-modal tuning curves with different phases and periods, which seems strikingly different from standard population codes with one or a few response peaks. We analytically and numerically calculate the mutual information between animal location and the GC for various tuning curve widths. In one grid network, narrowing tuning increases information up to a point and then is neutral. However, the multi-period GC generates an opposite pressure on tuning width. GC information drops precipitously to zero when the phase noise exceeds a threshold because of the nonlinear noise sensitivity of the multi-period GC. This threshold increases with the tuning curve width. Thus, broader tuning curves reduce the probability of total information loss. We find that unlike the results on unimodal tuning curves, the GC has a finite optimal tuning curve width, regardless of the dimensionality of the coded variable. This finite width should be of similar size to the amplitude of phase noise in the grid networks. We discuss this prediction in the context of the wide tuning curves found in neural recordings.

[15] Spike time-dependent synaptic plasticity can organize a recurrent network to generate grid cell responses

John Widloski and Ila Fiete
Center for Learning and Memory, The University of Texas at Austin

We describe a biologically plausible model for the development of a network that, after learning, reproduces the spatially periodic patterns of activity characteristic of grid cells. Further, the formed network can integrate velocity input to estimate animal location. The formed network displays the low-dimensional continuous attractor (CA) dynamics of models that successfully predict many features of the grid cell response. Our model uses a spike-time-dependent plasticity (STDP) rule with both symmetric and asymmetric components, applied to an initially unstructured network of spiking neurons that receive different velocity inputs and also randomly receive spatially local place cell-like inputs. The symmetric STDP term causes neurons firing at short time-lags to become recurrently connected, and those firing at intermediate time-lags to become negatively coupled. If the neurons are rearranged topographically according to their place inputs, this connectivity produces grid-like patterns on the neural sheet. The antisymmetric STDP term enhances connectivity in the movement directions of a simulated trajectory, causing slight asymmetries in the network weights based on both the location and velocity tuning of the cells. These asymmetries cause velocity inputs to drive movement of the network activity pattern in proportion to animal velocity, enabling path integration. The simplicity and plausibility of the developmental model should lay to rest critiques about the complexity of wiring in grid cell CA models. The model explains why the network need not be topographic, and generates predictions about inputs to and maturation of responses in the grid cell network during development.

[16] Assessing the role of feedback in spatially patterned grid cell responses

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[1]Center for Learning and Memory, The University of Texas at Austin, Austin, TX; [2]UCL Institute of Cognitive Neuroscience, London, United Kingdom; [3]UCL Institute of Behavioural Neuroscience, London, United Kingdom; [4]UCL Institute of Neurology, London, United Kingdom

An important class of models of grid cell activity is based on low-dimensional continuous attractor dynamics arising from recurrent connections within the grid system. A necessary prediction of these models is that the strong recurrent connections force the grid responses of different cells to maintain fixed relative spatial phases over long periods of time. We analyze the stability of pairwise neural correlations for experiments in which the spatial responses of single neurons change over time. The first such experiment involves resizing of a familiar enclosure, with the result that spatial responses rescale along the resized dimension. We show that the relative spatial phase between pairs of cells remains stable over time. This result is consistent with recurrent connectivity, but does not rule out the possibility that these relationships persist due to feed-forward input. In an attempt to address this, we analyze responses from animals' first exposure to novel environments. The spatial firing pattern expands, and relaxes back to the periodicity seen in familiar enclosures. During the relaxation, external sensory cues are static and thus likely not responsible for the changing grid responses. We analyze whether the constant phase relationships seen across familiar environments are present from first exposure or develop over time. Finally, we illustrate a generative model to predict grid cell spikes. The aim is to obtain the key determinants of grid cell firing, including animal location, velocity modulation, neural adaptation, and recurrent feedback in a Bayesian framework, and thus assess network contributions to grid cell activity.

[17] Regulation of GABAB as a mechanism for homeostasis

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Neurons change in response to global changes in their environment in order to stay within a homeostatic range that guides intrinsic excitability and accordingly firing properties. Changes to the composition and expression levels of synaptic receptors in response to activity are well characterized in AMPA glutamate receptors. Additionally, inhibition of NMDA glutamate receptors results in up regulation of the potassium channel, $K_v1.1$ through the suppression of the translational initiator, mTOR. With the increase $K_v1.1$ and mTOR suppressed, we asked how the composition of inhibitory channels would change to compensate for an expected decrease in excitability. Here we demonstrate that the GABAB receptor 1a (GABABR1a) surface expression in hippocampal neurons increases in proximal dendrites upon inhibition of GABAA receptors, but decreases in distal dendrites upon inhibition of NMDA receptors. Further, GABAB response to it agonist, baclofen, shifts toward excitability when NMDA receptors are inhibited. One of the molecular mediators for this may be the adaptor protein 14-3-3η, which decreases GABAB receptor surface expression in distal dendrites. Its protein level is reduced by NMDA receptors through a process that requires the 3′ untranslated region (UTR) of 14-3-3η mRNA. These results suggest a novel role for GABAB receptors in maintaining the homeostatic range of hippocampal neurons.

[18] $K_v1.1$ containing potassium channels regulate back propagation of bursts of action potentials in apical dendrites of CA1 pyramidal neurons

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The low-voltage activated K^+ current (I_D) activates and inactivates relatively slowly and is known to control the firing properties of neurons. Voltage-gated K^+ channels containing $K_v1.1$, 1.2 or 1.6 α subunits are thought to underlie I_D, and these channels are present in soma and dendrites of CA1 pyramidal neurons. I_D can regulate an after-depolarization (ADP) following an action potential (AP), the bursting of APs, and the threshold for initiation of APs. APs initiated at the initial segment of axons can back propagate to apical dendrites (bAPs) and play a role in the induction of synaptic plasticity. It has been demonstrated that the A-type K^+ current (I_A) regulates single bAPs in apical dendrites; however, it is not known whether I_D can play a similar role. To examine this, we used dendrotoxin-k (DTX-k), a specific blocker of Kv1.1 containing K^+ channels, which is a major subunit for I_D in CA1 neurons. With whole-cell recordings from the soma, we confirmed that DTX-k lowered threshold, shortened the latency to the first AP, and increased the firing rate and the size of the ADP. Using high-speed fluorescence imaging and 100 μM bis-fura 2 in the pipette, we also examined the Ca^{2+} signals from bAPs in apical dendrites with three protocols; a single bAP, a train of bAPs (five bAPs at 20 Hz), and a burst of bAPs (five bAPs at 100 Hz). Surprisingly, DTX-k significantly increased the Ca^{2+} signals in response to a burst of bAPs in dendrites 50~200 μm from soma, with little effect on Ca^{2+} signals from a single bAP or a train of bAPs at more proximal locations (0~50 μm from soma). These results suggest that while the slower activating and inactivating I_D (as compared to I_A) does not affect single bAPs, it does play a major role in regulating the propagation of a burst of APs into the dendrites. Because bursts of APs are critical for several forms of synaptic plasticity and have been suggested to have important physiological functions *in vivo*, $K_v1.1$ containing, DTX-k sensitive K^+ channels may be involved in these processes.

