

SENN/GASCNC 2010

TALK ABSTRACTS

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Glycemic Control Confers Cerebrovascular Protection and Improves Neurological Outcomes in Diabetic Ischemic Brain Injury

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We have previously shown that temporary focal ischemia by middle cerebral artery occlusion (MCAO) causes greater hemorrhagic transformation (HT) and neurological deficits in diabetic Goto-Kakizaki (GK) rats, a model that presents with increased cerebrovascular MMP activity and tortuosity.

Purpose: The goals of this study were to test the hypotheses that 1) diabetes-induced cerebrovascular remodeling is MMP-dependant, and 2) prevention of vascular remodeling by glucose control reduces HT and functional outcomes in diabetes after focal ischemia.

Methods: Male Wistar control and diabetic GK rats were treated with vehicle, metformin (300 mg/kg/day) from the onset of diabetes in GK rats (6 weeks) till they reached the weight for MCAO (11 weeks, 270-300 g). A cohort was then sacrificed after injection of the resin Pu4ii to visualize the pial vessels and vascular tortuosity index, lumen diameter, number of collaterals between MCA and ACA and number of anastomoses within the MCA tree were measured as indices of remodeling. In a second cohort, MMP activity was evaluated by zymography of isolated MCAs. A third cohort was subjected to 3h MCAO/21h reperfusion and infarct size and HT were evaluated as indices of neurovascular injury.

Results: All remodeling markers including MMP-9 activity were increased in diabetes and treatment with metformin prevented these changes (see Table). There was no change in infarct size by metformin yet both severity of HT and neurological deficits were significantly reduced.

Conclusions: Thus diabetes-mediated stimulation of MMP-9 activity promotes cerebrovascular remodeling and augmented remodeling contributes to increased HT in diabetes. Glycemic control through metformin offers vascular protection and improves behavioral outcomes that has important clinical implications for patients with diabetes who are at a 4 to 6-fold higher risk for stroke.

Parameters	Control (Vehicle)	Diabetes (Vehicle)	Diabetes + metformin
Tortuosity index	1.2±0.01 ^a	2±0.1	1.2±0.01 ^a
Collateral diameter	0.03±0.001 ^a	0.09±0.003	0.03±0.004 ^a
# of collaterals	71±6 ^a	154±18	96±8 ^a
# of anastomoses	71±9 ^a	276±33	122±13 ^a
MMP-9 activity (% standard)	60±33 ^a	460±150	175±64 ^a
Infarct size (% contralateral)	30.4±4.3 ^a	9.8±1.8	9.5±2.3
HT (ug/g)	4.2±0.7 ^a	22.5±8.8	8.9±1.9 ^a
Composite neurological score (higher score represents greater deficits)	3.15	4.85	2.87

^ap<0.05 vs GK vehicle, n=7-14/group

Activation of phenotypically distinct neurons in the amygdala of rats following exposure to ferret odor

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Exposure of rats to an odor of a predator can elicit an innate fear response. In addition, such exposure has been shown to activate limbic brain regions such as the amygdala. However, there is a paucity of data on the phenotypic characteristics of the activated amygdalar neurons following predator odor exposure. In the current experiments, rats were exposed to cloth which contained either ferret odor, low or high amounts of butyric acid (a noxious odor), or no odor for a duration of 30 minutes. Two hours following exposure, rats were sacrificed, perfused with PBS and paraformaldehyde (4%), and the brains were isolated. Sections of the brains were prepared for single- (cFOS) and dual-labeled immunohistochemistry and the number of single and dual-labeled neurons of the left and right basolateral (BLA), central (CeA), and medial amygdala were counted. To analyze some of the major cell populations of the amygdala, co-localization of cFOS and Ca²⁺/calmodulin-dependent protein kinase (CAMK) II, parvalbumin, and calbindin was assessed. Data were analyzed with 1- or 2-way ANOVA followed by Student-Newmann-Keul's post-hoc test. Behavior analysis showed an increase in defensive burying in ferret odor-exposed versus control rats during the behavior trial. Dual-labeled immunohistochemistry showed a significant increase in the percentage of CAMKII neurons co-localized with cFOS in both the left and right BLA and CeA of ferret odor-exposed compared to control and/or butyric acid-exposed rats. Further results suggested a decrease in the percentage of calbindin neurons that were also labeled with cFOS in the MeA of ferret-exposed versus low butyric acid-exposed rats. The results suggest that exposure to predator odor activates the glutamatergic projection neurons of the BLA, while potentially decreasing the activation of some inhibitory neuronal populations. These results enhance our understanding of the functioning of the amygdala following exposure to predator threats by showing phenotypic characteristics of activated amygdalar neurons. With this knowledge, specific neuronal populations could be targeted to further elucidate the fundamental underpinnings of anxiety or possibly for developing novel therapeutics.

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Bidirectional encoding of the positive and negative salient signals by majority VTA dopamine neurons

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Dopamine neurons in the ventral tegmental area (VTA) are traditionally believed to process reward information and addiction. Here we investigate whether and how VTA responds to fearful experiences or dangers. We report here that the VTA putative dopamine neurons, up to 96%, are, in fact, responsive to fearful events such as sudden elevator-drop and earthquake-shake. Vast majority of them exhibited suppression-and-rebound response, while a small percentage of them were excited by fearful events. Moreover, these VTA dopamine neurons encode fearful event-saliency parametrically in proportion to the stimulus duration and/or intensity. Finally, a neutral tone can become the conditioned cue for both food reward and fearful events in different contexts (Figure 1), that is, majority VTA dopamine neurons showed significant activation in response to the conditioned tone that predicts food reward (in the reward chamber), while they were significantly suppressed by the same conditioned tone that predicts fearful event (in the fear chamber). These results suggest that the majority VTA dopamine neurons are intrinsically capable of bidirectional encoding of both positive and negative salient signals.

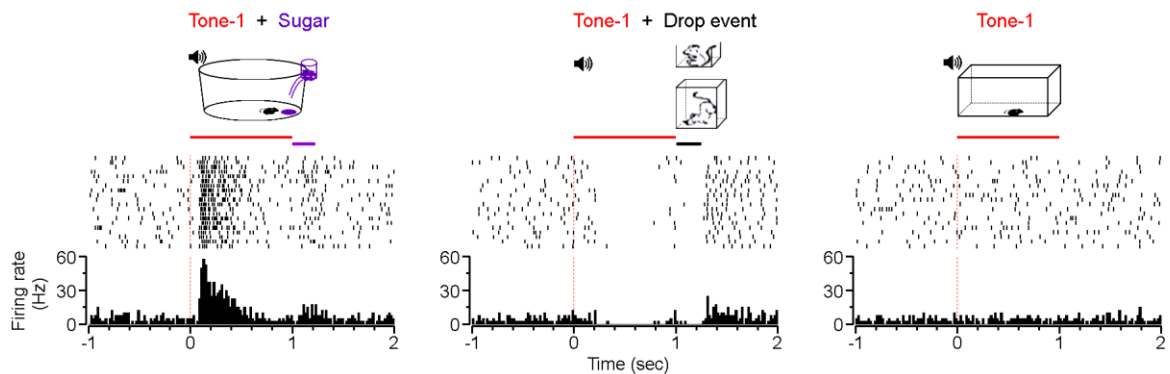


Figure 1. Peri-event rasters and histograms of one dopamine neuron in response to tone-1 (5 kHz, 1 sec, 85 dB) that predicts sugar pellet (left), that predicts drop event (middle) and that does not predict anything (right).

Characterization of the physiological- and genetic phenotype of CRF-expressing neurons in the bed nucleus of the stria terminalis

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Corticotrophin-releasing factor (CRF) and its receptors are regarded as a major mediator of an organisms' response to unexpected and stressful environmental challenges. Activation of neurons in the bed nucleus of the stria terminalis (BNST) plays a critical role in stress and anxiety-related behaviors. The BNST also has a high density of CRF-expressing neurons, suggesting that activation of these neurons may play a critical role in the behavioral response to stress. However, little is known about the cellular characteristics and neurochemistry of these neurons due to the difficulty in indentifying this cell population. We have overcome this obstacle by producing a novel transgenic mouse line expressing green fluorescent protein (GFP) driven by the CRF promoter. In this study, we have combined patch clamp recording, immunohistochemistry, and single cell RT-PCR (scRT-PCR) to examine the intrinsic membrane properties of CRF neurons in the BNST, and determine the genetic phenotype of these neurons.

We first used scRT-PCR analysis of GFP positive neurons to validate our transgenic CRF-GFP mouse line. As expected, all GFP neurons tested expressed CRF mRNA transcripts. This study also revealed that CRF-expressing neurons of the BNST also co-express mRNA transcript for GAD67, suggesting they are also GABAergic neurons. Consistent with this observation, our immunohistochemical studies showed that all CRF-immunoreactive neurons were also GAD67-immunoreactive. Having validated the CRF-GFP construct, we next characterized the physiological properties of these neurons. CRF-GFP cells of the mouse BNST have a mean resting membrane potential (V_m) of -67.4 ± 0.4 mV, a mean input resistance (R_m) of 337 ± 20 M Ω , and mean time constant of 26.7 ± 1.6 ms. In response to hyperpolarizing current injection, these neurons exhibited a fast time-independent rectification that was indicative of activation of the inwardly rectifying potassium current, $K_{(IR)}$, and a slower time-dependent depolarization which was indicative of activation of the hyperpolarization-activated cation current, I_h . Significantly, the intrinsic membrane properties of CRF neurons in the mouse BNST closely resemble those previously described as Type III neurons in the rat BNST (Hammack et al 2007). Consistent with these membrane properties, our scRT-PCR studies showed that CRF neurons expressed the mRNA transcripts for distinct ion channel subunits; including the I_A channel α subunits Kv4.2 and Kv4.3, the I_h channel subunit HCN4, and the $K_{(IR)}$ subunits Kir2.1, 2.2 and 2.3. Interestingly, we noticed that the baseline excitatory input onto the CRF-expressing neurons shows a higher amplitude and frequency of spontaneous EPSPs than that observed in non-CRF neurons, suggesting that these neurons are under tighter excitatory control than the neighboring non-CRF neurons. This study has shown for the first time that CRF-neurons of the mouse BNST CRF-expressing neurons have similar membrane properties to rat Type III BNST neurons and receive more prominent excitatory inputs than other BNST neurons. Moreover, the

Drugs that stimulate oxytocin release promote social bonding in an animal model relevant to autism

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Background: Oxytocin (OT) enhances prosocial behavior in animal models, and intranasal OT enhances some aspects of social cognition in humans, suggesting that the oxytocin system may be a viable target for pharmacological therapies in autism spectrum disorder. However the therapeutic potential of oxytocin is limited by its poor penetration of the blood-brain-barrier. An alternative approach to modulating the OT system is to pharmacologically enhance endogenous OT release. Here we use social bonding in the monogamous prairie vole to assess the prosocial effects of drugs known to stimulate OT release. Social bonding in voles is a complex social cognitive process which can be efficiently assayed in the laboratory using a partner preference paradigm. The formation of a partner preference in female prairie voles is dependent on OT. We propose that the partner preference paradigm in prairie voles may have face, construct, and predictive validity for screening drugs to enhance social cognition in disorders such as autism. Alpha-melanocyte stimulating (alpha-MSH) hormone and serotonin (5-HT) act on the oxytocinergic neurons of hypothalamus to stimulate OT release. To target these systems, we administered Melanotan I (MTI) and Melanotan II (MTII), which act on melanocortin 3/4 receptors, and busprione (BUS), a 5-HT1a receptor agonist, to female prairie voles. We hypothesized that administration of these drugs prior to pairing with a male would accelerate partner preference formation. If our hypothesis is correct, then we predict that a similar pharmacological approach may be useful to enhance social cognition in humans and potentially ameliorate some of the social deficits associated with autism.

Objectives: To determine the effect of drugs known to stimulate OT release on social bonding in prairie voles.

Methods: Melanotan I (MTI; 1 and 10 mg/kg), Melanotan II (MTII; 1 and 10 mg/kg), buspirone (BUS; 8 and 30 mg/kg) or vehicle were administered peripherally to female ovariectomized prairie voles. The non-receptive animals were cohabitated with a male partner for a period of time shorter than that typically necessary to stimulate a partner preference. Following the cohabitation, the females were tested for social bonding using the partner preference paradigm. Time spent in side-by-side immobile contact with the partner or a novel stimulus animal was quantified.

Results: Females receiving the high dose of MTII and the low dose of BUS formed robust partner preferences. MTI did effect partner preference formation in this paradigm.

Discussion: Drugs that targeted both the melanocortin and the serotonin systems via receptors on OT neurons induced partner preference formation under conditions in which bonding does not typically occur. As the receptors targeted by both of these compounds reside on OT neurons, we suggest that the prosocial effects of these drugs may be due to stimulation of endogenous OT release. Thus, targeting these receptor systems provides a potential mechanism to stimulate the OT system, which may have therapeutic value for

Providing virtual sensory feedback in an isolated nervous system

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Proprioceptors provide the nervous system with information about the state of the body and are thus vital for all animals. To test the response of the nervous system to proprioceptive feedback on the cellular level it is often inevitable to revert to reduced preparations. Yet, in these preparations it is difficult to provide adequate sensory feedback. Traditionally, predefined stimuli are used or time/phase-correct stimulations are applied. Both conditions do not reflect the activation of the proprioceptors in the animal and may not be adequate to study the effects of sensory feedback. We are addressing this issue by investigating the muscle tendon organ AGR (anterior gastric receptor) in the stomatogastric nervous system of the crab. AGR measures the tension of a muscle involved in the chewing of food, which is driven by the rhythmic activity of the gastric mill central pattern generator.

AGR's soma possesses intrinsic properties such as sag potential and spike frequency adaptation and spikes from the periphery have to pass the soma to reach their target circuits. AGR is typically activated phasically during teeth protraction and its firing frequency depends on the prevailing motor activity. Here, we test whether a simple sensory feedback is sufficient to elicit the adequate motor response or if a more complex feedback is required. For this, we used a combination of the isolated biological nervous system and real-time sensory feedback provided by a computer. We then compared the response of the gastric mill motor system in two conditions: (I) we activated AGR in a phase-correct way, but with constant frequency or (II) AGR activity was calculated by a model based on physiological data, which reproduced the known response of AGR. In the latter case, the activity of motor neurons was transformed into muscle tension, which then was used for current injection into the AGR model. The model output, in turn, drove the biological AGR.

We found that the resulting motor patterns showed great differences, for example in their period and phase relationships. On average, the cycle periods of rhythms with the AGR model were faster (about 10 s) than those of rhythms with constant AGR frequency (about 14 s), although the control period prior to AGR activation was similar.

Additionally, the protraction phase of the rhythm was significantly shorter when AGR was activated with constant frequency, while the retraction phase was prolonged.

Our results thus indicate that to provide adequate proprioceptive feedback it is necessary to consider the dynamics of the sensory response instead of just relying on predefined parameters. Supported by DFG STE 937/5-1 and 5-2.

Photoreceptor dystrophies result in the remodeling of bipolar cell morphology in the retina of the zebrafish (*Danio rerio*)

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In the zebrafish retina, little is known about the changes that occur in second order neurons in response to photoreceptor degeneration. The goal of this study was to use zebrafish genetic models that result in specific loss of rods or cones, in order to examine the morphological alterations occurring in the bipolar cells. Two photoreceptor degeneration lines were used; the XOPS-mCFP transgenic line causes rod degeneration (Morris et al. 2005), and the *pde6c* mutant line results in cone degeneration (Stearns et al. 2007). Bipolar cells were visualized either by immunolabeling for PKC α or by breeding each of the photoreceptor degeneration lines with the *nyx::MYFP* transgenic line, in which bipolar cells express YFP (Shroeter et al. 2006). Immunohistochemistry was also used to label photoreceptors, and confocal microscopy was utilized to quantify changes in bipolar cell morphology. Results indicated that retinas exhibiting cone photoreceptor degeneration show changes in ON-bipolar cell morphology compared to wild type retinas. Features affected include dendritic processes, axon terminal stratification, and localization of the cell body. Specifically, dendrites in retinas with photoreceptor degeneration often extended into the photoreceptor cell layer, showing longer processes than what is observed in the wild type retina. Additionally, axon terminals were seen to extend beyond the inner plexiform layer, reaching and terminating within the ganglion cell layer. In contrast, rod degeneration resulted in subtle alterations of bipolar cell morphology, particularly in the axon terminals. These data indicate that maintaining normal bipolar cell morphology in the zebrafish retina is more dependent on intact and functional cone photoreceptors than on intact rod photoreceptors. Further studies are proposed to investigate the developmental process of individual bipolar cells in response to photoreceptor degeneration. The study of putative changes in second order neurons provides insight into the integrity of inner retinal layers for carrying out cell transplants that aim to restore vision during photoreceptor degeneration.

Invasion of ectopic visual inputs compromises auditory function in primary auditory cortex

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It is known that brain trauma or sensory deprivation can cause rewiring of affected brain areas during the recovery phase. We reported previously that unilateral midbrain damage at birth in ferrets diverts retinal axons into auditory thalamus, inducing coexistence of auditory, visual and multimodal responses in auditory cortex (AC). Previous investigations of this cross-modal (XM) plasticity paradigm have described the novel visual functions in AC of XM animals, however to what extent the auditory function is affected remains unknown. We hypothesize that competition from visual inputs reduces the sensitivity of AC neurons to sound and alters the sound frequency map in AC. Using *in vivo* extracellular recording, we investigated sound frequency tuning and topographic mapping of auditory neurons and multimodal neurons in AC. We found that despite competition from visual inputs, auditory neurons in Xmodal AC were tuned to pure tones although their sensitivities were decreased and topographic order was disturbed. Furthermore, both the proportion and the location of low-frequency auditory neurons in Xmodal AC were significantly affected. In contrast to multisensory brain areas of normal animals, in which frequency tuning is not seen, multimodal neurons in XMAC did exhibit frequency tuning. Tuning characteristics of these neurons were similar to what is seen in unimodal auditory neurons of primary auditory cortex. In summary, although frequency tuning of auditory and multimodal neurons in Xmodal animals was largely intact, the tonotopic map of auditory neurons was misconfigured. These results suggest that sound discrimination may be impaired despite several months of recovery time. These findings provide insight into how a particular brain area could reorganize to manage multiple functions after brain damage, and provide further clues to understand how to encourage rewiring and minimize miswiring in the context of compensatory plasticity after trauma.

Schizophrenic patients display abnormal neural responses in auditory cortex to increasing rates of stimulation

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Auditory information processing is abnormal in schizophrenia patients [1]. Two well studied deficits involve amplitude reductions in 1) the n100m auditory-evoked field and 2) the auditory steady-state response (aSSR). While the n100m indexes early stimulus registration in secondary auditory cortices [2], the aSSR indexes a sustained ability of the auditory system to coherently entrain to and oscillate with a repetitive stimulus [3]. Insufficient glutamatergic and GABAergic transmission are theorized to underlie schizophrenia related abnormalities in these indices [4, 5, 6], and predict an abnormal functional relationship between neural responses and increasing stimulus frequency. These predictions have not been thoroughly investigated. Whole head magnetoencephalograms (MEG) were recorded while schizophrenia and healthy subjects heard broadband noise bursts amplitude modulated at 5, 20, 40, and 80 Hz. MEG data were co-registered with magnetic resonance images (MRI), and minimum norm source estimates of evoked fields were analysed in reconstructed individual brain space focusing exclusively on the neural responses in left and right auditory cortices.

Comparisons of transient responses reveal that, relative to healthy subjects, patients display: 1) reduced n100m amplitudes to 40Hz stimuli in both right and left auditory cortices, and 2) a left hemisphere specific deficit in n100m amplitude across all stimulus densities which exacerbates as frequency increases. Comparisons of steady-state responses (Figure 2) reveal that schizophrenia subjects display: 1) normal entrainment to 20Hz stimulation in both hemispheres, 2) reduced entrainment to 40Hz stimulation in right hemisphere only, and 3) reduced entrainment to 80Hz stimulation in both hemispheres. Overall, these results indicate that auditory processing deficits in schizophrenia are most pronounced as stimulus density increases and show distinct patterns in left vs right auditory cortices. Further, given ample time to develop the steady-state neural response (>500ms), some auditory deficits can be compensated for (such as the entrained response to 80Hz stimulation in right hemisphere), while other deficits appear to be resilient over time.

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Voltage Sensitive Dye Imaging of Spatiotemporal Dynamics of Somatosensory Cortex in the Rat Vibrissa Pathway

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TALK CANCELED, SEE POSTER P97

Intrinsic and Synaptic Dynamics have Separate Effects on Phase Locking for Weakly Coupled Oscillators

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A phase resetting curve (PRC) tabulates the change in the cycle period of a neural oscillator due to an arbitrary perturbation, and can be used to predict phase locking in networks of oscillators. For weak coupling, the PRC can be estimated by convolving the waveform of the perturbation with the infinitesimal PRC (iPRC). Two variants of this approach have been proposed in the literature in which one uses the iPRC computed with respect to small perturbations in current [1] and the other uses the iPRC computed with respect to small perturbations in conductance [2] for synaptic inputs where the reversal potential is a priori defined.

Perturbing the synaptic current produces a single iPRC that can be utilized for any synaptic perturbation, regardless of the reversal potential. We find that perturbing the synaptic conductance rather than the synaptic current produces an iPRC that separates the intrinsic response to a particular type of perturbation from the dynamics of the perturbation.

We will show that the iPRC with respect to conductance provides intuition regarding the effects of perturbations of varying duration, particularly if the slope at the locking point differs from that of the surrounding regions. On the other hand, the iPRC with respect to current provides a different type of insight into the PRC for neural oscillators near a saddle node bifurcation or a saddle node on an invariant circle bifurcation. In this case, the sign of the iPRC is mostly determined by the slope of the membrane potential waveform. The PRCs for this type of oscillator can consist of both advances and delays if the repolarizing phase of the action potential occupies a significant fraction of their cycle length. Thus while the use of the two types of iPRC is formally equivalent, they provide different types of insight into synchronization.

[1] Netoff et al., *J. Comput. Neurosci.*, 2005, 18, 287-295.

[2] Preyer and Butera, *Phys. Rev. Lett.*, 2005, 95, 138103.

Navigating parameter space between bifurcations to preserve the duty cycle of bursting activity

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We study the leech heart interneuron, and our model simulates activity in a pharmacologically reduced scenario. Central pattern generators control rhythmic activity in many animals, and neurons in these circuits often conserve functional characteristics, such as duty cycle, over a range of cycle periods. We present a mechanism through which coregulation of the half activation voltage of two conductances in a model of the leech heart interneuron maintains constant duty cycle over a wide range of cycle periods. These currents are a non-inactivating potassium current (I_K) and a hyperpolarization activated current (I_h). The proportionality is conserved if the location of two bifurcations is taken into account in coregulation: a saddle-node for periodic orbits and a saddle-node for stationary states. If the shift of the half activation of I_K brings the system close to the saddle-node for periodic orbits, the burst duration grows without bound. If the shift of the half activation of I_h brings the system close to the saddle-node for stationary states, the interburst interval grows without bound. In both cases, the cycle period grows proportionally to one over the square root of the control parameter. By varying the two parameters together, the system approaches both bifurcations and burst duration and interburst interval grow proportionally to one another. This mechanism of coregulation maintains constant duty cycle over a threefold range of cycle periods. Supported by NSF PHY-0750456.

Dopamine modulates I_h in a motor axon

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We studied the axons of the pyloric dilator (PD) neurons in the stomatogastric nervous system of the lobster. The several centimeters long portions of these axons in the motor nerves depolarize in response to low concentrations of dopamine (DA) and exhibit peripheral spike initiation in the absence of centrally generated activity. This effect is inhibited by blockers of hyperpolarization-activated inward current (I_h). Peripheral spike initiation was also elicited by D1-type receptor agonists and drugs that increase cAMP. This suggests that DA acts through a D1-type receptor mechanism to modulate hyperpolarization-activated cyclic nucleotide-gated channels. We used two-electrode voltage clamp of the axon to directly study the effect of DA on I_h . Surprisingly, DA decreased the maximal conductance. However, due to a shift of the activation curve to more depolarized potentials, and a change in the slope, conductance was increased at biologically relevant membrane potentials. These changes were solely due to modulation of I_h , as DA had no discernible effect when I_h was blocked. In addition, they were not induced by repeated activation and could be mimicked by application of drugs that increase cAMP concentration. DA modulation of I_h persisted when protein kinase A was blocked and is therefore likely mediated by a phosphorylation-independent direct effect of cAMP on the ion channel. A computer model of the axon showed that the changes in maximal conductance and voltage-dependence were not qualitatively affected by space clamp problems. DA and I_h contribute significantly to the temporal fidelity of axonal conduction. When I_h is blocked, the interspike interval structure of bursts traveling down the axon is more severely changed between the STG and the nerve terminals than in control saline, whereas DA modulation of I_h increases temporal fidelity.

