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SYMPOSIUM 1 – FROM MOLECULAR MOTORS TO NEURODEGENERATION

Sponsored by Brain and Mind Research Institute

SYM-01-01

REGULATION OF FAST AXONAL TRANSPORT AND ADULT-ONSET NEURODEGENERATIVE DISEASE

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The vast majority of cases of the major adult-onset neurodegenerative diseases are sporadic with no clear family history, including Alzheimer's (AD) and Parkinson's (PD) diseases as well as Amyotrophic Lateral Sclerosis (ALS). Although these diseases differ in the neuronal population affected, key pathological hallmarks, delayed neuronal cell death and age of onset, they also share some essential features. Specifically, these diseases exhibit the characteristics of a dying back neuropathy, where the earliest pathological changes appear to present as loss of synaptic function in affected neuronal populations and altered phosphorylation of neuronal proteins. Recent studies have shown that alterations in fast axonal transport are sufficient to produce a distal axonopathy in a select neuronal populations and that fast axonal transport is regulated by the coordinated action of a variety of kinases and phosphatases. An emerging theme in AD, PD and ALS research is that altered kinase activity and distribution is a primary cause of changes in fast axonal transport that in turn lead to the dying back neuropathy seen in these diseases. As a result, these diseases may be members of a class of neurodegenerative diseases. We propose the term "dysferopathy" (from the Greek "fero" meaning to carry or to transport) to describe pathologies associated with FAT dysregulation. The major pathways and mechanisms that link dysregulation of fast axonal transport to pathogenesis in sporadic forms of AD, PD and ALS will be discussed and summarized.

SYM-01-02

MOLECULAR MECHANISM OF IMPAIRED AXONAL TRANSPORT IN ALZHEIMER'S DISEASE MODELS

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There is emerging evidence that impaired axonal transport is not only causative for a range of motor disorders, but possibly also Alzheimer's disease (AD) and related neurodegenerative disorders. Support for this hypothesis comes from transgenic animal models (Gotz and Ittner, 2008). Dysregulation of hallmark proteins in AD, such as of the microtubule-associated protein tau, affects axonal transport early on in disease, causing impaired synaptic plasticity and reduced survival rates (Gotz et al., 2006). In AD and frontotemporal dementia (FTD), a dementia related to AD, tau becomes progressively hyperphosphorylated, eventually forming aggregates. However, how tau dysfunction is associated with functional impairment is only partly understood. K369I mutant tau transgenic K3 mice show axonal transport defects that suggests a cargo-selective impairment of kinesin-driven anterograde transport by tau (Ittner et al., 2008). We found further that kinesin motor complex formation is disturbed in the K3 mice. Under pathological conditions, hyperphosphorylated tau interacts with JNK interacting protein 1 (JIP1), which is associated with the kinesin motor protein complex (Ittner et al., 2009). As a result, transport of JIP1 into the axon is impaired causing JIP1 to accumulate in the cell body. Since JIP1 is involved in regulating cargo binding to kinesin motors, these findings may, at least in part, explain how hyperphosphorylated tau mediates impaired axonal transport in AD and FTD._Gotz J, Ittner LM, Kins S. (2006) J Neurochem 98:993-1006. _Gotz J, Ittner LM. (2008) Nat Rev Neurosci 9:532-544._Ittner LM, Fath T, Ke YD, Bi M, van Eersel J, Li KM, Gunning P, Gotz J. (2008) Proc Natl Acad Sci U S A 105:15997-16002._Ittner LM, Ke YD, Gotz J. (2009) J Biol Chem 284:20909-20916.

SYM-01-03

HYPEREXCITABILITY, PERSISTENT NA+ CONDUCTANCES AND NEURODEGENERATION IN MOTOR NEURONE DISEASE

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Establishing the presence of upper and lower motor neurone abnormalities affecting the same region is critical for the diagnosis of MND. In terms of the lower motor neurone, we have identified increased persistent Na+ conductances contributing to the peripheral hyperexcitability typical of MND, leading to the almost inevitable symptoms of fasciculations. When considering upper motor neuronal involvement, MND is characterised by progressive degeneration of the corticospinal tract. Using novel threshold tracking transcranial magnetic stimulation (TMS), we have established that cortical hyperexcitability was an early feature in sporadic MND. Cortical hyperexcitability was also evident in familial patients with mutations in superoxide dismutase (SOD-1), suggesting that similar pathophysiological processes operated in both sporadic and familial MND patients. Longitudinal studies in asymptomatic SOD-1 mutation carriers recently demonstrated that cortical hyperexcitability may develop prior to the clinical onset of MND. Reduction of short interval intracortical inhibition in MND appears to be determined by a combination of a loss of inhibitory cortical interneurone and glutamate-mediated downregulation. Our findings in SOD-1 mutation carriers were further supported in the G93A SOD-1 mouse model, where degeneration of spinal cord motor neurones occurred secondary to dysfunction within central nervous system motor pathways. Taken in total, these more recent findings lend support for a 'dying forward' hypothesis, with corticomotoneurones inducing anterograde excitotoxic motoneurone degeneration. From a therapeutic perspective, neuroprotective strategies aimed at preserving the integrity of intracortical inhibitory circuits, as well as antagonizing excitatory cortical circuits, may provide novel therapeutic targets for future MND treatment trials.

SYM-01-04

NEURODEGENERATION IN ALS AND AD: WHICH COMES FIRST THE AXON OR THE SOMA?

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There has been growing interest in the axon as the initial focus of pathological change in a number of neurodegenerative diseases of the central nervous system. Increasingly it has been proposed that neuronal degeneration is compartmentalized and that degeneration of the axon occurs in an active fashion, independently from apoptotic cell death. Our recent data indicates that the main pathological stimuli for both Alzheimer's Disease and Amyotrophic Lateral Sclerosis, stems from an initial perturbation of the axon and its cytoskeleton, which ultimately results in slow neuronal degeneration and loss of connectivity. The identification of a degenerative process initiated in the axon may provide new therapeutic targets for early intervention to inhibit the devastating outcomes related to the progression of these diseases.

SYMPOSIUM 2 – ION CHANNEL DYNAMICS

Sponsored by Victor Chang Cardiac Research Institute

SYM-02-01

MOLECULAR MECHANISMS OF K^+ CHANNEL ACTIVATION AND INACTIVATION GATING

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We aim to understand the molecular mechanisms of gating in voltage-dependent channels, by focusing on the analysis of K^+ channel gating in prokaryotic systems. Specifically we will address the following key questions: What are the molecular entities determining channel activity? How energy is transduced into protein motion? How different parts of the channel interact to define open channel activity? We have investigated these problems by combining spectroscopic techniques (EPR NMR and Fluorescence), X-ray crystallography and electrophysiological methods. Here we will discuss a series of new crystal structures of the K+ channel KcsA, trapped in a series of functional conformations that include the open/conductive and open/inactivated states, as well as a number of partial opening conformations. These structures, together with functional spectroscopic and computational data have defined the molecular basis of activation and inactivation gating in KcsA, and the coupling between the inner bundle gate and the selectivity filter. Our results clearly demonstrate that in KcsA, and most likely other K channels, a variety of gating transitions are determined by the conformational dynamics at the selectivity filter.

SYM-02-02

GATING IN INWARD RECTIFIER POTASSIUM CHANNELS

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Potassium currents across cell membranes form the basis of electrical signalling in multicellular organisms. Conduction occurs via the selective pore of K+ channels, and is switched on and off in response to regulatory signals. Electrophysiological studies have established that the switching process utilises gates in the permeation pathway, but understanding of the nature of the gates and gating process is limited. Present paradigms have been founded upon structural comparison of unrelated K+ channels, a situation that this study has sought to rectify. The study described here uses KirBac3.1 to investigate molecular reorganisation during K+ channel gating. The validity of using prokaryotic channels for investigating structural and molecular aspects of mammalian inward rectifier K+ (Kir; IRK) channels was established by the relatively seamless integration within a protein chimera comprising the pore of prokaryotic KirBac1.3 and cytoplasmic regions of mouse Kir3.1 (Nishida et al., 2007). In a series of eight structural snapshots of KirBac channels, correlated molecular changes consistent with gating have been identified. The series shows that changes in the selectivity filter region are correlated to the global conformational status of the assembly. Unexpectedly, the molecular transition appears largely independent of inner helix movement. We are now working toward verification of a gating hypothesis based on the series of structures, using point and truncation mutants of KirBac3.1, and will describe key elements of our model. Nishida, M., Cadene, M., Chait, B.T., and MacKinnon, R. (2007). Crystal structure of a Kir3.1-prokaryotic Kir channel chimera. EMBO J 26, 4005-4015.