[19] Smooth endoplasmic reticulum scales with synapse size and number along mature CA1 dendrites during long-term potentiation

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We recently found that 2 hours after the induction of long-term potentiation with theta burst stimulation (TBS-LTP), small spines were reduced in number and the remaining synapses were enlarged such that the total amount of summed synaptic area per unit length of dendrite remained the same as the control dendrites. We hypothesized that this coordination was mediated in part by the smooth endoplasmic reticulum (SER), an organelle important for regulation of intracellular calcium levels and distribution of integral transmembrane proteins, and polyribosomes, local indicators of protein synthesis. A significant reduction in the percentage of spines containing simple tubules of SER 2 hours after TBS-LTP was coupled with an increase in the presence of a spine apparatus (SA) in larger spines, suggesting that TBS-LTP may induce the elaboration of simple SER into the more complex structure of a SA. Spines containing both polyribosomes and SER/SA had PSDs that were 1.5x larger than PSDs on spines with SER/SA alone and 4.5x larger than PSDs on spines with polyribosomes alone 2 hr after TBS-LTP. This suggests that dendrites distribute resources such as SER and polyribosomes to spines depending on their level of synaptic activity.
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[20] The genetics of cognitive changes of older adults: An fMRI study of memory monitoring and the serotonin transporter

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23 healthy older (OA) and 17 younger adults (YA) participated in a two-part source and item memory task. At test, recognition memories for item and for the corresponding source were both reported along with low- or high-confidence ratings for each response. Memory monitoring was evaluated using accuracy for high-confidence items. The older adults were genotyped for the serotonin transporter (5-HTTLPR) gene, and split into those who are carriers of the short allele (CAR) and those who are not (NC). Behavioral results indicate there are no deficits for OA during sentence recognition monitoring but significant impairments of source memory monitoring. When the OA were split based on genotype (NC vs CAR) a significant interaction revealed that having a short allele resulted in greater impairment for source monitoring with no differences in recognition monitoring. Interestingly, the source monitoring performance of the NC was equivalent to that of the YA. Functional MRI analysis was done for all subjects, and an omnibus test of source memory versus a functional control task revealed a network of prefrontal cortex, medial temporal lobe, and parietal lobe structures. A comparison of accurate monitoring responses versus fixation was done for the OA; comparing NC to CAR. After using the cluster-corrected source memory map as a small volume correction, regions of the left lateral inferior frontal gyrus, the left dorsolateral prefrontal cortex, and the paracingulate gyrus showed significantly more activation for NC when accurately monitoring responses than for the CAR. For the same contrast, YA demonstrate a similar pattern of activation. These results indicate that preserved performance of the NC group for source memory monitoring is likely due to retained ability to recruit critical inferior frontal regions when making accurate memory assessments.

[21] Suppression of the protein kinase PKR promotes network hypersynchrony and enhanced cognition by interferon-γ mediated disinhibition

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The double stranded RNA-activated protein kinase (PKR) was originally identified as a sensor of virus infection. However, its function in the brain remains unknown. Here we report a unique mouse phenotype in which the lack of PKR leads to aberrant patterns of neuronal hypersynchronization yet enhances long-lasting synaptic potentiation (L-LTP) in hippocampal slices, and learning and memory in several behavioral tasks, including memory allocation. In addition, administration of a selective PKR inhibitor (PKRi) to WT mice replicates the *Pkr*$^{-/-}$ phenotype, namely enhanced network rhythmicity, L-LTP and memory storage. Surprisingly, we found that these effects are caused by a selective reduction in GABAergic synaptic transmission mediated by interferon-gamma (IFN-γ). Hence PKR controls the finely-tuned network activity that must be maintained while storing a given episode during learning without allowing pathological oscillations. Since PKR activity is altered in several neurological disorders, this kinase is a promising new target for the treatment of cognitive dysfunction.

[22] The new mTOR complex (mTORC2) is selectively required for actin-dynamics mediated long-term synaptic plasticity and long-term memory

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The reorganization of actin cytoskeleton is important for the late phase of long-term potentiation (LTP) and memory (LTM). However, the molecular mechanisms underlying actin dynamics-mediated changes in synaptic strength and memory storage remain poorly understood. The mammalian target of Rapamycin complex 2 (mTORC2), which contains mTOR and the key regulatory protein Rictor, was recently discovered. Thus, little is known about mTORC2's function, up-stream regulation and downstream targets in the brain. Here we show that in slices from *rictor* forebrain KO mice (*rictor* fb-KO) - where *rictor* is conditionally deleted and mTORC2 activity is consequently abrogated - the late phase of LTP (L-LTP), but not the early phase of LTP (E-LTP), is impaired. Furthermore, hippocampus-dependent LTM is selectively blocked in *rictor* fb-KO mice, whereas short-term memroy (STM) is spared. Finally we found i) the upstream synaptic events that activate mTORC2 and ii) that mTORC2 mediates these effects through regulation of Rac1-GTPase-mediated actin dynamics. In conclusion, our studies have identified a new regulatory complex (mTORC2) that accounts for the regulation of actin dynamics which controls the conversion from a short-term process (E-LTP and STM) into a long-term one (L-LTP and LTM).

[23] Selective pharmacogenetic inhibition of mTORc1 blocks long-term synaptic plasticity, memory consolidation and reconsolidation

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Both the formation of long-term memory (LTM) and late-long-term potentiation (L-LTP), which is thought to represent the cellular model of learning and memory, require *de novo* protein synthesis. The mammalian target of Rapamycin (mTOR) complex 1 (mTORC1) integrates information from various synaptic inputs and is best characterized function is the regulation of translation. Although initial studies have shown that rapamycin reduces L-LTP and partially blocks LTM, recent genetic and pharmacological evidence indicating that mTORC1 promotes L-LTP and LTM is controversial. Thus, the role of mTORC1 in L-LTP and LTM is unclear. To selectively inhibit mTORC1 activity in the adult brain, we employed a new "pharmacogenetic" approach that relies on the synergistic action of a drug (rapamycin) and a genetic manipulation (mTOR heterozygotes, *mTOR*$^{+/-}$ mice) on the same target (mTORC1). Although L-LTP and LTM are normal in *mTOR*$^{+/-}$ mice, application of a low concentration of rapamycin – one that is subthreshold for WT mice – prevented L-LTP and LTM only in *mTOR*$^{+/-}$ mice. Furthermore we found that mTORC1-mediated translational control is required for memory reconsolidation. We provide here the first direct genetic evidence supporting the role of mTORC1 in L-LTP and behavioral memory.

