

Getting a handle on neuropharmacology by targeting receptor-associated proteins

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Targeted therapy for neuropsychiatric disorders requires selective modulation of dysfunctional neuronal pathways. Receptors relevant to CNS disorders typically have associated proteins discretely expressed in specific neuronal pathways; these accessory proteins provide a new dimension for drug discovery. Recent studies show that targeting a TARP auxiliary subunit of AMPA receptors selectively modulates neuronal excitability in specific forebrain pathways relevant to epilepsy. Other medicinally important ion channels, gated by glutamate, g-aminobutyric acid (GABA), and acetylcholine, also have associated proteins, which may be druggable. This emerging pharmacology of receptor-associated proteins provides a new approach for improving drug efficacy while mitigating side effects.

An afferent subcortical white matter pathway to the amygdala facilitates fear recognition

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Our ability to rapidly detect threats is thought to be subserved by a subcortical pathway that quickly conveys visual information to the amygdala. This neural shortcut has been demonstrated in animals but has rarely been shown in the human brain. Importantly, it remains unclear whether such a pathway might influence neural activity and behaviour. We conducted a multimodal neuroimaging study of 622 participants from the Human Connectome Project. We applied probabilistic tractography to diffusion-weighted images, reconstructing a subcortical pathway to the amygdala from the superior colliculus via the pulvinar. We then computationally modelled the flow of neural activity, using functional magnetic resonance imaging, during a face-viewing task and found strong evidence for a functionally-afferent subcortical pathway. Critically, individuals with greater fibre density in this pathway also had stronger dynamic coupling and enhanced fearful face recognition. Our findings provide converging evidence for the recruitment of an afferent subcortical route to the amygdala in the human brain that facilitates fear recognition.

Profiling phytohormones in stroke brain – challenges and opportunities

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Stroke is a leading cause of death and disability in the world. However, protecting stroke-induced brain injury still represents one of the largest unmet medical needs. Despite of decades of efforts trying to find ways to protect neurons against ischemic insult, no clinical effective drugs are available. It is known that brain can indeed launch an internal protective response against ischemic insult, however, the exact endogenous protective mechanisms remain not well understood. Abscisic acid and phaseic acid (PA), are phytohormones regulating important physiological functions in higher plants. Here, we profiled the presence of plant stress hormones in ischemic brains and show the presence of naturally occurring (-)-PA in mouse and rat brains. (-)-PA is exclusively present in the choroid plexus and the cerebral vascular endothelial cells. Purified (-)-PA has no toxicity and protects cultured cortical neurons against glutamate toxicity through reversible inhibition of glutamate receptors. Focal occlusion of the middle cerebral artery (MCAO) elicited a significant induction in (-)-PA expression in the CSF, but not in the peripheral blood. Importantly, (-)-PA

induction only occurred in the penumbra area, indicating a protective role of PA in the brain. Indeed, elevating (-)-PA level in the brain reduced ischemic brain injury, while reducing (-)-PA level using a monoclonal antibody against (-)-PA increased ischemic injury. Collectively, these studies showed for the first time that (-)-PA is an endogenous neuroprotective molecule capable of reversibly inhibiting glutamate receptors during ischemic brain injury. Further understating of the internal defense system would be extremely beneficial to develop appropriate drugs against stroke in humans.

Inhibitory synapses and plasticity in the ventral tegmental area

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Drug-induced persistent changes in the reward pathway, including the ventral tegmental area (VTA), may precede the transition to addiction. Drugs of abuse share the common mechanism of increasing dopamine release from VTA dopamine neurons, and drug exposure fundamentally alters synaptic transmission in the VTA by enhancing excitatory and reducing inhibitory drive. For example, drug exposure induces long-term potentiation (LTP) at excitatory synapses on VTA dopamine cells, and our understanding of this mechanism has prompted possible intervention strategies for treatment of addiction. However, a second important component of dopamine cell firing rate is GABAergic inhibition, a strong brake on these spontaneously firing neurons. Our lab has been exploring how drugs of abuse and stress interact with synaptic plasticity at GABAergic VTA synapses (LTP_{GABA}); several distinct drugs of abuse block LTP_{GABA}. There are multiple long-range GABAergic projections to the VTA, and using optogenetics we have begun to unravel their control of the local VTA circuit and modification by drugs and acute stress.