SYM-02-03

THE TRPC6 NON-SELECTIVE CATION CHANNEL AND CARDIAC α_1-ADRENERGIC RECEPTOR SIGNALLING

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Sympathetic regulation of cardiac contractility can be mediated by alpha$_1$-adrenergic receptors (ARs) and the alpha$_{1A}$-subtype has been implicated in the pathogenesis of cardiac hypertrophy. However, little is known about alpha$_{1A}$-AR signaling pathways in ventricular myocardium and a transgenic mouse model we developed did not show hypertrophy, but rather hypercontractility and, with marked (170-fold; alpha$_{1A}$-AR-H) but not moderate (66-fold; alpha$_{1A}$-AR-M) cardiac-restricted receptor overexpression, died of sudden death. Using this model of enhanced cardiac alpha$_{1A}$-AR signaling and various molecular, biochemical and physiological tools (bioactive substances, specific pore inhibitory antibodies, shRNA-coding adenovirus, ratiometric Ca^{2+} binding fluorescent dye, Langendorff hearts), we identified a novel signaling pathway that mediates receptor-coupled Ca^{2+} entry in cardiomyocytes (CMs) and myocardium. This pathway involves receptor-activated translocation of the scaffold protein Snapin and the non-selective cation channel TRPC6 to the plasma membrane to form a ternary complex with alpha$_{1A}$-AR. In CMs from adult alpha$_{1A}$-AR-H, alpha$_{1A}$-AR-M and non-transgenic littermate mice, the alpha$_{1A}$-AR-Snapin-TRPC6 complex results in increased $[Ca^{2+}]_i$ with stimulation by the alpha$_{1A}$-AR specific agonist A61603, which is dose related and proportional to the level of receptor expression, and can be blocked by the alpha$_{1A}$-AR-selective antagonist, KMD3213, by shRNA-mediated Snapin knockdown, by the non-selective cation channel blockers, gentamicin and La^{3+}, by PLC inhibition with U-73122, and by anti-TRPC6-specific blocking antibody, but not by the store-operated channel blocker 2-aminoethyoxyphenyl borate, the IP_3 receptor blocker xestospongin C or the PKC inhibitor Go6976. Further, we show that Ca^{2+} entry via this receptor-operated channel is independent of store-operated Ca^{2+} entry, and that antibody-mediated blockade of TRPC6 channels not only inhibits alpha$_{1A}$-AR-mediated increases in cardiac contractility in wild type hearts, but also prevents $_{1A}$-AR stimulated Ca^{2+} overload-induced sudden death in $_{1A}$-AR-H hearts. We have thus delineated a novel myocardial $_{1A}$-AR signaling-pathway that provides significant insights into receptor-regulated cardiac contractility and sudden death.

SYM-02-04

Abstract unavailable at time of printing

SYM-03-01

THE ACTIN CYTOSKELETON AND SYNAPTIC PLASTICITY

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Dendritic spines form the postsynaptic contact elements for most excitatory synapses in the central nervous system. Using time-lapse imaging of living neurons expressing proteins tagged with green fluorescent protein (GFP) we discovered that dendritic spines undergo rapid changes in shape thus identifying them as the major sites of morphological plasticity in neuronal circuits of the brain. This motility is driven by dynamic actin filaments and is differentially regulated by various subtypes of postsynaptic glutamate receptors. Activation of AMPA receptors produces an immediate blockade of spine motility which is reversed as soon as the stimulus is withdrawn. By contrast, blockade of spine motility via NMDA receptors requires 30 min to develop and persists for hours after the stimulus is withdrawn. To understand the cellular mechanisms underlying these effects we have examined the role of various actin binding proteins in dendritic spines. Profilin shows activity-dependent targeting to spine heads which depends on activation of NMDA receptors and is induced by electrical stimulation patterns associated with changes in synaptic strength such as LTP. Simulatneously actin dynamics are suppressed and spine motility is blocked for periods of hours. Together these observations suggest a mechanism by which the immediate events of sensory perception are transformed into lasting memories.

SYM-03-02

SEIZURE-RELATED GENE 6 AFFECTS FILOPODIAL DYNAMICS AND DENDRITIC ARBOR DEVELOPMENT

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The development of appropriate dendritic arbors relies on intrinsic molecular programs as well as extrinsic signals and afferent activity. The activity-regulated gene, Seizure-related gene 6 (sez-6), is implicated in neuronal development and the activity-dependent plasticity of learning and memory. Using immunocytochemistry and immunogold electron microscopy, we have shown that Sez-6 protein expression is restricted to the somatodendritic compartment of cortical neurons. To determine the function of sez-6 during cortical development, we generated sez-6 null mutant mice. In the absence of Sez-6, cortical neurons display abnormal dendritic arbors with excess short dendritic branches. Overexpression of individual Sez-6 isoforms in knockout neurons reveals opposing actions of membrane-bound and secreted Sez-6 proteins with membrane-bound Sez-6 exerting an anti-branching effect under both basal and depolarizing conditions. Layer V pyramidal neurons in knockout brain slices show reduced excitatory post-synaptic potentials and dendritic spine densities on their apical dendritic arbors correlating with a reduction in punctate staining for the asymmetric synapse marker post-synaptic density 95 (PSD-95). Lower levels of active CaMKII are observed in post-natal Sez-6 null mouse brains and we provide evidence for the involvement of CaMKII and Erk pathways in Sez-6 signalling. Furthermore, co-immunoprecipitation of Sez-6 with actin cytoskeletal regulators indicates a role for Sez-6 in modulating the actin cytoskeleton and this is borne out in time-lapse imaging of filopodial dynamics. In conclusion, Sez-6 is required for the development of normal dendritic arbors and appropriate excitatory synaptic connectivity.

SYM-03-03

IDENTIFICATION OF THE PROLINE RICH INOSITOL POLYPHOSPHATE 5-PHOSPHATASE (PIPP): CRMP2 POLARITY COMPLEX THAT REGULATES NEURITE OUTGROWTH

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Neuron polarization is essential for the formation of one axon and multiple dendrites, establishing the neuronal circuitry. Here we report the proline-rich inositol-polyphosphate 5-phosphatase (PIPP), which degrades the phosphoinositide-3-kinase signal PtdIns(3,4,5) P3, is a negative regulator of neuronal polarization. PIPP siRNA knock-down in hippocampal neurons led to polarization defects, with multiple hyperelongated axons. CRMP2 promotes axon selection and elongation by regulating Kinesin-1-dependent cargo trafficking. We identify CRMP2 in complex with PIPP by Y2H screening, direct binding of CRMP2 to PIPP, colocalisation of these proteins in the axon shaft and co-immunoprecipitation of PIPP with CRMP2 and the motor protein, Kinesin-1 from brain lysates. CRMP2 is widely and highly expressed in normal human brain, whereas PIPP shows a more restricted expression which includes the pyramidal neurons of the hippocampus. PIPP and CRMP2 showed co-dependent function. PIPP regulated CRMP2-dependent neurite outgrowth by facilitating the localization of Kinesin-1 and its cargo, WAVE, to the growth cone. However, PIPP is also a CRMP2 cargo, and could not access the growth cone in the absence of CRMP2. Therefore this study identifies a novel PIPP:CRMP2 polarity complex that functions to regulate neurite outgrowth and neuron polarization.

SYM-03-04

REGULATION OF CALCIUM HOMEOSTASIS IN GROWTH CONE MOTILITY

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Homer proteins are post-synaptic density proteins with known functions in receptor trafficking and calcium homeostasis. They are key mediators of synaptic plasticity and are known to function in axon guidance. Homer proteins couple extracellular receptors such as metabotropic glutamate receptors (mGluRs) and transient receptor potential canonical (TRPC) family of cation channels to intracellular receptors such as inositol triphosphate (IP3) and ryanodine receptors on intracellular calcium stores, and hence are well placed to regulate of calcium dynamics within the neural growth cone. Using protein knockdown in dorsal root ganglia (DRG) neurons and a growth cone turning assay, we demonstrated that Homer1 functions in axon guidance, converting growth cone turning from attraction to repulsion in response to the calcium dependent guidance cues, brain derived neurotrophic factor (BDNF) and netrin-1. Conversely, Homer1 knockdown had no effect on repulsion to the calcium independent guidance cue, sema-3a. The reversal of attractive turning indicated a requirement for Homer1 in calcium signalling. Calcium imaging of motile growth cones revealed that Homer1 is intimately associated with the regulation of multiple aspects of calcium signalling, including basal cytosolic calcium, calcium-induced-calcium release and store operated calcium entry. Current experiments are focused on the role of the store operated calcium entry protein, STIM1 in growth cone turning. STIM proteins are highly sensitive calcium-sensing proteins and we demonstrate that STIM1 is necessary in growth cone turning. Calcium is a key second messenger in axon pathfinding and deciphering the underlying homeostatic mechanisms that control calcium in the growth cone, and allow calcium to signal multiple, discrete events has direct implications for a wide variety of developmental and neurodegenerative conditions.