{24} The effect of synaptic plasticity on place field stability under graded environmental perturbations

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We developed a parallelized computational model of networks of entorhinal and hippocampal cells influenced by synaptic plasticity to examine the stability of place fields under environmental perturbations. Place cells in the CA1 and CA3 form single firing fields within an environment. They receive the majority of their spatial input from cells in the medial entorhinal cortex (MEC) called grid cells, which fire in hexagonal patterns (Witter and Moser, 2006). We use the model to analyze results from an experiment in which a rat circles a track with various local and distal cues that rotate in opposite directions. The experiment demonstrates functional differences between CA1 and CA3 place cells, where the CA3 cells' response is much more coherent and cannot be explained by grid cell input (Lee et al., 2004). In the model, place fields form due to grid cell input and rate-based plasticity (Savelli and Knierim, 2009). The place fields then shift backward, opposite to the rat's movement, as seen experimentally. This backward shift is correlated to a feed-forward structure in recurrent CA3 connections due to synaptic plasticity. We are currently using the model to better understand the respective roles of entorhinal and recurrent CA3 input for place field stability.

[25] Rosiglitazone reversal of Tg2576 cognitive deficits is independent of peripheral gluco-regulatory status

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Converging lines of evidence associate gluco-regulatory abnormalities and peroxisome-proliferator-activated receptor (PPAR) gamma function with increased risk for Alzheimer's disease (AD). In this study, we used the Tg2576 AD mouse model to test the hypothesis that cognitive improvement following 1 month of PPAR gamma agonism with rosiglitazone (RTZ) correlates with peripheral gluco-regulatory status. We assessed cognition and peripheral gluco-regulatory status of Tg2576 mice following 1 month treatment with RTZ initiated prior to, coincident with, or after, the onset of peripheral gluco-regulatory abnormalities (4, 8, and 12 months of age, respectively). Whereas 5 months old (MO) and 13 MO Tg2576 did not gain cognitive improvement after 1 month treatment with RTZ, 9 MO Tg2576 mice exhibited reversal of associative learning and memory deficits. Peripheral gluco-regulatory abnormalities were improved in 9 and 13 MO Tg2576 with RTZ treatment; RTZ treatment had no effect on the normal glucose status of 5 MO Tg2576 mice. These findings suggest that RTZ-mediated cognitive improvement does not correlate with peripheral gluco-regulatory abnormalities per se, but reflects the age-dependent mechanistic differences that underlie cognitive decline in this mouse model.

[26] PPAR gamma as a pharmacological modulator in drug abuse

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Psychostimulant abuse, addiction, and relapse during abstinence remains a confounding public health issue in the United States and safe, effective pharmacotherapies are still needed for treatment. Here we explore a novel therapeutic target, peroxisome proliferator-activated receptor (PPAR), using a preclinical model of addiction in vivo. This ligand activated transcription factor belongs to the nuclear receptor family and its gamma isoform (PPARγ) plays a vital role as a primary lipid sensor and regulator of lipid metabolism. Thus, there are several FDA approved ligands that are clinically used for the treatment of diseases such as type 2 diabetes. However PPARγ is also widely distributed in the CNS and is highly expressed in neurons. Our lab has already demonstrated that PPARγ rescues hippocampal cognitive impairment in an animal model of Alzheimer's. This rescue partly involves the recruitment of hippocampal ERK MAPK activity to the nucleus (Rodriguez et al., 2010). Given the important role for learning and memory in the process for which drug abuse transitions into addiction, and our recent evidence that neuronal PPARγ is involved in restoring cognitive deficits through ERK MAPK, we hypothesize that neuronal PPARγ represents a potential therapeutic target for maintaining drug abstinence during stimulant withdrawal.

[27] Deletion of TRIP8b decreases h-current in hippocampal CA1 pyramidal neurons

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Hyperpolarization-activated cyclic nucleotide gated non-selective cation channels (I_h, HCN) are important regulators of neuronal physiology. Despite their physiological importance, little is known regarding the molecular mechanisms underlying the proper expression and localization of I_h in the mammalian brain. The tetratricopeptide containing Rab8b-interacting protein (TRIP8b) is a recently identified auxillary subunit of HCN channels. Studies have shown that decrease in the interaction between HCN subunits and TRIP8b protein following *status epilepticus* is accompanied by loss of dendritic I_h suggesting that TRIP8b is important for expression and maintenance of HCN channels *in vivo*. As previous studies describing the interaction of TRIP8b and HCN channels have been limited to *in vitro* systems, we sought out to characterize the role of TRIP8b in regulating I_h *in vivo* by electrophysiologically characterizing I_h-based membrane properties in mice lacking TRIP8b expression due to genetic deletion. We find that loss of TRIP8b leads to a significant downregulation of constitutive I_h in the soma and dendrites of CA1 neurons lacking TRIP8b, suggesting that TRIP8b is essential for functional expression of HCN channels *in vivo*. We also demonstrate that mice lacking TRIP8b have impaired I_h-dependent intrinsic plasticity associated with LTP suggesting that TRIP8b deficient mice have intact synaptic plasticity mechanisms but lack ability to regulate surface expression of HCN channels specifically.

[28] The gradient of HCN channels normalizes the timing of incoming inputs across the apical dendrite in CA1 pyramidal neurons

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A crucial aspect of information processing in a single neuron is the real-time integration of co-incident inputs across its dendritic arbor. However, this seemingly simple task is an arduous challenge for the neuron when one considers its magnitude and complexity. For example, a CA1 neuron in the rodent hippocampus has to integrate roughly 20,000 inputs from the CA3/CA2 region, which are spread across 350 microns at varying distances from the axo-somatic integration site. So how does a CA1 neuron overcome the distance-dependent variability in the timing of incoming inputs? In this study, we show that the CA1 neuron has intrinsic mechanisms to counteract the propagative delay of distal inputs and thus normalize the timing of incoming inputs at the integration site for natural rhythmic inputs. These neurons, like many other principal cells in the mammalian CNS, exhibit a gradient distribution of HCN channels along their apical dendrite. The HCN channels, due to their tendency to resist membrane change, impart inductive properties to the membrane, which counteract the local capacitive delay and thus advance the timing of the local voltage response to natural rhythmic inputs. As this advance of local response is higher for distal inputs than proximal inputs because of the gradient distribution of HCN channels, we show that in a CA1 neuron, natural rhythmic inputs across the apical dendrite are co-incident at the axo-somatic integration site, irrespective of their location along the dendritic arbor.