Protease-activated receptor-1 (PAR1) function in memory formation and synaptic plasticity

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Protease-activated receptor-1 (PAR1) is a member of a family of G-protein coupled receptors (GPCRs) that is activated by proteolytic cleavage of its amino terminus by serine proteases, such as thrombin and plasmin. While previous work has shown that inhibiting PAR1 activation is neuroprotective in models of ischemia, traumatic injury, and neurotoxicity, surprisingly little is known about PAR1's roles in normal brain function. In the CNS, PAR1 is expressed in the amygdala and the hippocampus, which are two brain regions critical for memory formation. Within the hippocampus, PAR1 is expressed predominantly in astrocytes. Mounting evidence indicates that activation of certain G α q-coupled GPCRs in astrocytes results in the release of various neurotransmitters, a process which can modulate synaptic activity. Prior studies have shown that PAR1 activity results in gliotransmission, which leads to activation of downstream effectors known to influence memory formation. We have previously demonstrated that PAR1 knockout mice have impaired performance in passive avoidance and cued fear-conditioning tasks, suggesting an important and specific role for PAR1 in memory formation. Here we report that PAR1 knockout mice show decreased levels of long-term potentiation at Schaffer collateral-CA1 synapses, while having normal baseline synaptic transmission at these same inputs. These data suggest that normal PAR1 function is important for glial-neuronal interactions subserving learning and memory.

***Drosophila* p38 MAP kinase controls lifespan and locomotor coordination**

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Determining how the genetic elements that contribute to neurodegenerative disease interact with environmental factors is imperative for our understanding of disease causality and progression. For instance, a key environmental factor that has been associated with Alzheimer's disease, Parkinson's disease, and ALS is oxidative stress, which is mediated by a variety of signaling pathways. One such genetically determined signaling pathway engaged by oxidative stress is the p38 MAP Kinase (p38K) cascade. However, mechanistic understanding of how p38K and oxidative stress contribute to neurodegenerative disease is sparse, due in part, to the complexity of the mammalian genome. Therefore, in my studies I have generated and utilized mutations in the two p38 kinase genes in the simpler genetic model system, *Drosophila*. My results show that lack of either p38Ka or p38Kb do not compromise viability, while complete loss of both is lethal. However, a strongly hypomorphic combination of p38Kb and p38Ka mutations results in decreased viability and a severely reduced lifespan. Interestingly, these defects can be rescued by ubiquitous or muscle specific expression of wild type p38Kb, or by the detoxifying enzyme SOD2. Furthermore, over expression of wild type p38Kb in muscles or neurons confers lifespan extension suggesting that p38K controls aging in flies, by regulating oxidative stress. Consistent with this, loss of p38K signaling results in age dependent locomotor behavior defects. Finally, I observe strong genetic interaction between p38K and *parkin* mutants. This, in addition to increased loss of dopaminergic neurons in p38K mutant brains, suggests a role for p38K signaling in PD, and supports the hypothesis that p38K signaling is a point of convergence for environmental stress and genetic factors that predispose towards neurodegeneration.

Properties of the M3-M4 intracellular loop of the GABA_A receptor revealed through site-directed mutagenesis

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The γ -amino butyric acid receptor type A (GABA_AR) functions as a chemical to voltage transducer in the central nervous system, converting inhibitory neurotransmitter signals to changes in postsynaptic membrane potential. GABA_AR is a member of the cys-loop family of ligand-gated ion channels along with nAChR, GlyR, and 5-HT₃R. GABA_AR is functionally hetero-pentameric and this study focuses on the most common synaptic subunit arrangement of $\alpha 1:\beta 2:\alpha 1:\beta 2:\gamma 2$ s. Each subunit is comprised of a large extracellular domain, four alpha helical transmembrane domains (M1-M4), and a variable intracellular loop (IL) between M3 and M4. The current theory of ion permeation relies on rings of charged residues contributed by each subunit that line the central pore and interact with permeating anions. These residues are located in M2 and the extracellular vestibule. Traditionally, the IL is thought to control channel function via interactions with trafficking and scaffold proteins. However, a partial structure of nAChR [1] shows an intracellular vestibule which may also contribute a portion of the permeation pathway. Therefore, we performed a site-directed mutagenesis scan assaying individual $\alpha 1$ IL charge switch mutations for GABA concentration response properties, ion selectivity, and properties of the current-voltage relationship. Results show a definite role for IL charged residues in controlling anion permeation. Mutagenesis produced significant changes in GABA apparent affinity, current magnitude, ion selectivity, rectification, and hysteresis of channel activation. These properties are regionally localized, which suggests different critical roles for subdomains of the IL. Overall, these findings show that the intracellular loop of the $\alpha 1$ subunit of GABA_AR does contribute to the functional properties of channel activity.

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Is categorizing objects in natural contexts achievable?

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Humans can recognize and categorize objects in complex natural scenes within 100-200 ms. This amazing ability of rapid categorization has motivated several models of natural vision and object recognition. A common feature of these models is that a large set of image features are extracted and decision rules are trained to categorize objects in complex contexts (e.g., scenes in which cars are present vs. scenes in which animals are present). The conclusion of these models is rather far-reaching: humans achieve rapid categorization in a way similar to these models. Since understanding the computations underlying rapid categorization is important for achieving natural vision, we have re-examined several of these models. In particular, we trained the models and tested them with scenes in which the objects to be categorized were replaced with uniform ellipses. We found that the models categorized most of the scenes with ellipses as having the objects. Therefore, these models do not categorize objects but rather the contexts in which the objects are imbedded and thus provide little clue on how humans achieve rapid categorization. To overcome this impasse, we proposed a statistical framework of rapid categorization in natural scenes. Natural visual scenes consist of objects of various physical properties that are arranged in three dimensional space in a variety of ways. When projected onto the retina, visual scenes entail highly structured statistics, occurring over the full range of natural variations in the world. Instantiating these highly structured statistics is a paramount requirement for natural vision. Therefore, in this framework, instead of image features, we use statistical models of objects and scenes and represent scenes and objects as hierarchically organized, structured probability distributions. Object recognition/categorization is performed as statistical inference. We tested this framework on several large datasets. After trained on fully labeled scenes, the framework generates great performance on object recognition/categorization both in isolation and in natural scenes and thus achieves rapid categorization in natural contexts. These results suggest that natural vision operates as a statistical machine on a large set of hierarchically organized, structured probability distributions of natural scenes and objects. The neural mechanisms underlying this fast, efficient, large-scale statistical inference remain to be discovered.

Operation span capacity and attentional modulation during the antisaccade task: An EEG study

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Performance on measures of cognitive control (e.g., working memory span tasks) predicts behavior on measures of a variety of complex cognitive operations, including reading comprehension [1], and performance on the Stroop [2] and antisaccade tasks [3,4]. Lowered performance on these measures may indicate failure to maintain a task goal, or failure in top-down control [5], however this theory has yet to be directly tested. In this study, participants completed an operation span task in which they were required to alternate between memorizing a string of letters and solving math problems. Participants scoring in the extremes (high and low span) then completed a prosaccade and antisaccade task while having their neural activity recorded with dense-array EEG. Previous work has shown that individuals with poor working memory capacity demonstrate poorer performance on the antisaccade task [3,4]. Prosaccade and antisaccade tasks require differing levels of top-down attentional control. Accurate performance on the antisaccade tasks requires ability to maintain a goal state to inhibit the prepotent response to make a saccade toward the cue and instead make a saccade to the mirror image location of that cue. Fast and efficient performance in the pro- and antisaccade tasks necessitates modulation of attentional mechanisms according to task demands. Stimuli for the saccade task consisted of 3 flickering checkerboards which oscillated at unique frequencies. Data were analyzed with complex demodulation, which yields power of oscillatory biosignals as a function of time for frequencies of interest. Measures of single trial power for neural activation to the central stimulus suggest that individuals scoring in the high operation span range exhibit good top-down control and are able to modulate their attention according to task demands (higher power to central stimulus during the antisaccade task than in the prosaccade task) while individuals scoring in the low operation span range show a failure of top-down attentional modulation to variations in task demand. This study provides a complement to previous behavioral work in that it gives a direct measure of top-down control as a function of working memory capacity.

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Differential degree of learning induced plasticity in primate lateral prefrontal cortex (IPFC) during auditory working memory task

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The sensory cortex has shown that learning causes a non-transient increase in cortical response strength to the target stimulus, and differential degrees of cortical strength have been observed throughout learning progression. The current study investigates the learning-induced plasticity in the primate lateral prefrontal cortex (IPFC) in response to various task demands. We studied these issues using chronic cortical implants, allowing continued recordings over a period of months. The implants have 64 electrodes, each of which can be vertically positioned independently. The cortical implant was surgically placed lateral to the principal sulcus, sampling a 4x4-mm surface area. Single unit & multiunit recordings were sampled from more than 40 electrodes daily. As a control plasticity study, broad responses to acoustic stimuli were first collected before any behavioral training. We observed few responses to tone stimuli and macaque vocal calls during passive listening and before training. Animals were subsequently trained to hold a lever for 2 to 3 seconds while task-irrelevant tone stimuli were added at random times during the trial. Still, no tone-evoked action potential responses were observed, although task-evoked activity related to the press & release of the lever was observed. Then, shaping was initiated in a working memory task requiring the comparison of two stimuli presented in sequence with an intervening delay period. The animals then trained to perform the working memory task with a variety of intervening delays (1600ms, 800ms, 400ms). When the tonal stimuli became task-contingent and were delivered at predictable times during the lever-hold, IPFC neuronal responses were greatly elevated during the presentation of acoustic sound stimuli on some sessions, but not on others. The same sites could show acoustically-driven responses or not on the following days, with no apparent change in the recording quality. We also observed a degree of IPFC response strength where the high variance of firing rate correlated with a longer delay between the stimuli. Our results demonstrate that learning induced-plasticity is evident by the presence of tone-evoked responses only after behavioral training, and differential recruitment of the IPFC is dependant on the level of cognitive procedure.

Neural activation for identifying matching versus mismatching tool-object pairs

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Knowledge of relationships between objects in our environment is a critical aspect of our daily lives. Tool use is an example which requires understanding the functional relationship of a tool (e.g. key) with its associated object (e.g. lock). Because the mechanisms of this pairing process remain unclear, we recorded 64-channel electroencephalography to determine the neural correlates of identification of tool-object matches and mismatches. Subjects (n=15) were shown a target tool (S1t, e.g. spoon) later paired with an object (S2t) that was either a conceptual match (e.g. bowl) or mismatch (e.g. wood). To verify that activity was not related to general concept of match-mismatch, in a second condition subjects saw non-tool environmental items (S1e, e.g. bird) later paired with a (S2e) conceptual match (e.g. nest) or mismatch (e.g. spider web). Analysis was focused on time bins after each picture, using standardized low-resolution brain electromagnetic tomography (sLORETA). The S1t showed significantly greater activation than S1e across widespread occipital, posterior parietal and temporal cortex. Tool-object match versus mismatch revealed significant differences in the posterior cingulate, precuneus, left insula and superior temporal gyrus (STG). These patterns were not present for environmental match versus mismatch, which showed bilateral activation differences, and involvement of anterior frontal areas. This work defines a specific network in comprehending tool-based pairings, but not extensive to other pairings. The posterior cingulate, precuneus, insula and STG preferentially differentiates tool-object matching and mismatching, identifying a potential locus related to impairments in comprehending appropriate and inappropriate tool-object relationships that arise after neural injury.

Organization and function of lateral line afferent neurons in larval zebrafish

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Afferent neurons of the zebrafish posterior lateral line relay hydrodynamic signals sensed along the body to the hindbrain. We labeled individual neurons to reveal their connections to single and multiple neuromasts along the body. Electroporation of Alexa 647 in HUC-Kaede fish, a transgenic line expressing a photo-convertible protein under control of a pan-neuronal promoter, indicates that single and multiple-neuromast afferent neurons correspond to later and early-born cells, respectively. We normalized ganglion area across individuals and plotted position of afferent neurons to show that early-born cells are located centrally in the ganglion, with newly developing cells added to the periphery. Whole-cell recordings of afferent neurons show an inverse relationship between soma area and input resistance, where input resistance is a proxy for excitability. Taken together, a picture is emerging that large, early-born cells are less excitable and may therefore fire only to strong hydrodynamic stimuli across the whole body, while small, later-born cells that are more excitable sense local flows. We hypothesize that large, coarse coding afferents innervating multiple hair cells are critical for initiating powerful escape responses while small, fine coding afferents are responsible for modulating routine motor behaviors such as swimming.

Synaptic properties predict individual susceptibility to and recovery from lesion of a central pattern generator

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Motor patterns produced by central pattern generator (CPG) circuits can be similar among individuals despite individual variability in the cellular and synaptic properties of the CPG neurons. This has led to the idea that homeostatic mechanisms maintain network function in the face of varying cellular and synaptic properties. Such network flexibility may also play a role in adaptive responses to neural injury. Here we found that there was significant animal-to-animal variability in the synaptic properties between the CPG neurons. This variability does not seem to have functional consequences for normal network function but correlates well with susceptibility to and recovery from the lesion of the CPG circuit.

The system we study is the CPG for the escape swim of the mollusc *Tritonia diomedea*. The CPG consists of three neuronal types: VSI, C2, and DSI, all of which project axons in the pedal-pedal commissure (PPC) and make functional connections in both pedal ganglia. We have previously shown that C2 excites VSI in the pedal ganglion distal to the VSI soma, evoking antidromic spikes that travel in the PPC. Cutting the PPC impairs the swim behavior, possibly by disconnecting the C2-evoked excitation of VSI in the distal pedal ganglion. In the proximal pedal ganglion C2 produces a biphasic synaptic potential in VSI with a weak excitation followed by a stronger inhibition.

In this study, we found that individual animals varied in the extent to which the proximal site was excitatory. Furthermore, there was an inverse correlation between the magnitude of excitation in the proximal pedal ganglion and the extent to which the swim was disrupted by the PPC lesion; the larger the excitatory component, the less the impairment. Within 20 hours of the PPC lesion, the swim motor pattern functionally recovered as determined by the number of burst cycles, but the extent of recovery was variable among preparations. The excitatory component of the C2-evoked response increased in parallel to the recovery, but the extent of recovery was better correlated with the amplitude of the synaptic potential evoked by stimulating both C2 and DSI together. DSI is known to evoke serotonergic neuromodulatory actions within the CPG and is co-active with C2 during a swim motor pattern. This suggests that serotonergic mechanisms may also be involved in the network reorganization after injury. Thus, individual variability in specific synaptic actions reflects the relative susceptibility of the CPG to injury, whereas its recovery is correlated more with the extent to which the network functions as a whole. Although the cellular mechanisms underlying this synaptic change are not known, further study may provide insight into mechanisms of adaptive plasticity that are applicable to other neural circuits.

Two modalities of bursting in inspiratory pacemakers: an integrative model

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The respiratory rhythm, generated by the network of inspiratory neurons in the pre-Bötzinger complex (pBC), has two, seemingly contradictory properties. It is robust and persists under highly variable neuronal input and, at the same time, it is easily adjusted to metabolic demand and environmental conditions. Even when isolated from the network, inspiratory pacemakers generate a stable bursting with intrinsic mechanisms dependent on either a persistent sodium current or changes in intracellular Ca^{2+} . Motivated by these experimental evidences, we present a two-compartmental mathematical model of the pBC pacemaker with two independent bursting mechanisms. The somatic compartment has a bursting based on inactivation of a persistent sodium current, and the dendritic compartment relies on Ca^{2+} oscillations. The model explains a number of contradictory experimental results and is able to generate a robust bursting rhythm, over a large range of parameters with a frequency easily adjusted by neuromodulators.

POSTER ABSTRACTS

Brain activity and network in self-generated and externally driven metronome rhythms

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Self-paced and external metronome-driven movement rhythms are everyday phenomena: tapping, clapping, dancing and beating drums with or without explicit external sensory cues are some of the examples. We are not only capable of creating but also maintaining and changing these coordinated movements. Past neuroimaging studies have shown a distributed network of brain areas is involved in movement rhythms. How the brain achieves precise timing and what patterns of brain network are at work in complex rhythmic movements are not completely understood. Using rhythmic finger-tapping tasks with and without external metronomes, we conducted neuroimaging experiments to look at the brain activity and brain functional network in movement rhythms. We found that, in self-paced rhythms, the rhythm complexity, defined based on the patterns of movement timing, modulated the brain activity in the cerebellum- thalamus- basal ganglia network. In external metronome-driven rhythms, we identified the brain areas associated with synchronization and variable rhythm rates. A decreasing rate of rhythms recruited more brain resources than an increasing rate. These results provide more comprehensive understanding of the brain origin of movement rhythms.

Sensory mechanisms of chemical deterrence by sea hare ink against predatory blue crabs

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The sea hare *Aplysia californica* releases a purple ink when attacked by predators, and this ink is a powerful feeding deterrent against the blue crab *Callinectes sapidus*. This deterrence is expressed either by rejection of food or an increase in the time the animals handle the food with their mouthparts before ingesting. Towards understanding the mechanism of this chemical deterrence, we used behavioral and electrophysiological techniques to identify the location and characterize the sensitivity of the chemoreceptors responsible for ink deterrence. Using selective ablation of mouthparts and behavioral assays, we showed that the chemoreceptors mediating deterrence are not located on the legs or restricted to a specific mouthpart. We then focused our electrophysiological efforts to record single-unit responses from mouthpart chemoreceptor neurons using as a chemical deterrent a fraction of ink highly enriched in aplysiotoxin and phycoerythrobilin (= APV-PEB fraction). Our results indicate that there is not a population of cells specific for these chemical deterrents. Single-unit chemoreceptor responses are neither intense nor specific to this fraction; instead APV-PEB evokes a unique across fiber pattern of activity. Additionally, cross-adaptation experiments revealed no effect of the APV-PEB fraction on the responses of mouthpart chemoreceptors to other chemical stimuli. We conclude that the deterrent effect of APV-PEB is mediated, at least partially, through chemoreceptor neurons in the mouthparts that encode these signals not via deterrent-specific neurons but through a distributed neural code. Funded by NSF IBN-0614685.

I_h modulation of neocortical network activity is altered in a model of cortical dysplasia

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Cortical dysplasia is commonly observed in cases of epilepsy. Epilepsies associated with dysplasia are often resistant to anti-epileptic drug treatment. Freeze lesions during the early post-natal period in rats have been extensively used as a model of cortical dysplasia. These lesions reproduce many of the anatomical and electrophysiological characteristics of human focal cortical dysplasia including a region of hyperexcitability surrounding the malformation. Furthermore, animals with freeze-lesions have a reduced seizure threshold. The mechanisms behind hyperexcitability in cortical dysplasia are poorly understood. I_h is a non-specific cation current which activates upon membrane hyperpolarization. This current is mediated by hyperpolarization activated non-specific cation (HCN) channels. Alterations in I_h have been implicated in epilepsy. HCN channels are predominantly present in the apical dendrites of pyramidal neurons and have been strongly implicated in modulation of synaptic inputs. Loss of I_h leads to an increase in intrinsic excitability while enhanced I_h reduces excitability. Additionally, the anti-epileptic drug lamotrigine works via enhancement of I_h . We hypothesized that the role of I_h in intrinsic excitability of individual cells translates to an effect on network activity within the neocortex. We also hypothesized that this effect would be altered in the freeze lesion model of cortical dysplasia. Using voltage sensitive dye imaging, we have shown that I_h blockade increases the duration of cortical network activity. In contrast, I_h enhancement with the antiepileptic drug lamotrigine decreases the spread of activity across the cortex. Voltage sensitive dye imaging also revealed increased spread and amplitude of activity in the area near the freeze lesion. Dysplastic cortex also showed a reduced response to lamotrigine. These results suggest that I_h normally modulates network activity in the neocortex. This modulation is altered in cortical dysplasia.

Rod photoreceptor number is regulated by the locus *lots-of-rods-junior* (*lrj*) in the zebrafish retina

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We are interested in the patterning and development of the vertebrate retina, and use the zebrafish (*Danio rerio*) embryo as a model. We have undertaken a large-scale genetics screen to identify mutations that alter photoreceptor cell number and mosaic pattern in free-swimming larvae. The locus *lots-of-rods-junior* (*lrj*) was identified in a screen of chemically mutagenized zebrafish. This study focuses on the characterization of the *lrj* mutant phenotype and the identification of the mutant gene.

The number and distribution of the cones, rods, and most retinal cells in wild-type and *lrj* mutant larvae were analyzed in immunolabeled retinal sections. Cell cycle was examined by phospho-histone 3 (PH3) immunolabeling and BrdU incorporation in mutants and wild-types. Chromosome linkage was assigned using SSLP markers. The generation of high-resolution mapping was performed by generating additional polymorphic markers in the mutation interval. Cell autonomy was assayed by generating genetic chimeras by blastula cell transplants.

lrj was identified by a four-fold increase in the number of rods at 4 dpf, when compared to wild-type siblings. Other photoreceptor and retinal cell types seem unaffected and no other morphologic alterations are evident in *lrj* homozygous mutant larvae or adults.

PH3 immunolabeling and BrdU incorporation at different time-points do not reveal alteration in the mutants. *lrj* complemented *lor*, consistent with a mutation in a different locus. Linkage analysis positioned the *lrj* locus within a 0.5-Mb interval on chromosome 7. In genetics chimeras, *lrj* mutant cells fail to produce the increased number of rods phenotype in a wild-type host, suggesting a non-cell-autonomous function of the gene.

This study validates the power of screening for alterations in photoreceptor patterning to uncover genes related to photoreceptor development. *lrj* is the second locus identified in zebrafish, after *lor*, which display an alteration in the photoreceptor cell number without cell death. Future investigations will comprise the fine mapping to identify the loci mutated in *lrj*.

The Effects of Maternal Depression during Pregnancy on the Neurodevelopment of Social Cognition in Kindergarten-Aged Children

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Children exposed to prenatal maternal depression have increased risk for anxiety, depression, and other developmental and affective problems. The specific impact of prenatal maternal depression on the neurodevelopmental processes that underlie this risk remains poorly characterized. I explored the behavioral and neurodevelopmental trajectories of these children to test the overarching hypothesis that prenatal maternal depression represents a neurodevelopmental teratogen that disrupts child emotional, social and cognitive development and thus confers risk for psychological and developmental problems later in the child's life. In collaboration with the Emory Women's Mental Health Program, I identified ~50 children whose mothers' depression severity and medication were monitored during pregnancy and in the post-partum period. I used functional magnetic resonance imaging (fMRI) during a joint attention task to define the neurodevelopment of social cognition for 4.5-6 year old offspring of these mothers. Offspring were categorized by the severity and gestational timing of prenatal maternal depression, based on 4-16 separate clinical interviews during pregnancy. Current clinical status and behavior of the offspring are measured via parent questionnaires and a clinical interview with the mother.

To date, we have collected and analyzed complete fMRI and behavioral data for ten children (ages 58-78 months, 4 boys/6 girls, 5 low/5 high depression). The preliminary task-related neural responses were consistent with the children's processing of social signals and affect regulation (vACC/subgenual ACC), effortful control of joint attention (right IFC, pSTS), and the processing of happy facial expressions (right fusiform gyrus, dmPFC). All results are shown at $p < 0.005$, $k > 5$ voxels and were not significantly changed when age and IQ were controlled for. In addition, a regression analysis of the impact of the severity of prenatal maternal depression on task-related neural responses indicated that a more severe prenatal burden of MDD was associated with lesser right dlPFC response to cognitive interference in the joint attention task in offspring ($p < 0.01$, $k > 5$ voxels). The results of these regressions with individual prenatal maternal MDD severity were unaffected after adjusting for differences in child age or IQ. However, task performance (reaction times) was not related to maternal depression burden. In addition, emotional, social, and behavior problems from the behavioral questionnaires were not correlated with maternal depression burden.

While preliminary, these results suggest that prenatal maternal depression dramatically compromises the childhood maturation of self-regulatory functions of the dlPFC. This neurological deficit is not manifested in task performance or current child behavior, thus it may represent a plausible neurodevelopmental risk factor for future psychological and developmental problems seen in children exposed to maternal depression during pregnancy. This demonstrates the importance of identifying biological markers of risk, which may appear developmentally prior to observable symptoms.

Analysis of GluR1 expression in Basigin null mouse brains

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Basigin null mice are characterized as having learning and memory deficiencies, as well as other neurological abnormalities. GluR1 is a member of the glutamate-gated ion channel family and functions as a subunit of AMPA receptors, which are involved in long-term potentiation. The purpose of this study was to examine membrane-expression of GluR1 in Basigin null mice and littermate controls. Enzyme Linked Immunosorbent Assays (ELISAs) were performed on detergent lysates from normal and Basigin null mouse brains using an antibody specific for GluR1. It was determined that membrane-associated expression of GluR1 in Basigin null mouse samples was similar to that of the controls. The data suggest that synaptic plasticity mediated via AMPA receptors is not impaired in Basigin null mouse brains. Future studies will examine other molecular components of synaptic plasticity in Basigin null mouse brains in an attempt to determine the mechanism underlying the learning and memory deficiencies observed in those animals.