Striatal circuit dysfunction underlies motor deficits in a model of human dyskinesia

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Abnormal involuntary movements, or dyskinesias, are seen in many neurological diseases, including disorders where the brain appears grossly normal, suggesting circuit dysfunction may be a root cause. Using unsupervised gene coexpression analysis, we identify a dyskinesia/dystonia gene module in striatum that is enriched with markers of indirect-pathway striatal projection neurons. To understand how indirect pathway neurons might contribute to dyskinesia symptoms, we used electrophysiological, optogenetic, and chemogenetic techniques in awake behaving animals to examine basal ganglia circuit function in a transgenic mouse model of human dyskinesia based on a gene within this module, paroxysmal nonkinesigenic dyskinesia (PNKD). We show that dyskinesia bouts in PNKD mice are caused by a transient loss of indirect pathway activity, which appears to be driven by alterations in excitatory synaptic input. These data provide both genetic and functional evidence for dysfunction of striatal indirect pathway neurons in the etiology of dyskinesia, and may guide development of new treatments for dyskinesias based on selective modulation of basal ganglia circuitry.

Cell-specific splicing of neuronal calcium channels: mechanism, function and disease

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Voltage-gated calcium ion (Ca_v) channels are critical for numerous neuronal functions including triggering neurotransmitter release, rebound bursting, pacemaking, and excitation-dependent gene transcription. Ca_v channel activity is tightly controlled – from gene expression to membrane trafficking – and by the action of several signaling molecules via G protein coupled receptor activation. Mammalian Ca_v channel encoding genes are complex, containing 50+ exons and each having the capacity to generate hundreds of unique splice isoforms. Transcriptome analyses of functionally unique subsets of neurons have shown that each express a distinct pattern of ion channel splice isoforms that contribute to cell-phenotype and that change depending on cell state. A family of RNA binding proteins, including Rbfox and Nova, orchestrate cell-specific splicing across a number of genes including Ca_v channels. We have shown that cell-specific exon selection during alternative pre-mRNA splicing regulates ion channel biophysics, G protein inhibition, and drug sensitivity. Of particular interest, Trpv1-lineage nociceptors express a Ca_v2.2 mRNA that contains an exon that is found rarely in other neurons. This splicing event in Trpv1-lineage neurons, enhances the sensitivity of Ca_v channels to mu-opioid receptor inhibition, is disrupted following peripheral nerve injury, and when disrupted is associated with reduced effectiveness of morphine as an analgesic. In this case, the unique Ca_v2.2 mRNA exon composition in nociceptors is regulated by methylation of gDNA. Thus, cell-specific composition of Ca_v channel mRNAs across the nervous system is controlled by cell-specific epigenetic modification of gDNA and by a family of RNA binding proteins that collectively determine Ca_v channel function, subcellular distribution, and sensitivity to G protein modulation. This critical RNA processing step, between gene expression and mRNA export, expands the proteome to generate a deep, rich array of calcium-dependent cell functions. Supported by NIH grant NS055251

Neural mechanisms of social reward

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Positive prosocial interactions contribute to the development and maintenance of a range of adaptive, cooperative behaviors. Conversely, inability to participate in normal social interactions is a debilitating symptom of several prominent neuropsychiatric disorders. Although the role of neuromodulators in social behaviors, in particular oxytocin, is an active area of investigation, relatively little is known about the detailed neural mechanisms that influence sociability. We have pursued the hypothesis that the release of serotonin (5-HT) from dorsal raphe (DR) neurons in the mouse nucleus accumbens (NAc), a key node of classic reward circuitry, is critical for promoting non-aggressive prosocial interactions. We find that bidirectional modulation of 5-HT release in the NAc robustly modifies sociability in opposing directions, while having minimal effects on control behaviors. We test the importance of this mechanism in a mouse model of a relatively common genetic cause of autism spectrum disorders, a copy number variation on human chromosome 16p11.2. Genetic deletion of chromosome 7F3, which is syntenic to human 16p11.2, specifically from 5-HT neurons induces deficits in social behavior and decreases DR 5-HT neuron excitability. The decrease in sociability in 16p11.2 deletion mice can be rescued by optogenetic activation of DR 5-HT neurons, an effect requiring activation of 5-HT_{1b} receptors in the NAc. Consistent with these results, the drug MDMA (3,4-methylenedioxy-methamphetamine), well known for its effects on promoting positive social interactions in humans, promotes sociability in mice via a mechanism that

requires targeting the serotonin transporter in the NAc. These results demonstrate a surprisingly robust role for 5-HT release in the NAc in social behaviors and suggest that targeting this mechanism may prove therapeutically beneficial.