[29] Persistent Na+ current mediates neuronal excitability in hippocampal CA1 pyramidal neurons

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The biophysical properties and distribution of voltage gated ion channels of the neuron determines the input-output function of the neuron. Persistent Na^+ current (I_{NaP}) is of interest because of its activation near rest, slow inactivation kinetics, and consequent effects on excitability. Overshadowed by transient Na^+ current (I_{NaT}) of large amplitude and fast inactivation, various quantitative characterizations of I_{NaP} have not provided clear understanding. We addressed this question using quantitative electrophysiology to study somatic I_{NaP} in 4–7 week old rat hippocampal CA1 pyramidal neurons. Compared to I_{NaT}, it has much smaller amplitude (2.38 % of I_{NaT}) and distinct voltage dependence of activation: 16.7 mV lower half maximal activation voltage and 41.3 % smaller slope factor than those of I_{NaT}. Hexanol has an anesthetic effect and preferentially blocks I_{NaP} compared to I_{NaT}, and causes a significant voltage threshold elevation (4.6 mV) and reduced neuronal excitability. The differential blocking of I_{NaP} compared to I_{NaT} by halothane, a commonly used volatile anesthetic, further supports the critical role of I_{NaP} in setting voltage threshold. Taken together, our results suggest that a lower voltage threshold for action potential initiation in the axon initial segment can be ascribed to the different biophysical properties of the Na^+ channels, not the high density of the channel in the region.

[30] Knockdown of HCN1 channels in dorsal hippocampus leads to antidepressant-like behavior

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Membrane currents that are generated by hyperpolarization-activated, cyclic nucleotide gated nonselective cation channels (h-channels), also known as I_h, are characterized by 1) cyclic nucleotide-mediated modulation, 2) Na^+ and K^+ permeability, and 3) activation by membrane hyperpolarization. In the brain, I_h is important for rhythmic oscillation, synchronization of neuronal activity through temporal summation, and the regulation of synaptic transmission. Although h-channels are highly expressed in the hippocampal CA1 region, the behavioral phenotypes of knockout mice missing subunits HCN1 or HCN2 have been subtle. Furthermore, there are no available subtype-specific blockers or genetic animal models that offer a region-specific manipulation of HCN1 channels in the brain. To explore further the behavioral role of h-channels, a subtype specific knock-down of HCN1 by lentivirus-RNAi system was developed. The efficiency of lentivirus expressing shRNA in the dorsal CA1 pyramidal neurons was tested with whole-cell current clamp recordings, western blot, and immunohistochemistry. ShRNA-infected pyramidal neurons from dorsal hippocampus displayed altered intrinsic membrane properties consistent with a loss of functional I_h. HCN1 protein expression was significantly decreased in the dorsal CA1 region. Given that genetic knockout mice missing HCN1 in the brain exhibited antidepressant effect, we tested further whether reduction of I_h in the dorsal CA1 region exhibits an anti-depressant-like effect using a forced swim test paradigm. Reduction of I_h by stable gene knockdown in the dorsal CA1 region produced a robust antidepressant effect without a significant change of basal locomoter activity. We also confirmed that knockdown of I_h in the dorsal hippocampal CA1 region led to increased hippocampal activity by voltage sensitive dye imaging. These findings suggest that HCN1 is involved in modulating antidepressant-like effect in the dorsal hippocampus of CA1.

[31] Age-dependent dorso-ventral gradient of calcium store depletion induced intrinsic plasticity in CA1 pyramidal neurons of the hippocampus

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Disruption of the calcium homeostasis within the endoplasmic reticulum is commonly associated with pathological conditions in the brain. One postulated neuroprotective mechanism to deal with ER calcium disruptions in the hippocampus is Store Depletion Induced (SDI) Intrinsic plasticity. While the dorsal and ventral regions of the hippocampus are distinct in terms of their susceptibility to multiple neurological disorders, it is unknown whether these two regions are also subdivided in terms of cellular competence for dealing with insults such as calcium store depletion. In this study, we demonstrate that there is an age-dependent emergence of a dorso-ventral gradient in SDI intrinsic plasticity. In young-adult animals, the hippocampus appears homogeneous in its levels of SDI Intrinsic plasticity. Into adulthood, SDI intrinsic plasticity dwindles in the dorsal hippocampus and is maintained, however in a seemingly altered form in the ventral hippocampus. While young-adult SDI plasticity resembles plasticity of the h channel, adult SDI plasticity is suggestive of alteration in another channel. These results indicate a region-specific maturation of intrinsic plasticity mechanisms in response to internal calcium store disruptions. We suggest that this dorso-ventral gradient of plasticity may be related to differential susceptibilities to aging-related diseases in the dorsal and ventral hippocampus.

[32] Differential contribution of I_h to the resting membrane potential of CA1 neurons from the rat dorsal and ventral hippocampus

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The hippocampus is widely recognized as a critical structure for the formation and consolidation of memories. Based on lesion-defect behavioral assays and anatomical connectivity, it has become clear that the rodent hippocampus can be divided into as least two major parts: the dorsal hippocampus (DHC) and the ventral hippocampus (VHC). The DHC is associated with spatial learning and memory, whereas the VHC is associated with emotional learning and memory. Although there has been a great deal of work describing differences between the DHC and VHC at the behavioral, anatomical, and biochemical levels, the intrinsic physiological properties of individual neurons from these two hippocampal regions has received less attention. Using the whole-cell current-clamp method, we investigated the subthreshold electrophysiological properties of CA1 pyramidal neurons from the DHC and VHC and found significant differences in both the resting membrane potential (RMP) and input resistance (R_{in}). Specifically, the RMP was ~8 mV more depolarized, and the R_{in} was 155% larger in VHC CA1 pyramidal neurons than their DHC counterparts. Bath application of the hyperpolarization-activated cation non-selective current (I_h) blocker ZD7288 (20 μM) eliminated this difference in RMP, but not R_{in}. Direct measurement of I_h using dendritic cell-attached patches revealed that the midpoint of activation for I_h was ~15 mV more depolarized in VHC neurons than in DHC neurons. These results indicate that I_h plays a major role in determining the RMP of CA1 pyramidal neurons from the VHC, but not the DHC. Together, these observations highlight different physiological roles for I_h in DHC and VHC CA1 pyramidal neurons, which may contribute to the behavioral phenotypes generally associated with these two hippocampal regions.