Inducible Deletion of Amygdalar Glucocorticoid Receptor to Examine Early-Life Stress-Induced Anxiety and Depression

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Stress experienced in early life leads to sustained changes in gene expression and behavior, making people less able to cope with stress and more vulnerable to anxiety and depression later in life. The mechanisms by which early-life stress can lead to the development of these major mood disorders remains unknown. An important component of the stress response is mediated through stress hormones (glucocorticoids) binding to the glucocorticoid receptor (GR). GRs are ubiquitously expressed throughout the brain, displaying higher expression in a number of important limbic areas, including the central nucleus of the amygdala (CeA), a key nodal forebrain structure for integrating behavioral responses to stress. Moreover, glucocorticoid activity in the CeA has been implicated in mediating a positive feedback loop that potentiates heightened stress reactivity. The objective of this proposal is to elucidate the role of CeA GR activity in the etiology of anxiety and depression fostered through ELS as a novel therapeutic target to remedy these major mood disorders. We hypothesize that CeA GR-dependent mechanisms are critical links between early-life adversity and the subsequent development of anxiety and depression that persist over the lifespan. The proposed studies utilize a novel model of selective GR deletion in the CeA to determine if CeA GR signaling is required for maintenance of the maladaptive stress response resulting from early-life adversity. To mimic early-life stress, mice will undergo maternal separation for 3 hours/day during the first 2 weeks of postnatal life. GR expression is then deleted by injecting lentiviral vectors containing Cre-recombinase into the CeA of floxed-GR mice in a 2x2 design (+/- GR and +/- maternal separation). To determine if loss of CeA GR signaling can reverse the persistent negative effects of early-life stress, mice will perform a series of behavioral tests thought to correspond to human depression (e.g. tail suspension and forced swim tests) and anxiety (e.g. the open-field test, novel object recognition test and elevated-zero maze). The physiological effects of disrupting CeA GR activity will be quantified by measuring plasma levels of corticosterone, the principle glucocorticoid in mice. The results of our planned studies on the role of GR function in the amygdala hold promise for new insights into the molecular mechanisms regulating the stress response. Ultimately, our efforts aim to further the understanding of neural circuits relevant for anxiety and depression and promote development of novel therapeutic approaches for treatment of these major health disorders.

Nitric oxide synthase controls spontaneous firing activity and growth cone morphology via upregulation of a persistent sodium current

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Nitric oxide (NO) is a gaseous molecule that controls neuronal electrical activity. We found that the NO-producing enzyme, nitric oxide synthase (NOS), controls the intrinsic neuronal activity of identified buccal neuron B5 of the pond snail *Helisoma trivolvis* via the selective modulation of ion channels. NOS is expressed in a small number of buccal neurons, including B5 neurons. Mature B5 neurons that have been grown in culture for 2-3 days and that have developed distinct neurites tipped with growth cones exhibit spontaneous tonic firing activity. This firing activity is under the control of intrinsic activity of NOS, as pharmacological inhibition of intrinsic NOS activity blocks spontaneous spiking. The spontaneous firing activity requires the presence of depolarizing currents that bring the membrane potential to the action potential threshold. We found that one such depolarizing current, a persistent sodium current (PNaC), is expressed in mature B5 neurons, and riluzole, a specific inhibitor of PNaC blocks the spontaneous firing activity. Moreover, NOS inhibitors also block the PNaC. Thus, intrinsic activity of NOS is necessary for the spontaneous tonic action potentials in B5 neurons. Interestingly, PNaC is developmentally regulated, because it is not found in immature cultured B5 neurons. The inhibition of intrinsic NOS activity by NOS inhibitors and treatment of neurons with *Helisoma* RNAi both result in a reduction in the size of growth cones. Riluzole also decreases the size of B5 growth cones, suggesting that intrinsic NO acts through PNaC to regulate growth cone morphology. Thus, the intrinsic activity of NOS controls the spontaneous tonic firing activity and growth cone morphology via upregulation of a PNaC in B5 neurons.

Gene expression in hypothalamus, liver and adipose tissues and feed intake response to melanocortin-4 receptor (MC4R) agonist in pigs expressing (MC4R) mutations

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Transcriptional profiling was used to identify genes and pathways that responded to intracerebroventricular (ICV) injection of melanocortin-4 receptor (MC4R) agonist, NDP-MSH, in pigs homozygous for the missense mutation in the MC4R, D298 allele (n = 12), N298 allele (n = 12) or heterozygous (n = 12). Feed intake (FI) was measured at 12 and 24 hr after treatment. All pigs were sacrificed at 24 hr after treatment and hypothalamus, liver and backfat tissue was collected. NDP-MSH suppressed ($P < 0.004$) FI at 12 and 24 hr in all animals after treatment. In response to NDP-MSH, 278 genes in hypothalamus ($q \leq 0.07$, $P \leq 0.001$), 249 genes in liver ($q \leq 0.07$, $P \leq 0.001$) and 5066 genes in fat ($q \leq 0.07$, $P \leq 0.015$) were differentially expressed. Pathway analysis of NDP-MSH-induced differentially expressed genes indicated that genes involved in cell communication, nucleotide metabolism, and signal transduction were prominently down-regulated in the hypothalamus. In both liver and adipose tissue, energy-intensive biosynthetic and catabolic processes were down-regulated in response to NDP-MSH. This included genes encoding for biosynthetic pathways such as steroid and lipid biosynthesis, fatty acid synthesis, and amino acid synthesis. Genes involved in direct energy-generating processes, such as oxidative phosphorylation, electron transport, ATP synthesis were up-regulated, whereas TCA associated genes, were prominently down-regulated in NDP-MSH treated pigs. Our data also indicate a metabolic switch toward energy conservation since genes involved in energy-intensive biosynthetic and catabolic processes were down-regulated in NDP-MSH treated pigs.

Manipulation of the prairie vole genome

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Prairie voles' (*Microtus ochrogaster*) rich sociosexual repertoire of behavior characterized by lifelong social attachments and biparental care make them ideal model organisms for studying the genetic and neurobiological mechanism regulating behavior. Molecular mechanisms mediating this social organization may shed light on social behavioral disorders such as autism and schizophrenia. However, the field is limited in its understanding of the mechanistic control of social behavior as no routine transgenic manipulations exist for nontraditional organisms. Our lab has recently created a line of voles expressing a GFP transgene, and we are beginning to extend this technology to more detailed research of behavioral genetics. Here we present our preliminary attempts at generating transgenic voles with altered vasopressin (V1a) receptor expression using RNA interference, in which short hairpin RNAs chosen based on their knockdown efficacy *in vitro* are inserted into the genome of single-cell embryos using lentiviral vector gene transfer. This manipulation will allow us to investigate the causal relationship between V1a receptor expression and social behavioral outcomes. Methods, preliminary results, and potential complications of *in vivo* shRNA expression are discussed. The ability to manipulate the genome of prairie voles will open the door for a wide variety of investigations using nontraditional organisms chosen based on their relevance to specific behavioral questions rather than their ease of study.

Perceived sense of effort and dual-task performance for fluent and nonfluent individuals with aphasia

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Attention deficits in aphasia have been explained as an inability to appropriately allocate attentional resources, or a reduction in available resources [1]. Inaccurate sense of effort (SOE) has also been speculated as a source for decreased performance on divided attention tasks, as SOE reflects, and may influence, the amount of cognitive resources expended [2, 3].

While it is thought that the attention deficits are due to damage of a “common” attention network [4], there is little information regarding how aphasia type influences perceived task demand and subsequent performance. IWA (fluent=4, nonfluent=3) were assessed for SOE on a dual-task procedure (card sorting with tone identification) [5]. Results (Figures 1 and 2) indicate significant group differences in the mean percentage of tones identified correctly. Nonfluent individuals demonstrated greater performance [$F(1, 5) = 16.15, p = 0.01, \eta^2 = 0.764$], $M = 87.52\%$ (nonfluent), 38.10% (fluent). There were no other significant group differences.

Findings suggest that task evaluation and attention allocation may differ between fluent and nonfluent IWA. Results conflict with previous findings of similar performance between aphasia type [4]. It is possible that the attention system is in fact disrupted differently in individuals with fluent and nonfluent aphasia, or that perception of task demand influences dual-task performance differently in these populations.

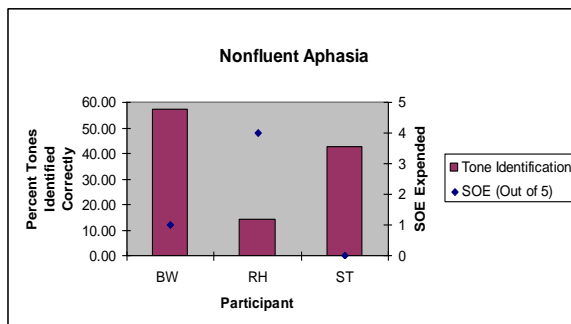


Figure 1.

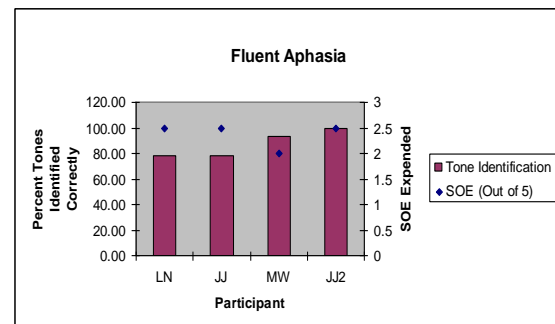


Figure 2.

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Investigating Roles of LRRK2: Neurite Outgrowth and Filopodia in Midbrain Dopamine Primary Neurons

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Amongst several currently identified genes that contribute to Parkinson's disease (PD), mutations in the leucine-rich repeat kinase 2 gene (*LRRK2*) are the most commonly known cause of autosomal dominant PD. *LRRK2* encodes a multi-domain protein consisting of a leucine-rich repeat domain, Ras in complex proteins (ROC) domain, C-terminal of ROC (COR) domain, a kinase domain related to mitogen-activated protein kinase kinase kinases (MAPKKKs) and a WD40 protein interaction domain. The G2019S mutation in the kinase domain is the most prevalent mutation in *LRRK2* in both familial and sporadic cases. Current literature suggests this is a gain-of-function mutation resulting in higher kinase activity.

Using *LRRK2* G2019S Knock In (KI) mice and murine *LRRK2* Knock Out (KO) mice, we investigated the role of endogenous *Lrrk2* and the effect of the G2019S mutation on neurite outgrowth in tyrosine-hydroxylase positive midbrain primary cultures. We observed an increase in total neurite outgrowth in KO midbrain dopamine neurons compared to those from their heterozygous (HT) littermates possessing one copy of the *LRRK2* gene. In KI primary cultures however we observed no difference in neurite outgrowth between homozygous KI and wild-type (WT) midbrain dopamine neurons.

A study of the more subtle phenotype of dendritic filopodia in primary cultures showed an increase in the number of filopodia in KO midbrain dopamine neurons compared to WT neurons, whilst KI cultures exhibited a decrease in filopodia numbers compared to WT neurons. These studies suggest that regulation of dendritic filopodia and branches is an inherent function of *Lrrk2*. Further, these studies support that a pathogenic mutation in *Lrrk2* increases this negative regulation of process and filopodia formation, lending support to the idea that this is a gain of function mutation.

NFAT modulates expression of CG42340 to alter plasticity at the pre-synapse

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Transcriptional mechanisms regulate long-term neuronal plasticity and underlie behaviors such as learning and addiction. Although most transcription factors studied to date, such as Fos and CREB, are positive regulators of plasticity, much less is known about proteins that constrain long-term changes in the structure and function of neurons. Using the model organism *Drosophila melanogaster*, our laboratory has determined that NFAT (Nuclear Factor of Activated T-cells) is expressed in the nervous system, inhibits pre-synaptic growth and transmitter release, and negatively regulates activity-dependent pre-synaptic plasticity. While NFAT-mediated regulation of synapse growth engages the microtubule based cytoskeleton, NFAT also alters calcium dependence of transmitter release. To derive mechanistic understanding of the role of NFAT in activity-dependent plasticity, we have focused on CG42340, a gene that encodes a dual-pore potassium leak channel and is regulated by neural activity. Western blot analysis from adult brain extracts suggests that NFAT could be a key controller of CG42340 protein levels in the brain; CG42340 protein levels are elevated following over-expression of NFAT and reduced in the NFAT^{ΔAB} deletion mutant. Functional analysis of CG42340 also reveals an increase in synapse size for larvae lacking the entire coding region of CG42340. We propose a model in which neuronal activity induces expression of CG42340 in an NFAT dependent manner to constraint synaptic plasticity.

Effects of heterogeneity in synaptic conductance between weakly coupled identical neurons

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A significant degree of heterogeneity in synaptic conductance is present in neuron to neuron connections. We study the dynamics of weakly coupled pairs of neurons with heterogeneities in synaptic conductance using Wang-Buzsaki and Hodgkin-Huxley model neurons which have Type I and Type II excitability, respectively. This type of heterogeneity causes a symmetry breaking in the bifurcation diagrams of equilibrium phase difference versus synaptic time constant when compared to the identical case. For weakly coupled neurons coupled with identical values of synaptic conductance a phase locked solution exists for all values of the synaptic time constant, α . In particular, in-phase and anti-phase solutions are guaranteed to exist for all α . Heterogeneity in synaptic conductance results in regions where no phase locked solution exists and the general loss of the ubiquitous in-phase and anti-phase solutions of the identically coupled case. We explain these results through examination of interaction functions using the weak coupling approximation and an in depth analysis of the underlying multiple cusp bifurcation structure of the systems of coupled neurons.

Mu-opioid receptors in the caudate-putamen modulate pair bonding in female prairie voles

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Socially monogamous prairie voles (*Microtus ochrogaster*) form enduring pair bonds between mates, and have become an informative animal model for exploring the neurobiology of social attachment. The opiate system has long been implicated in the regulation of infant-mother attachment, and mu-opioid receptors in the nucleus accumbens (NAcc) mediate the reinforcing properties of many natural rewarding stimuli. However, the role of the opiate system in partner preference formation, the laboratory proxy for pair bonding, has not been explored. The NAcc plays a critical role in pair bonding in female prairie voles. In contrast, the caudate-putamen (C-P), an anatomically parallel structure involved in reward- and cue-based learning, has not been implicated in pair bonding and has traditionally been used as a control injection site. We hypothesized that endogenous opioids play a role in partner preference formation in female prairie voles via activation of mu-opioid receptors in the NAcc. To test this hypothesis, we first administered the opioid antagonist naltrexone (NTX) (IP, 7.5 mg/kg, Q6H) or saline to adult female prairie voles during an 18-hour mating period with a male partner. NTX injected females mated significantly less during the first 4 hours of cohabitation ($p < 0.01$) and displayed a significant preference for the stranger ($p < 0.01$), suggesting that the non-selective antagonist effectively blocks partner preference formation. We then explored the role of mu-opioid receptors within the brain more specifically by site-specific administration of the mu-opioid selective antagonist CTAP. We administered CTAP or saline to the NAcc (1 $\mu\text{g}/0.2 \mu\text{L}$ bilateral), the C-P (1 $\mu\text{g}/0.2 \mu\text{L}$ bilateral), or i.c.v. (2 $\mu\text{g}/0.4 \mu\text{L}$ unilateral) prior to a 24-hour mating period. Control females receiving vehicle in the NAcc, C-P, or i.c.v. all displayed a significant partner preference ($p < 0.01$ for all groups). Females receiving CTAP in the NAcc ($p = 0.03$) or i.c.v. ($p < 0.01$) also displayed significant partner preferences. Only CTAP injected into the C-P was effective in blocking partner preference formation ($p > 0.05$). Furthermore, the central antagonist treatments did not affect mating behavior during the cohabitation period. These data strongly implicate the opiate system in pair bond formation in female prairie voles, and suggest that mu-opioid receptors in the C-P mediate this effect.

Arc expression and neuroplasticity in primary auditory cortex during initial learning are inversely related to neural activity

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Models of learning--dependent sensory cortex plasticity require local activity and reinforcement. An alternative proposes that neural activity involved in anticipation of a sensory stimulus, or the preparatory set, can direct plasticity, so that changes could occur in regions of sensory cortex lacking activity. To test the necessity of target--induced activity for initial sensory learning, we trained rats to detect a Low Frequency sound. After learning, *Arc* expression and physiologically--measured neuroplasticity were strong in a High Frequency auditory cortex region that lacked target--induced activity in control animals. After 14 sessions, *Arc* and neuroplasticity were aligned with target--induced activity. The temporal and topographic correspondence between *Arc* and neuroplasticity suggests *Arc* may be intrinsic to the neuroplasticity underlying perceptual learning. Furthermore, not all neuroplasticity could be explained by activity--dependent models, but can be explained if the neural activity involved in the preparatory set directs plasticity.

Proliferative, adherent neural progenitors derived from human induced pluripotent stem cells.

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Induced pluripotent stem cells (iPSCs) derived from immunocompatible, patient-specific donor cells have garnered attention for their therapeutic potential in cell replacement strategies. Previous studies show that somatic fibroblasts reprogrammed into induced pluripotent stem cells are indistinguishable in their epigenetic state and developmental potential from blastocyst-derived embryonic stem cells (ESCs). However, the capacity of iPSCs to differentiate into therapeutically relevant and functional cell types in comparison to ESC-derived cell types is largely uninvestigated. Here we have generated human iPSCs by transducing IMR-90 human lung fibroblasts with lentiviral vectors encoding all 6 previously used pluripotency factors, Oct-3/4, Nanog, Sox2, Klf4, Lin28, and c-Myc, under the EF1 α promoter, along with an additional EF1 α -eGFP lentiviral vector to monitor transgene expression during differentiation. In these studies we induced iPSCs to differentiate *in vitro* into neural progenitors and their neuronal derivatives. Resulting progenitor cells were characterized by immunocytochemistry, flow cytometry and then assayed for their differentiation potential to their respective cell lineages. We found that the morphology, time frame for differentiation and marker expression of iPSC-derived progenitors is similar to hESC-derived progenitors for neural differentiation *in vitro*. We also observed no reemergence of silenced lentiviral transgene expression in iPSC-derived differentiated cell types (i.e. GFP fluorescence). In addition, we developed robust, proliferative iPSC neural progenitors capable of being maintained as adherent monolayer cultures for multiple passages. Our results show that iPSCs have the potential to generate progenitor cell types for future regenerative medicine therapies.

Puberty increases excitatory synapse markers in the medial amygdala

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Puberty is a phase of mammalian reproductive development marked by dramatic phenotypic plasticity. In addition to physical changes, social behaviors such as courtship, coitus, and territorial aggressiveness emerge at puberty. The neurobiological mechanisms that underlie the development of these behaviors, however, are unclear. In the Siberian hamster, *P. sungorus*, the initiation of puberty is controlled by photoperiod. We used summer- and winter-like photoperiods to induce puberty in one cohort of hamsters and to inhibit puberty in another. All hamsters were perfused at 40 days of age. We then used double label immunofluorescence in conjunction with stereology to estimate several parameters in the posterodorsal subnucleus of the medial amygdala (MePD), an important site for the steroid hormone regulation of social behavior. We report here that post-pubertal male Siberian hamsters have 50% more puncta immunoreactive for vesicular glutamate transporter-2 and postsynaptic density-95, both of which are markers of glutamatergic synapses. There was also a non-significant trend toward 25% more puncta immunoreactive for glutamic acid decarboxylase (GAD). Confirming our previous findings, we observed that post-pubertal hamsters had a larger MePD regional volume as well as larger neuronal somata. Taken together, these data indicate that puberty is accompanied by excitatory synaptogenesis in the medial amygdala, which may contribute to the behavioral changes that occur at this time.

Distinct cortical areas encoding decision making and motor preparation in the posterior parietal cortex

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Traditional psychological theories have considered decision making as a separate neural process occurring before action planning. Recently, neurophysiological studies suggest that plan selection and movement preparation involve the same brain regions and are performed in an integrated manner. However, nearly all previous studies of decision making have emphasized the spatial aspects, so dissociation of decision making from motor planning has only been tested for spatial target selection, which involves spatial attention and in turn engages numerous cortical areas. It still remains unclear whether plan selection and action preparation are represented in segregated areas for other kinds of decision making. In the present study, we recorded single-neuron activity from the dorsal area 5 (area 5d) and the adjacent parietal reach region (PRR) of the posterior parietal cortex while monkeys performed a non-spatial, effector-choice between saccade and reach. Results demonstrated that PRR forms potential movement plans in ambiguous situations and then only the reach plan remains once the monkeys make a decision. On the other hand, area 5d does not form potential plans and carries only the movement plan once the decision has been made. The absence of activity related to potential plans in area 5d suggest a distinct neural correlate of motor preparation downstream to plan selection, in favor of the serial hypothesis that decision making occurs before action planning.

STEP (Striatal-enriched protein tyrosine phosphatase) is a critical component of the CRF system signaling in the extended amygdala of the rat brain.

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STEP (Striatal-enriched protein tyrosine phosphatase), a recently discovered neuron-specific tyrosine phosphatase, is widely distributed in the rodent brain. STEP has been reported to be critically involved in the glutamate (NMDA receptor)-mediated regulation of the ERK (extracellular signal-regulated kinase) signaling cascade in rat neurons. Activation of the ERK cascade is thought to be an important signal transduction factor that regulates synaptic function, long-term potentiation (LTP), and consequently learning and memory. STEP has been shown to downregulate the activity of ERK by dephosphorylation of ERK tyrosine residue. We have found robust somatodendritic expression of STEP in the extended amygdala: primarily in the anterolateral BNST (alBNST) and central amygdala (CeA). Because STEP distribution was highest in those regions of the extended amygdala that also show high corticotrophin releasing factor (CRF) expression, we performed dual immunofluorescence experiments to determine the relative co-localization of CRF and STEP. Dual labeling experiments revealed almost complete co-localization of CRF and STEP in alBNST and CeA. In contrast to CRF neurons of the extended amygdala, the population of CRF-positive neurons in paraventricular nucleus of the hypothalamus (PVN) did not express STEP. These data suggest that CRF neurons in the extended amygdala can utilize distinct second messenger pathways from CRF neurons of the PVN. CRF-expressing neurons in the extended amygdala are believed to be associated with the chronic affective (emotional) component of the stress response, in contrast to CRF neurons in the PVN, which are associated with acute mobilization of the hypothalamic-pituitary-adrenal (HPA) axis. Here, we used repeated stress paradigm to examine the effects of chronic stress on STEP expression in the alBNST. Rats were subjected to one-hour restraint stress for four consecutive days. Six days after the final stress manipulation (day 10), BNST samples from control and stressed animals were collected for protein assay (Western Blot), mRNA expression (RT-PCR) and immunohistochemistry. We have observed decreased levels of STEP in the BNST of stressed rats at the level of protein expression (STEP total protein content and STEP-positive neurons' expression) as well as significantly decreased mRNA expression. We conclude that STEP might be directly involved in stress-induced regulation of CRF signaling in the BNST, and deficits in STEP may therefore modulate the long-lasting effects of stress that are associated with states of fear and anxiety as well as etiology of PTSD, panic attacks and depression.

Synergistic efficacy of ha14-1 and apigenin in inhibiting angiogenic factors and increasing apoptosis in neuroblastoma cells

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Neuroblastoma is the most common extracranial solid tumor in children. Current therapies are insufficient in treating this complex malignancy. There is an urgent need for novel treatment of neuroblastoma. In this study, we examined the effects of combination of Bcl-2 inhibitor HA14-1 (HA) and apigenin (APG) on malignant neuroblastoma SH-SY5Y (non-N-Myc amplified) and SK-N-BE2 (N-Myc amplified) cells. Dose-response studies indicated that treatment with HA + APG for 24 h synergistically reduced cell viability in SH-SY5Y. SK-N-BE2 showed no significant response to the drugs during treatment for 24h but displayed synergism during treatment for 48 h for decreased cell viability. Further studies were focused on SH-SY5Y cells using the treatments: 2.5 μ M HA, 100 μ M APG, and 2.5 μ M HA + 100 μ M APG. Western blotting showed that HA+APG inhibited angiogenic (VEGF and EGFR) and survival (NF κ B and N-Myc) factors. Wright staining showed capability of combination therapy in increasing morphological features of apoptosis. Cell cycle analysis and Annexin V-FITC/PI binding assay showed that combination therapy was more effective than monotherapy in inducing extrinsic apoptotic pathway with activation of caspase-8 and BID cleavage to tBID. Activities of calpain and caspase-3 generated 145kD spectrin break down product (SBDP) and 120kD SBDP, respectively. Thus, HA and APG is a promising therapy against human malignant neuroblastoma having N-Myc non-amplification or amplification. This work was supported by R01 grant (NS-57811) from the NINDS.

Comparative expression of insulin-like peptides and the insulin receptor in the brain of a mosquito

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Eight insulin-like peptides (ILPs) and one insulin receptor (IR) homolog are encoded in the yellow fever mosquito, *Aedes aegypti*. In this study, we show that seven of the eight ILPs and the IR are specifically expressed in female brains, as determined by RT-PCR, *in situ* hybridization, and immunoassays. After immunocytochemistry with a specific antiserum, ILP3 was localized to the medial neurosecretory cells in the brain, and these cells have axons extending out of the brain to a neurohemal organ and along the gut. Published studies show that this ILP is a high affinity ligand for the IR and has gonadotropic activity in blood-fed, decapitated females and insulin-like activity in sugar-fed, decapitated females. Roles for the other ILPs expressed in the brain are not known, nor is their affinity for the IR established. In this study, we show that IR RNAi blocks expression in specific tissues, but not the brain, and slows the feeding response in female mosquitoes. This result suggests that ILPs released from the brain may regulate feeding behavior in mosquitoes in the same way as known for insulin in mammals.