Brain circuits controlling the suppression and relapse of traumatic fear memories

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While it is generally adaptive to rapidly learn about threats in the environment, this form of learning can lead to psychopathology including post-traumatic stress disorder (PTSD). In the clinic, exposure therapy is an effective method for suppressing pathological fear, but relief can be transient and prone to relapse. Recent work from my laboratory has explored the neural mechanisms underlying fear relapse after extinction, a form of learning that models exposure therapy in humans. Interestingly, extinction memories are labile and fear relapses after either the passage of time or a change in context. The return of fear after extinction training reveals that extinction results in a new inhibitory memory that is formed alongside the excitatory fear memory. We have now identified a network of brain structures in the rat including the amygdala, hippocampus, and prefrontal cortex that contribute to regulation of fear responses after extinction. In particular, we show using electrophysiological, pharmacogenetic, and cellular imaging methods that hippocampal projections to parvalbumin-positive inhibitory interneurons in the medial prefrontal cortex regulate amygdala circuits involved in fear expression. The identification of this circuit presents novel opportunities for therapeutics directed at cortical inhibitory networks in the treatment of anxiety and PTSD.

Neuronal pentraxins control glutamate receptor driven development of hippocampal inhibitory circuits in health and disease

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Circuit computation requires precision in the timing, extent, and synchrony of principal cell firing that is largely enforced by parvalbumin-expressing, fast-spiking interneurons (PVFSIs). To reliably coordinate network activity, PVFSIs exhibit specialized synaptic and membrane properties that promote efficient afferent recruitment such as expression of high-conductance, rapidly gating, GluA4-containing AMPA receptors. We found that PVFSIs upregulate GluA4 during the second postnatal week coincident with increases in the AMPAR clustering proteins neuronal pentraxins, NPTX2 and NPTXR. Moreover, GluA4 is dramatically reduced in NPTX2(-)/NPTXR(-) mice with consequent reductions in PVFSI AMPAR function. Early postnatal NPTX2(-)/NPTXR(-) mice exhibit delayed circuit maturation with a prolonged critical period permissive for giant depolarizing potentials. Juvenile NPTX2(-)/NPTXR(-) mice display reduced feedforward inhibition yielding a circuit deficient in rhythmogenesis and prone to epileptiform discharges. Memory loss in Alzheimer's disease (AD) is attributed to pervasive weakening and loss of synapses. In a mouse model of AD amyloidosis, *Nptx2*^{-/-} results in reduced GluA4 expression, disrupted rhythmicity, and increased pyramidal neuron excitability. Postmortem human AD cortex shows profound reductions of NPTX2 and coordinate reductions of GluA4. NPTX2 in human CSF is reduced in subjects with AD and shows robust correlations with cognitive performance and hippocampal volume. These findings implicate failure of adaptive control of pyramidal neuron-PV circuits as a pathophysiological mechanism

contributing to cognitive failure in AD. Our findings demonstrate an essential role for NPTXs in controlling network dynamics highlighting potential therapeutic targets for disorders with inhibition/excitation imbalances such as schizophrenia and (AD).

NMDA receptor regulation: clues from rare variants implicated in disease

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NMDA receptors are tetramers composed of GluN1 and GluN2 subunits. These receptors are critical for neuronal development and synaptic plasticity. GluN2A and GluN2B are highly expressed in hippocampus and cortex and are intolerant to genetic variation in the human population. In recent years, rare variants identified in GluN2 subunits have been identified in patients with a variety of neurological disorders, including autism spectrum disorders (ASDs), schizophrenia (SCZ) and epilepsy. Specifically, a large number of rare variants of GluN2B have been identified in ASD probands, whereas GluN2A variants are more commonly identified in patients with epilepsy. We have been studying variants identified within the C-terminal domain of GluN2A and GluN2B to better understand receptor structure/function in this domain and also characterize effects on binding proteins, phosphorylation and synaptic expression of receptors. Using this approach, we hope to reveal insights into the pathophysiology of neurological disorders.

Molecular and cellular mechanisms of synapses loss in Alzheimer's disease and tauopathy

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Synaptic dysfunction and synapse loss are hallmarks of Alzheimer's disease (AD) and other tauopathies, yet the underlying molecular pathomechanism remains largely undefined. Here, we used unbiased proteomic analysis of postsynaptic density (PSD) proteins from wild-type versus Tau-P301S transgenic mice before the onset of overt neurodegeneration to identify early tau-dependent changes in the synapse. We identified that C1q, initiator of the classical complement cascade, is highly increased in PSDs purified from Tau-P301S hippocampus, and that C1q is localized at synapses. Tau-P301S brains showed increased engulfment of synaptic material by microglia. Moreover, C1q-neutralizing antibodies suppressed microglial synapse clearance in neuron-microglia co-cultures and *in vivo* in Tau-P301S mice. These findings suggest that tau pathology induces tagging of synapses by C1q, leading to removal of synapses by microglia, and raise the possibility that C1q-neutralizing antibodies might be a potential approach to mitigate synapse loss in AD.