[33] Anticipatory hippocampal responses predict individual differences in reward-based modulation of memory

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Emerging evidence suggests that medial temporal lobe (MTL) memory processing is modulated by reward, resulting in enhanced encoding of episodic information—long-term memory for events. Recent neuroimaging research has further revealed activation in hippocampus prior to stimulus presentation that predicts later memory performance, suggesting that modulatory processes such as reward may influence encoding processes in anticipation of upcoming events. Moreover, individual differences in neural responses to reward predict performance in reinforcement learning paradigms. Such individual differences in reward sensitivity may similarly influence the degree to which reward impacts MTL encoding. Using high-resolution functional magnetic resonance imaging (fMRI), the present study examines (1) how cues indicating future rewards influence MTL subregional activation prior to associative encoding and (2) how individual differences in reward sensitivity are reflected in MTL subregional activation. A high-value or low-value monetary cue preceded a pair of objects indicating potential reward for successful retrieval of the association. Memory was tested using a two-alternative forced-choice paradigm. Behaviorally, memory was superior for high-value associations relative to low-value associations. fMRI analysis revealed anticipatory responses within the hippocampus predicting memory formation that were further modulated by reward. Importantly, the observed enhancement of anticipatory activation for high-value compared to low-value pairs correlated with individual differences in behavioral reward sensitivity (hit rate for high-value pairs – hit rate for low-value pairs). The results suggest that reward-based motivation influences memory by facilitating hippocampal encoding processes prior to stimulus presentation, and that increased behavioral sensitivity to reward is reflected by increases in reward effects within the hippocampus.

[34] Detection of sequence violations in the medial temporal lobe: Subregional contributions to memory-based prediction through high-resolution fMRI

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Current research proposes that medial temporal lobe (MTL) subregions perform distinct computations to enable comparison between past and present experience. However, it is unclear how hippocampal subregions and surrounding MTL cortex differentially contribute to the detection of sequence novelty, wherein associated items appear in a new order. Here, we used high-resolution fMRI to measure MTL responses to temporal sequence violations while subjects performed an incidental 1-back detection task. Trials consisted of two consecutive sequence presentations. In the first, participants observed a sequence of four object-scene pairs. In the second presentation, the same set of object-scene pairs were presented again in a modified order. In the half condition (H), the order of the third and fourth pairs was switched. In the object-half (OH) and scene-half (SH) conditions, only the order of the objects or scenes was switched. In the novel (N) condition, the orders of all objects and scenes were scrambled. We identified distinct populations of voxels in hippocampus that were sensitive to violations of object order (OH>N), scene order (SH>N), and overall temporal order (H>N). By contrast, MTL cortical regions showed sensitivity to novelty that was predominantly related to the amount of previous exposure. These results expand on previous findings to suggest that hippocampal responses to associative novelty can be distinguished by the class of content being violated, and that MTL cortical regions show sensitivity to the item-level novelty status of stimulus configurations.

[35] Long-term recordings from purkinje and interpositus nucleus cells during eyelid conditioning

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Eyelid conditioning is a type of motor learning that engages the cerebellum and allows systematic investigation of the cerebellar processes that support learning. Learning-related changes in the cerebellar cortex, which help induce learning-related changes in the deep cerebellar nuclei, are the sites of plasticity necessary for learning within the cerebellum. For eyelid conditioning learning-related plasticity occurs at Purkinje cells in the anterior lobe of the cerebellar cortex that receive an inferior olive driven climbing fiber input (complex spike), and also at the anterior lateral region of the interpositus nucleus that maps onto the eyelid muscles. Acquisition, expression, and extinction of eyelid responses have been correlated with learning-related changes in these regions of the cerebellum but studies using electrophysiology have not been able to follow individual cerebellar neurons across many different training manipulations to examine how computation within the cerebellum accomplishes these different tasks. This study used tetrode recordings with a hyperdrive array to follow Purkinje cells that receive a complex spike elicited from eyelid stimulaiton and interpositus nucleus cells in the eyelid region for many consecutive days (up to 25) to determine how individual neurons in key areas of the cerebellum change during different task demands. Results indicate that neurons in the cerebellar cortex and interpositus nucleus show learning-related changes in a predictable way under a verity of training conditions. These results are important as they rule out many different hypotheses about the contribution of the cerebellum during motor learning.

[36] Protein kinase Mzeta inhibitor (ZIP) alters cocaine sensitization initiation and expression and decreases VTA AMPA mediated currents

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Repetitive cocaine administration results in a progressive and lasting increase of behavioral responses induced by a subsequent drug challenge. In certain regions of the reward circuit, like the ventral tegmental area (VTA) and the nucleus accumbens (Nacc), excitatory dopaminergic synapses undergo a robust and long-lasting AMPA-receptor-mediated potentiation after cocaine administration. Protein kinase Mζ (PKMζ) is involved in synaptic plasticity maintaining LTP in the hippocampus. Inhibition of PKMζ has been shown to erase spatial, instrumental, and classically conditioned long-term memories. If the presence of LTP-like changes induced by addictive drugs maintains addiction, then it should be possible to modulate or even reverse this condition with application of LTP/PKMζ inhibitors. Male Sprague-Dawley rats (250g) received 15mg/kg of cocaine ip for 5 days. Intra-VTA ZIP microinfusions were given after the last day of cocaine administration. A cocaine challenge (15mg/kg) 24 hours later showed that ZIP infusions significantly decreased locomotor activity ($p<0.05$) but had no effect on the expression measured after a 7 day withdrawal period. This evidence suggests that the persistent phosphorylation by PKMζ in the VTA mediates a necessary process for maintaining sensitization. To elucidate the mechanism for the previously observed behavioral response, we employed voltage-clamp recordings of VTA DA cells in slices prepared from chronically cocaine- or saline-injected rats. Pharmacologically isolated EPSC's were electrically evoked. Once a stable EPSC was achieved 5μM ZIP was superfused into the slice. ZIP superfusion decreased AMPA current EPSC's (~25% from baseline, $p<0.05$, $n=8$) in cocaine-treated compared to saline-treated rats, suggesting that the persistent activity of PKMζ can maintain drug-induced late-LTP. Intra Nacc PKMζ infusions on days 6 and 7 of the withdrawal period decreased expression of sensitization on a different group of sensitized rats. These results support the hypothesis that addiction involves aberrant learning that can be reversed by a PKMζ inhibitor. (Support GM-08224 CAJR)

{37} Back to continuous EEG: Colorimetric technique reveals fine spatiotemporal organization across different scales

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Continuous (unaveraged) electrophysiological signals contain functional information that is lost at the stage of temporal aggregation present in most quantitative analysis methods. The development of a 4-D spatiotemporal mapping by our team allows the assessment of continuous EEG signals, and brings a new framework to investigate the dynamics of brain-behavior relationships. Colorimetric mapping reveals sequences of coordinated oscillatory patterns appearing and disappearing in relation to underlying behavior. In our first application of this method, we inspected the 7-14 Hz frequency band in the context of an experiment designed to investigate how two brains interact during social behavior. We found a representative sequence of EEG patterns that significantly marked the transition from social to individual behavior. Here we illustrate the method and detail three principal stages involved in the parsing of continuous EEG into spatiotemporal patterns and further into functional brain processes: segmentation, classification and temporal organization. We discuss the implications of this new framework for the investigation of cognitive and especially memory processes. Recurrent and/or periodic dynamics within the temporal organization of EEG signals may reveal how specific chains of brain patterns are related to the storage and recovery of specific behavioral information.