SYMPOSIUM 4 – AUDITORY NEUROSCIENCE – NEURONAL SIGNALLING IN DEVELOPMENT, REPAIR AND INNOVATION IN BIONICS

Sponsored by Garvan Institute of Medical Research

SYM-04-01

FROM PLASTICITY TO STABILITY OF BRAIN CIRCUITRY: A LESSON FROM STUDIES OF CELL DEATH AND CELL SURVIVAL IN THE COCHLEAR NUCLEUS

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Since the classic experiments of Hamburger & Levi-Montalcini, and Hubel & Wiesel, a large variety of studies have shown that manipulations of peripheral input and sensory experience can have profound influences on the development of sensory encoding pathways of the central nervous system. Yet, relatively little is known about the cellular mechanisms whereby changes in sensory system function influence the structure or integrity of CNS elements. We have used the brainstem auditory pathways of birds and mammals to investigate the early cellular events underlying deprivation- and deafferentation-induced changes in the structure and integrity of neurons and glial cells. Our work in this area uses a variety of methodologies on in vivo and in vitro preparations of the brainstem to address three issues related to activity-regulated development and maintenance of auditory brainstem neurons. What is the nature of the intercellular signals regulating structural integrity of postsynaptic neurons? What are some of the intracellular cascades of events underlying deprivation-induced changes in neuronal integrity? What biological mechanisms may underlie developmental differences in responses to peripheral manipulations (critical periods)? I will briefly summarize our approach to these problems and then discuss recent and ongoing experiments focused toward understanding the differential susceptibility of neonatal and adult sensory systems to neuronal death due to deprivation of afferent activity (a critical period) using normal and transgenic mice, and microarray technology. I will present results indicating that downstream regulators of cell death can dramatically modulate the critical period response and that the switch from deprivation sensitive to deprivation insensitive probably involves the orchestrated changes transcription of many cell death regulatory genes. These experiments emphasize the developmental transition from selection pressures to optimize plasticity of the growing nervous system to selection pressures emphasizing stability.

SYM-04-02

MICROFLUIDICS AND MICROPATTERNING FOR THE IN VITRO EVALUATION OF NEURITE TARGETING IN THE SPIRAL GANGLION

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During development, the neurons of the cochlea develop highly regular projections to linear arrays of hair cells within the sensory epithelium. The spatial distribution of neurite guidance cues plays a major role in the development of innervation patterns throughout the nervous system, and presumably does so in the inner ear. A variety of methods have been developed for modeling spatial patterns of soluble and surface- or cell-bound guidance cues in vitro. We employed photolithographic techniques to evaluate the responses of developing cochlear ganglion neurites to spatially distributed cues. Fibronectin forms linear patterns beneath the developing cochlear sensory cell array, against a more general background of laminin. When presented with substrate patterns, neonatal cochlear neurites avoided stripes of fibronectin and exhibited dose-dependent preference/avoidance of laminin on culture surfaces. In competition, neurites show a preference for laminin over fibronectin. While cochlear neurites showed no response to EphA1, strong avoidance of EphA4 was negated by antibodies against ephrins B2 and B3. Since both NT-3 and BDNF are present in the developing sensory epithelium, neonatal neurons were cultured in microfluidic chambers that presented strong gradients of these neurotrophins. Their neurites exhibited directional responses to gradients of NT-3, but not of BDNF. In a competition assay, neurites not surprisingly chose NT-3. The responses of developing cochlear neurites to guidance cues provide potential mechanisms for shaping their trajectories within the sensory epithelium.

SYM-04-03

COCHLEAR PATHOLOGIES: FROM ANIMAL MODELS TO FEASABLE CLINICAL TREATMENT

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The knowledge of intimate molecular mechanisms of dysfunction of the cochlear sensory and neural structures is the first step in the development of a new therapeutic strategy. We will summarize experimental protocols and recent results obtained in Montpellier by Jean-Luc Puel and colleagues. Clearly, a round window delivery of very specific molecules proves to be an asset in protecting cochlear hair cells and neurons prior to or immediately after injury (ototoxicity, acoustic trauma). A similar protocol targeting NMDA receptors sounds to be a promising therapeutic strategy for tinnitus treatment. The translation of these experimental approaches to clinic will be discussed.

SYM-04-04

MEDICAL BIONICS IN DEAFNESS: CURRENT AND FUTURE DEVELOPMENTS

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Medical bionics is the replacement or monitoring of damaged organs through engineered devices that interface with the body to improve health outcomes. This presentation will concentrate on neural prostheses designed to restore or supplement hearing lost during disease or injury. Existing commercially available cochlear prostheses will be described after which a review of current research performed in this area around the world – including cochlear implantation in partially deafened cochleae and recent developments in electrically stimulating sites within the central auditory pathway – will be presented. Experimental studies performed in my laboratory associated with (i) combining prosthetic devices with therapeutic drug delivery to minimize the degeneration of the target neural population; and (ii) the plasticity of the auditory cortex in neonatally deafened animals in response to long-term cochlear implantation will be described. Finally I will give a brief overview of the work being performed in other areas of medical bionics including the development of a bionic eye, the use of functional electrical stimulation for standing and gait, the application of deep brain stimulation for movement disorders and the application of brain/machine interfaces to control simple tasks via a computer cursor but ultimately control devices as sophisticated as prosthetic limbs.

SYM-05-01

CENTRAL NEURAL CONTROL OF BROWN ADIPOSE TISSUE. IMPLICATIONS FOR THERAPEUTIC STRATEGIES

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Recent developments showing the presence of functional and physiologically relevant brown adipose tissue (BAT) in adult humans have refocused attention on this organ as an anti obesity target. While some information is available as to the nature of its innervation, the central neural mediators of its activity are poorly defined. We describe here the importance of specific endogenous peptides in the control of BAT activity. Furthermore, we examine the contribution of BAT thermogenesis to the determination of changes in body weight associated with the administration to rats of drugs which either increase body weight in the case of the anti psychotic drug olanzapine or the anti obesity therapeutic, rimonabant. It is hoped that insights derived from this animal work will inform studies, using recently described techniques, of the involvement of BAT in body weight changes following pharmacological treatment in humans.

SYM-05-02

BROWN ADIPOSE TISSUE AND VARIATIONS IN BASAL METABOLIC RATE

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The amplitude of a number of autonomic variables (eg temperature, metabolic rate, arterial pressure, heart rate) suddenly increases in an irregular episodic manner approximately every 1-2 hours during the waking phase of the circadian cycle. Since these episodes recur at intervals of less than 24 hours they are defined as ultradian events. The amplitude of the episodic increases may be quite large. The amplitude of episodic increases in brain temperature, for example, may be as high as 1°C in rats, with increases lasting for approximately 20-30 min. Although the amplitude of ultradian rhythms is comparable with the amplitude of corresponding circadian rhythms, the physiological significance of ultradian rhythms has remained obscure. In rats, episodic increases in brown adipose tissue (BAT) thermogenesis, occurring approximately every 70-130 minutes, contribute to corresponding increases in brain temperature occurring together with increases in EEG indices of active engagement with the environment and with behavioural activity (1). The brain warming could facilitate the complex brain functioning necessary for interacting with the environment. The cerebral cortical EEG during ultradian troughs in brain temperature is similar to that occurring during slow wave sleep, a pattern that) also accompanies torpor and hibernation, states of reduced vigilance and reduced metabolic rate that conserve energy supplies. Ultradian troughs in BAT thermogenesis might also serve to conserve bodily fuel supplies. 1. Ootsuka Y, et al. Brown adipose tissue thermogenesis heats brain and body as part of the brain-coordinated ultradian basic rest-activity cycle. Neuroscience 2009:164;849-861.

SYM-05-03

NEW INSIGHTS INTO THE REGULATION OF ENERGY EXPENDITURE IN SKELETAL MUSCLE

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Body weight is determined not only by energy intake, but also by the rate at which energy is expended. Skeletal muscle is an important site of energy expenditure. Our recent work has demonstrated that skeletal muscle exhibits an increase in heat production after meal feeding. Molecular studies indicate that this increase in heat production is caused by enhanced adaptive thermogenesis in muscle. Thus, skeletal muscle displays physiological properties similar to brown adipose tissue and can expend energy via thermogenic processes. These changes in heat production are associated with changes in cellular metabolism or function and not due to changes in blood flow, since manipulated excursions in one parameter do not correlate with changes in the other. Central administration of leptin markedly enhances thermogeneis in muscle and fat tissues of the sheep. In this case, the primary drivers appear to be uncoupling proteins 2/3 and not uncoupling protein 1. In addition to the central control of thermogenesis, we have shown that direct infusion of alpha-melanocyte stimulating hormone (αMSH) into the femoral artery increases post-prandial thermogenesis. This work provides new insight into how the body expends energy, and is fundamental to our understanding of weight regulation. Furthermore, our studies clearly indicate a strong potential for skeletal muscle thermogenesis as a novel target in the development of anti-obesity drugs.

SYM-05-04

THE ROLE OF AMPK IN THE REGULATION OF FAT OXIDATION AND ENERGY EXPENDITURE

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Changing body weight relies on altering the balance between energy intakea and energy expenditure. A prolonged period of positive energy balance results in obesity and it is often presumed that increasing fat oxidation will result in fat loss. There is however, little mechanistic evidence showing that a chronic switch to fatty acid oxidation is sufficient to reduce adiposity. AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase 2 (ACC2) have become major drug targets for weight loss and improved insulin action because activation of AMPK, via inhibition of ACC2 promotes fatty acid oxidation. We have used both pharmacological (administration of the AMPK agonist 5-aminoimidazole-4-carboxamide-riboside (AICAR)) and genetic means (deletion of the ACC2 gene product in mice) to manipulate fatty acid oxidation to determine if this is sufficient to promote leanness. Both of these strategies increased whole body fatty acid oxidation without altering energy expenditure or adiposity. In the absence of any change in energy expenditure, the increase in fat oxidation appeared to be compensated for by the diversion of alternate energy substrates into glycogen and lipid synthesis. We conclude that increased energy expenditure is a pre-requisite for weight reduction and increasing fatty acid oxidation alone has little, if any, effect to reduce adiposity.