Multi-modal optimization techniques for improving qualitative features of biophysical neural models

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We present a novel computational technique that enables more efficient optimization of qualitative features in biophysical neural models. In particular, we extend the idea of multiple objective optimization for a single comparison modality (such as comparison of individual voltage traces) [1] into multiple modalities. For instance, features may be defined in terms of measurements from different experimental scenarios, and might include not only action potential spike shape characteristics (Cf. [1, 2]), but also complex features such as the frequency response curve (as a function of injected current), and phase response curve (as a function of perturbation time). The benefit of multiple feature modalities is to provide additional ways to distinguish solutions that might otherwise appear similarly fit in the restrictive view of a single modality. This is often due to relative insensitivities of some parameters to features in a single modality, or parameter co-variation/modulation [2], which creates an ill-posed optimization problem (poor gradients, local minima, or multiple global minima in the fitness landscape). Thus, for any given parameter choice, an evaluation of model fitness might involve multiple test scenarios to be executed.

We use an implementation in the PyDSTool software [3] to demonstrate the ease with which this multi-modal approach can be prepared and embedded in conventional gradient-based or global optimization algorithms. We show that the technique improves detailed models with many parameters over heterogeneous data sets that contain noise and variability in underlying conditions, for which methods based on single modalities fail.

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Effect of muscarinic receptor activation on dendritic morphology of honey bee Kenyon cells in age-matched foragers

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The honey bee (*Apis mellifera*) brain is a model system for the study of experience-dependent neuronal plasticity. Young worker bees perform in-hive tasks while older bees exit the hive to forage. Experienced foragers have a larger volume of neuropil associated with the mushroom bodies than do younger workers [1]. We hypothesize that sensory cues associated with foraging increase cholinergic signaling in the mushroom body calyces which ultimately results in increased dendritic complexity. In the present study, we used pharmacological manipulations to simulate the increased cholinergic signaling presumed to accompany foraging. Age-matched bees with 1 week of foraging experience were caged in the laboratory and treated with pilocarpine (muscarinic agonist) or scopolamine (muscarinic antagonist) for one week. The Golgi method was used for detailed studies of Kenyon cell arborizations. Because the increase in mushroom body volume induced by foraging is correlated with increased complexity of Kenyon cell dendritic arbors, we predicted that pilocarpine would increase measures of Kenyon cell complexity and that scopolamine would have the opposite effect. This research was supported by NIH R01 Award to SEF and GER (GM073644).

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Potential role of heme oxygenase-1 in neuroinflammation caused by manganese

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In humans, excess exposure to manganese (Mn) is associated with a Parkinsonian type neurological disorder. While Mn can be directly neurotoxic to neurons (dopaminergic and GABAergic) in the midbrain, it can also increase glial (microglia and astrocytes) derived inflammatory products that contribute to the development of Mn neurotoxicity. Several studies report that Mn potentiates the production of inflammatory cytokines, reactive oxygen species, nitric oxide, and prostaglandins induced by inflammagens, such as lipopolysaccharide (LPS) in microglia cells. Inducible heme oxygenase (HO-1), an enzyme responsible for the cleavage of the oxidant heme into biliverdin, carbon monoxide, and iron (Fe), is increased in response to oxidative stress caused by various toxicants, including metals, and has been reported to play a role in the regulation of inflammation. Expression of HO-1 is increased in response to LPS. However, at present, the effect of Mn on HO-1 has yet to be determined. The first study was designed to examine the effect of Mn on HO-1 induction with and without the presence of LPS in microglia (N9) and dopaminergic neuronal (N27) cells and to uncover the role of HO-1 on cytokine potentiation by Mn. N9 microglia and N27 neuronal cells were exposed to either LPS (100 ng/ml), Mn (100 μ M), or combined Mn+LPS for 24 hours. In microglia, while Mn had minimal effects on its own, induction of HO-1 (protein and mRNA) by LPS was potentiated by Mn. The increase in HO-1 was not due to increased intracellular Mn, but was accompanied by a small but significant increase in Fe concentration and an increase in mRNA and protein for iNOS, and the inflammatory cytokines TNF- α and IL-6. Mn also potentiated the increase in COX-2 levels caused by LPS. In contrast, neither LPS nor Mn had any effect on HO-1 in the N27 neuronal cells. Moreover, combined Mn+LPS treatment caused a small, but significant decrease in HO-1. These results indicate that Mn potentiates the induction of HO-1 by LPS in microglia, but not in neuronal cells. Because the microglial increase in HO-1 is accompanied by an increase in inflammatory mediators as well as Fe, there is a possibility that in the presence of Mn, HO-1 acts as a pro-oxidant and is involved in the enhanced cytokine production by Mn-exposed activated microglia that has been reported previously. To further investigate, N9 cells were pretreated (3 hrs) with an HO-1 inhibitor (tin protoporphyrin; SnPP), prior to Mn, LPS, and Mn+LPS exposure. In the presence of SnPP, Mn caused even greater potentiation of TNF- α and IL-6 levels in the culture medium, suggesting that increased HO-1 by Mn+LPS is not contributing to the potentiating effects of Mn on cytokine production. In fact, it has an anti-inflammatory role. Because H₂O₂ is a possible inducer of HO-1 and it has been reported to be increased in microglia exposed to Mn, we looked at the role of H₂O₂. As reported, Mn exposure increased H₂O₂ (6h post treatment). However this effect was not potentiated by LPS. Furthermore, the presence of the HO-1 inhibitor further increased H₂O₂ output by microglia exposed to Mn, as well as from control and LPS exposed microglia. Thus it appears that H₂O₂ is not responsible for the increased HO-1. On the contrary, HO-1 serves in an antioxidant capacity in part by controlling the redox status of microglial cells. Supported by: R01ES016965 (NIEHS)

Self-administration of heroin and incubation of heroin-seeking in adolescent vs. adult male rats.

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Heroin abuse is prevalent among human adolescents. Yet few laboratory experiments explore adolescent sensitivity to heroin using animal models, such as intravenous drug self-administration and reinstatement of drug-seeking after abstinence. In this study, adolescent (postnatal day 35 start) and adult (postnatal day 86 at start) male Sprague-Dawley rats spontaneously acquired lever-pressing maintained by heroin. In Experiment 1, 13 days of self-administration on fixed ratio 1 (FR1) schedule of reinforcement (0.05, then 0.025 mg/kg/infusion; 3 hr-sessions) were followed by a reinstatement test after 1 or 12 days of abstinence. Adolescents took more heroin and exhibited higher rates of non-reinforced responding, compared to adults. In reinstatement, adolescent-onset groups exhibited less heroin-seeking than older adults, and levels of reinstatement increased between 1 and 12 days of abstinence for both age groups (incubation). In Experiment 2, 9 days of acquisition (0.05 mg/kg/infusion; 3 days each on FR1, 2, and 5) were followed by 9 days of testing on a progressive ratio (PR) schedule (0.0125, 0.05, or 0.1 mg/kg/infusion; max 9-hr sessions), and a reinstatement test after 12 days of abstinence. No age differences in self-administration or reinstatement were observed. Body weight and fecal boli were quantified, and heroin affected these somatic signs less in adolescents than adults. Overall, this study suggests that younger rats may be less sensitive than adults to some acute and long-term effects of heroin. Thus, further investigation of adolescent rats may reveal neuroprotective factors that could be mimicked for relapse prevention in humans.

Analyzing how varying neuronal parameters influence network activity using a database of computational models of a half-center oscillator

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The rhythmic activity of the heartbeat neuronal network of the leech is based on pairs of inhibitory interneurons that make reciprocal spike-mediated and graded synapses across the ganglion midline. Here, we modeled such a pair of HN(4) reciprocally inhibitory interneurons, known as a half-center oscillator model [1]. We aim at investigating the changes in this model's oscillatory activity and bursting characteristics based on cellular and synaptic parameters. To achieve this, we varied selected parameters in all combinations by using a brute-force approach and built a database of the resulting model characteristics.

For our parameter search, we varied eight parameters in both neurons: the maximal conductances of the spike-mediated and of graded transmission, and of the persistent Na⁺, slow Ca²⁺, leak, hyperpolarization-activated (h), and persistent K⁺ currents, across of 0, 25, 50, 100, 125, 150, and 175 percent of their canonical values, and the leak reversal potential across -70 mV, -65 mV, -60 mV, -55 mV, and -50 mV, resulting in a parameter space of 10,485,760 models. After changing a parameter, a model was run for 100 s to allow the system to establish stable activity, and then, it was run for another 100 s, from which the data were recorded and analyzed. The cycle period was measured as the time between the middle spikes of two consecutive bursts.

We performed all the simulations and we built a SQL database table for their firing characteristics [2,3]. We used the entire database to ask fundamental questions about the activity of half-center oscillators. First, we subdivided the models in to those in which the component cells are intrinsically silent, spiking or bursting, and then, asked whether or not oscillators of these different types responded to parameter changes similarly.

The results we have so far show that in approximately 36.46% (3,823,240 simulations) of the models both cells were silent, in 27.72% (2,906,249) of them both cells were spiking, and in 22.03% (2,310,359) both cells were bursting. The rest of the simulations (13.79% or 1,445,912) did not have symmetric activity in the two model cells. Out of the bursting models, in 18.96% (438,041) both cells showed irregular activity, in 18.19% (420,307) the component cells produced spikeless plateau potentials, in 8.18% (189,041) the two cells showed asymmetric bursting activity, and in 54.67% (1,262,970) both cells were bursting with standard bursting activity. We will now use this last sample of bursting models and then the entire database to ask mechanistic questions about their alternating activity. We will be particularly interested in parameter changes which correspond to known neuromodulations such as the modulation of h current by myomodulin [4].

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Parkinsonian brain activity patterns in urethane-anesthetized VMAT2-deficient mice

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Parkinson's disease (PD) is a devastating neurological disorder involving the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc). SNc neurons project principally to the striatum and other regions of the basal ganglia, and the loss of dopaminergic modulation in this circuit results in akinesia, rigidity and tremor – the movement symptoms that are the hallmarks of PD. In addition to the motor symptoms of PD, there are a number of non-motor symptoms that typically do not respond as well to dopamine replacement therapy. It has been proposed that these non-motor symptoms may involve the noradrenergic and serotonergic neurotransmitter systems, which also show significant degeneration in PD. In support of this hypothesis, a recent study found that mice expressing only 5% of the normal level of the vesicular monoamine transporter 2 (VMAT2 LO mice), resulting in severely depleted levels of DA, NE and 5-HT, display both an age-dependent motor phenotype and several of the non-motor symptoms of PD [1].

Neurophysiological studies of dopamine-depleted primate and rodent models of PD have found altered neuronal firing rates and patterns at multiple basal ganglia sites including the output nuclei. Three pattern changes have been emphasized as consistent features of Parkinsonian brain activity: increased burst firing, abnormal 8 – 45 Hz oscillations, and elevated synchrony between neurons. To determine the neurophysiological correlates of the Parkinsonian phenotype observed in VMAT2 LO mice, we have compared local field potential and single-unit activity between these mice and wild-type controls. We recorded simultaneously from three brain regions: the substantia nigra pars reticulata (SNr), which is a major basal ganglia output nucleus composed of GABAergic projection neurons; the ventral medial thalamus (VM), which is one of the principal targets of the SNr in mice; and motor cortex, which is reciprocally connected with VM and also a major source of input to the basal ganglia circuit. Preliminary results show markedly increased oscillatory power at about 10 Hz in the VMAT2 LO mice under urethane anesthesia. The 10 Hz oscillations were highly coherent between motor cortex, SNr and VM, consistent with increased synchrony across the cortico-basal ganglia network. The elevated oscillations were not observed under isoflurane anesthesia, and were observed under urethane only during periods of cortical activation induced by a toe-pinch stimulus. These results indicate that VMAT2 LO mice show some of the same neuronal activity pattern abnormalities that are characteristic of more established animal models of PD.

[] Taylor TN et al. 2009. J Neurosci, 29, 8103-13.

Midbrain Periaqueductal Gray-mediated morphine tolerance and glial cell expression is altered in the absence and presence of persistent pain

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Opioid-based narcotics are the most prevalent therapeutic treatment for chronic pain management with morphine being the most heavily prescribed drug. In a persistent pain state morphine sensitivity and the latency to develop morphine tolerance increases. Conversely, tolerance develops rapidly in the absence of pain. Our lab has shown that the midbrain Periaqueductal Gray (PAG) is an important site for the development of morphine tolerance. Up until relatively recently, glial cells were thought to be involved in neuronal support alone. It has now become clear that glial cells communicate with, and influence neuronal activity in several important ways, including influencing the development of morphine tolerance. Surprisingly, there have been no studies directly assessing the role of PAG-glia in this phenomenon. Male Sprague Dawley rats were given a priming dose of bilateral, intra-PAG glial cell inhibitor, Propentofylline, or a vehicle control followed by three consecutive days of subcutaneous (SC) injections of morphine (5mg/kg) preceded by bilateral intra-PAG injections of Propentofylline (1, 10 or 100fmol) or vehicle. On the fifth day morphine tolerance was assessed with a cumulative challenge doses of SC morphine (1.8 to 10mg/kg). Latency to withdrawal their paw from a noxious thermal stimulus was recorded 15 minutes after each SC morphine injection. Rats that received intra-PAG Propentofylline had increased paw withdrawal latencies as compared to vehicle controls, indicating that morphine was more effective in animals with inhibited PAG-glia. These results indicate a possible role for PAG-glia in the development of morphine tolerance.

Carbon monoxide as a modulator of growth cone motility

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Gaseous molecules have been shown to play a role in a variety of physiological functions including development and are capable of diffusing across cell membranes to modulate multiple cells at a given time. While nitric oxide (NO) is well established as a messenger molecule, recent studies have shown that carbon monoxide (CO), another cellularly produced gas, is capable of modifying cellular activity in a variety of model systems; however, the role of CO in development is poorly understood. We have previously demonstrated that NO can modulate the morphology and physiology of B5 neurons, resulting in an increase in filopodial length and a decrease in the rate of neurite outgrowth. These phenomena, termed slow-down and search behavior, resemble growth cone behavior at decision points during pathfinding. NO's effects have been shown to be mediated through soluble guanylyl cyclase (sGC), resulting in activation of PKG and an increase in the intracellular calcium concentration [1,2]. The objective of this study was twofold: to investigate the effects of CO exposure on growth cone dynamics and to uncover the downstream pathway through which CO affects neurons in the buccal ganglion of *Helisoma trivolvis*. Bath application of the CO-releasing molecule (CORM) resulted in an increase in filopodial length in growth cones of both B5 and B19 neurons within minutes. We show evidence that the effects of CO are mediated through the soluble guanylyl cyclase (sGC)/cGMP/PKG pathway and result in an increase of intracellular calcium. Our preliminary results indicate that NO and CO apparently share the same signaling pathway to affect growth cone motility. Taken together, these results suggest that CO could act as a physiological modulator of growth cone motility, and that CO production in the nervous system might affect neuronal path-finding behavior during development.

[1] Trimm & Rehder. 2004. *Eur Neurosci.*, 19, 809–818

[2] Van Wagenen & Rehder. 1999. *Neurobiol.*, 39, 168–185

Clinical and pathological heterogeneity in patients with the p.C139R missense mutation in Progranulin

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Frontotemporal lobar degeneration (FTLD) and Alzheimer's disease (AD) are two of the most common forms of dementia. AD usually affects individuals over 85 years of age and clinically those individuals present with memory and cognitive deficits. FTLT typically affects individuals in their early 60s and clinically presents with speech impairment, behavioral and personality changes before any memory and cognitive deficits are noticed. The majority of patients with FTLT have a positive family history of dementia indicating a strong genetic component to this disease. Recently, we identified loss-of-function mutations in the gene encoding the secreted growth factor progranulin (*GRN*) as a major novel cause of FTLT. Missense mutations in *GRN* have also been reported but their role in the development of neurodegenerative diseases still remains largely unknown. One particular missense mutation, p.C139R, showed evidence for a partial loss-of function. To further study the clinical, pathological and functional characteristics of p.C139R we screened a large cohort of neurodegenerative disease patients and controls for the presence of this missense mutation using a custom designed Taqman single-nucleotide polymorphism genotyping assay. Functional analyses were performed to determine the effect of p.C139R on *GRN* stability and secretion in a cell culture model. We identified three patients with the p.C139R missense mutation in our cohort, one patient with a clinical diagnosis of FTLT, one with a pathological diagnosis of FTLT and one patient with a pathological diagnosis of AD. Two research groups from Italy and Belgium also reported a clinical FTLT and AD patient with this mutation. Haplotype sharing analysis showed that the p.C139R mutation arose twice independently from two common founders. Western Blot analysis showed that expression of GRN p.C139R in cell culture after treatment with cyclohexamide resulted in more GRN in the pellet than in the media compared to cells transfected with wildtype GRN. A time-course experiment confirmed the delayed and reduced secretion of p.C139R GRN compared to wildtype GRN. Our data suggest that GRN p.C139R may cause a partial loss-of-function and that this may be a susceptibility factor for both FTLT and AD.

Stimulus-specific theta-band gating of auditory cortical spiking in awake mice

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The spiking activity of sensory cortical neurons *in-vivo* is not exclusively driven by external stimulus inputs, but also by other mechanisms such as top-down inputs and local interactions with the surrounded network. For example, some cells prefer to fire at particular phases of the local field potential (LFP), which is a coarse grain sample of the synaptic and spiking activity in the local network. This effect is known as *phase-locking*. Although network driven modulations of single neuron activity have been previously documented in several brain regions, the interaction between stimulus-driven and network-driven inputs is still not fully understood. One way to address this problem is to ask whether the state of the network (e.g. as measured by the instantaneous phase of the LFP signal) before stimulus onset affects how a single neuron within this network responds. Here we investigate this issue in an awake, restrained mouse preparation, recording single neuron spiking along with the LFP in the auditory cortex while presenting natural communication sounds to the animals. We classified trials into two groups based on whether the LFP at specific times before the stimulus onset was near a local peak in the signal (phase of $\phi = 0$) or near a local valley ($\phi = \pm\pi$). We considered the LFP in different frequency bands, including the theta (4-10 Hz), gamma (50-90 Hz) and wide (4-100 Hz) bands. Our preliminary data show that the theta-band LFP at stimulus onset, but not the gamma or wide band LFPs, modulates the evoked spiking response in a stimulus-specific manner.

Intrinsic microcircuitry of GABAergic inputs from direct and indirect striatofugal neurons on striatal cholinergic interneurons in the primate putamen

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Cholinergic interneurons in the striatum display complex, inhibitory responses following electrical stimulation of the centromedian thalamic nucleus in monkeys [1]. The source of this inhibition remains unknown, although intrinsic striatal GABAergic microcircuits are important regulators of acetylcholine release following parafascicular nucleus stimulation in rats [1,2]. In order to better understand the potential sources of GABAergic inputs that regulate the electrical activities of striatal cholinergic interneurons in primates, we undertook a comparative analysis of the synaptology of intrinsic GABAergic axon collaterals from direct (substance P-immunoreactive) or indirect (enkephalin-immunoreactive) striatofugal neurons onto cholinergic interneurons in the monkey striatum.

In single labeled tissue, both substance P (SP) and enkephalin (ENK) immunoreactivity was expressed predominantly in axon terminals, dendrites and perikarya. Data obtained from the analysis of 87 SP-labeled boutons and 91 ENK-immunoreactive terminals revealed that medium sized (0.5-1.0 μm in diameter) dendritic shafts are, by far, the predominant target of both populations of intrinsic GABAergic afferents from axon collaterals of medium spiny neurons. Less than 10% of these terminals contact spines or perikarya. Pre-embedding immunogold localization of SP or ENK combined with peroxidase immunostaining for choline acetyltransferase (ChAT) showed that about one-third of 93 SP-labeled boutons make symmetric synaptic contacts with ChAT-labeled dendrites, of which 61% have a medium sized diameter. On the other hand, preliminary observations showed that 21% of 38 ENK-immunoreactive terminals form symmetric synapses with ChAT-labeled dendrites; 50% of them being in the large-diameter category (larger than 1.0 μm). These preliminary findings provide evidence that intrinsic GABAergic axon collaterals from both direct and indirect striatofugal neurons are located to subserve important regulation of cholinergic interneuron activity in the primate striatum.

[1] Nanda et al. 2008. EJNS, 29:588

[2] Zackheim et al., 2005, Neurosci, 131:423

Role of the tyrosine kinase receptor EphA5 in recovery from perinatal brain injury

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Traumatic brain injury (TBI) is a serious public health problem and one of the major causes of death and disability in young people. The topographically ordered projection between the retina and midbrain superior colliculus (SC) in rodents is an excellent model system in which to approach this difficult problem. The retina makes a map of visual space on the midbrain superior colliculus. This retinotopic map is established during development in part by a family of graded molecular guidance cues in the SC called ephrin-As, recognized by their EphA tyrosine kinase receptors in the retina. Damage to the caudal part of SC at birth, instead of deleting part of the map, causes it to compress *in toto* onto the remaining SC. This process of recovery from injury preserves visual function, but the compensatory mechanism underlying map compression is unknown. Our previous results showed that in compressed maps the rostrocaudal gradient of ephrinA expression is steeper than normal. We proposed that the change in the slope of the gradient plays an instructive role in map compression. Here we test the hypothesis that TBI to SC leads to changes in retinal EphA5 receptor expression that are complementary to the changes in collicular ephrinA expression. We measured EphA5 mRNA and protein levels after SC damage, and found that the nasotemporal gradient of EphA5 expression steepens in the retina and overall expression levels change dynamically. Importantly, the alterations in retinal EphA5 expression occur after the change in collicular ephrinA5 expression. Taken together, these findings, suggest that neonatal TBI triggers changes in the ephrinA gradient and then in the retinal EphA gradient. Map compression could be instructed directly by the change in ligand expression or by the subsequent change in receptor expression, guiding the recovery of this visual pathway from early injury. Understanding what molecular signals direct for the response to TBI is important for developing clinical approaches to rehabilitation and for maximizing the potential for recovery.

Reduced pharmacoresistance to stiripentol during prolonged status epilepticus

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Status epilepticus (SE) is characterized as a prolonged seizure accompanied by a loss of consciousness, and is capable of resulting in severe neuronal damage and even death. Benzodiazepines (BZDs), which act on GABAA receptors, are often the first line of treatment for patients who experience SE. The actions of diazepam, one of the most common BZDs, rely on the presence of the $\gamma 2$ subunit of the GABAA receptors to potentiate inhibition. However, following seizure activity GABAA receptors containing the $\gamma 2$ subunit internalize, thus quickly and significantly decreasing the efficacy of diazepam. Stiripentol (STP) is an antiepileptic drug currently approved for treatment of Dravet syndrome, a severe form of epilepsy in infancy, characterized by prolonged generalized tonic-clonic seizures. STP is a positive allosteric modulator of the GABAA receptor, and potentiates inhibition through GABAA receptors with a variety of different subunit combinations, with its greatest impact on $\alpha 3$ or δ -containing receptors. GABAA receptors containing the $\alpha 3$ subunits are widely distributed throughout the embryonic brain, but their expression is markedly reduced during development. Neither $\alpha 3$ nor the δ subunit containing GABAA receptors internalize following a prolonged seizure event. This led to the hypothesis that unlike diazepam, STP would have similar efficacy during both brief and prolonged SE. We used the lithium-pilocarpine model of SE to compare the ability of diazepam and stiripentol to terminate behavioral seizures during brief and prolonged SE in postnatal day 21 male rats. After pilocarpine treatment, brief SE was defined as the onset of stage 3 seizure activity, while prolonged SE was defined as 45 minutes of SE following the first stage 3 behavioral seizure. The pharmacoresistance was evaluated by the ratio between the effective dose protecting 50% of animals (ED50), via the intraperitoneal route, determined at the brief and prolonged time points for each compound. As has been previously reported, we found an approximate 12 fold decrease in the efficacy of diazepam during prolonged SE. However, STP yielded only a 2.7 fold decrease in efficacy over the same SE duration. These findings suggest less pharmacoresistance to STP than diazepam during prolonged SE. Overall, these preliminary results support a continued investigation into the use of stiripentol for the treatment of status epilepticus, as well as the development of anti-epileptic drugs targeting the $\alpha 3$ or δ -containing subunits of the GABAA receptor. Supported by Biocodex.

Modeling *Drosophila* motoneurons to examine the functional effect of Na channel splice variants

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Neurons have diverse electrophysiological characteristics controlled by voltage-gated ion channels. It is not known how much of the diversity of neuronal activity is caused by differential channel gene expression as opposed to alternate splicing of these genes.

The contribution of alternate splicing to neural activity and therefore neuronal function can be addressed more easily in invertebrates because of their smaller genome.

Specifically, the fruitfly *Drosophila melanogaster* represents a very powerful molecular genetic model system that has been instrumental for our understanding of early development of the nervous system. Recently in *Drosophila*, several voltage-gated sodium channel (DmNav) splice variants have been identified [1].

However, the expression of DmNav splice variants cannot yet be controlled in *Drosophila*, preventing the analysis of their effect on neuronal function. Instead, splice variants can be expressed in the oocytes of the South African clawed frog *Xenopus Laevis*. The expression of these channels in *Xenopus* oocytes allows the electrophysiological characterization and the construction of computational ion channel models. If these models are built with sufficient detail, the functional effect of the splice variants on neuronal activity thus can be analyzed.