Impaired metabolic capacity and cellular resilience in antidepressant resistance: implications and opportunities for treatment refractory psychiatric disorders

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Deficits in synaptic plasticity contribute to treatment resistance in psychiatric illnesses, including mood, anxiety and stress disorders. Our work aims to determine the neurobiological mechanisms that functionally limit therapeutic neuroadaptations to first line antidepressant treatments in preclinical (rodent) models of stress pathophysiology. Using a genome-wide transcriptomics approach we identified molecular pathways contributing to antidepressant resistance in rats.

Antidepressant-resistance was induced via chronic adrenocorticotrophic hormone (ACTH; 100µg; i.p.; 14 days) treatment. Animals were allocated to forced swim test (FST) or stress-naïve conditions. The infralimbic cortex, implicated in regulation of FST responses, was dissected and global gene expression profiles obtained (Agilent). Gene set enrichment analysis was performed (DAVID) following Bonferroni correction and KEGG pathways identified (Fisher exact score $p < 0.05$). Pivotal genes were validated in independent groups by RT-PCR and/or immunoblotting. The effects of deep brain stimulation, ketamine (10mg/kg), and lithium (100 mg/kg) on these behavioural and molecular responses were determined. Significant alterations were observed in key sensors of energy demand, cell division/growth, apoptosis, protein synthesis, and glucose/glycogen regulation following stress. Significantly less genes were differentially expressed following stress for ACTH pretreated animals relative to saline controls; suggestive of reduced capacity to respond under supplemental stress. Markers of oxidative stress, hypoxia, endoplasmic reticulum stress, pro-inflammatory cytokines, nutrient deprivation and DNA damage were, however, increased in the ACTH group. Mitochondria deficiency was confirmed in separate experiments and modulation of mTOR signalling was demonstrated to be associated with treatment response to deep brain stimulation, ketamine, and lithium. These data suggest that deficits in metabolic capacity and cellular resilience contribute to antidepressant resistance. Moreover, these deficits can be functionally overcome with deep brain stimulation, ketamine or lithium. Such actions may be critical for initiating longer-term neural adaptations to enable recovery from psychiatric disorders resistant to first line antidepressant treatments.

A novel auxiliary protein that regulates the function of NMDA receptors

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NMDA receptors (NMDARs) are a subtype of postsynaptic ionotropic glutamate receptors that have critical roles in models of learning, and are associated with a variety of neurological and psychiatric disorders, including schizophrenia, depression and Alzheimer's disease. We recently identified the first auxiliary protein (NRAP-1) for NMDARs. NRAP-1 is a presynaptic secreted protein that binds to postsynaptic NMDARs and modifies receptor gating. Our studies have revealed a novel mechanism for the regulation of neurotransmission and synaptic plasticity.

Optogenetic dissection of neural circuits underlying processing of innate fear

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The ability of animals to detect and generate emotional responses to natural threats is highly innate and conserved cross-species. Appreciated adaptive behavioral responses to different environmental cues are crucial for animal survival. Recent studies have shown that an overhead looming stimulation to mimic an approaching danger can trigger mouse flight behavior and escape to their nest (Yilmaz et al., 2013) or unlearned freezing in the open-field where is "no place to hide" (Wei et al., 2015). Our previous work proved the superior colliculus (SC) is the crucial brain structure for rapid responding to this looming threat signal and initialing the visually guided innate defensive responses. By using optogenetic, electrophysiology recording in free moving animal, we also identified a subcortical pathway from the glutamatergic projecting neurons in the medial region of the

intermediate layers of the SC (ILSCm) to the lateral posterior nucleus of the thalamus (LP) mediating the innate freezing behavior. We further identified a cell specific pathway triggered by visual inputs, promoting appropriate defensive response to overhead visual threats. While expression of these responses is considered to be instinctive, their magnitude may be affected by environmental cues. However, the neural circuits underlying this modulation are still largely unknown. In current study, we found that repeated stress evoked an anxiety-like state in mice and accelerated defensive responses to looming. Stress also induced c-fos activation in locus coeruleus (LC) TH⁺ neurons and modified adrenergic receptor expression in SC, suggesting a possible Th::LC-SC projection that may be involved in the accelerated defensive responses. Indeed, both anterograde and retrograde neural tracing confirmed the anatomical Th::LC-SC projection and that the SC-projecting TH⁺ neurons in LC were activated by repeated stress. Optogenetic stimulation of either LC TH⁺ neurons or the Th::LC-SC fibers also caused anxiety-like behaviors and accelerated defensive responses to looming. Meanwhile, chemogenetic inhibition of LC TH⁺ neurons and the infusion of an adrenergic receptor antagonist in SC abolished the enhanced looming defensive responses after repeated stress, confirming the necessity of this pathway. These findings suggest that the Th::LC-SC pathway plays a key role in the sophisticated adjustments of defensive behaviors induced by changes in physiological states.