SYM-06-01

PRECISE AND PATTERNED ELECTRICAL STIMULATION OF THE RETINA FOR PROSTHETIC DESIGN

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Epiretinal prostheses are designed to replace the function of retinas damaged by disease by stimulating surviving retinal ganglion cells (RGCs), producing patterns of electrical activity that mimic visual responses normally transmitted to the brain. This goal raises a major engineering challenge: eliciting the desired patterns of electrical activity with high precision in many RGCs simultaneously. Using arrays of small, densely spaced extracellular electrodes and a unique stimulation and recording approach, we have shown that injection of brief current pulses well within safety limits can produce a single, directly elicited spike in a target RGC with sub-millisecond timing precision, while eliciting few or no spikes in neighboring RGCs of the same type. This approach to stimulation succeeds in the 5 major RGC types in primate retina which form the large majority of fibers in the optic nerve. Electrical stimulation of RGCs in degenerating (P23H) rat retina produces results very similar to those in healthy retina. Recently we have examined the impact of simultaneous stimulation with 2 or more electrodes. The results suggest that the spatial arrangement of electrical stimulation may be useful in avoiding the stimulation of bypassing axons, and that the activity of any given cell caused by patterned stimulation may be understood in a relatively simple mathematical framework.

SYM-06-02

VISION PROSTHESIS - WHERE ENGINEERING MEETS NEUROSCIENCE

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Implantable bionics for restoration of visual function to the blind is a field of research that requires a unique combination of neuroscience and biomedical engineering. This presentation will describe the experiences over the past 12 years of research into a retinal neurostimulator for treatment of blindness caused by degenerative disorders of the retina such as age-related macular degeneration and retinitis pigmentosa. It will highlight the limiting factors that have thus far precluded a truly beneficial prosthesis from being devised and will aim to illustrate where a deeper understanding of the neuroscience associated with neural stimulation of surviving retinal neurons may help overcome these factors. In particular, the characteristics of the electrode-tissue interface in light of the absence or reduction of retinal processing will be discussed, as well as the potential roles that the on and off pathways play in eliciting visual perceptions by way retinal neurostimulation.

SYM-06-03

ANALOGUE VLSI MODELLING OF SIGNAL PROCESSING IN THE AUDITORY PATHWAY

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The brain uses nerve pulses (spikes) for its signal processing. These spikes can be considered as signals that are discrete in amplitude, but continuous in time. This is different from digital signal processing, which is discrete in both time and amplitude, and analogue signal processing, which is continuous in both time and amplitude. The question we are trying to answer in this work is if there are certain types of signal processing operations for which spike based computation offers clear advantages. This could offer more efficient signal processing systems and shed light on some of the brain's computational mechanisms. The answer seems to lie in the ease with which temporal and spatial correlations are extracted in such systems. In this talk, the auditory system is used as an example of a neural system. We will briefly introduce it and then present several systems that use spike based computation to extract auditory information. Analogue VLSI has been used to implement our models, which are as a result low-power, scalable, and real-time, while having to cope with similar constraints as the neural system, such as component variability and noise. In this talk we will present measurements of the aVLSI systems, but no circuit details will be given - the focus is on computation with spikes, not on the actual implementations.

SYM-06-04

NEURAL INTERFACE SYSTEMS TO RESTORE MOVEMENT IN PARALYSIS

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Neurotechnology is an emerging field that is beginning to provide new medical devices to treat nervous system disorders and to restore lost functions. The creation of these unique technologies is being driven by impressive advances in neuroscience, in mathematics and computer science, in engineering and electronics, and in clinical disciplines. Neural interface systems (NIS) that sense neural signals are a newer neurotechology, now in early-stage human trials, that are designed to restore lost function for those with movement disabilities. NIS, also called a brain computer interface (BCI) or neural prosthesis, aim to restore communication, control and potentially reanimate muscles for people with paralysis. BrainGate is a sensing NIS being developed by our group at Brown University and Massachusetts General Hospital to allow paralyzed humans to use their own neural signals to operate external devices ranging from computer software to robotic assistants to muscles. Control signals are derived from the electrical activity of motor areas ordinarily used for arm movement. Neural signals related to movement are detected through a unique, baby aspirin-sized 100 channel neural sensor that is implanted onto the surface of the motor cortex. Signals from the sensor pass to external signal processors and computers that decode neural activity patterns into a simple control signal that is operated by intention alone, thus allowing thought to become action. BrainGate is now in early stage human clinical trials. Results from four tetraplegic participants indicate that arm-related motor cortical signals remain years after stroke, spinal cord injury or ALS (Lou Gehrig's disease) in the absence of movement. Further, using BrainGate people with paralysis can generate useful control signals merely by thinking about moving. Our participants have been able to demonstrate the ability to operate various assistive devices, including a computer cursor to open email, a robotic arm to grasp objects, and a powered wheelchair. Next-step advances toward fully automated and wireless implanted systems are underway. These steps are essential to make the system continuously available, reliable, and portable. These initial advances in neurotechnology promise to eventually provide new devices that would substantially improve the lives of those with a wide range of nervous system disorders.

SYM-07-01

ROLE OF CALCIUM SIGNALLING IN PROSTATE CANCER

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Major clinical problem with prostate cancer is the cell's ability to survive and proliferate upon androgen withdrawal. Indeed, deregulated cell proliferation together with the suppression of apoptosis provides the condition for abnormal tissue growth. Alterations in Ca2+ homeostasis have been described to increase proliferation, to induce differentiation or apoptosis. During the last years it has emerged that several members of the TRP family could play an important role in prostate carcinogenesis and even more, some of them have been suggested as a prognostic markers for PCa especially useful in the differential diagnosis. 1. We have demonstrated that a calcium signal can promote either cell proliferation or apoptosis, depending on the type of channel involved: calcium entry via TRPC6 or TRPV6 channels stimulates cell proliferation whereas store-operated channels (represented by ORAI1 and TRPC1) are mostly involved in apoptosis induction. 2. We were particularly interested by TRPM8 channels since TRPM8 is a target gene of the androgen receptor and its expression strongly increases in prostate cancer. Recent evidence we have obtained indicate that TRPM8 may be expressed not just in the plasma membrane, but also in the endoplasmic reticulum (ER) membrane where TRPM8 may operate as an ER Ca2+ release channel. The "preferred" TRPM8 localization depends on epithelial cell phenotype (differentiated apical cells vs. non-differentiated basal cells) and on androgen status (androgen-dependent vs. hormone refractory).

SYM-07-02

TRP CHANNELS IN MECHANOSENSATION AND CHEMOSENSATION IN VISCERAL AFFERENTS

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Chronic visceral pain and discomfort of unknown origin represent a large unmet need for treatment and consequent economic impact. In order to understand how symptoms are generated and transmitted to the central nervous system, we need to know at least three principles of extrinsic sensory nerve function – first, what types are there and what do they signal? – second, what is the molecular basis of sensory transduction? – and third, how does all of this change in disease? These questions are a key focus of our group, with a particular emphasis on the role of TRP channels in each case. TRPV1 is highly expressed in visceral afferents, but its role in mechanotransduction is controversial. It clearly plays a role in detection of low pH which occurs in inflammatory and painful conditions. TRPV4 plays a highly selective role in mechanotransduction in high-threshold afferents from the lower viscera where it is preferentially expressed. TRPA1 interacts with TRPV1 and also has a selective role in mechanotransduction, but in a broader range of afferent subtypes. TRPM8 also interacts with TRPV1 selectively in high-threshold afferents, and induces cross-desensitization – a possible mechanism for its analgesic properties (e.g. in response to menthol). Evidence is also emerging for TRPs as effectors following activation of G-protein coupled receptors, in some cases with distinct relationships (e.g. PAR-2 / TRPV4, and BK1 / TRPA1 interactions). Although there are no drugs yet available for clinical use that target TRP channels, some of the early pointers are identified that hold promise for their use in pharmacotherapy of chronic visceral pain.

SYM-07-03

CA2+ INFLUX IN DUCHENNE MUSCULAR DYSTROPHY - MEMBRANE TEARS, SACS, SOCS, TRPC1, OR TRPV2?

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It is established that degenerating muscle fibres in Duchenne muscular dystrophy (DMD) are loaded with Ca2+ and it is widely believed that this elevated Ca2+ has an important role in the pathology of the disease through activation of proteases (calpains), damaging mictochondria and activating phospholipases leading to membrane damage. However the source of this additional Ca2+ is uncertain. Many authorities state that dystrophin is a cytoskeletal protein connecting the contractile machinery to the membrane and the extracellular matrix and that in its absence the membrane is more fragile and susceptible to "membrane tears". These tears would be an important source of Ca2+ influx and the membrane repair process would have a key role in limiting this source of Ca2+. In our view the evidence for this mechanism is limited. Many studies have found evidence for increased activity of various channels in DMD, particularly stretch-activated channels (SACs) and store-operated channels (SOCs) which are Ca2+ permeable and could contribute to Ca2+ influx. Our work has focused on stretch-activated channels whose activity is enhanced by contractions in which the muscle is stretched. We show that blocking these channels with either streptomycin, Gd3+ or the spider venom peptide GsMTx-4 prevents the rise of Ca2+ and reduces many aspects of muscle damage. However the molecular basis of these channels remains uncertain with TRPC1 and TRPV2 current contenders. Establishing the molecular basis of the Ca2+ influx channels and the signaling pathways that regulate their activity are critical steps for understanding the early pathology of DMD.