As a first step in this task, we build a full computational model of the *Drosophila* motoneuron, which, to our knowledge, has not been accomplished before. To build this model, we combine channel data recorded from *Drosophila* and oocytes. In the oocytes, recordings show a space clamp problem because of the oocytes' large size, which is required for expressing DmNav splice variants. We address this problem with a spatial model of leak current in the oocyte. We present solutions to several obstacles in modeling fast kinetics of DmNav channels and also putting them together in a full model of a *Drosophila* motoneuron.

[1] W.-H. Lin et al. 2009. J. Neurophysiol., 102(3), 1994-2006

Cytisine and diarylpropionitrile may not modulate depressive behaviors in female WKY rats

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Nicotine and estrogen may regulate mood and influence depressive behaviors. Results from rodent studies suggest the potential involvement of nicotinic cholinergic receptors and estrogen receptors in antidepressant-like behavior. Specifically, blockade of nicotinic receptors containing the β_2 subunit may be responsible for antidepressant effects [1]. Furthermore, selective estrogen receptor β (ER β) agonists have been shown to reduce depressive behaviors in female ovariectomized rats [2].

In this study, we evaluated potential anti-anxiety and anti-depressant effects of a nicotinic agent, cytisine, and a selective estrogen receptor modulator, diarylpropionitrile (DPN), in an animal model of depression in female rats. Cytisine is a partial agonist at $\alpha_4\beta_2$ receptors and a full agonist at $\alpha_3\beta_4$ and α_7 receptors. DPN is a selective ER β agonist. The Wistar Kyoto (WKY) rat strain is a putative model of endogenous depression. Compared to their control, the Wistar rat, WKY rats demonstrate hormonal, behavioral and physiological parameters that are similar to symptoms found in depressed patients [3]. WKY rats appear to be resistant to standard SSRI antidepressants. Interestingly, a subset of depressed patients does not respond to SSRI drugs. Therefore, this model could be useful in identifying novel agents for antidepressant therapy. Female WKY and Wistar rats (n=6-8/group) received subcutaneous injections of cytisine (0.5, 1, 1.5 mg/kg), DPN (10 μ g, 0.5 mg/kg, 1 mg/kg) or vehicle prior to being tested in the elevated plus maze (EPM), locomotor activity chamber (LCA) and forced swim test (FST). Dosages and latencies for drug administration were chosen based on previous reports and data from preliminary studies.

Our results show that WKY rats displayed significantly more depressive behaviors compared to Wistar rats. WKY rats had significantly more immobility counts and also significantly less locomotor activity compared to Wistar rats.

No differences were detected in the EPM with cytisine or DPN in either strain.

In addition, none of the doses of DPN produced significant effects on behavior in the other tested paradigms. Because the rats had intact reproductive systems, their behavior could be influenced by the estrous cycle.

At the tested doses of cytisine, the 1.5 mg/kg dose significantly increased immobility in the FST in Wistar and WKY rats. There was a trend for WKY rats treated with 1.5 mg/kg of cytisine to exhibit reduced locomotor activity; however, this did not reach statistical significance. At this dose of cytisine there appears to be an exaggeration of depressive behaviors. This observation could be due to activation of nicotinic receptors in the peripheral ganglia, which may cause autonomic dysregulation and account for the decrease of locomotor activity and increase in immobility in the FST.

In conclusion, cytisine and DPN do not appear to have anti-anxiety or anti-depressant effects in the female WKY rat. Additional experiments that test a wider range of doses, combination of drugs, and consider the phase of estrous cycle are necessary.

[1] Mineur et al, 2007. [2] Walf et al, 2004. [3] Will et al, 2003.

Synchronization in a bursting half-center oscillator with slow-to-fast reciprocal inhibition

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Our modeling studies reveal that a pair of busters reciprocally coupled by fast non-delayed inhibition can synchronize, contrary to the conventional belief. Various measures are computed to quantify the synchrony between the specific models of leech heart interneurons. We also discuss the bistability of co-existing in-phase and anti-phase synchronous bursting patters in the half-center oscillator (HCO). In addition, we examine temporal characteristics of inhibitory synaptic connections, varying from fast to slow, to establish synchronization within the HCO. Our study of inhibitory synchronization and co-existing dynamical rhythms may help one better understand switching mechanisms between different neuronal rhythms of a CPG upon various dynamical conditions and inputs.

Effects of aging on morphine analgesia and associated changes at the μ -opioid receptor

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Both clinical and basic science studies have established that morphine produces a differential degree of analgesia in males and females. In particular, in models of orofacial, visceral, or persistent inflammatory pain, females require 2x the amount of morphine to produce a level of analgesia comparable to males. To date, however, research on morphine analgesia has been carried out primarily in adult rats. Few studies to date have examined the effects of advanced age on morphine analgesia, and none have examined the impact of advanced age on opiate receptor expression and function.

To characterize the impact of age on morphine analgesia, male and female Sprague Dawley rats (adult: 3 mos; aged 18-24 mos) received an intraplantar injection of the inflammatory agent Complete Freund's Adjuvant (CFA) to induce persistent inflammation. Twenty-four hours later, morphine was administered using a cumulative dosing paradigm (0, 1.8, 3.2, 5.6, 8, 10, and 18 mg/kg; ip) and analgesia was assessed using the paw thermal stimulator. No significant differences were noted in baseline paw withdrawal latencies (PWLs) or in CFA-induced hyperalgesia for aged vs adult, male and female rats. A significant impact of age and sex was observed for morphine analgesia. In particular, a significant rightward shift in the morphine dose response curve was noted for aged rats, as well as adult females. ED₅₀ values for these groups were 2x higher (7.0 mg/kg) than those for adult males (3.5 mg/kg). Subsequent anatomical studies demonstrated reduced mu opioid receptor protein and binding in the midbrain periaqueductal gray, an essential brain region for morphine action. The finding of reduced MOR levels in the PAG may provide the biological bases for the decreased morphine sensitivity observed in aged animals.

Effect of high frequency AC stimulation on the compound action potential of the sciatic nerve of frogs

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Conduction block using high frequency alternating current (HFAC) stimulation has been shown to be completely effective, repeatable and quickly reversible in various amphibian and mammalian animal models. Prior experimental work in our lab on the sea-slug, *Aplysia*, has shown that complete, reversible block through HFAC stimulation can also occur in unmyelinated nerves. However, unlike simulations and experiments on myelinated nerves, a non-monotonic relationship between frequency and blocking thresholds was found in the unmyelinated fibers. To further investigate this difference in the response of myelinated and unmyelinated nerves to conduction block induced by HFAC stimulation, we studied the effect of HFAC stimulation on the compound action potential of the sciatic nerve of frogs. Maximal stimulation of the sciatic nerve produces a compound action potential consisting of the A-fiber and C-fiber components corresponding to the myelinated and unmyelinated nerve fibers' response. The A-fiber and C-fiber components of the compound action potential were found to be blocked at different blocking amplitudes of HFAC stimulation. This property of differential thresholds for inducing block in myelinated and unmyelinated nerves has potential clinical implications, especially in the field of selective stimulation.

Parasympathetic nervous system responses and cardiovascular traits predict PTSD symptoms

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Posttraumatic stress disorder (PTSD) is a heterogeneous disorder that is defined by three major symptom clusters: intrusive, avoidance, and hyper-arousal symptoms. Individual patients can vary in the degree to which they present with the different symptoms. The purpose of this study was to examine the relationship between physiological responses and specific PTSD symptoms to gain insight into biological markers of PTSD. We measured psychophysiological responding during a fear conditioning discrimination task. This paradigm, termed AX+/BX-, independently assesses responses to danger (CS+) and safety (CS-) cues. We compared resting heart-rate (HR) and HR variability (HRV) as a measure of sympathetic and parasympathetic tone. We used these measures to predict ratings on individual items on the Modified PTSD symptom scale (PSS), and the DES-T (Dissociative Experiences Scale – Short Version) using linear regression analyses. The study sample (n=76) was recruited from a highly traumatized civilian population seeking treatment at Grady Memorial Hospital in Atlanta, GA. Results show that PTSD subjects had higher HRV than controls, and high-frequency HRV in the presence of safety cues predicted severity of avoidance and hyper-arousal PTSD symptoms significantly in males ($F(1,29)=3.67$, $p=0.065$) and in females ($F(1,42)=4.86$, $p=0.033$.) Resting heart-rate was not associated with symptoms; however, resting blood pressure was negatively associated with PTSD.

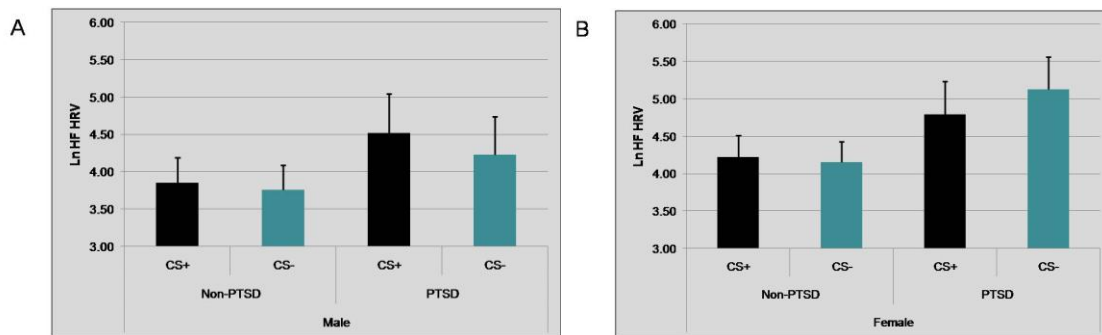


Figure 2

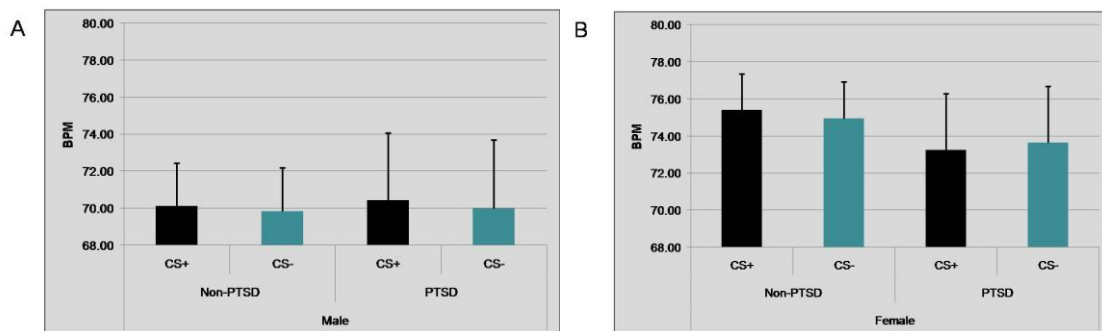


Figure 3

Influence of Antiepileptic Drugs on the basic characteristics of EEG

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Understanding the correlation between clinical and neurophysiological effects has been receiving much more appreciation in clinical epileptology more recently. Therefore electroencephalography is thought to be an efficient tool not only controlling the ongoing effectiveness of the treatment but also to be an efficient tool to predict potential clinical and psychocognitive adverse effects of the treatment. The drugs- Vallproate acid (Depakine-D) and carbamazepine (CBZ) are amongst the most widely used Antiepileptic Drugs (AED). Although, the selection of antiepileptic drugs is highly determined by the type of seizures, the effect of these drugs on basic neurophysiological processes of CNS is not fully investigated. Therefore, the aim of the present study was to compare the effect of CBZ and D on those variables of EEG that assess both epileptic activity and overall functional state of the brain.

53 patients aged 3 to 9 years treated by D, 48 patients same aged- by CBZ. All patients underwent EEG recording for three- times: 1st visit, before the administration of AED, in a 3-4 months (2nd visit) and 6-8 months (3rd visit) after the initiation of treatment. EEG signals were digitally recorded using a set of 19 scalp electrodes, 10-15 fragments for EEG were performed for the evaluation of the specificity of the background activity as well as the spectral analysis was performed to calculate absolute value of power (AVP) EEG in different frequency spectra.

D revealed that the drugs from this class reduce the degree of disorganization of basic rhythmicity of EEG at the expense of reduction of high amplitude mono- and poly-morph waves in low frequency. It is considered as the sign of increased seizure readiness of CNS. D appears significantly reduce AVP spectra practically in all zones recorded from the brain surface especially occipital areas. During the treatment with CBZ deceleration of the background EEG activity and decrease in the mean frequency of the alpha rhythm find. Deceleration of AVP arises at the expense of the low frequency range, predominantly in the parietal and occipital zones.

The difference between D and CBZ effect on electrogenesis of the brain is mainly revealed in the low frequency spectra of EEG and for more typical epileptiform elements such as spike-wave. On the one hand, D markedly decreased the ratio of high amplitude waves of Delta and Theta range. Contrary, under CBZ there was significant increment of the given variables. Moreover, D was more efficient in eliminating spike-wave.

In summary, the difference in the effect of CBZ and D on bioelectrical activity of the brain could be related to region-specific differences within the loci of maximal neuropharmacological effect of these drugs: CBZ increases low wave activity within the AVP spectra, that is generated by mostly by neural mechanism of cortex.

D decreases the signs of exaggerated synchronization at the expense of reduction of low frequency spectra within the AVP. In addition, D appeared more efficient to suppress epileptic complex spike-wave with substrate in thalamus.

Lipopolysaccharide increases aquaporin-4 expression in mouse cortical astrocytes

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Cerebral edema is a frequent and life-threatening neurological complication following brain trauma and stroke. Unfortunately, medical therapeutics to reduce brain swelling are currently lacking, in part, due to the absence of viable drug targets. Recent work by our laboratory and others suggest neuroinflammatory mediators promote cerebral edema via the induction of the astrocytic water channel, aquaporin-4 (AQP4); however, the precise cellular mechanisms underlying AQP4 regulation remain poorly defined. In the present study, we hypothesized that activation of toll-like receptor 4 (TLR4), a key mediator of innate immunity, promotes AQP4 expression in cultured astrocytes. The exogenous TLR4 ligand, lipopolysaccharide (LPS), concentration (10-1000 ng/mL) and time-dependently stimulated AQP4 mRNA and protein expression in mouse cortical astrocytes. A peak effect was observed following a 3 hour treatment with 100ng/mL LPS. LPS-induced AQP4 expression was attenuated by co-treatment with curcumin, helenalin, or PDTC which inhibit the pro-inflammatory transcription factor, NF κ B. Similarly, LPS increased the activation of the p50 and p65 subunits of NF κ B. Together, these data identify a possible functional role for TLR4 activation in promoting AQP4 and cerebral edema following stroke or TBI. Supported by grants from the National Institute of Health (NS065172) and the American Heart Association (2300135) to KMD.

The Effect of Exercise on Executive Control in Overweight Children

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Evidence provides support for the beneficial effects of exercise on the cognition of overweight children. The current study evaluates whether exercise benefits children's executive control (EC), processes which include inhibition and management of conflicting information. EC was assessed using an antisaccade task (requiring suppression of a prepotent glance to a cue and the generation of a glance to the cue's mirror image) and an Eriksen flanker task (requiring a response to a central stimulus in the presence of congruent or incongruent peripheral stimuli). A prosaccade task (requiring a glance to a target) was also used as a baseline to evaluate antisaccade performance. Participants were sedentary, overweight (BMI \geq 85th percentile) children ages 8 – 11 years old. Participants were placed randomly into either an attention control group (N=37 at time 1), who engaged in instructor-led sedentary activities, or an exercise intervention group (N=38 at time 1), who participated in aerobic training for 40 minutes/day, 5 days/week over the entire school year. It was hypothesized that exercise would improve performance on both antisaccade and flanker tasks. Data were collected at three time points: at start, at 9 weeks, and at 18 weeks. Data will be collected from a final testing in April when the impact from the full training complement can be evaluated.

A novel behavioral paradigm for genetic analysis of the concept of nests in mice

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Abstract concepts in the brain enable humans to efficiently and correctly recognize and categorize a seemingly infinite amount of objects and daily events. Such abstract generalization abilities are traditionally considered to be unique to humans and perhaps non-human primates. However, emerging neurophysiological recordings indicate the existence of neural correlates for the abstract concept of nests in the mouse brain.

To facilitate the molecular and genetic analyses of concepts in the mouse model, we have developed a nest generalization test based on mice's natural behavior. We show that inducible and forebrain-specific NMDA receptor knockout results in pronounced impairment in this test. (Fig. 1) Interestingly, this generalization deficit could be gradually compensated for over time by repeated experiences even in face of the continued deficit in object recognition memory. Therefore, our study not only establishes a quantitative method for assessing the nest concept in mice, but also demonstrates its great potential in combining powerful mouse genetics for dissecting the molecular basis of concept formation in the brain.

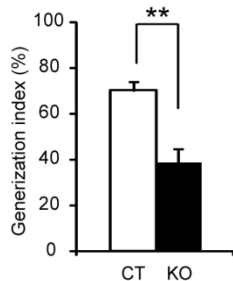


Fig. 1

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Object familiarity modulates effective connectivity during haptic shape perception.

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Visual cortical areas are routinely active in normally sighted individuals during haptic shape perception but there is debate about whether this is due to visual imagery, mediated by top-down pathways from prefrontal areas, or engagement of multisensory representations via bottom-up pathways from somatosensory areas. In two functional magnetic resonance imaging experiments, participants haptically discriminated the shape of unfamiliar (Experiment 1) or familiar (Experiment 2) objects (HS task). In separate sessions, the same participants in each experiment made shape judgments on visual images of objects represented by words they heard (VI task). Regardless of familiarity, VI- and HS-related activations overlapped in the lateral occipital complex (LOC) bilaterally, left ventral premotor cortex, left ventral intraparietal sulcus, and left pulvinar. For familiar objects, there were additional overlap zones in left prefrontal and posterior parietal cortex. We then performed multivariate Granger causality analyses of effective connectivity on task-specific time series data from these experiments, using a novel method that eliminates zero-lag correlations (correlation-purged Granger causality) thus yielding purer estimates of effective connectivity. These analyses showed that the VI and familiar HS tasks activated similar networks involving top-down paths into the LOC, consistent with the use of visual imagery during haptic perception of the shape of familiar objects. However, the unfamiliar HS task activated a different network characterized by bottom-up, somatosensory cortical inputs into the LOC. We conclude that LOC activation during haptic shape perception reflects visual imagery, but that this is mediated by object familiarity. Thus, shape representations in the LOC are flexibly accessible, either top-down or bottom-up, according to task demands.

Activation of NR2B mediates neuronal release of high mobility group box protein 1 (HMGB1): a novel link to cerebral edema?

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Acute neuronal necrosis clinically correlates with the development of cerebral edema, an important cause of patient mortality following traumatic brain injury (TBI). Given the established link between over-stimulation of NMDA-type glutamate receptors (NMDA-R) and neuronal excitotoxicity, we hypothesized that glutamatergic signaling may promote the development of brain edema. To test this hypothesis, we explored whether selective inhibition of individual NR2 subunits of the NMDA-R would reduce acute brain injury after a moderate TBI in mice. Selective inhibition of NR2B containing NMDA-R using Ro25-6981 (6 mg/kg), significantly attenuated cerebral edema at 24h post-TBI, as compared to placebo-treated mice, and reduced neuronal cell death. In contrast, selective inhibition of NR2A containing NMDA-R with NVP-AAM077 (5 mg/kg) did not significantly influence neuronal viability or TBI-induced brain swelling. Neuronal cell death within the pericontusional cortex was paralleled by a concomitant decrease in immunoreactivity for high mobility group box protein 1 (HMGB1), a predominantly neuronal nuclear protein that is released into the parenchyma to activate toll-like receptor 4 (TLR4)-dependent inflammatory response after a necrotic injury. As was observed with NR2B antagonism, inhibition of the HMGB1 activity using glycyrrhic acid (600 mg/kg) reduced the development of cerebral edema following TBI in mice. Cellular edema, which is characterized by glial swelling, is regarded as the major cause of brain swelling following TBI. Thus, functional studies to establish whether neuronally-derived HMGB1 affects astrocytes were performed. Consistent with a detrimental role in brain edema, treatment of cultured murine astrocytes with lipopolysaccharide, a specific TLR4 agonist, or HMGB1 increased the expression of aquaporin-4 (AQP4), a water channel implicated in TBI-induced cellular edema. Similarly, conditioned media from NMDA-treated neuronal cultures also stimulated AQP4 expression in cultured glia and this effect was prevented by co-treatment with glycyrrhic acid (200 μ M). Together, these studies support an important mechanistic role for neuronal NR2B activation in the development of cerebral edema following TBI.

Brain Activity and Network in Economic Decision-Making

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Much of our everyday life involves making decisions and choices. Using behavioral paradigms from game theory, recent neuroimaging studies have begun to probe the underlying brain processes associated with human decision-making in the context of social interactions. These studies identified the prefrontal cortex as a decision-making hub that incorporates emotional biases from affect-related brain regions. Using the Ultimatum Game, Impunity Game and Fixed Decided Offers, we conducted behavioral and neuroimaging (fMRI) studies to further elucidate the roles of the network of cognition- and affect-related brain regions in economic decision-making in situations of fair, undercompensated and overcompensated (unfair) monetary offers. The behavioral data showed that the unfair undercompensated offers were much more likely to be rejected than fair (nearly equal) offers. Most of the unfair overcompensated offers were accepted. The standard univariate analysis of brain responses in these games provided new insights into the neural bases of maintaining punishment goal, accepting overcompensated offers, and protesting an unfair offer. Our connectivity analysis further showed how the cognition- and affect-related brain regions coordinate activity in economic decision-making in situations of equity. These findings support the emerging view of brain organization that complex cognitive-emotional behaviors are the results of dynamic interactions of brain areas currently attributed to different functional specializations.

Alcohol exposure during development and its impact on the nucleus accumbens

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Previously, we have shown that alcohol exposure during development robustly alters c-fos gene expression as measured by immunoreactivity in the nucleus accumbens after both the opportunity to play and exposure to cocaine-associated cues. These effects were sexually dimorphic. In order to aid in the interpretation of the findings, it is critical to understand the neuroanatomical changes induced by alcohol exposure during development. A rat model of Fetal Alcohol Spectrum Disorders (FASD) was used and it entailed exposure to alcohol from gestational days 1 through 22 and from postnatal days 2 through 10. Alcohol was administered via intra-gastric intubations in both dams and pups. Control groups consisted of a non-treated group and a pair-fed group exposed to the procedures to administer alcohol. Peak blood alcohol concentrations were between 300 and 400 mg/dl in both the prenatal and postnatal period and there were no sex differences. The Golgi stain was used to examine dendritic structure in the nucleus accumbens on postnatal day 21 using NeuroLucida software (MicroBrightfield, Inc.) and the Rapid Golgi Kit (FD Neurotechnologies, Inc.). Nissl stain was used to stain neurons and glia on postnatal day 90 and a stereological cell count in the nucleus accumbens was conducted using the Optical Fractionator Workflow method from the Stereo Investigator software (MBF Bioscience). Alcohol exposure during development did not impact either spine density or dendritic structure of the medium spiny neurons in the nucleus accumbens. Also, there was no impact of alcohol exposure on either glial or neuronal cell number in the nucleus accumbens. No sex differences or interaction of sex with treatment were found. These negative findings are very important in the context of changes in neuronal activity in the nucleus accumbens (as measured by c-fos gene expression) in alcohol-treated rats exposed to cues with motivational significance. Together, the findings suggest that alcohol exposure during development causes either alterations in the neuronal inputs into the nucleus accumbens or that the neurons within the nucleus accumbens have altered reactivity to those inputs and that these alcohol-induced changes must be sexually dimorphic.

D1 dopamine receptor-mediated enhancement of LTP in the basolateral amygdala via activation of the pre-synaptic adenylate cyclase pathway

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Evidence from behavioral and electrophysiological studies indicates that the induction of long term potentiation (LTP) in principal neurons of the basolateral amygdala (BLA) may underlie the acquisition of fear conditioning. Moreover, activation of dopaminergic afferents to the amygdala is thought to facilitate fear memory, and local activation of the D1 family of dopamine receptors (D1R) also enhances fear memory. The D1R family is comprised of D1 and D5 receptors, and evidence suggests that these receptors may couple through different effectors systems. Hence, D1 may preferentially couple to PKA, whereas D5 may couple through PLC. To date nothing is known about how activation of these receptors may affect the induction of LTP in the BLA. The goals of this study were to determine if D1R facilitate LTP in BLA, and if so, what signaling cascade/s is/are involved.