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[38] Olfactory conditioning in the third instar larvae of drosophila melanogaster using heat shock reinforcement

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The adult *Drosophila melanogaster* has been a popular model for learning and memory studies. Now, using both positive and negative reinforcements, the larval stage of the fruit fly is also being used in an increasing number of classical conditioning studies. In this study, we employed heat shock as a novel negative reinforcement for larvae and obtained high learning scores following just one training trial. We demonstrated heat shock olfactory conditioning in both reciprocal and non-reciprocal paradigms and observed that the window of association between the odorants and the heat shock is much wider than seen previously using other negative reinforcements, possibly due to lingering effects of high temperature. Neither heat shock alone nor odor exposure alone altered the olfactory response in wild type larvae or the classic learning mutant *dnc1*. The *dunce* mutation did however, impair learning scores following the odor-heat association and we confirmed this learning defect using non-complementation analysis. Given that heat shock has not been employed as reinforcement in past studies, we explored learning as a function of heat intensity and found that optimal learning occurred around 41° C, with both higher and lower temperatures resulting in suboptimal reinforcement. In summary, we developed a very simple, robust paradigm of learning in fruit fly larvae using heat shock reinforcement.

[39] Delivery of RNAi to the CNS, as a potential treatment for alcohol addiction

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One of the key obstacles in treating neurological disorders is the lack of a robust mechanism for drug-delivery to the brain. This obstacle is primarily constituted by the blood-brain barrier, which prevents the passage of therapeutic molecules from blood to brain. In this report, we describe the use of a Myristoylated Polyarginine Peptide (MPAP) as a mechanism for RNAi delivery into neuronal cells, in vitro. MPAP is a cell-penetrating peptide that utilizes its cationic charge to be internalized via electrostatic interaction with the cell membrane. We first determined the optimal concentrations of MPAP and siRNA required for the formation of MPAP/siRNA complexes. The optimal ratio of MPAP to siRNA for efficient complexation was determined by gel shift assay and was found to be 7:1 respectively. Flow cytometry and fluorescent microscopy were used to determine that MPAP was capable and necessary for the delivery of siRNA into neuro2a and HEK293 cell cultures. These findings corroborate previous research, and may provide a noninvasive approach for siRNA delivery across the blood-brain barrier, to study and treat alcoholism.

[40] Neural network activity dynamics in the rodent barrel cortex

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Sensory inputs are represented and propagated by the spatiotemporal spiking activity of neural populations in the CNS to lead to behavioral outcomes. Spiking activity within neural networks may contain specific spatial and temporal relationships (activity patterns and correlations) that may be important for information signaling. Thus it is essential to examine network activity at the mesoscale to understand neural mechanisms underlying learning and memory. Here we studied properties of propagation and plasticity of spiking activity between recurrent networks in the rodent barrel cortex.

Observing propagation and plasticity in neural networks requires recording spiking activity of several neurons from multiple populations, simultaneously, with single-cell, single-spike resolution. To accomplish this, we used dithered random-access functional calcium imaging to record spiking activity from a large sample of up to 40 neurons from cortical neural populations in layers 4 (L4) and 2/3 (L2/3), simultaneously, as activity propagated from L4 to L2/3. This optical technique in conjunction with maximum likelihood deconvolution has been shown to detect spiking activity with high efficiency and temporal precision. We found that two kinds of population activity; a sparse, adapted and a wave-type, unadapted; were evoked in the neural networks of the juvenile mouse barrel cortex. These states of activity differed in their extent of propagation, content of fine-scale information and change in correlations with propagation. Also, the impact of cortical plasticity on sparse activity was spatially specific in terms of patterns of neurons activated. These results suggest that cortical networks signal and propagate information and undergo plasticity in a highly specific manner that is dependent on the state of network activity.

[41] The alpha2 agonist guanfacine reinstates amphetamine-disrupted latent inhibition in the rat

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Recent evidence of norepinephrine (NE) playing a role in the attention processing gave incentive to reexamine NE modulation of Latent Inhibition(LI). We examined guanfacine's ability to reverse the effects amphetamine on Latent Inhibition(LI). The inability to recondition a previously non-reinforced stimulus to a reinforced response is termed LI. This phenomenon is a robust marker for learned ignorance of irrelevant stimuli, a function required for selective attention. To measure the phenomenon we attempted to condition a tone to the emotional response of a foot shock. The association is tested by recording the licking of a rat and then playing the tone without a foot shock. A cessation of licking shortly after the tone indicates the association was made. Rats previously pre-exposed to the tone demonstrate much lower rates of association. The difference between the pre-exposed and non-prexposed group represents LI. Amphetamine (1mg/kg) administered, 30 min before conditioning and before pre-exposure (or non-preexposure) can abolish the difference between the two groups. The present study found that the NE alpha-2 agonist guanfacine (0.3mg/kg) can reverse the amphetamine-induced disruption of LI in the rat. These findings imply that NE has a modulatory role in low level association formation.

[42] Two-fold MeCP2 overexpression alters dentate synaptic plasticity and inhibitory function *in vivo*

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MeCP2, a protein that binds to methyl-CpG dinucleotides, is thought to modulate gene expression through chromatin modification. Loss-of-function mutations as well as increased expression of MeCP2 cause a spectrum of postnatal disorders including Rett syndrome, nonsyndromic mental retardation, learning disability, and autism. MeCP2 overexpression in mice has been previously shown to impair or enhance hippocampus-dependent learning and memory as well as synaptic plasticity in the CA1 region of hippocampal slices. However, it is not clear if increased MeCP2 levels are directly linked to altered synaptic plasticity *in vivo*. Here we examined the long-term potentiation (LTP) of synaptic transmission in awake, freely moving MeCP2^{Tg1} mice that express MeCP2 at twice the normal levels, and in wild type controls. Field recordings from the hilar region of the dentate were measured in response to electrical stimulation in the medial part of the perforant path. The recording and stimulating electrodes were implanted 2-3 weeks before the tests. Theta burst stimulation induces 400% potentiation in MeCP2^{Tg1} mice, compared with ~200% in controls. The enhanced LTP lasted for 3-4 days. To assess the possible mechanism underlying the LTP enhancement in MeCP2^{Tg1} mice, we analyzed the local inhibitory function in the dentate. Feed-forward inhibition in MeCP2^{Tg1} mice is significantly less than in wild type control mice. These results suggest that MeCP2 regulation of inhibition is critical for hippocampal synaptic plasticity and hippocampus-dependent memory.