SYM-07-04

THE ROLE OF TRP CHANNELS IN OXIDATIVE STRESS DAMAGE IN LIVER

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In many diseased states, including ischemia reperfusion injury, hepatocytes are damaged by reactive oxygen species (ROS). The mechanisms involved are complex and are not fully understood. However, one important initial event is the enhancement of Ca^{2+} entry across the plasma membrane. This leads to an increase in the concentration of free Ca^{2+} in the cytoplasmic space (Ca^{2+}_{cyt}), the uptake of Ca^{2+} by mitochondria, and opening of the mitochondrial transition pore, ultimately resulting in the loss of mitochondrial function and cell death. The nature of the Ca^{2+} entry pathway(s) activated by ROS in hepatocytes is not known. A major hepatocyte Ca^{2+} entry pathway activated by hormones and growth factors is the store-operated Ca^{2+} channel (SOC). In addition, a number of transient receptor potential (TRP) channels are expressed in hepatocytes. Several of these, including TRPM2 and TRPM7, are involved in ROS-induced Ca_{2+} entry in other cell types. We have recently shown, using quantitative PCR, that mRNA encoding TRPC1, C3 and C6, TRPV4, and TRPM2, M4 and M7 is expressed in rat hepatocytes. H4-IIE rat liver cells express most of these TRP channels with the exception of TRPV4 and TRPM2. Pre-incubation of hepatocytes with H_2O_2, which generates ROS, leads to a substantial enhancement of Ca^{2+} entry (measured using fura-2 and patch clamp recording) and subsequent cell death. Under the conditions of these experiments, the concentration of H_2O_2 which gave half-maximal stimulation of the initial rate of Ca^{2+} entry was approximately 2 mM. The increase in $[Ca^{2+}_{cyt}]$ induced by H2O2 was inhibited by relatively high concentrations of 2-aminoethyldiphenylborate (2-APB). It is concluded that ROS induced Ca^{2+} entry to liver cells is mediated by one or more TRP channels with the possible additional involvement of SOCs.

SYMPOSIUM 8 – GET MOVING – CELL MIGRATION DURING DEVELOPMENT

Sponsored by Florey Neuroscience Institutes

SYM-08-01

INSIGHT INTO THE ROLES OF ELONGATOR IN CEREBRAL CORTICAL NEUROGENESIS

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The generation of cortical projection neurons is a complex process that relies on the decision of progenitors to leave the cell cycle, migrate to appropriate laminar locations and differentiate into neurons that are stably positioned and are actively extending axonal and dendrite branches. Importantly, these concurrent steps imply dynamic cell shape remodelling which largely depends on the regulation of cytoskeleton components. Thus, identification of new cytoskeleton regulators is essential to shed more light on the molecular mechanisms responsible for the generation of fully differentiated cortical neurons. Here we report that the multi-subunit histone acetyltransferase Elongator complex, which contributes to transcript elongation and which is disrupted in patients suffering from familial dysautonomia, regulates the maturation of projection neurons. Indeed, silencing of its scaffold (Elp1) or catalytic subunit (Elp3) cell-autonomously delays the migration and impairs the branching of projection neurons. Strikingly, neurons defective in Elongator show reduced levels of acetylated α tubulin. A direct reduction of α tubulin acetylation leads to comparable defects in cortical neurons and suggests that α tubulin is a target of Elp3. This is further supported by the demonstration that Elp3 promotes acetylation and counteracts HDAC6-mediated deacetylation of this substrate in vitro. Thus, our results uncover α tubulin as a target of the Elongator complex and suggest that a tight regulation of its acetylation by Elp3 underlies the maturation of cortical projection neurons.

SYM-08-02

COORDINATING NEURAL CREST STEM CELL MIGRATION WITH CELL SPECIFICATION

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Trunk neural crest cells (NCCs) emigrate from dorsal neural folds at the border between the neural plate and the non-neural ectoderm to give rise to an immense variety of derivatives. Their choice of migration path is intimately linked to their developmental potential. Thus, the firstborn NCCs travel ventrally alongside intersomitic blood vessels and form sympathetic neuronal progenitors near the dorsal aorta (early NCC wave), whereas the lastborn NCCs migrate superficially beneath the epidermis to form melanocytes in the skin (late NCC wave). Yet, most NCCs travel ventromedially into the anterior sclerotome of each somite (intermediate wave). These NCCs either traverse the sclerotome to give rise to sympathetic neurons at the dorsal aorta, or they stall within the somite to differentiate into sensory neurons. A fundamental, yet unanswered question therefore is how NCC migration is directed into the three temporally and spatially distinct pathways. Here we present data detailing the molecular mechanism that controls the choice between the intersomitic (early wave) and sclerotome routes (intermediate wave). Thus, the Neuropilin receptors (NRP1 and NRP2) are expressed in separate pools of NCCs and their disruption impacts on neuronal gangliogenesis. Our preliminary in vitro analysis of NCC migration identifies novel mechanisms controlling NRP mediated guidance that underpin differences between mutant phenotypes.

SYM-08-03

MASTER CONTROL OF CORTICAL NEURON MIGRATION AND MATURATION

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During brain development, proneural bHLH transcription factors such as Neurogenin2 and Mash1 act as master regulatory switches to drive cortical neuron production and maturation. Within the developing cerebral cortex, neuroprogenitor cells express the proneural bHLH factor Neurogenin2 (Neurog2) in order to initiate their neurodifferentiation and then undergo active cell migration to their final destination before terminally differentiating into glutamatergic cortical projection neurons. While Neurog2 has been shown to drive neurogenesis as well as the subtype specification of cortical projection neurons, very little is known as to whether it also plays an active role in the control of neuroprogenitor cell cycle exit, or if the Neurog2-signalling cascade is tempered by negative regulators of gene expression. To address these issues, we have identified the zinc finger transcriptional repressor Znf238 to be a downstream target of Neurog2; and preliminary experiments suggest that Znf238 controls the differentiation of newborn cortical neurons as well as their migration. These RNAi experiments suggest that knockdown of Znf238 in cortical progenitors resulted in defects in cell cycle exit as well as a perturbation of cell migration. Remarkably, luciferase reporter assays for the transcriptional activity of Znf238 revealed its antagonism for signalling through Neurog2-type E-box binding sites. We conclude that Neurog2 coordinates the temporal progression for neurodevelopment through stimulation of a Znf238-dependent negative feedback loop for the consolidation of cell cycle exit, as well as for controlling the migration of newborn cortical neurons.

SYM-08-04

ACTIVATION OF SIGNAL TRANSDUCTION PATHWAYS DURING NEURONAL MIGRATION IN CORTICOGENESIS

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Neuronal migration is integral to the development of the cerebral cortex and normal higher brain function. Interaction of newly born neurons with their extracellular environment, including matrix proteins, regulates these events through cell surface receptors. However, it is less clear how the signals from extracellular matrix proteins are transduced to facilitate migration. We have shown that mouse embryos lacking the Ras family guanine nucleotide exchange factor, C3G (Rapgef1, Grf2), exhibit impaired neuronal migration resulting in a failure of cortical preplate splitting and impaired radial glia attachment to the basement membrane in vivo. Neural precursor cells in vivo, in brain slice cultures and isolated from C3G-deficient embryos exhibit a failure of neuronal migration. C3G-deficient neurons fail to attach to extracellular matrix proteins. In contrast, proliferation of neural precursor cells is not impaired, but enhanced. In conclusion, C3G is an integral part of the intracellular signaling events that govern neuronal migration during cerebral cortex development. New results concerning signal transduction during neuronal migration will be presented.

SYMPOSIUM 9 – INNOVATIVE MICROSCOPY; NEW APPROACHES TO FLUORESCENCE MICROSCOPY

Sponsored by Carl Zeiss Pty Ltd

SYM-09-01

SUPER-RESOLUTION FLUORESCENCE MICROSCOPY FOR CELL SIGNALLING

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We employ single molecule imaging techniques to understand how activation of the T cell receptor on the cell surface leads to an intracellular signalling response. To function in an immune response, T cells become activates when a highly specific pathogen-derived peptide binds to the T cell receptor (TCR). However, the concomitant signaling events are not specific to the TCR raising the question how T cells recognize specific signals for activation. The organization of signalling proteins in time and space may establish hierarchies and, ultimately, control signalling outcomes that determine cell function in health and disease. Our previous data revealed that membrane lipids and proteins co-operate to form stable membrane domains and protein clusters that are necessary for full T cell activation. To understand the underlying principles of the organization of signalling proteins in the T cell membrane, we established a super-resolution microscopy approach based on photo-activation localization microscopy (PALM). This is a single molecule imaging technique that allows us to quantify the number of proteins participating in signalling clusters, the number of clusters and the ratio of proteins within clusters. In other words, we are able to quantify signalling efficiency and thus determine how lipids influence the organization and regulation of signalling process.