Here we used whole cell patch clamp recording from BLA projection neurons to investigate the role D1R activation in LTP. LTP at cortical inputs to the BLA was induced with HFS using $5 \times 100\text{Hz}/1\text{s}$ trains administered at 20s intervals ($146 \pm 6\%$ of baseline, $n=5$, 30min following LTP induction), and was blocked following NMDA receptor antagonism and intracellular calcium chelation. Significantly, induction of LTP was completely abolished in slices pretreated with the D1R antagonist, SCH23390 ($10\mu\text{M}$; $106 \pm 4\%$ of baseline, $n=6$), and facilitated by inclusion of the specific dopamine (DA) uptake blocker GBR12783 in the ACSF ($10\mu\text{M}$; control: $151 \pm 3\%$ of base line, $n=5$; GBR12783: $228 \pm 2\%$ of baseline, $n=6$; $P < 0.05$). Hence, release of endogenous DA is both sufficient and necessary for the induction of LTP in BLA slices. Similarly, magnitude of LTP was significantly enhanced slices treated with D1R agonist SKF38393 ($50\mu\text{M}$; control: $154 \pm 7\%$ of base line, $n=5$; SKF38393: $182 \pm 4\%$ of baseline, $n=6$; $P < 0.05$), an effect that was blocked by pretreatment with the D1R selective antagonist SCH23390 ($10\mu\text{M}$; $161 \pm 5\%$, $n=5$). However, neither exogenous DA ($50\mu\text{M}$) nor the D2 agonist quinpirole ($10\mu\text{M}$) facilitate LTP in BLA slices (control: $157 \pm 3\%$ of base line, $n=5$; DA: $161 \pm 5\%$ of baseline, $n=6$).

Inhibition of PKA anchoring to scaffolding proteins by intracellular application of AKAP St-Ht31 ($1\mu\text{M}$) 30min prior to HFS fully blocked LTP induction ($100 \pm 8\%$ of base line, $n=5$; 30min following LTP induction), suggesting that activation of the D1R-cAMP cascade is critical for LTP induction. Importantly, application of the membrane permeable PLC inhibitor, U73122 ($10\mu\text{M}$), unmasked a DA facilitation of LTP ($185 \pm 6\%$ of baseline $n=5$). Finally, pretreatment with the membrane permeable AC inhibitor, MDL-12330A ($30\mu\text{M}$), fully blocked SKF38393 facilitation of LTP (SKF38393: $173 \pm 5\%$ f baseline, $n=5$; MDL-12330A+SKF: $143 \pm 6\%$ of baseline, $n=5$; $P < 0.05$). Together, these data suggest that the D1 and D5 receptor subtypes may play diametrically opposing roles in regulating LTP in the BLA. D1 receptor activation may enhance LTP in BLA through activating presynaptic adenylyl cyclase.

Species differences in serotonergic neuromodulation related to locomotor behavior

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We address the question of whether neuromodulatory actions are conserved across species. In the nudibranch mollusc, *Tritonia diomedea*, serotonin (5-HT) increases the strength of synapses made by cerebral neuron 2 (C2). C2 is a member of the central pattern generator (CPG) underlying swimming in *Tritonia*. Multiple lines of evidence suggest that serotonergic neuromodulation of C2's synapses is necessary for the CPG to generate the swim motor pattern. The pleurobranchomorph, *Pleurobranchaea californica* swims in the same manner as *Tritonia* and its swim CPG contains a homologue of C2 that exhibits a similar firing pattern and synaptic connectivity [1],[2]. We found that unlike in *Tritonia*, 5-HT did not alter the amplitude of synaptic potentials evoked by the *Pleurobranchaea* C2. We examined a more closely-related species, the nudibranch, *Hermisenda crassicornis*, which does not swim in the same manner as *Tritonia*. We identified a homologue of C2 in this species based on white soma coloration, FMRFamide-like immunoreactivity, and a contralaterally projecting axon. Bath applied 5-HT, once again did not have any effect on the amplitude of C2-evoked synaptic potentials. In contrast to the lack of serotonergic neuromodulation, we found that C2 inhibition of a large pedal neuron involved in ciliary crawling was shared by 4 different nudibranch species including *Hermisenda*. Thus, the neural circuitry for control of crawling appears to be highly conserved across species, but the neuromodulatory actions that are thought to play an important role in swimming in *Tritonia* are absent – even in species that swim as *Tritonia* does. This suggests scenarios for the evolution of behavior that could be generalized to other systems.

This work was supported by a grant from NSF.

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

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We have found that 3 μM CCh, a muscarinic agonist presynaptically inhibits evoked IPSCs recorded from hilar mossy cells. Intriguingly, while CCh inhibits evoked IPSCs in several other systems presynaptically through direct muscarinic activation or indirect CB1 receptor activation, evidence has indicated that this effect on hilar mossy cells is independent of inhibition through M2 type mAChRs or CB1 receptors, but is dependent on activation of M1/3 type mAChRs which are usually coupled to. Previous evidence in the lab has indicated that the same concentration of CCh increases levels of ambient GABA that acts in an activity dependent manner to inhibit mossy fiber transmission through presynaptic GABAB receptors; however, CGP52432, a GABAB antagonist, had no effect on either 3 or 10 μM CCh-mediated inhibition in mossy cells. Thus we hypothesized that CCh-mediated inhibition of evoked IPSCs in mossy cells may be produced by activity dependent increases in ambient GABA that target presynaptic GABAA receptors. In support of this hypothesis, we found first, there was a correlation between increases in spontaneous IPSCs and decreases in evoked IPSCs with bath application of CCh, and second, that CCh has no effect on evoked IPSCs reported by postsynaptic GABAB receptors in the presence of GABAA receptor antagonist, picrotoxin. While together these provide a strong argument in favor of our hypothesis, the observation that a GAT-1 transport blocker, NO-711, inhibits evoked IPSCs similar to CCh but is completely CGP sensitive raises several questions. To answer these questions, we have proposed a hypothetical model that attempts to reconcile these differences by suggesting an activity dependent switch from primarily GABAB and primarily GABAA mediated autoreceptor feedback mechanisms. From this model we have made several predictions that hold up to experimental evaluation and together suggests a complex relationship between two functionally distinct and activity dependent mechanisms for autoreceptor inhibition of GABAergic transmission to hilar mossy cells.

Characterization of Basigin expression in mouse brain

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Basigin gene products are members of the Immunoglobulin gene superfamily (IgSF) that function as cell adhesion molecules. Two Basigin gene products exist in the mammalian nervous system, Basigin and Basigin-2. Basigin-2 is a retina-specific gene product expressed by photoreceptor cells. Basigin is found on epithelial layers throughout the body and has been found on Müller glial cells of the retina and in the brain. The purpose of this study was to examine the distribution of Basigin gene products in the mouse brain. Once expression is localized, functional studies of the gene products can then be performed. Normal mouse brains were dissected into several sections, including cortex, cerebellum, midbrain, and hindbrain. Proteins were extracted from the different regions and subjected to ELISA and immunoblotting analyses using an antibody that recognizes both Basigin gene products. Preliminary data indicate that Basigin gene products are found in every region of the mouse brain tested. The data suggest that Basigin gene products participate in cell-cell interactions throughout the brain and nervous system, although additional studies are necessary to determine the purpose and function of these molecular interactions.

Complex phase resetting of bursting neurons in response to excitation implies slow outward current and spatial segregation of burst generating mechanism

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Central pattern generators (CPGs) frequently include bursting neurons that serve as pacemakers for rhythm generation. Phase resetting curves (PRCs) can provide insight into mechanisms underlying phase locking in such circuits. PRCs were constructed for a pacemaker bursting complex in the pyloric circuit in the stomatogastric ganglion of the lobster and crab. This complex is comprised of the anterior burster (AB) neuron and two pyloric dilator (PD) neurons that are all electrically coupled. Artificial excitatory synaptic conductance pulses of different strengths and durations were injected into one of the AB or PD somata using the Dynamic Clamp. Previously, we characterized the inhibitory PRCs by assuming a single slow process that enabled synaptic inputs to trigger switches between an up state in which spiking occurs and a down state in which it does not. Excitation produced five different PRC shapes, which could not be explained with such a simple model. Instead, a four compartment model of the AB/PD complex with two slow processes was required. The primary neurite of the model was electrotonically coupled to its soma, an axon in which spikes were initiated and a lumped dendritic compartment in which the burst envelope was generated. The spatial separation of spike and burst generation combined with a second slow process caused somatic spiking to be independent of the up and down states of the burst generator. Phase resetting of bursting neurons in response to excitation is much more complex than to inhibition, and the injection of excitatory synaptic conductances in the soma mimics dendritic activation less accurately than inhibitory.

Uncovering the neurogenetic basis of male pair bonding behavior in the prairie vole (*Microtus ochrogaster*)

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The prairie vole (*Microtus ochrogaster*) is a socially monogamous rodent that unlike the majority of mammalian species, forms long-term relationships between sexual partners, or pair bonds. While the roles of the neurotransmitters arginine vasopressin (AVP) and dopamine (DA), as well as their respective receptors, in mediating and modulating male pair bonding have been demonstrated, little is known about other genes and pathways affecting this behavior. To begin to expand our understanding of the genetic architecture of male pair bonding behavior, we have begun a selective breeding regime to generate two lines of prairie voles where males display either a high propensity to form pair bonds or do not form pair bonds at all. After five generations of experimental evolution, we have already observed divergence in behaviors between lines. In parallel with the selective breeding regime, we have also begun to develop a number of genomic resources for the prairie vole including a 10x coverage BAC library, a vole-mouse cytogenetic map, a comprehensive gene catalogue and a panel of ~750 single nucleotide polymorphisms (SNPs) which are being employed to generate a first generation genetic linkage map. These genomic resources will be used to perform quantitative trait loci (QTL) mapping and comparative transcriptome profiling to identify genetic loci and gene expression patterns that contribute to differences in the selectively bred lines.

Characterization of the interaction between Basigin protein and L1cam

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Cell-adhesion molecules of the immunoglobulin superfamily (IgSF) play integral roles in cell-cell interactions throughout the body. Recent studies by this laboratory indicate that Basigin gene products (Basigin/Basigin-2) form important interactions between Müller glial cells and photoreceptor neurons in the mammalian retina. Basigin-2, a member of the IgSF found only in the retina, contains significant amino acid sequence similarity with L1cam, an IgSF neural cell adhesion molecule that is a known binding partner for Basigin in the brain. We hypothesize that the region of high amino acid conservation between Basigin-2 and L1cam is used as a Basigin-binding domain. The purpose of this research was to characterize the Basigin-binding domain on L1cam and determine if the conserved sequence is used in the interaction. A synthetic peptide of the conserved sequence within L1cam fused to a FLAG epitope (L1cam-FLAG) was used as a probe for binding to endogenous mouse retina Basigin via sandwich ELISA analyses. The synthetic L1cam-FLAG peptide was also used to compete with a recombinant version of the conserved sequence in Basigin-2 (B2C2-L1-6XHis) for binding to endogenous mouse retina Basigin. It was determined that L1cam-FLAG does bind Basigin, with moderate affinity (~2 nM). In addition, L1cam competes with B2C2-L1-6XHis for binding to Basigin, but only at 10-fold molar excess and greater. The data suggest that the conserved amino acid sequences in the extracellular domains of Basigin-2 and L1cam compose the Basigin-binding domain on both molecules and that the affinity of the Basigin/Basigin-2 interaction is stronger than that of Basigin/L1cam. The protein database will be searched with the Basigin-binding domain sequence to identify other potential binding partners for Basigin. In addition, analyses of the individual amino acids within the conserved domains will be performed to determine the exact Basigin-binding motif.

A novel cell integrates sensory input through multiple spike initiation zones

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Several components of the leech swim system are well characterized, including trigger neurons in the subesophageal ganglion and gating neurons located in midbody segments M9-M16. Trigger neurons are excited by sensory input, and in turn synapse onto gating neurons, cell 204, to elicit swimming. However, trigger neuron elicited excitation in cell 204 can be blocked with glutamate antagonists, while other methods of swim-initiation remain effective [1]. This implies that other swim-initiation pathways must exist.

We introduce a new neuron, cell E21, which provides a secondary pathway between sensory input and cell 204. Cell E21, whose soma is located in midbody ganglion M21, strongly excites swimming and exhibits properties resembling both gating and trigger neurons. The presence of both antidromic and orthrodromic spikes in cell E21 prompted us to investigate the location of its spike initiation zones (SIZ). We found that cell E21 processes can initiate spikes in all midbody ganglia examined (6 total), providing strong evidence that it possesses a SIZ in most or all midbody ganglia. These distributed SIZs enable cell E21 to respond to sensory inputs anywhere along the body and to propagate information quickly to gating neurons. Thus, cell E21 might function to rapidly initiate or modulate swimming in response to sensory input. This work is supported by the NSF (award number IBN-0615631).

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Calcyon interacts with clathrin adaptor proteins AP-1, AP-2, and AP-3 and accelerates post-Golgi trafficking of VSVG

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Calcyon is a single transmembrane protein that interacts with clathrin light chain and stimulates clathrin mediated endocytosis [1]. The clathrin adaptor protein (AP) complexes AP-1, AP-2, AP-3, and AP-4 are hetero-tetramers. Their ‘mu’ subunits interact with YXXØ type motifs. As the calcyon cytoplasmic domain contains two such tyrosine motifs we tested whether AP mu subunits bind to calcyon. GST pull down assays suggests that calcyon can directly interact with mu1, mu2 as well as the ubiquitous and neuronal isoforms of mu3, 3A and 3B, respectively. Yeast 2 hybrid assays confirmed the direct interaction between calcyon and mu1, mu2, mu3A and 3B subunits of AP. Further GST-calcyon effectively pulled down AP-1, AP-2, and AP-3 from wild type brain lysates, and FLAG-calcyon immunoprecipitated the γ subunit of AP-1, α subunit of AP-2, and δ subunit of AP-3 from Cal^{OE} [2] brain lysate. Pull down experiments with tyrosine motif point mutations or C terminal truncations suggest that the second tyrosine motif of calcyon is essential for interacting with AP. AP’s regulate internalization of cargo as well as the trafficking of cargo from the trans-Golgi network to vesicles. Neuronal endocytic protein of 21kD (NEEP21), a member of NEEP21/calcyon/P19 gene family [3], regulates transcytosis of cargos from the dendritic to axonal compartment of neurons. Given the evidence that NEEP21 is involved in neuronal transcytosis and that calcyon interacts with clathrin as well as AP-1, AP-2, and AP-3, I tested whether calcyon plays a role in transcytosis. Chasing the trafficking of the G protein of vesicular stomatitis virus (VSVG) by ‘temperature block and release assay’ suggests that calcyon accelerates post-Golgi trafficking of VSVG. Taken together my data suggests that calcyon directly interacts with AP via a YXXØ type motif and accelerates post-Golgi trafficking.

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CaMKIV mediates dendrite arborization and filopodia formation

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Calcium/Calmodulin-dependent protein kinase IV (CaMKIV) is found predominantly in neuronal nuclei and has been implicated in synaptic plasticity and memory. CaMKIV mediates calcium induced dendritic growth and is upregulated during postnatal development. Together this data suggests CaMKIV may be essential for developmental events, such as filopodia formation and synaptogenesis. Further, CaMKIV is expressed in a subset of cortical neurons in vivo and in vitro that are non-GABAergic. To examine the role of CaMKIV, we analyzed the dendrite morphology of E18 rat cortical neurons that expressed CaMKIV and those that did not. Dendrite complexity, as measured by number of branch points, primary processes and dendrite length was significantly greater in CaMKIV positive neurons at 8 DIV in basal conditions. However, In addition, the number and density of filopodia was greater in CaMKIV expressing neurons. To test if CaMKIV is sufficient for filopodia formation constitutively active (ca) and wild type (wt) CaMKIV was coexpressed with GFP in cortical neurons. Neurons that expressed CaMKIVwt and CaMKIVca showed potentiated increases in filopodia number and density as well as dendrite complexity. Furthermore, knockdown of CaMKIV expression by siRNA showed that CaMKIV is necessary for filopodia formation and increasing dendrite complexity. Taken together, these results indicate that CaMKIV promotes dendrite arborization and filopodia formation.

Predicting post traumatic stress disorder like behaviors

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Post traumatic stress disorder (PTSD) is a debilitating anxiety disorder. Some people are susceptible to developing this disease and similar susceptibilities have been observed in animals. Once established, PTSD is difficult to treat and therefore it is important to identify ways to prevent it from developing or treat it at the earliest stage possible. Studies on humans with established PTSD and animal models of similar behavior have shed light on various aspects of the disease but how PTSD develops is still unknown. Investigating the development of PTSD in humans is very restricted therefore it is important to have an animal model that a priori classifies animals as susceptible and resistant to developing PTSD-like symptoms.

Here, we report that rats can be pre-classified as susceptible and resistant to developing PTSD-like behaviors after a traumatic event (contextual fear conditioning) based on their pre-trauma behavior in the elevated plus maze and their acoustic startle response (ASR). This pre-classification is reliable only after an initial mild stressor (exposure to cat hair). The animals that were pre-classified as susceptible showed sustained elevation in ASR and impaired extinction after contextual fear conditioning.

This model can be used to investigate and compare the physiologic, cellular and molecular processes in susceptible and resistant rats, occurring during the traumatic event.

Unbiased clustering of true neural components to reveal task specific brain activations

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We sought to evaluate whether clustering independent components (ICs) of EEG neural activation can be a measure of quantifying the networks involved in planning skilled hand movements.

Fifteen normal right-handed subjects were instructed to perform simple movements and complex tool use pantomime movements while recording 64-channel electroencephalography (EEG) and electromyography (EMG) from forearm and hand muscles. Both movements were similar in kinematics but differed in context (naively performing a twist gesture vs. pantomiming a twist screwdriver; naively performing a push gesture vs. pantomiming a novel “yankee” style push screwdriver). Subjects were first instructed to perform the naïve movements, and then perform the pantomimes after practicing with the two types of screwdrivers. Trials were marked for EMG onset and epoched 3.5s – EMG – 0.5s. We then decomposed the EEG signal into its statistically most independent components, using the popular ICA algorithm. We identified the strongest component that contributed to power of EEG signal just before movement [500ms to EMG onset] in a pantomime twist condition, and sought significant correlations ($r > 0.9$) to other components in all subjects and conditions. The expectation is that we can find unique, condition specific activations in single subjects based on this unbiased, automated technique.

Preliminary results revealed a cluster of highly correlated ($r > 0.9$) left hemispheric parietal components for the pantomime twist condition, across all subjects. This is in line with prior research, indicating that the parietal areas store representations of previously learned tool movements. This component was not well correlated in other conditions, across subjects. Neuroanatomical correlates of the identified components were calculated by source localization and estimating dipoles of neural activity. This enabled us to characterize True Neural Components (TNC) in the data.

Correlating ICs appears to be a robust method of blindly clustering TNC in multivariable datasets. We will further test this method by correlating template ICs for all conditions to find neural evidence of other exclusive motor related components.

Microembolism infarcts alter affective behaviors in rats

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Small cerebral infarcts (SCIs) are diffuse brain lesions caused by vascular occlusions. SCIs commonly occur in the aged brain and correlate with the presence of depressive disorders. Although underreported due to their asymptomatic nature, SCIs are estimated to affect 30% of the population over age 65, 60% of individuals with pre-senile onset depression and 94% of people with senile onset depression. In addition to being precursors for symptomatic stroke and dementia, SCI-induced “vascular depression” is highly resistant to antidepressant treatments. Links between ischemia and disrupted mood have long been suggested; however, neither a causal relationship nor an underlying mechanism has been established.

The present study uses a unique rodent model to address SCI-induced changes in affective behavior. To do this, adult Wistar rats (3 months) received either ischemia-inducing microspheres or SHAM treatment to the left or right hemisphere. Following a two-week recovery, behavioral tests were used to assess depressive-like and anxiety-like symptoms. The Cavalieri Principle was used as an unbiased stereological approach to quantify SCI damage in the affected hemisphere as compared to both the control side and SHAM animals.

The generation of SCIs result in both depressive and anxiety-like behaviors in treated versus SHAM animals without affecting baseline activity. As compared to SHAM animals, treated animals consume less sucrose over a two day period in the sucrose-preference test, indicating a decreased sensitivity to reward. Similarly, SCI animals spent less time in the center of the open field paradigm, suggesting an increase of anxiety-like behaviors. These findings support the hypothesis that diffuse ischemic damage can alter affective behavior in adult rats. Future work will focus on the mechanisms that underlie SCI-induced behavioral changes.

Detection of whisker deflection in awake behaving rats

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The ability of an animal to detect a stimulus of varying strength, or to discriminate between stimuli with varying features, indicates that the ascending sensory pathway is carrying essential information about the stimuli. In order to study and potentially interact with this sensory system in awake behaving animals, a stable and well controlled behavioral assay must first be designed to probe the animal's ability to detect stimuli and/or to discriminate between stimuli with varying characteristics. To this end, we have designed a task in which head-fixed rats are trained to respond to an external stimulus consisting of a short puff of air delivered to their large mystacial vibrissae. The motion of the whiskers is monitored and quantified on a subset of trials using high speed video. Preliminary behavioral results indicate that the response probability varies with stimulus strength in a characteristic sigmoidal fashion. Given a 500ms response window, the animals tended to display a response latency that falls within a physiologically plausible sensorimotor integration period. High speed video analysis demonstrates that the stimuli tested to date correspond to mean deflection velocities ranging from zero to approximately 350 deg/s measured at the whisker base. In addition, preliminary studies have been performed in an acute setting using voltage sensitive dye measurements to characterize the layer 2/3 cortical response to the same range of stimuli.

Examination of mitochondrial health in the basigin null mouse retina

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Basigin null mice are blind from the time of eye opening, despite normal retina architecture at that age. At eight weeks of age, photoreceptor degeneration begins and proceeds until the entire photoreceptor layer is ablated. Basigin gene products are members of the immunoglobulin superfamily found in the retina that are known to interact with the lactate transporter monocarboxylate transporter 1 (MCT1). MCT1 protein expression does not localize correctly in the absence of Basigin gene products and therefore function is inhibited. It has been proposed that lactate transport and utilization are essential for photoreceptor maturation and function and that faulty lactate metabolism underlies blindness in Basigin null mice. The purpose of this study was to assess the metabolic integrity of mitochondria in the retinas of Basigin null mice. An ELISA (Enzyme-Linked Immunosorbant Assay) was performed to determine the levels of cytochrome C in retina cell extracts from Basigin null mice and littermate controls at visual maturity (three weeks of age) and at three months. It was determined that the concentration of cytochrome C was significantly lower in Basigin null mice compared to controls at both of the ages tested. The low cytochrome C concentrations observed in Basigin null mouse retinas may be due to the sequestration and degradation of cytochrome C or the whole mitochondria. We propose that the sequestration of mitochondria in Basigin null mouse retinas occurs due to autophagy, a degradation pathway for damaged organelles that is induced by cellular stress during neurodegenerative diseases. Future studies are aimed at examining mitochondrial activity and autophagy in the Basigin null mouse retina.

Investigating cis-SNP/mRNA level associations of candidate Alzheimer's disease genes in different brain regions of patients

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The levels of many gene transcripts have been shown to be influenced by genetic variants. We hypothesize that some Alzheimer's disease (AD) risk variants may act via modifying gene expression levels in the brain. To test the presence of AD risk variants that modify transcript levels, we measured mRNA levels of 13 AD candidate genes in the cerebellum and temporal cortex of pathologically-confirmed AD subjects and performed association studies with their *cis*-SNP genotypes residing within $\pm 1\text{cM}$ flanking regions of these genes. Transcript levels were measured in the cerebella of 197 AD patients and in the temporal cortex of 88 ADs, using quantitative PCR. Genotypes of the *cis*-SNPs were obtained from the genome-wide associate study (GWAS) of these subjects was performed on the Illumina Hap 300 platform. Multivariate linear regression analyses was performed within PLINK using age, gender and ApoE genotypes as covariates. We identified 195 *cis*-SNP/mRNA associations in the cerebellum of ADs and 153 from their temporal cortex with nominally significant p -values < 0.05 . Our results suggest that the selected AD candidate genes may contain variants that influence their expression in the brain.

⁺: equal contribution

Characterization of MCT1 expression in the Basigin null mouse brain

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Basigin null mice are characterized as having neuronal deficiencies related to vision and learning and memory. Basigin null mice are blind from the time of eye opening and have reduced short- and long-term memory when compared to littermate controls. Basigin gene products are members of the immunoglobulin superfamily (IgSF) and are known to interact with monocarboxylate transporter 1 (MCT1) *in cis* in the plasma membrane of cells in which the proteins are expressed. In the retina, MCT1 does not assemble at the plasma membrane in the absence of Basigin gene products and remains in intracellular vesicles. It has been proposed that deficient lactate transport from Müller glial cells to photoreceptor neurons is the mechanism underlying blindness in Basigin null mice. The purpose of this study was to determine if MCT1 expression is altered in the Basigin null mouse brain as well. Immunoblotting and ELISA analyses for MCT1 were performed on normal and Basigin null mouse brain extracts. Preliminary data indicate that membrane-associated and total MCT1 protein expression is unchanged in null mouse brain as compared to the controls. This suggests that, unlike in the retina, metabolism does not account for the neuronal abnormalities observed in the Basigin null mouse brain.

Sympathetic nervous system responses during fear conditioning predict PTSD avoidance symptoms

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Posttraumatic stress disorder (PTSD) occurs in some people after exposure to traumatic events. This is a heterogeneous disorder that is defined by three major symptom clusters: intrusive symptoms, avoidance of reminders of the event, and hyper-arousal. Individual patients can vary in the degree to which they present with the different symptoms. The purpose of this study was to examine the relationship between physiological responses and specific PTSD symptoms to gain insight into biological endophenotypes of PTSD. We measured psychophysiological responding during a fear conditioning discrimination task. This paradigm independently assesses responses to danger and safety cues using electrodermal activity (EDA) and skin conductance responses (SCR). We used these measures to predict ratings on individual items on the PTSD symptom scale (PSS) using linear regression analyses. The study sample (n=104) was recruited from a highly traumatized civilian population seeking treatment at the Grady Health system in Atlanta, GA. Results show that PTSD subjects have lower skin conductance responses than controls, $F(1,102)=11.91$, $p<0.001$, with PTSD subjects showing exaggerated habituation after the first block of conditioning. Higher SCR during the last block of conditioning predicted avoidance symptoms on the PSS $F(1,102)=13.31$, $p<0.001$, accounting for 11.5% of the variance in PTSD symptoms.