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[43] Proteomic analysis of AMPA receptor complex

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AMPA receptor (AMPA-R) is a major excitatory neurotransmitter receptor in the brain. The regulation of the activity and trafficking of the AMPA-R is a major mechanism for synaptic plasticity. Previous studies with several AMPA-R interactors could explain only a part of AMPA-R function for the synaptic plasticity, indicating that there could be more numbers of synaptic proteins involved in the regulation of AMPA-R activity and trafficking under different neurophysiological situations. In this study, to identify AMPA-R interactors *in vivo*, we performed proteomic analysis of AMPA-R complex from the brain. AMPA-R complexes were isolated from the brain through various combinations of biochemical techniques for solubilization, enrichment, and immunoprecipitation of AMPA-R complex. Mass spectrometry of these complexes identified several novel components of AMPA-R complex as well as known components. Identification of these novel components followed by functional characterization could contribute to understanding of the synaptic plasticity, a main mechanism for the cognitive function of the brain such as learning and memory.

[44] Elevated hippocampal dendritic I_h in Fragile X Syndrome

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Fragile X syndrome (FXS) is the most common form of inherited mental retardation. Patients with FXS display a variety of phenotypes related to the central nervous system including impaired cognitive ability (IQ between 20 and 70), problems with working memory, autistic behavior, and increased incidence of epilepsy. Although the synaptic and anatomical underpinnings of FXS have been extensively investigated, the contribution of altered ion channel function to the neurological impairments associated with FXS remains largely unexplored. One ion channel in particular, the h-channel (which mediates I_h), is expressed at high density in dendrites and has been implicated in several neurological and psychiatric disorders.

Electrophysiological recordings from hippocampal CA1 pyramidal neurons suggest that the dendritic excitability is lower in a mouse model of FXS (*fmr1*$^{-/-}$) compared to wildtype mice. We found that the dendritic input resistance was significantly lower in *fmr1*$^{-/-}$ mice compared to wildtype mice. Additionally, rebound slope, voltage sag, and resonance frequency measured in the dendrites were significantly higher in *fmr1*$^{-/-}$ mice compared to wildtype mice. Somatic measures of these same parameters were not significantly different. These data suggest that dendritic, but not somatic, I_h is significantly higher in *fmr1*$^{-/-}$ mice. To compliment our physiological data, we used immunohistochemical techniques to label the h-channel subunits HCN1 and HCN2 and the auxiliary h-channel protein TRIP8b. We found that the immunofluorescence for HCN1 was higher in the distal dendrites of *fmr1*$^{-/-}$ mice compared to wildtype mice. There was no difference in HCN2 or TRIP8b labeling. These results suggest that the higher dendritic I_h in fmr1$^{-/-}$ mice is due to an increase in the density of the HCN1 h-channel subunit. These results support our working hypothesis that a dendritic h-channel dysfunction occurs in the *fmr1*$^{-/-}$ mouse model of FXS.

[45] Microtubule number as a predictor of spine density in the dendritic arbors of hippocampal dentate granule cells

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Long-term potentiation (LTP) and long-term depression (LTD) are differentially expressed throughout the granule cell dendritic arbor when the perforant pathway is activated. To investigate altered synaptic structure associated with LTP and LTD we must determine whether dendritic properties influence baseline synaptic densities. Previous studies in hippocampal area CA1 reveal spine densities are greater along thicker dendrites that contain more microtubules. We used 3D reconstruction from serial EM to determine whether this microtubule to spine density relationship holds in the granule cell dendritic arbor. Adult rats (121-179 days) were rapidly perfused with aldehydes, and processed for serial EM. Dendritic segments from inner, middle and outer molecular layers of the dentate gyrus were reconstructed and spine density (spine / μm) and microtubule number (number / cross section of the dendrite) were measured. Spine density decreased systematically and correlated with decreasing microtubule number and dendrite taper with distance from the granule cell bodies. We hypothesize that microtubule number serves to regulate the distribution of dendritic resources including polyribosomes, SER, and endosomes, and limits the number or size of synapses that can be supported per dendrite. Hence, dendrites used for comparison across LTP and LTD conditions must be controlled for intrinsic structural properties.

[46] Correction of electron microscope imaging distortion for serial section alignment by analysis of calibration replicas

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Section to section alignment is a preliminary step in three dimensional reconstruction from serial section electron micrographs. Typically, the micrograph of one section is aligned to its neighbors by analyzing a set of fiducial points to calculate an appropriate polynomial transform. This transform is then used to map all of the pixels of the micrograph into alignment. The higher the order of this polynomial, the more susceptible it is to gross distortion due to small errors in fiducial estimation. This limits the maximum order of such transforms. Unfortunately, magnetic-lens-distortion (TEM) and scan-distortion (STEM) may not be modeled accurately with such constraints. These distortions, however, are inherent to imaging systems, and are therefore static and directly calculable. Thus we can calculate the inverse transforms once and may remove this large-order distortion from all sections. Here, we propose a method for modeling lens- or scan-distortion from analysis of calibration grating replicas. We show that while these distortions have few implications for geometric measurements, removing them improves the section-to-section alignment. We also show the benefits of STEM imaging versus TEM imaging in this respect.

[47] SynapticDB, effective web-based management and sharing of data from serial electron microscopy

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Serial section electron microscopy (ssEM) is rapidly expanding to investigate synaptic circuitry and plasticity. The ultrastructural images collected through ssEM are content rich and their comprehensive analysis is beyond the capacity of an individual laboratory. Hence, sharing ultrastructural data is becoming crucial to visualize, analyze, and discover the structural basis of synaptic circuitry and function in the brain. We devised a web-based management system called SynapticDB (http://synapses.clm.utexas.edu/synapticdb/) that catalogues and shares experimental data from ssEM. The management strategy involves a library with check-in, checkout and experimental tracking mechanisms, a series of spreadsheet templates guiding users in methods of data collection, structural identification, and quantitative analysis through ssEM, and research progress tracking system with experimental note management and dynamic PDF forms that allow new investigators to follow standard protocols and experienced researchers to expand the range of data collected and shared. The combined use of templates and tracking notes ensures that the supporting experimental information is populated into the database and associated with the appropriate ssEM images and analyses. We anticipate that SynapticDB will serve future meta-analyses towards new discoveries about the composition and circuitry of neurons and glia, and new understanding about structural plasticity during development, behavior, learning, memory, and neuropathology.