SYM-09-02

INFLUENCE OF CAVEOLIN-3 UPON MEMBRANE RAFT LIPIDS AND ITS IMPLICATIONS FOR TRAFFICKING IN MUSCLE

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Lipid rafts are subdomains of the plasma membrane that contain high concentrations of cholesterol and glycosphingolipids. Caveolae are a subtype of lipid rafts present in some cells expressing proteins of the caveolin family. Since caveolins are associated with cholesterol/sphingolipid-enriched membrane microdomains, we investigated the effect of caveolin-3 expression upon the mobility and density of lipid rafts using Fluorescence Recovery After Photobleaching (FRAP) and Fluorescence Resonance Energy Transfer (FRET) methods respectively [both techniques using fluorescent-tagged cholera toxin subunit B (CT-B)]. FRAP confirmed that in the absence of transfected caveolin-3, the raft marker was highly mobile on the plasma membrane (mobile fraction 0.77). However, when caveolin-3-YFP was transfected into the cells, the mobility of the lipid raft marker was decreased compared to non-transfected cells (mobile fraction 0.27; $p<0.05$). Next we developed a new FRET method to measure the assembly of the raft marker into molecular lattices (using CT-B-Alexa555 and CT-B-Alexa647 as FRET donor and acceptor respectively). Interestingly, cavaeolin-YFP-rich regions of the plasma membrane showed the tightest CT-B packing density (highest FRET) ($p<0.05$) compared to non-transfected cells. We propose that the convex shape of the caveolae may contribute to higher FRET efficiency in those microdomains, as a result of closer proximity of neighboring CT-Bs. Using 4 dimensional laser scanning confocal microscopy analysis (Z stack plus time), we observed that mechanical stretch was able to induce trafficking of caveolin-3 to the plasma membrane in some cells. The ability of mechanical stretch to induce remodelling of such microdomains suggests a mechanism whereby cation channels, and other caveolin/lipid rafts bound proteins, might be shuttled to the plasma membrane in response to stretch.

SYM-09-03

IMAGING TUBULAR SYSTEM, SARCOPLASMIC RETICULUM AND MYOPLASMIC CALCIUM WITH NOVEL FLUORESCENCE METHODS

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The precise control of contraction in skeletal muscle critically depends on the rapid and precise delivery of Ca^{2+} from the specialized internal store, the sarcoplasmic reticulum (SR), which is under voltage control from the tightly apposed tubular (t-) system. Regulation of Ca^{2+} in the SR and cytoplasm during periods of muscle work is complex and involves influxes of Ca^{2+} from the t-system. A major problem is that measurements of voltage and store-dependent Ca^{2+} fluxes from the t-system of skeletal muscle are not possible with standard electrophysiological techniques. Methods to simultaneously image Ca^{2+} in two subcellular compartments of single muscle fibres using laser scanning confocal microscopy have recently been developed. This has largely involved trapping one Ca^{2+} sensitive dye in the t-system of a mechanically skinned fibre and introducing a second spectrally separate Ca^{2+} indicator to the cytoplasm. By stimulating release of Ca^{2+} directly from SR or via t-system depolarization, voltage dependent and independent fluxes across the t-system can be spatiotemporally resolved against the release flux of Ca^{2+} from SR. These measurements have identified an action potential-induced Ca^{2+} flux across the t-system and an ultra-rapid store-operated Ca^{2+} entry (SOCE) mechanism in skeletal muscle. This has led to the development of a working model for SOCE that is relevant within the large Ca^{2+} release fluxes initiated by excitation-contraction coupling. Potential roles of the action potential-induced Ca^{2+} flux in skeletal muscle remain speculative.

SYM-09-04

NEW INSIGHTS INTO THE EXPERIENCE-DEPENDENT EMERGENCE OF FUNCTIONAL CIRCUITS IN VISUAL CORTEX DERIVED FROM IN VIVO 2-PHOTON MICROSCOPY

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The onset of vision occurs when neural circuits in the visual cortex are immature, lacking both the full complement of connections, and the response selectivity that defines functional maturity. Direction-selective responses are particularly vulnerable to the effects of early visual deprivation, but it remains unclear how stimulus-driven neural activity guides the emergence of cortical direction selectivity. Here we report observations from a motion training protocol that allowed us to monitor the impact of experience on the development of direction-selective responses in visually naive ferrets. Using intrinsic signal imaging techniques, we found that training with a single axis of motion induced the rapid emergence of direction columns that were confined to cortical regions preferentially activated by the training stimulus. To probe alterations in the response properties of single cells that underlie the emergence of direction columns, we used in vivo two-photon imaging following injections of the calcium indicator Oregon Green BAPTA. In visually naive animals, single neurons exhibited strong selectivity for orientation, but only weak directional biases and they lacked the strong local coherence in the spatial organization of direction preference that was evident in mature animals. Training with a bidirectional moving stimulus rapidly strengthened the direction-selective responses of individual neurons by building on the weak initial biases and producing a significant increase in local coherence. These changes were absent when animals were trained with a flashing grating stimulus or when no training stimulus was employed. We conclude that early experience with moving visual stimuli drives the rapid emergence of direction-selective responses in the visual cortex.

SYM-10-01

THE FIRST INTESTINAL MOTILITY PATTERNS IN FETAL MICE ARE NOT MEDIATED BY NEURONS OR INTERSTITIAL CELLS OF CAJAL

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In mature animals, neurons and interstitial cells of Cajal (ICC) are essential for organized intestinal motility patterns. We investigated the types of motility patterns present, and the roles of neurons and ICC, in the duodenum and colon of developing mice. Spatiotemporal mapping revealed organized, regular contractions that propagated in both directions from E13.5 in the duodenum and E14.5 in the colon. The propagating contractions were unaffected by tetrodotoxin, and were present in the intestine of embryonic Ret -/- mice, which lack enteric neurons. Neurally mediated motility patterns were not observed in the duodenum until E18.5, and in the colon until P10. Intracellular electrical recordings from the circular muscle of the duodenum did not detect slow wave activity at E16.5, but regular slow waves were observed in 4/11 preparations of E18.5 duodenum and in 8/8 P0 preparations. Immunohistochemical studies showed that Kit-immunoreactive cells with the morphological characteristics of myenteric ICC (ICC-MY) were first observed at E18.5. Previous studies have shown that in late embryonic W/Wv mice, there is an absence of Kit+ ICC-MY and slow waves. Motility patterns were examined in 3 litters (one at E13.5 and two at E16.5) from matings of a W/+ female and a Wv/+ male. All offspring, including W/Wv embryos, showed organized propagating contractions in the duodenum. Thus, the first intestinal motility patterns are myogenic, and do not require neurons or ICC.

SYM-10-02

ROLE OF INTERSTITIAL CELLS OF CAJAL IN GASTROINTESTINAL MOTILITY

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Many visceral organs display spontaneous electrical and mechanical activity which have been described as myogenic. Now it appears that in the gastrointestinal tract, activity arises from a specialized group of cells, termed interstitial cells of Cajal (ICC). In the gastrointestinal tract, ICC generate pacemaker potentials that are conducted passively into the adjacent muscle where they produce rhythmical membrane potential changes. The mechanical activity of GI muscles can also be altered by enteric nerves innervating those tissues where it was thought that neuroeffector transmission occurred because released transmitters directly acted on smooth muscle cells. However in several tissues, rather than communicating directly with smooth muscle cells (SMC), nerve terminals preferentially form synapses with ICC and these relay information to SMC. Thus ICC, distributed amongst the SMC, are the targets of transmitters released by enteric nerve terminals. Therefore ICC act as integrators of nerve signals in the gastrointestinal tract. ICC networks are reported to become disrupted in a variety of gastrointestinal motility disorders including diabetic patients suffering from gastroparesis, delayed gastrointestinal transit, diarrhea or constipation. Disorders associated with DM have been linked to enteric neuropathies. However, the data supporting this is conflicting. Studies have reported increases rather than decreases in nitrergic and cholinergic nerves. The finding that ICC are affected by type II DM suggests that dysmotility and neuromuscular dysfunction associated with DM could be due to changes in ICC pacemaker activity and the 'in-series' relationship between enteric nerves and ICC. This symposium talk will describe the the role of ICC in pacemaker activity and in enteric neurotransmission. It will also describe the disruption of ICC and decreased motility in animal models with type II DM.

SYM-10-03

COLONIC PERISTALSIS: HOW DOES IT WORK ?

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The mechanisms underlying the generation and propagation of colonic peristalsis are incompletely understood. For some time, it has been proposed that enterochromaffin cells (EC cells) in the intestinal mucosa have been suggested to act as mechanotransducers of the gut wall, by secreting 5-HT in response to mechanical distortion of the mucosa. It has been thought that this release of 5-HT then activates a population of mucosally-projecting intrinsic sensory neurons which initiates peristalsis. However, no recordings of 5-HT release have been made from EC cells during colonic peristalsis to verify these hypotheses. We used real time amperometry to record the dynamic release of serotonin from EC cells. The aim was to determine whether the 5-HT release from EC cells was required for distension-evoked peristalsis in the isolated guinea-pig distal colon. Insertion of an artificial fecal pellet into the oral end of an isolated segment of guinea-pig distal colon a evoked peristaltic wave that propelled an artificial pellet anally at a velocity of 1-2 mm.sec-1. No sudden release in 5-HT release from EC cells was detected prior to the initiation of peristalsis. When the colon was inverted and the mucosa and submucosal plexus removed, 5-HT release could no longer be detected, but peristalsis and fecal pellet propulsion still occurred at similar velocities to control animals (n=13). These results show that the mechanotransduction and generation of peristalsis and fecal pellet propulsion does not require any release of 5-HT from EC cells, nor activation of any sensory nerve endings in the mucosa, as originally assumed. The mechanoreceptors that respond to physiological levels of distension to initiate peristalsis must lie in the myenteric plexus, or muscularis externa.