Integration of sensory signals during escape behavior in crayfish

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Juvenile crayfish that approach a food odor release point in an aquatic tank respond to visual threat stimuli with one of two discrete and mutually exclusive escape behaviors. Shadows elicit either an immediate freezing response or a tail-flip that propels the animals backwards away from the approaching visual stimulus. The frequency of each behavioral response is dependent on shadow velocity. Slower shadows evoke more tail-flips and faster shadows elicit more freezing behavior. All tail-flips are mediated by the medial giant (MG) interneurons (Liden & Herberholz [2008] *J. Exp. Biol.* 211). By combining non-invasive recordings of MG activation with photodiode measurements of shadow speed and position, we found that tail-flipping is typically inhibited and freezing dominates when shadows become inescapable, i.e. when tail-flips cannot be generated quickly enough to avoid collision with the approaching shadow. Under these conditions, tail-flipping may be less beneficial than freezing because it moves the animal away from the food source. To determine whether the intensity of food odor in the tank affects the responses to shadows, we are now testing different food odor concentrations in combination with different shadow velocities. Our preliminary results show that crayfish exposed to food odors of higher intensity tail-flip less and freeze more as compared to control animals. This implies that olfactory signals may play an important role in setting the threshold for visual activation of the MG tail-flip circuit. Moreover, it appears that the increase in MG threshold under high food odor conditions is less pronounced when the animals are exposed to slow shadows. This suggests that the relative strengths of olfactory (food) and visual (threat) signals are assessed and integrated to produce adaptive behavioral output.

TGF-beta: A retrograde signaling molecule involved in the formation of neuromuscular junctions in *C. elegans*

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The aim of this research is to investigate the role of Transforming Growth Factor Beta (TGF-B) in the formation of neuromuscular junctions in the nematode. Laser ablation of selected embryonic myoblasts removes guidance markers used by post-embryonic muscles (1). The absence of these guideposts results in migrating muscle losing their way and differentiating in ectopic locations. These ectopic muscle strands become innervated by processes from the DD motor neurons (mns). We postulate that a retrograde signaling molecule released by the muscles induces neurite sprouting from the DD mns, resulting in the formation of ectopic neuromuscular junctions (nmjs). An earlier report, combined with our own observations focusing on post-embryonic muscle, indicate that the transient expression pattern of TGF- B corresponds to the nmj formation in the DD mns (2). Current experiments combining laser ablations in various mutant backgrounds, with green fluorescent protein expression patterns should shed light onto the communication between presynaptic and the post-synaptic elements at the nmj.

1. Plunkett, JA, Simmons, RB & Walthall, WW (1996). *Dev.Bio.* 15, 154-165.
2. A Colavita, S Krishna, H Zheng, R W Padgett, J G Culotti: 1998 *Science* 281(5377):706-9.

A restricted pool of abnormal Purkinje cells within a normal cohort causes focal dystonia

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The abnormal movements in dystonia can exhibit a broad range of body-part distribution. Whereas generalized dystonias involve multiple regions, focal dystonias are isolated to a single body part. Substantial evidence points to the cerebellum as a source of some dystonias, but the precise relationship between cerebellar dysfunction and the distribution of dystonia is unknown. To delineate the contribution of the cerebellum to dystonia, output through Purkinje cells was eliminated with a transgene over several months in *tottering* mice, which exhibit generalized dystonia that likely arises from dysfunctional Purkinje cells. This approach revealed that the volume of cerebellar dysfunction mediates the severity of dystonia, as we detected significant correlations between the distribution of dystonic movements in these mice and the number of abnormal Purkinje cells remaining. Furthermore, only 15% of the abnormal Purkinje cells were sufficient to express focal dystonias of the limbs. However, we are currently exploring whether a small pool of dysfunctional Purkinje cells admixed within an otherwise normal cohort can generate focal dystonias using lentiviral constructs to generate dysfunction within an isolated region of the cerebella of normal mice.

17 β -Estradiol Attenuates Proapoptotic p53/PUMA Signaling Pathway in Hippocampus CA1 following Global Ischemia

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17 β -Estradiol (E₂) has been implicated to be neuroprotective against a variety of neurodegenerative diseases, including stroke. The current study examined whether E₂ neuroprotection following global cerebral ischemia may involve an epigenetic mechanism to modulate acetylation/methylation (and thus activation) of the proapoptotic protein p53, and whether this could alter expression of the p53 downstream target, PUMA, a BH3 proapoptotic protein. Our results revealed that simultaneous epigenetic modifications of p53, including Lys acetylation (K373/K382) and Lys methylation (K372), are markedly *increased* in hippocampal CA1 neurons in placebo-treated animals 24h after reperfusion, as compared to sham controls. Intriguingly, E₂ strongly attenuated the increase of both p53 post-translational modifications in CA1 neurons 24h after reperfusion, without affecting total p53 protein levels. The E₂-induced attenuation of p53 acetylation/methylation suggests that p53 transcriptional activation may be diminished by E₂ treatment. In support of this suggestion, Western blot and immunohistochemical studies revealed that protein expression of the p53 target gene, PUMA, was significantly enhanced in placebo-treated animals at 24h after reperfusion (as compared to sham controls), and that E₂ strongly attenuated PUMA upregulation. As a whole, our studies suggest a *novel* epigenetic regulatory effect of E₂ in stroke to modulate p53 activation and attenuate expression of the downstream proapoptotic target gene, PUMA, thereby providing an antiapoptotic mechanism for E₂ neuroprotection. NINDS Grant # NS050730.

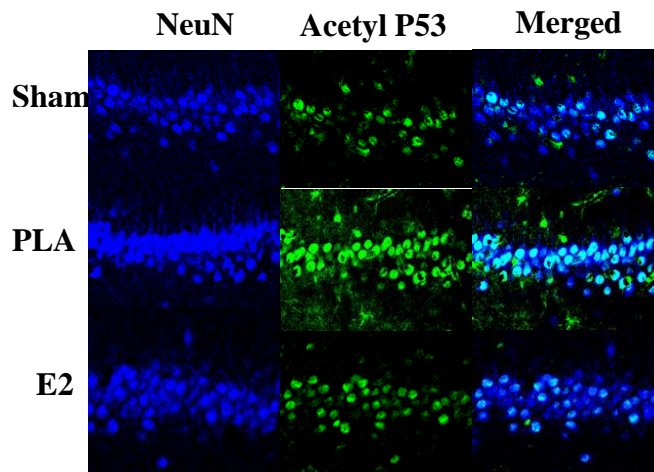


Figure 1: Confocal staining of hippocampal CA1 sections shows E2 profound attenuation of p53 acetylation at 24h following global cerebral ischemia.

Two-photon imaging reveals astroglial injury alongside neuronal damage during stroke-induced ischemic depolarizations

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Two-photon laser scanning microscopy (2PLSM) enables real-time visualization of functioning cells expressing Green Fluorescent Protein (GFP) deep within living neocortex *in vivo*. Using 2PLSM we have shown that cardiac arrest-induced global ischemia elicits astroglial soma and process swelling, indicating acute damage [1]. We also have evidence that peri-infarct depolarizations (PIDs) cause acute damage to neuronal dendrites and spines in the ischemic penumbra [2]. Here we used 2PLSM to monitor changes in astroglial volume concurrently with signs of neuronal injury in two different models of ischemic stroke. The first method was transient bilateral common carotid artery occlusion (CCAO), which allowed for the induction of global ischemia and subsequent reperfusion. The second method was modified photothrombotic microcirculatory occlusion, in which a square-shaped ischemic lesion was made to surround a penumbra-like “area at risk” with PIDs recurring for minutes to hours following photothrombosis. With both methods, neurons showed a relatively high rate of recovery for several hours after insult. However, astroglial recovery was much more variable, with many somata and processes remaining swollen well after the induction of ischemia. Thus the fate of astrocytes may be a more critical determinant of the final outcome of ischemic stroke than was previously thought.

[1] Risher et al. 2009. *Glia* 2009, 57,207-221

[2] Risher et al. 2009. Society for Neuroscience Annual Meeting Abstract 620.6

Finding balance amidst variability: synaptic strength in the leech heartbeat CPG

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The middle premotor interneurons of the leech heartbeat CPG provide synaptic input to mid-body heart motor neurons (Norris et al, 2006). The phase of these interneurons relative to the oscillator interneurons shifts with a switch from synchronous to peristaltic coordination mode. This shift in phase of the middle premotor interneurons is critical in setting up the coordination modes in the rear of the animal. These cells receive inputs from multiple different presynaptic partners in the timing circuit of the CPG: electrical excitation from the ipsilateral oscillator interneurons and chemical inhibition from both the ipsilateral and contralateral switch interneuron. The balance between these two opposing inputs is critically important in generating the appropriate phasing of each coordination mode.

Previous modeling studies (Weaver et al, 2003, 2005) showed that a specific balance must exist between electrical excitation and chemical inhibition onto the middle premotor interneurons for the network to produce its stereotypical phasing. In constructing the model, two simplifying assumptions were made that are confirmed here experimentally: 1) Electrical coupling from a given ipsilateral oscillator interneuron onto a given middle premotor interneuron is the same on each side i.e. there is neither a systematic left-right asymmetry in the nerve cord nor any mechanism to alter coupling with changes in coordination mode. 2) Electrical coupling from each ipsilateral oscillator interneuron is the same onto both pairs of middle premotor interneurons.

A CPG model with these assumptions importantly predicts that each of the four synapses from the switch interneuron onto the middle premotor heart interneurons will have different relative strengths. In order to mimic the proper output of the network, the synapses onto the ipsilateral middle premotor interneuron had to be stronger than the synapses onto the contralateral middle premotor interneuron. The model also predicts that synapses onto the interneurons in ganglion 7 be stronger than the corresponding synapses onto the interneurons in ganglion 6. Here we confirm these predictions experimentally in the living system.

The large two-to-four fold animal-to-animal variability in synaptic strength previously reported in this system (Norris et al, 2007, Seaman and Calabrese, 2008) is observed here as well. We will also show this large variability present on the inputs to the important switch interneurons that control the switching behavior. Despite this large variability, the correct relative strengths of all of these synapses are maintained within an animal, producing the proper balance between inhibition and excitation that is necessary to produce the observed phasing in network output despite wide ranging variability in synaptic strength between animals. Supported by NS024072 to RLC and NS064682 to RCR.

Pathogenicity of Indel variants in the fused in sarcoma (FUS) gene in amyotrophic lateral sclerosis

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Introduction

In the past year, numerous pathogenic missense mutations have been reported in the *fused in sarcoma (FUS)* gene as a novel cause of amyotrophic lateral sclerosis (ALS). Small insertion/deletion variants (indel) in *FUS* were also reported as being causative of the disease, however a number of indels within glycine-rich regions of the FUS protein were recently identified in controls. In this study, we investigated the pathogenicity of previously published and novel indels in *FUS* in an extensive cohort of ALS cases and controls.

Methods

We performed PCR amplification of each of the 15 exons of *FUS*, using one fluorescently labeled primer, in 631 ALS cases and 1063 controls followed by fragment length analysis on an automated ABI3730 DNA-analyzer. To identify the indel variants in *FUS*, direct sequencing was performed on each sample with abnormal allele sizes. Where available, the effect of *FUS* exonic indels on the nuclear localization of FUS was investigated in patient lymphoblastoid cell lines.

Results

We detected 29 variants in patients (4.6%) of which 6 were exonic (0.95%), and 38 variants in controls (3.6%) of which 8 were exonic (0.75%). Exonic variants occurred in exons 5, 6, 12 and 14, with 3 occurring within, and 3 outside of glycine stretches. The most common exonic variant was c.521_523+3delGAGgtg, predicted to result in p.Gly174del, which was found in 3 cases and 4 controls. One case was found to have c.1422_1424delTGG, occurring in exon 14, predicted to result in p.Gly475del and is potentially pathogenic. Other interesting indels detected include c.412_429delGGACAGCAGCAAAGCTAT in exon 5, predicted to lead to p.Gly138_Tyr143del, and c.1204_1206delAGT in exon 12, expected to result in p.Ser402del, both of which were detected in controls.

Discussion

Although mutations in *FUS* have been shown to be pathogenic in ALS, our data suggest that not all genetic variants in *FUS* cause disease. We identified indels in four *FUS* exons, with two exons showing variation in both cases and controls. Exonic indels were identified at a slightly higher frequency in patients compared to controls, suggesting that these may confer susceptibility to ALS. Subcellular localization studies of FUS in lymphoblast cell lines derived from *FUS* indel carriers are currently ongoing.

***Anolis carolinensis* male-male agonistic encounters: a three year study of the best predictors for determining dominant/subordinate status**

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Agonistic encounters can play an important role in the allocation of reproductive resources and the consequent fitness of an individual. The green anole lizard has an easily recognizable suite of behavioral displays that allow the determination and establishment of dominance and subordination. To investigate the predictive value of different displays during the agonistic encounter, male *Anolis carolinensis* were paired with similar-sized males and observed as they established social status during a ninety-minute session. In real time, observers recorded bob A, B, and C, eyespot presence, dewlap displays, lateral displays, open mouth displays, wrestles, pushups, nips, and body color changes. Anole individuals that became dominant were green for a greater duration during the observation period. Dominant males also had a higher bob A and C count, more dewlap displays, and a longer total duration of dewlap displays. In fights that escalated to open mouth displays, dominants had higher frequencies of open mouth displays.

In contrast to previous reports, eyespot latency in this study was not predictive of winning or losing, and subordinates most often displayed aggressively first, usually as bob A displays. The latency differences between this study and others may be due to different geographic sources of anoles, smaller differences in size between competitors, or housing males with females as opposed to isolation before encounters. This suggests that displays that are predictive of outcomes may vary depending on the source populations and encounter environment.

Cytoarchitecture of neuroblasts and their stem cell niche maintaining adult neurogenesis in the brain of spiny lobsters

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Adult neurogenesis persists in the olfactory midbrain of decapod crustaceans including spiny lobsters, *Panulirus argus*. Neuronal precursor cells are located in a small proliferation zone (PZ) in each of the four soma clusters of local and projection neurons. One neuronal stem cell – a large adult neuroblast (aNB) - is located close to each PZ and is itself associated with a unique clump of cells constituting a putative stem cell niche (Schmidt, *J. Comp. Neurol.* 503:64-84, 2007). We analyzed the cytoarchitecture of the aNB and the clump of cells with immunocytochemistry and TEM. These analyses showed that the clump is comprised of small cells (clump cells) whose somata form a dense mantle around a nucleus-free core and that the aNB has a unique hourglass-like shape. The peripheral part of the aNB contains a large nucleus and is connected via a thin cytoplasmic bridge to a bulb-shaped ‘foot’ extending into core of the clump of cells. The clump cells are bipolar with a long outward-facing process and a shorter process reaching into the core of the clump. The outward-facing processes form a strand that surrounds the peripheral part of the aNB and projects further to the PZ. The shorter processes are convoluted and completely cover the bulbous foot of the aNB. Processes of multipolar, soma-associated glial cells envelope clump and strand in several layers and separate them from neighboring neuronal somata and arterioles. We conclude that the clump of cells has morphological features of a protected stem cell niche, in which clump cells constitute the microenvironment of the aNB and insulate it from the surrounding tissue. Since the clump cells differ from glial cells in immunocytochemical properties and in overt morphology, we hypothesize that they maintain embryonic characters, a common feature of stem cell niches in adult tissues.

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Basigin gene products form a novel cell adhesion system between Muller cells and photoreceptors in the mouse retina

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Basigin gene products are members of the immunoglobulin super family and are important for cellular recognition, and adhesion processes. Two Basigin gene products have been identified in the mouse retina and are thought to play a role in retinal function, as Basigin null mice are blind from the time of eye opening. Basigin protein is expressed on the surface of Muller glial cells, whereas Basigin-2 protein is expressed on the surface of photoreceptor cell bodies and inner segments. The gene products are generated by splice variation and are identical in amino acid sequence, with the exception of an additional extracellular immunoglobulin loop in the Basigin-2 variant. The amino acid sequence of the Basigin-2-specific loop is highly conserved across many species and there is also high sequence similarity between the extracellular domains of Basigin-2 and neural cell adhesion molecule L1. The focus of this study was to determine whether Basigin-2 binds to Basigin, and if so, whether this interaction involves the region of Basigin-2 that is highly similar to L1. Sandwich ELISA analyses were performed in which recombinant six-histidine-tagged proteins of the Basigin-2 specific loop were generated and used to test for binding to endogenous mouse retina Basigin. Site directed mutagenesis was performed to alter the amino acid sequence of the recombinant Basigin-2-specific loop proteins in the region highly similar to L1 to assess their participation in the interaction via ELISA analyses. It was determined that the Basigin-2-specific loop does bind to Basigin, using the region with high sequence homology for L1. The data suggested that Basigin gene products interact within the retina to adhere supportive Muller glial cells to photoreceptor neurons. This interaction appears to be necessary for photoreceptor maturation and/or function, but not development, as Basigin null mouse retinas are functionally blind, yet architecturally normal at the time of eye opening.

Cyclooxygenase-2 in principal neurons modulates brain inflammation after status epilepticus.

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Temporal lobe epilepsy (TLE) is a type of epilepsy often associated with brain inflammation. In the murine model of status epilepticus (SE), systemically administered pilocarpine induces prolonged SE resulting in robust astrogliosis, microglial activation, leukocyte infiltration, and rapid induction of numerous mediators of inflammation as observed in human TLE. One of the inflammatory mediators that is rapidly induced after SE is the multifunctional neuronal modulator, cyclooxygenase-2 (COX2). Its induction occurs mainly in principal neurons of the hippocampus, cortex, and amygdaloid complex. In this study we explore the role of neuron-derived COX2 in seizure-induced pathology using a conditional knockout (cKO) mouse created in the laboratory that lacks COX2 in the principal forebrain neurons after P15. In this study, we used the conditional knockout to investigate the involvement of neuron-derived COX2 in the inflammatory reactions observed after SE. We explored the changes of the principal brain cells involved in inflammation using immunohistochemical techniques, and the regulation of inflammatory cytokines and their receptor expression using RT-PCR. Mice experienced SE for one hour and were sacrificed one day later. After brain dissection, half of the forebrain was fixed overnight with 4% paraformaldehyde and processed with rabbit anti-GFAP or rabbit anti-Iba1. The other half was used for RNA extraction and first strand DNA synthesis for RT-PCR. An array of 84 inflammatory genes from SA-Bioscience was used for each animal. The latency to reach SE, the behavior before and during SE, and the percentage mortality were similar in wt and cKO animals. Nevertheless, RNA expression from several inflammatory factors, as well as the number of reactive astrocytes, were up-regulated after SE to a higher extent in wt mice than in cKO. These results underline an important neuronal component during seizure-induced brain inflammation. In addition, we previously demonstrated that COX2 induction after SE results in degeneration of somatostatin-labeled GABAergic interneurons in the dentate hilus and CA1stratum oriens. This together with the present findings demonstrates a detrimental outcome of neuron-derived COX2 induction after SE. We conclude that neuron-derived COX2 induction after SE strongly contributes to the pro-inflammatory events observed after SE that might lead to neuronal degeneration and/or synaptic remodeling.

Glucocorticoid receptors distribution across the development of the avian song system

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When male zebra finches (*Taeniopygia guttata*) experience early developmental stress they exhibit reduced song complexity and a decreased HVC size in adulthood. Song nucleus HVC is required for learning and production of song and song complexity is important for mate choice. This reduction in song complexity and song nucleus size suggests a direct link between the stress response, brain and behavior, however the mechanisms underlying these effects are unknown. We are investigating the role of glucocorticoid receptors and stress on the development of the avian song system. Glucocorticoid receptor-like immunoreactive-neurons (GR-like ir-neurons) were localized in the brains of male zebra finches collected on P1 (post-hatch day 1, song nuclei not yet formed), P10 (post-hatch day 10, song nuclei formed), and adult birds (post-hatch day 90 or older, sexually mature and singing crystallized songs). In adults, high density of glucocorticoid receptor-like immunoreactivity was found in the HVC, tubero-infundibular area, nucleus paraventricularis (PVN), posteromedialis and lateralis hypothalami, nucleus septi lateralis, tectum opticum, and brainstem nuclei including the locus coeruleus and raphe nucleus. The cerebellar cortex and hippocampal formation were also immunopositive. In addition, adult birds that were stressed from P5-26, via chronic corticosterone treatments, had significantly smaller HVC volumes and altered regulation of GR-like ir-neurons in several telencephalic and diencephalic brain regions when measured in adulthood. Plasma corticosterone concentrations were measured using radioimmunoassay to validate the treatments and compare treated versus control birds. The mechanism of how chronic stress influences HVC size is yet to be determined.

Analysis of CREB expression in the Basigin null mouse brain

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Behavioral studies of Basigin null mice indicate that these animals have impaired learning and memory. Both short- and long-term memory deficiencies were noted. CREB (cAMP response element binding) protein is a transcription factor implicated in the molecular events of learning and memory. Therefore, the purpose of this study was to determine whether CREB expression is altered in Basigin null mice. Enzyme-Linked Immunosorbent Assay (ELISA) was performed to analyze brain proteins extracted from normal and Basigin null mice using an antibody specific for CREB. The preliminary results indicate that there is no statistically significant difference in the expression of CREB in Basigin null mouse brains, as compared to littermate controls. The data suggest that the molecular components for learning and memory mediated via CREB are intact in Basigin null mouse brains; and that there is no correlation between Basigin and CREB expression.

Behavioral Effects of Continuous Corticotropin-Releasing Factor Overexpression within the Bed Nucleus of the Stria Terminalis

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The bed nucleus of the stria terminalis (BNST), a limbic structure implicated in stress coping and affective disorders such as anxiety and depression, contains numerous corticotropin-releasing factor (CRF)-expressing neurons projecting to target structures that modulate neural and behavioral sequelae of stress. It has been shown that stress increases CRF mRNA within the BNST. However, it is still unclear how enhanced CRF expression within these BNST neurons may contribute to stress-related affective behavior. Therefore, the present studies assessed the behavioral effects of CRF over-expression within CRF-expressing cells of the BNST using a lentiviral vector containing a CRF promoter to drive expression of CRF. Pilot data revealed no significant effects of intra-BNST CRF over-expression on elevated plus maze behavior or on tests of baseline startle or fear-potentiated startle in response to a conditioned 3.7-second clicker cue. However, compared to controls, intra-BNST CRF over-expression suppressed conditioned fear-potentiated startle in response to an 8-minute clicker cue. These results suggest a complex role of CRF expression within the BNST on stress-related affective behavior, which may be effectuated through differential feedback or compensatory action in target structures.

Significant Decline of the Force of Contraction in Tendon Reflexes as Early as Three Weeks Following the Onset of Diabetes in STZ Diabetic Rats.

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Diabetic Neuropathy-resulting in: pain, a lost of sensation, balance, and change in gait-has an effect on proprioceptor function and the force of contraction through the medial gastrocnemious muscle in rats with STZ induced diabetes. An evaluation of proprioceptor function was made stimulating a monosynaptic pathway through the medial gastrocnemious, then recording the muscle's force of contraction, and electromyogram. The study used four groups of Wistar female rats: untreated, three week, six week, and vehicle-injected. The achilles tendon was attached to a servomotor that vibrated the muscle at frequencies of ten, twenty, fifty, one hundred, one hundred sixty-seven, two hundred fifty, and five hundred hertz, with amplitude of eighty microns. The experiments showed an overall decrease in the force of contraction through the medial gastrocnemious with distinct effects on the muscle at the various vibrations through other measurements. This loss of force contraction was noticed as early as three weeks, and as late as six weeks following the onset of diabetes. The implications of the study show how soon diabetes can have an effect on the nervous system, and may provide a foundation for further investigation into the finite functioning of muscle fibers, proprioceptors, motor neurons and their interrelations, in STZ diabetic rats.

A bidirectional mechanism for CPEB1-mediated regulation of synaptic mRNA and dendritic spine morphology

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Neural activity affects synaptic morphology and plasticity through several mechanisms including postsynaptic protein synthesis. Dendritic protein synthesis is necessary for learning and memory as well as activity-induced alterations in synapse structure and function, but the underlying molecular mechanisms remain poorly understood. Cytoplasmic polyadenylation element binding protein 1 (CPEB1) is an mRNA binding protein that is important for synaptic plasticity as well as synaptic mRNA polyadenylation and translation, although the mechanism for this regulation is unclear. We have discovered a neuronal mRNA-protein (mRNP) complex that consists of CPEB1, a poly(A) polymerase GLD2, a poly(A) ribonuclease PARN, and a scaffolding protein symplekin. These molecules are localized to hippocampal synapses and colocalize in dendritic poly(A) mRNA granules. NMDA stimulation increases synaptic CPEB1 phosphorylation and extrudes PARN from the mRNP complex. Moreover, NMDA stimulation increases dendritic poly(A) mRNA levels, and both GLD2 knockdown (KD) and PARN KD alter dendritic poly(A) mRNA levels. GLD2 KD leads to an immature spine phenotype, and PARN KD increases the proportion of mature spines. Taken together, these data suggest that this CPEB mRNP complex provides a reversible mechanism to modulate dendritic mRNA polyadenylation and spine structure.