[48] Automated high resolution imaging of large volumes of neuropil using SEM-based scanning transmission electron microscopy and serial ultrasections

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We developed automated SEM-based STEM (Scanning Transmission Electron Microscopy) with gigapixel frame store for an efficient and cost-effective way to image circuit-scale volumes of neuropil at ultrastructural resolution. Individual images are of optimum TEM image pixel dimension yet have more than sixty times greater area. The large individual images can also be tiled as mosaics that span the extent of the tissue section in a reasonable time. The entire serially-collected volume of tissue can be loaded at once in the specimen chamber and is available for unattended image acquisition. We are able to continue to use high quality ssTEM (serial section TEM) preparations and archival material in a non-nondestructive way that thus can be available for labeling and subsequent imaging with correlative light and electron microscopy. Compared to conventional TEM, this method has: reduced distortions, aberrations, drift and beam damage; an approximately linear contrast history during scanning; and greater dynamic range. We combine the large field of view with a sample mounting method that allows access to both the dendrite cross sections and the length of the dendritic arbor from the same CA1 or dentate gyrus tissue.

[49] Maturation of smooth endoplasmic reticulum within developing and adult CA1 dendrites.

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The network of smooth endoplasmic reticulum (SER) is well-known for its role as an intracellular calcium store and regulator of membrane protein trafficking. We investigated the organization of SER in hippocampal spines and dendrites using serial section electron microscopy and three-dimensional reconstruction of developing (P15) and adult dendrites. Overall, the number of spines with SER was low and did not differ between ages; however, the presence of a spine apparatus, an organelle containing stacks of SER, was only seen in adult spines. The network of SER in the dendrite shaft can be distinguished between regions of 'simple SER' that consist of SER tubules relative to regions of 'complex SER' that consist of bridging elements and cisterns of SER. Even though adult dendrites had more SER relative to P15 dendrites, they had a similar organization. We discovered that 'complex ER' correlates with spiny dendrite segments; whereas, 'simple ER' is more common in aspiny dendritic segments in both P15 and adult dendrite segments. The volume of SER in the shaft only correlates with the summed excitatory synapse area in adult hippocampal dendrites, but not in P15 dendrites. These results support the hypothesis that SER complexity is controlled locally in dendrites.

[50] Theta-burst stimulation advances the developmental onset age for enduring LTP

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In adult brain excitatory synapses are mainly located on dendritic spines, which play an essential role in synaptic transmission and long-term synaptic plasticity. In hippocampal area CA1, synapses are located primarily on dendritic shafts and filopodia during the first postnatal week, and then at about P11-12 dendritic spines begin to form (Fiala et al., 1998; Kirov et al., 2004). Previous reports had indicated that synaptic transmission in Schaffer collateral-CA1 synapses is depressed by low-frequency test pulses at P5 to P12 and high-frequency stimulation merely reverses this response decrement (Abrahamsson et al., 2008). Here we show that test pulse depression was both age- and frequency-dependent with less depression at lower stimulus frequency and decreasing in effect from P8 to P15. At ages <P12, theta-burst stimulation (TBS) only reversed the depression; then abruptly at P12 TBS both reversed the response decrement and caused an enduring LTP (L-LTP, lasting > 3Hr). At P10 and P11 a single TBS only reversed the response decrement; but surprisingly, additional TBS applied 60-120 minutes after the first TBS resulted in L-LTP. These results suggest a need for dendritic spines in order to elicit L-LTP during development at P12. In addition, they suggest an early developmental onset for metaplasticity at P10-11 when the first TBS might produce the needed spines, the subject of ongoing studies.

[51] Three-dimensional analysis of the spine apparatus in the molecular layer of rat hippocampal dentate gyrus

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The spine apparatus (SA) is an organelle composed of two or more cisterns of smooth endoplasmic reticulum (SER) interdigitated by electron-opaque "dense plates". The SA is located in a subset of large dendritic spines in forebrain areas including the hippocampus, and may participate in calcium signaling, local synthesis and post-translational modification of proteins, as well as regulation of synaptic plasticity. Here we used serial section transmission electron microscopy to determine whether spine and synapse dimensions in the hippocampal dentate gyrus were correlated with the presence of this organelle. Six dendritic segments (8-12 µm in length) were reconstructed from the dentate middle molecular layer of a perfusion-fixed adult male rat. SER occurred in 44% of all spines analyzed, of which 12% had a SA. On average, one SA-containing spine was found in every 1.9 µm of dendritic length. SA-containing spines had significantly larger volumes and their postsynaptic densities were larger than spines without SA. SER was found in 82% of the spines that were deemed large enough to contain a SA. These observations show that the amount and distribution of SER in dentate granule cell dendrites is greater than in the hippocampal area CA1 dendrites, where only about 20% of all spines contain SER. This differential presence of SER across hippocampal areas may play important roles in regulating synaptic plasticity in these two areas.

[52] Bilateral activation of the basolateral amygdala is necessary for trace, delay, and contextual fear conditioning

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The basolateral amygdala (BLA) is necessary for the association of an auditory conditional stimulus (CS) and a fear-producing shock unconditional stimulus (UCS). This structure is also required for the association of the training context with the UCS. This critical role of the BLA in multiple fear conditioning paradigms has led to the hypothesis that the BLA is also necessary for trace fear conditioning, which requires the subject to associate the CS and UCS when they are separated by an empty trace interval. Trace fear conditioning is dependent upon an intact hippocampus (McEchron et al., 1998, 2000; Quinn et al., 2002; Czerniawski et al., 2009) and the prelimbic area of the medial prefrontal cortex (mPFC; Gilmartin & Helmstetter, 2010). It is currently unclear whether these additional structures can support TFC in the absence of the amygdala. This study addressed this question using targeted bilateral or unilateral inactivation of the BLA prior to TFC using infusions of muscimol. We found that both unilateral and bilateral inactivation of the BLA impaired the formation of trace fear memory for the CS and context measured 24 hours later, compared with saline-infused control rats. This study also showed that unilateral temporary inactivation of the BLA was sufficient to impair CS and context memory during delay fear conditioning, a finding that contrasts with unilateral BLA lesions prior to DFC, which failed to significantly impair memory (Goosens & Maren, 2001). Taken together with previous findings in the lab (Gilmartin et al., SFN 2010), these results suggest that the acquisition of trace and contextual fear memories requires bilateral activation of the BLA and ventral hippocampus, and unilateral activation of the mPFC.

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