SYM-10-04

INTEGRATION OF SENSORY INFORMATION BY MULTI-FUNCTIONAL ENTERIC NEURONS

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The motility of the gastrointestinal tract comprises a diverse set of behaviours which are primarily controlled by the neural circuits of the enteric nervous system (ENS). The ENS contains all functional classes of neuron required for complete reflex arcs, however, recent data suggests that many neurons play several roles and could best be thought of as multi-functional enteric neurons (MUFENs). One of the most important and intriguing kinds of MUFEN are those that respond both to sensory stimuli and synaptic input. Thus far these include mono-axonal neurons in the colon which adapt slowly to maintained stretch, another population in the ileum that adapt rapidly to stretch, and the multi-axonal AH/Dogiel type II neurons found in both the ileum and colon. The AH/Dogiel type II neurons have three outstanding features which provide a useful template for understanding the function of MUFENs. They have reciprocal slow synaptic inputs with other neurons demonstrating an interneuron role; they respond to mechanical deformation and chemical stimuli demonstrating a sensory neuron role; and they have a large after-hyperpolarising potential (AHP) at the soma which allows synapse independent shaping of sensory input. Their ability to fire APs, and hence, drive outputs, is strongly determined by the recent firing history of the neuron (through the AHP) and network activity (through slow synaptic transmission). Positive feedback within the population of AH/Dogiel type II neurons means that the network is able to drive outputs well beyond the duration of the stimuli. In general, it could be speculated that this processing of sensory information is integral to the functions of the ENS. Thus, MUFENs contribute another layer of complexity to the control of the gastrointestinal tract.

SYM-11-01

SUGAR AND AMINO ACID SYMPORTERS: COMMON STRUCTURE AND MECHANISM

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Symporters are responsible for the active accumulation of glucose, amino acids and other solutes in epithelial cells and other cells throughout the body including neurons. Over the past twenty years considerable success has been achieved in cloning solute transporters and the human genome contains over 360 in 55 gene families, e.g. the sodium symporters in the glucose (SSF, SLC5) and neurotransmitter (SNF, SLC6) gene families. Although there is no amino acid sequence homology between the SSF and SNF transporters, these genes are often expressed in the same cell type, epithelia and neurons, and have very similar transport kinetics despite the fact that there is no overlap in substrate specificity. Recent progress in solving the atomic structure of transport proteins (LeuT, vSGLT, Mhp1, & BetP) has provided a dramatic new perspective on the transport mechanism. Namely, that genetically diverse transporters have a common structure that is also shared with a proton/amino acid symporter (ApcT) and a Na+-independent amino acid antiporter (AdiC). Despite the lack of amino acid homology these structures can be aligned (RMSD <5 Å) and their substrates can be placed in a common occluded binding site. Of course the coordinating residues for each substrate are different. Despite the overlap of the 6 structures they are in slightly different conformations, e.g. open outward facing, closed outward facing with substrate, and closed inward facing with substrate. These different conformations provide the first structural basis for the alternating access mechanism of solute transport. In addition, these advances demonstrate that the structural fold of membrane proteins has to be included in the classification of transporters into families.

SYM-11-02

IDENTIFICATION OF A DISTAL GLUT4 TRAFFICKING EVENT CONTROLLED BY ACTIN POLYMERISATION

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The insulin-stimulated trafficking of GLUT4 to the plasma membrane in muscle and fat tissue constitutes a central process in blood glucose homeostasis. The tethering, docking and fusion of GLUT4 vesicles with the plasma membrane represent the most distal steps in this pathway and have been recently shown to be key targets of insulin action. However, it remains unclear how insulin influences these processes to promote the insertion of the glucose transporter into the plasma membrane. In this study we have identified a previously uncharacterised role for cortical actin in the distal trafficking of GLUT4. Using high frequency total internal reflection fluorescence microscopy (TIRFM) imaging we show that insulin increases actin polymerisation near the plasma membrane and that disruption of this process inhibited GLUT4 exocytosis. Using TIRFM in combination with probes that could distinguish between vesicle transport and fusion we found that defective actin remodelling was accompanied by normal insulin-regulated accumulation of GLUT4 vesicles close to the PM, but the final exocytotic fusion step was impaired. These data resolve multiple steps of the final stages of GLUT4 trafficking, demonstrating a crucial role for actin in the final stage of this process. We are now using super resolution TIRF microscopy to begin the characterize the carriers that deposit GLUT4 at the plasma membrane and this has revealed multiple classes of carriers thus further adding to our ability to distinguish between discrete steps in this important process.

SYM-11-03

TRANSPORT METABOLONS IN THE APICAL MEMBRANE

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Australian National University.

Protein absorption in the small intestine is an efficient process, assimilating 95% of protein amino acids. To achieve complete digestion, pancreatic and brush-border peptidases work together with a variety of amino acid and peptide transporters. The aim of this project was to identify whether peptidases and transporters form complexes in the membrane to optimize protein absorption. The major transporter for neutral amino acids in the intestine and kidney is called B0AT1 or SLC6A19 (Broad neutral (0) Amino acid Transporter1). Expression of B0AT1 at the cell surface requires coexpression of the carboxypeptidase angiotensin-converting enzyme 2 (ACE2). Coexpression of B0AT1 together with ACE2 increases transport activity more than 20-fold. ACE2 preferentially hydrolyses neutral amino acids from the carboxyterminus of small peptides, which then become substrates of B0AT1. However, kinetic properties of B0AT1 remain unaltered in the presence of ACE2 and both proteins could not be coimmunoprecipitated suggesting that they do not form a tight complex. Coexpression of B0AT1 with aminopeptidase N, by contrast, increased surface expression and changed the kinetic parameters of the transporter. Similarly, ACE2 increased surface expression and the kinetic parameters of the related transporter B0AT3 (SLC6A18), which is coexpressed together with ACE2 in the kidney. Thus, members of the SLC6 family require brush-border membrane peptidases for surface expression and to optimize transport of neutral amino acids. A model is proposed whereby peptidases act as binding proteins for neutral amino acid transporters. Binding proteins are known to be involved in bacterial ABC transporters, but have not been demonstrated in higher cells.

SYM-11-04

NAS1 SULFATE TRANSPORTER, HYPOSULFATAEMIA AND AUTISM

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Sulfate is involved in many metabolic and cellular processes, and is essential for normal growth and development. Circulating sulfate levels are maintained by the NaS1 sulfate transporter, which is expressed in the kidney where it facilitates renal sulfate reabsorption. We generated NaS1 knock out (Nas1-/-) mice, which exhibit hyposulfataemia, reduced sulfonation capacity, seizures, gastrointestinal disturbances and behavioural abnormalities. A consistent autistic disorder (AD) susceptibility locus is on chromosome 7q31-33, which contains the NaS1 gene that we have cloned. Some AD patients have sulfonation defects, for which the aetiology is yet unknown. Due to phenotypic similarities between AD individuals and our Nas1-/- mice, the aims of this study were to explore the involvement of NaS1 and sulfate homeostasis in AD. We developed a highly specific assay for measuring sulfate, which we used to calculate the fractional excretion index (FEI) for sulfate (normal range 0.32-0.47) in a selected clinical cohort of children meeting autism diagnostic observation schedule (ADOS) criteria. FEI sulfate levels were increased (>0.50) in some of the autistic individuals, indicating reduced renal sulfate reabsorption. Sequence analysis of the NaS1 gene in these AD individuals revealed 2 single nucleotide polymorphisms, R12X and N174S, which lead to 100% and 60% loss of NaS1 function, respectively. These findings demonstrate that loss of NaS1 function is associated with hyposulfataemia in some autistic individuals. The significance of this study is relevant to humans with hyposulfataemia and prompts future assessment of autistic individuals with altered sulfate homeostasis.

SYM-12-01

GENETIC CLUES TO THE PATHOGENESIS OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is a common progressive neurodegenerative disorder affecting greater than two percent of individuals over the age of 65. The motor features of PD, including rigidity and resting tremor, are well recognised and predominantly due to the selective degeneration of dopaminergic neurons of the substantia nigra. While dopamine-replacement therapy can ameliorate these symptoms, it is not effective in slowing or halting disease progression. In addition, it has become increasingly apparent that autonomic and cognitive disturbances, not amenable to dopaminergic therapy, are an integral component of the disease. The recent advances in understanding the molecular pathogenesis of PD have been facilitated by the identification and analysis of familial PD genes. While gene mutations can be detected in less than 5% of all patients with PD, the similar clinicopathological presentation of idiopathic and monogenic forms of PD suggests that disruption of a common metabolic pathway(s) underlies disease pathogenesis. Our research has focussed on the parkin gene. Mutations in parkin are the most common cause of autosomal recessive early onset PD. Parkin encodes an E3 ubiquitin-protein ligase that functions in the ubiquitin proteasome system (UPS). Using genetic studies, coupled with cell and animal models, we have investigated the function of parkin and its contribution to protein turnover and disease pathogenesis. Most recently, we have demonstrated that the parkin promoter is bi-directional and regulates the expression of the parkin co-regulated gene (PACRG). Our data suggests that the two proteins directly interact, and elevated PACRG promotes aggresome formation and activation of the autophagic protein degradation pathway. We hypothesise that upregulation of the parkin-PACRG locus and increased autophagy may be beneficial in PD and other age-associated disorders characterised by compromised UPS function.