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The effect of sensory stimulation on recovery of an evoked motor behavior after neuronal injury

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Neural circuits can undergo spontaneous or training-induced changes that allow them to recover from neuronal injury. We have been studying the neural basis for recovery from injury in the escape swim response of the nudibranch mollusc, *Tritonia diomedea* (Sakurai and Katz, J.Neurosci., v29, 2009). In response to noxious sensory stimuli, such as 0.5ml 5M NaCl, *Tritonia* produces a swim response consisting of a series of dorsal and ventral whole body flexions. The swim response is disabled by cutting a commissure that connects the two halves of the brain. The animal can recover the ability to swim after commissure cut, but what role sensory stimuli play in the ability to recover is unknown. To determine the extent to which the recovery is dependent upon stimulation that might elicit a response, we tested animals with salt stimuli at the following intervals: every 2 hours, 4 hours, 12 hours, and 24 hours. Animals were paired with sham controls of similar size and swimming tendency. The experimenter was blind to the identity of the experimental animal. All sham controls recovered by 6 hours as judged by the number of body flexion cycles. Experimental animals that were tested every 24 hours exhibited no apparent loss (*i.e.* the swim response recovered spontaneously in the interval between surgery and the first test.) Stimulating animals every 12 hours delayed the recovery until 36 hours. Animals that were stimulated every 4 hours recovered within 24 hours, but animals that were stimulated every 2 hours did not fully recover by 24 hours. These results show that sensory stimulation is not necessary for recovery from injury; however, it may interact with the recovery process in a complicated fashion.

Confocal fluorescence microscopy of cobalt-filled, silver intensified insect neurons.

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For years, studies of insects have relied upon utilizing cobalt chloride or hexamminecobaltic chloride solutions to label neurons either by backfill or intracellular iontophoretic injection. Ganglia with cobalt filled cells are bathed in a hydrogen sulfide solution to precipitate the cobalt as CoS_2 and then fixed in Bouin's solution. They then undergo a "Timm's silver-intensification" procedure to produce brownish-black neurons in a pale yellow ganglionic background. The neurons are well-stained, including the finest of branches, but light micrographs of these preparations fail to represent the clarity and thoroughness of cellular neural anatomy because of the limited depth of focus. Therefore, drawing tubes are used to trace the detailed anatomy onto paper. While examining ganglia with cells stained with a fluorescent dye backfill, we also looked at some cobalt-filled preparations.

Surprisingly, these ganglia can be examined to great advantage with confocal microscopy. Background fluorescence is exceedingly low even though the ganglia fixation and mounting procedures are very different from standard confocal fluorescence microscopy protocols. Furthermore, the labeled neurons selectively fluoresce beautifully when viewed using excitation and emission spectra in the long-red range. Preparations embedded in Canada Balsam for over ten years can be used to make Z-stacks for three-dimensional viewing.

In this study, a Nikon AIR confocal microscope with a 633nm excitation laser and a 622-737 bandpass filter for emission were used (equivalent to Alexa Fluor 633 data sheet from Molecular Probes). Images in all other emission ranges tested were found to be poor, with high background and low signal. Use of this method compensates for the lack of depth of field obtained with light microscopy and micrographs. It allows for perfect 3D reconstruction using new and readily available image reconstructions software (e.g., NIH's IMAGE J and macros) and allows for 3D renderings of bouton density, absolute axonal length measurements in wild type and genetic mutant backgrounds, using confocal microscopy across an entire ganglion. It enables permanent storage of filled neurons, and thus is useful for both back-filling experiments and intracellular filling.

Combined Modulatory Effects of Dopamine and Serotonin in a Reflex Circuit

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Dopamine and serotonin are amine neuromodulators that have generally opposing modulatory effects in many vertebrate and invertebrate systems, and are believed to counteract one another to equilibrate circuits involved in movement and behavior. In crayfish, sensory signals transmitted to the lateral giant (LG) interneuron, a functionally and morphologically identified cell involved in a tail flip reflex, are depressed by dopamine. Serotonin, however, may elicit depression or facilitation of LG's synaptic responses depending on the dose and rate of application. Interestingly, the summation of serotonergic and dopaminergic effects on LG is nonlinear, i.e. applying a facilitatory dose of serotonin with dopamine coincidentally results in an enhancement of the facilitation. This synergistic facilitation persists for hours after application and subsequent washout of the amines. All three of these modulatory effects are inhibited by the calcium chelator EGTA, an indication that calcium signaling pathways influence both states of modulation, and may be involved in mediating the synergistic effects observed when both modulators are applied simultaneously. Applying dopamine before a facilitatory dose of serotonin has different effects that depend on the length of dopamine exposure. Facilitation is blocked by long exposure to dopamine (45 minutes), but not by an exposure lasting 15 minutes. These results together suggest that combined physiological effects of neuroactive substances can not be reliably predicted based on the influences of each acting individually.

Immune activation and neurotoxicity in patients with malignant melanoma undergoing IL-2 immunotherapy

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We sought to determine whether IL-2 stimulated increases in peripheral IFN-g plasma concentrations would be associated with increases in IL-6 plasma levels and decreases in FACT/GOG-Ntx Scores in stage IV malignant melanoma patients undergoing high-dose IL-2 therapy. IL-2 treatment is the standard of care in the United States for patients with stage IV melanoma. Although in 10-15% of cases it is effective in extending the survival of the patient it also induces many detrimental side effects including depressive, neurotoxic and even psychotic symptoms [1]. A lot of the times, these symptoms interfere with the continuation of this potentially life-saving therapy. These complications of IL-2 therapy have been posited to be caused by the activation of “secondary” cytokines through IL-2-induced stimulation of both the T-cells and B-cells of the immune system [2]. We believe that release of IFN-g, induced by IL-2 treatment, plays a significant role in the incidence of these symptoms as IFN-g activates the catabolism of the protein tryptophan. After giving informed consent, patients with Stage IV malignant melanoma were recruited. Prior to, during, and after IL-2 administration we measured plasma concentrations of IFN-g, neurobehavioral symptoms, cognitive function, and tolerance of IL-2 treatment. The Friedman test, Wilcoxon signed-rank test and Spearman’s correlation were used in the analysis. IFN-g levels were undetectable until about two hours post injection. The neurotoxicity scores and IFN-g levels correlate during cycle 4 and the Ntx scores reach a low at the same time point that the IFN-g levels reach their peak. Our findings support our proposed model but further analysis of levels of TNF-a, tryptophan and quinolinic acid are still needed. Recruitment of more patients will also allow for more accurate and visible correlations.

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A Potassium Channel Expressed in the Olfactory Bulb Subserves as a Metabolic Sensor

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Naturally occurring modulators of the Kv1.3 ion channel expressed in mitral cells located in the olfactory bulb (OB), include the insulin receptor and the TrkB neurotrophin receptor - both of which have been implicated in other parts of the CNS to modulate metabolism. We now demonstrate via OB slice electrophysiology that mitral cell action potential firing properties are sensitive to another metabolically important substance, glucose. Like the hypothalamus, we have found the olfactory bulb contains two populations of glucose-sensitive mitral cells; glucose excited and glucose inhibited. Gene-targeted deletion or acute suppression of Kv1.3 results in an overall increased mitral cell sensitivity via increased-current evoked spiking frequency, decreased latency to first spike, and a more depolarized resting membrane potential. To explore the correlation between Kv1.3, metabolism, and olfaction, 11 week old mice were maintained on a moderately high fat diet (MHF, 32% fat) for 26 weeks and then systems physiological parameters of body weight, oxygen consumption, locomotion, and ingestive behaviors were quantified in a custom designed, computer interfaced, metabolic chamber. Diet-induced obese (DIO) mice exhibited a 47% increase in body weight, a 32% increase in serum insulin, and a loss of 52% of M72-expressing olfactory sensory neurons (OSNs). Kv1.3-null mice were resistant to DIO with a weight gain of only 10% and no change in adiposity as a result of a significant increase in basal metabolic rate linked to the MHF challenge. Bilateral olfactory bulbectomy (OBX) in a Kv1.3-null background yielded mice that were no longer resistant to DIO. The mice exhibited a 30% increase in body weight by preventing the increase in basal metabolic rate in response to the MHF challenge and decreased activity-dependent metabolism resulting in decreased total energy expenditure.

Pharmacological treatment of repetitive behavior using the deer mouse model: targeting adenosine, dopamine, and glutamate heteromeric receptor complexes

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Repetitive, stereotypic behaviors are extremely common in neurodevelopmental disorders. The deer mouse (*Peromyscus maniculatus*) model of this repetitive behavior is a particularly valid model because the stereotypy is spontaneously expressed (i.e. not induced by drugs, neuronal, or genetic insults). Preliminary evidence from our laboratory indicates that stereotypy is a result of a neurobiological imbalance of activation between the direct and indirect pathways of the basal ganglia [1,2]. This imbalance seems to be caused by decreased activation of the indirect pathway that allows direct pathway activation to over-excite the cortex. On neurons of the direct and indirect pathways there are heteromeric complexes of receptors that exhibit antagonistic relationships [3-6]. These receptor complexes include dopamine D1 and adenosine A1 receptors on direct pathway neurons and dopamine D2, adenosine A2A, and glutamate mGluR5 receptors on indirect pathway neurons. In this study we evaluated the efficacy of drugs that affect the heteromeric receptor complexes on the indirect pathway neurons to reduce repetitive behavior in deer mice.

We examined the effects of an adenosine A2A agonist (CGS21680), a dopamine D2 antagonist (L-741,626), and a glutamate mGluR5 positive allosteric modulator (PAM; CDPPB) individually and in combination.

Our data suggest that when administered individually the adenosine A2A agonist, the dopamine D2 antagonist, and the glutamate mGluR5 PAM do not significantly reduce stereotypy in deer mice. However, when co-administered, the adenosine A2A agonist and the dopamine D2 antagonist are effective at significantly decreasing the rate of stereotypy in deer mice. We are currently evaluating whether the addition of the glutamate mGluR5 PAM will further reduce stereotypy. In addition, we will explore whether the cell surface expression of these three receptor types differ between high and low stereotypy deer mice.

We continue to explore the hypothesis that drug cocktails will be more effective than any of the treatments individually because of the nature of the heteromeric receptor complex. Our data suggest that targeting these receptor complexes may offer pharmacotherapeutic benefit for individuals with neurodevelopmental disorders who exhibit restrictive repetitive behavior.

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Resveratrol Inhibits the Activity of Kv1.3 Potassium Channels Expressed in HEK 293 Cells

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Resveratrol, a polyphenol present in red grapes and wine, is associated with beneficial effects including cardiovascular protection, anti-cancer action and prolonged life-span in several experimental models. Recently, it has been shown that Resveratrol modulates various types of potassium channels. Interestingly, deletion of the Shaker Kv1.3 potassium channel increases longevity in a rodent model of obesity [1] suggesting that Resveratrol alters longevity by modulating the activity of this voltage-dependent potassium channel. To evaluate this hypothesis, we studied the action of Resveratrol on the electrophysiological properties of Kv1.3 transiently expressed in HEK 293 cells. We found that 200 μ M Resveratrol, introduced via the patch pipette, causes approximately 40% inhibition of the Kv1.3 macroscopic current measured under cell-attached conditions. A single channel experiment suggests that whole-cell current inhibition is achieved by reduction of single-channel open probability rather than by reduction of unitary conductance. Additionally, Resveratrol causes a significant slowness of activation and deactivation kinetics without changes in inactivation kinetics or voltage dependency of conductance. Thus, we have unequivocally demonstrated that Kv1.3 is a molecular target for Resveratrol action and that inhibition of Kv1.3 activity might explain some of the pharmacological actions attributed to Resveratrol, including prolonged life-span.

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Sex differences in the impact of neonatal inflammatory injury on long-term stress responsivity in rats

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We have previously reported in rats that neonatal inflammatory pain experienced on the day of birth results in an increase in endogenous opioid tone, resulting in long-term decreases in pain sensitivity. These reports parallel recent clinical studies documenting decreased pain sensitivity in children with prior experience in the Neonatal Intensive Care Unit (NICU). As endogenous opioids contribute to HPA activity, we examined whether neonatally injured animals display altered stress responsivity in adulthood. On the day of birth (PN0), male and female Sprague-Dawley rats received an intraplantar injection of the acute inflammatory agent carrageenan (CGN; 1%); control rats were handled in an identical manner. In adulthood (PN60), animals were tested for their response in the Elevated Plus Maze, Open Field and Forced Swim tests. In the open field test, animals that were injured on the day of birth entered the open arena more frequently than uninjured controls, suggesting decreased anxiety. Similarly, in the elevated plus maze, injured females spent more time in the open arm versus controls. These results suggest that neonatal injury results in a long-term reduction in anxiety-like behaviors. By contrast, in the forced swim test, neonatally injured males and female exhibited significantly shorter latencies to immobility, suggesting that these animals are *hyper*-responsive to a physiological stressor. Basal, restraint stress-induced and recovery corticosterone (CORT) levels were significantly blunted in injured females. Finally, neonatally injured animals showed increased glucocorticoid receptor immunoreactivity (GR-ir) in the PVN but decreased GR-ir in CA1. Together, these studies indicate that neonatal injury alters adulthood stress and anxiety-related behaviors in adulthood in a sexually dimorphic manner and contributes to mounting evidence that neonatal trauma in the absence of analgesics has long-term polysystemic adverse effects.

Bioinformatics analysis of mouse cytoplasmic polyadenylation element binding protein-3 (cpeb3)

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Cytoplasmic polyadenylation element binding proteins (CPEBs) regulate translation of defined mRNA targets by binding to the regulatory motifs in their 3' UTRs. CPEBs and their target mRNAs may be stored over long period of time, or localized in specific subcellular locations, awaiting temporally or spatially specific stimuli. This translational control mechanism has been demonstrated to play an essential role in oocyte maturation, in the establishment of body polarity during early development, and even in memory formation in the hippocampus. Little is known about the presence or functions of CPEBs in the retina. A particular CPEB, CPEB3, has a putative role of regulating the translation of GluR2 mRNA. The purpose of the current study is to investigate the alternative splicing isoforms of CPEB3 based on current databases, and to characterize the expression of CPEB3 in the retina.

In this study, we characterized CPEB3 whose putative role is to regulate the translation of GluR2 mRNA. We identified the presence of multiple alternative splicing isoforms of CPEB3 transcripts and proteins in the current databases. We also discovered a novel isoform of CPEB3 experimentally. The tissue distribution of the alternative splicing patterns of CPEB3 was characterized. The majority of the patterns appeared to be uof the patterns quantitatively in the retina. We also showed that CPEB3 expression is increased in a time-dependent manner during the course of postnatal development, and CPEB3 is localized predominantly in the inner retina, including retinal ganglion cells.

This study demonstrated for the first time that CPEB3 mRNA and protein are present in the mouse retina. The developmental-regulated expression of CPEB3 occurs in concert with known tissue modeling events in the postnatal mouse retina. This demonstration implies that translational regulation mediated by CPEB3 plays an important role in this tissue. The wide tissue distribution of CPEB3 suggests that CPEB3-regulated translational control is a ubiquitous mechanism. The presence of multiple transcripts and protein variants of CPEB3 indicates a high degree of complexity in the regulation and function of CPEB3.

Poincaré return mapping for models of elliptic neurons

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We propose a novel computer assisted method for the effective construction and accurate examination of families of Poincaré return mappings for voltage maxima in models of elliptic bursting neurons. Such busters are adequately described by dynamical systems with two characteristic time scales: slow and fast. A feature of a slow-fast dynamical model is that its solutions stay close to the so-called slow motion manifolds, comprised of equilibria and limit cycles of its fast subsystem. In the context of neurodynamics they are respectively called quiescent and tonic spiking equilibria.

We reveal the topology of the manifolds in the phase space of an elliptic neuron model to define the Poincaré mappings for oscillatory dynamics of the fast membrane potential. The algorithms discussed allow us to create a full family of Poincaré mappings to get insight into driving forces on the dynamics of a model in question over an entire parameter range.

Of special interests are these mappings for nonlocal bifurcations at transitions between states of the model. This includes mechanisms of transitions between tonic spiking and bursting, between quiescence and tonic spiking, bifurcations of bursting, the emergence of various mixed mode oscillations, and their interactions with bursting and quiescence etc.

The examination of nonlocal bifurcation at these transitions is accomplished through reduction of the multidimensional model to a one-dimensional voltage next amplitude mapping: $T: V_n \rightarrow V_{n+1}$. Such mappings allow us to identify and study bifurcations and complex dynamics of the model at such transitions. We note that the reduction to next amplitude mappings for maximal values of oscillatory voltage alone is highly advantageous as the behavior of any neuron model are manifest through the evolution of accumulative voltage dynamics which can only be accessed in experimental studies in most cases.

We examine the mathematical Fitzhugh-Nagumo-Rinzel model, a bursting modification of the classical Hodgkin-Huxley model, as well as the Terman-Rubin model for the external segment of globus pallidus. We begin by creation of the respective slow motion manifolds through parameter continuation techniques. Next we are then able to extrapolate a set of maxima's, for each limit cycle of the manifold, to be used as initial conditions for the solution set for a given parameter. We then find the next maxima for the set of initial conditions creating a next amplitude map. Varying the parameter yields a family of next amplitude maps that allow for the identification of periodic and chaotic attractors, as well system repellers and homoclinic solutions that globally organize the dynamics of the model. We are then able to predict and/or identify the mechanisms for state transitions in the models, before the actual transition.

Variance Across the Temporal Contrast Sensitivity Function Curve

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Temporal processing can be influenced by differences in retinal and cortical processing, age, and neurodegenerative diseases. These effects can be characterized by measuring the temporal contrast sensitivity function (TCSF). The lower frequencies of the TCSF curve appear to reflect retinal processes while the higher frequencies are influenced by cortical processes. We used a novel device to measure TCSF in the foveal retina. Eighty young and healthy subjects were tested (age range = 18-37). This novel LED-device uses a 1-degree, 660nm test centered with a 10-degree 660nm surround. We found larger amounts of variance at both the lower (0.4-1.0 log Hz) and higher frequencies (1.5 log Hz) of the TCSF curve. Contrastingly, the middle frequencies (1.2-1.4 log Hz) displayed smaller amounts of variance. Across ages, we could say that differences in temporal processing could result from age-related neuronal degeneration, however, even in young healthy subjects, we find these differences in temporal processing. It may be that individual differences in retinal and cortical processing as opposed to age-related neuronal deficits could be the forces driving this variation in temporal processing.

The role of motor neuron intrinsic properties in leech heartbeat fictive motor pattern generation

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The movement of blood through the circulatory system of the leech is driven by the bilaterally asymmetric constriction of two lateral heart tubes. One (the peristaltic heart) constricts with a rear to front progression while the other (the synchronous heart) constricts nearly synchronously along its length. The leech heartbeat central pattern generator (CPG) drives these constriction patterns by generating a bilaterally asymmetric synaptic input pattern to heart motor neurons (HE) which innervate these heart tubes in a segment-specific manner. Previous work in our lab developed a canonical model of the ensemble of motor neurons (heart motor neurons, HEs) responsible for the output of the leech heartbeat CPG. We then introduced the spatiotemporal pattern of synaptic inputs to this model that we derived from the living system. While the model captures the gross features of the living system, the phasing of the activity pattern of the model motor neurons with respect to their inputs was substantially different from that found in the living system. Because the input pattern to the model came from the living system, we hypothesize that the discrepancy between the model and the living system may be due to the intrinsic properties of the motor neurons in the living system. We attempt to address this hypothesis using the dynamic clamp technique. We introduce segment-specific patterns of inhibitory input to motor neurons and ask the question: what is the phasing acquired by the motor neurons in response to this input? We compared the phasing observed in the hybrid system to that observed in the living system and the model. We show that, in the hybrid system, motor neurons receiving the synchronous input pattern acquire phasing more consistent with the living system, and different from the model, while motor neurons receiving the peristaltic input pattern acquire phasing more like the model, yet show burst onsets and offsets consistent with the living system and different from the model. These results suggest that the intrinsic properties of the heart motor neurons contribute to the heartbeat fictive motor pattern, though for the peristaltic mode the impact is unclear.

Natural scenes statistics and visual saliency

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Visual saliency is the perceptual quality that makes some items in visual scenes stand out from their immediate contexts. Visual saliency plays important roles in natural vision in that saliency can direct eye movement, deploy attention, and facilitate object detection and scene understanding.

Natural visual scenes consist of objects of various physical properties that are arranged in three dimensional space in a variety of ways. When projected onto the retina, visual scenes entail highly structured statistics, occurring over the full range natural variation in the world. Thus, a given visual feature could appear in many different ways and in a variety of contexts in natural scenes. Dealing effectively with these enormous variations in visual feature and their contexts is a paramount requirement for routinely successful behaviors. Thus, for visual saliency to have any biological utility for natural vision, it has to tie to the statistics of natural variations of visual features and the statistics of co-occurrences of natural contexts. Therefore, we propose to explore and test a novel, broad hypothesis that visual saliency is based on efficient neural representations of the probability distributions (PDs) of visual variables in specific contexts in natural scenes, referred to as context-mediated PDs in natural scenes.

We first develop efficient representations of context-mediated PDs of a range of basic visual variables in natural scenes. We derive these PDs from the Netherland database of natural scenes and the McGill dataset of natural color images using independent component analysis. We then derive a measure of visual saliency based on context-mediated PDs in natural scenes. Experimental results show that visual saliency derived in this way predicts a wide range of perceptual observations related to texture perception, pop-out, saliency-based attention, and visual search in natural scenes.

Voltage Sensitive Dye Imaging of Spatiotemporal Dynamics of Somatosensory Cortex in the Rat Vibrissa Pathway

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Voltage-sensitive dye (VSD) imaging is a cutting-edge technique that captures *in vivo* population neuronal activity from a relatively large cortical area with both high spatial and high temporal resolutions. Capturing cortical activation with high spatial and temporal resolution is crucial in the study of somatosensory information encoding because the cortical response to tactile stimuli is highly dynamic and spatially widespread. We employed VSD imaging to characterize the spatiotemporal dynamics of neuronal activity in the rat primary somatosensory cortex in response to computer controlled deflections of facial vibrissae, or whiskers. Single whisker deflections induced focused somatotopic cortical activation. Cortical responses demonstrated stronger activation and wider spatial spread as the velocity of whisker deflection was increased. Paired deflections with short inter-stimulus intervals (ISI) evoked a first response that was excitatory, followed by a suppressed second response. The second response gradually recovered for larger ISIs, consistent with previous electrophysiological recordings from our laboratory. Taken together, our preliminary results suggest that the dynamics of the interplay between excitation and inhibition previously demonstrated with single unit recordings in our laboratory extend to spatiotemporal cortical activation, which may have important implications for cortical representations of more complex tactile stimuli in the natural environment.

Action potential broadening modulates neuronal growth cone dynamics

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Electrical activity in neurons plays important roles during neuronal development. Electrical stimulation suppresses neurite outgrowth and changes growth cone motility, suggesting that spiking activity could affect neuronal pathfinding. The effects of neuronal firing activity on neurite outgrowth are mediated by increases in the intracellular Ca^{2+} concentration, either via depolarization-induced entry through Ca^{2+} channels or through intracellular Ca^{2+} release. K^+ channels are important targets of neuromodulation, which can result in changes in action potential shape and thereby affect Ca^{2+} influx. Whether the modulation of K^+ channels can affect neurite outgrowth and growth cone motility, however, has not been studied systematically. Here we address this question in an identified neuron (B5) from the buccal ganglion of the pond snail *Helisoma trivolvis*, which produces mixed Na^+ and Ca^{2+} action potentials and fires spontaneously with stable frequencies ranging from 0.3 to 2 Hz. By combining optical imaging, calcium imaging and electrophysiological techniques, we report that modulating the action potential shape of cultured B5 neurons has striking effects on one aspect of growth cone motility, namely filopodial dynamics. Filopodia, spike-like projection at the leading edge of growth cones, function as sensory and motor structures and are necessary for neuronal pathfinding. We describe that broadening action potentials by inhibiting K^+ channels with tetraethylammonium (TEA) increased Ca^{2+} influx and resulted in an elevation in the intracellular Ca^{2+} concentration in growth cones and subsequent lengthening of filopodia. Therefore, action potential duration, controlled by K^+ conductances, critically influences the actin-based cytoskeleton and can thus control neurite outgrowth and potentially neuronal pathfinding. Forskolin, a potent adenylate cyclase activator, also led to spike broadening, an increase in the intracellular Ca^{2+} concentration and filopodial elongation, as seen with TEA treatment. The effects of forskolin were diminished in the presence of TEA, suggesting that forskolin might affect filopodia length via inhibition of TEA-sensitive K^+ channels. Our data support the hypothesis that growth cone motility is sensitive to action potential shape and that the modulation of K^+ channels could be a determining factor in the outcome of neuronal pathfinding.