SYM-12-02

NFIA CONTROLS NEURAL PROGENITOR CELL DIFFERENTIATION THROUGH DIRECT REPRESSION OF THE NOTCH EFFECTOR HES1

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Notch signaling plays a central role in regulating the self-renewal of progenitors. Nuclear Factor I A (Nfia) has been implicated downstream of Notch in mid-gestation telencephalic development, regulating the switch towards astrocytic differentiation from radial progenitors. However, a crucial step in this process is the simultaneous repression of Notch signaling which enables subsequent differentiation to occur. How this is regulated is unknown. Here we demonstrate that, in addition to regulating astrocyte-specific genes, Nfia represses expression of the Notch effector Hes1. During hippocampal development, we find that Nfia-deficient mice exhibit delays in both neuronal and glial development. Hes1 is significantly up-regulated in Nfia mutant hippocampi, and is a direct transcriptional target of Nfia as shown by both bioinformatic and chromatin immunoprecipitation analyses. Thus, Nfia promotes differentiation of progenitors via complementary mechanisms, through activation of neuronal- and glial-specific genes and via repression of Notch signaling.

SYM-12-03

FOLLISTATIN-MEDIATED MUSCLE HYPERTROPHY - A MODEL FOR STUDYING MUSCLE ADAPTATION

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Many serious medical conditions are caused or aggravated by reduced skeletal muscle function or consequent metabolic complications. Interventions that prevent or treat muscle-related illness could considerably improve human health, but a more complete understanding of the mechanisms that govern muscle adaptation is required to accelerate intervention development. Utilising recombinant viral vectors as a means to specifically modify gene expression in skeletal muscle cells in vitro and in vivo, we have begun to explore the mechanisms and physiological effects of muscle adaptation associated with manipulation of the TGF-β signalling pathway. In adult mice, local intramuscular, or systemic administration of recombinant adeno-associated viral vectors can elicit robust expression of a transgene such as follistatin (which can inhibit specific TGF-β family ligands) within 7 days of administration, which is consequently maintained for greater than 24 months without re-treatment. Local follistatin expression by this mode can promote a 100% increase in muscle mass and >50% increase in maximum force producing capacity within 4 weeks. Mice treated via systemic administration exhibit a similar hypertrophic response body wide, which at 24+months of age can compensate for the loss of muscle mass otherwise observed in old mice. As a prospective approach for combating the loss of muscle mass and function in disease, we have examined the effects of this intervention in animal models of cancer-related cachexia, muscular dystrophy, and neurodegenerative disorders. We have observed that different disease states exhibit differing responsiveness to acute follistatin expression, which we propose reflects upon the involvement of the TGF-β signaling pathway as a regulator of muscle adaptation. Our findings and related work demonstrate that the use of viral vector-based gene delivery technology to modify gene expression in muscle as described here is a powerful approach for dissecting the cellular processes that modify muscle phenotype in health and disease.

SYM-12-04

THE POSITION OF AN ARGININE RESIDUE INFLUENCES SUBSTRATE AFFINITY AND POTASSIUM COUPLING IN THE HUMAN GLUTAMATE TRANSPORTER, EAAT1

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Glutamate is the predominant excitatory neurotransmitter in the mammalian central nervous system and extracellular glutamate levels are controlled by a family of transporters known as Excitatory Amino Acid Transporters (EAATs). The EAATs transport glutamate and aspartate with similar micromolar affinities and this transport is coupled to the movement of Na^+, K^+, and H^+. The crystal structure of a prokaryotic homologue of the EAATs, Glt_{Ph}, has yielded important insights into the architecture of this transporter family. Glt_{Ph} is a Na^+-dependent transporter that has significantly higher affinity for aspartate over glutamate and is not coupled to H^+ or K^+. The highly conserved carboxy-terminal domains of the EAATs and Glt_{Ph} contain the substrate and ion binding sites, however, there are some striking differences in this region that we have investigated to better understand the transport mechanism. An arginine residue is in close proximity to the substrate binding site of both Glt_{Ph} and the EAATs, but is located in transmembrane domain 8 (TM8) in the EAATs and hairpin loop 1 (HP1) of Glt_{Ph}. We show that the position of this arginine residue can partially explain the functional differences observed between the EAATs and Glt_{Ph}. Moving the arginine residue from TM8 to HP1 in EAAT1 results in a transporter that has significantly increased affinity for both glutamate and aspartate and is K^+ independent. Conversely, moving the arginine residue from HP1 to TM8 in Glt_{Ph} results in a transporter that has reduced affinity for aspartate.

SYMPOSIUM 13 – IBRO/ANS SYMPOSIUM: CURRENT NEUROSCIENCE RESEARCH ACROSS THE ASIA-PACIFIC

Sponsored by the International Brain Research Organisation and the Australian Neuroscience Society

SYM-13-01

CREATING THE CORTEX AND THE HIPPOCAMPAL ORGANIZER

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The earliest step in creating the cerebral cortex is the specification of telencephalic neuroepithelium to a cortical fate. To date, several transcription factors have been identified which are critical for particular aspects of cortical development. Of these, Lhx2 appears to be act at the earliest stage of specifying the cortical primordium. We find that in the Lhx2 standard knockout, the entire cortical primordium is deleted. Instead, there is an expansion of the hem and the antihem, tissue that normally flank the cortical primordium. Using embryonic stem cell chimeras, we tested whether this is due to a cell-autonomous role of Lhx2 in the dorsal telencephalic neuroepithelium. We find that Lhx2 mutant patches take on either of two alternative fates in the chimeric dorsal telencephalon: laterally, they take on the identity of the antihem, whereas medially they differentiate into cortical hem tissue. Lhx2, therefore, is required to create the cortex, functioning as a "cortical selector" gene. The ectopic patches of cortical hem act as "hippocampal organizers," patterning the adjacent wild-type cortex into multiple ectopic hippocampi. Each of these show appropriate field-specific molecular and structural features, including additional radial glial palisades, which are essential for proper cell migration. This provides definitive functional evidence for the cortical hem as a secondary "organizer" in the telencephalon. References: Mangale et al., Science, 2008 Funding support: A Wellcome Trust Senior International Fellowship, a Swarnajayanti Award (Department of Science and Technology, Govt. of India), intramural funds from TIFR.

SYM-13-02

Sponsored by the Queensland Brain Institute

NON-APOPTOTIC ROLE OF CASPASE-3 IN SYNAPSE REFINEMENT

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During the development of vertebrate neuromuscular junction, the formation and maintenance of acetylcholine receptor (AChR) clusters are regulated by both positive and negative factors. Agrin stabilizes, whereas acetylcholine (ACh) destabilizes AChR clusters. The mechanisms underlying this counteractive interaction are not completely understood. Here we show that caspase-3, an effector caspase involved in apoptosis, negatively regulates AChR clustering. We found that caspase-3 was activated by cholinergic stimulation of cultured muscle cells. This activation did not depend on calpain, a calcium-dependent protease which is involved in the dispersion of AChR clusters. Agrin treatment prevented the activation of caspase-3. Interestingly, inhibition or down-regulation of caspase-3 attenuated ACh agonist-induced dispersion of AChR clusters. Furthermore, the loss of AChR clusters in agrin mutant mice was partially rescued by the inhibition of caspase-3 or genetic ablation of caspase-3. Taken together, these results suggest a novel role of caspase-3 in the refinement of synapse.

SYM-13-03

GENE-ENVIRONMENT INTERACTIONS MEDIATING EXPERIENCE-DEPENDENT PLASTICITY IN THE HEALTHY AND DISEASED BRAIN

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Huntington's disease (HD) is caused by a CAG trinucleotide repeat expansion encoding a polyglutamine tract in the huntingtin protein. HD patients exhibit psychiatric symptoms (e.g. depression), cognitive deficits and motor abnormalities. In a transgenic mouse model of HD we have correlated early molecular and cellular deficits, particularly in the neocortex and hippocampus, with onset of cognitive and sexually dimorphic affective (depression-like) abnormalities. We have also demonstrated that altered sensory, cognitive and motor stimulation can modify the disease process in HD mice. The modulatory effects of environmental enrichment, and associated enhancement of mental and physical activity levels, may be mediated by experience-dependent changes in gene expression, synaptic plasticity and adult neurogenesis. These findings have been extended by our group, and others, to a variety of different mouse models of brain disorders. For example, we have identified behavioural changes in knockout mice suggesting that specific neuronal signaling pathways implicated in cortical maturation are crucial for development of various cognitive and sensorimotor functions, which are known to be disrupted in schizophrenia. We have identified behavioural deficits that can be rescued by increased levels of environmental stimulation, providing evidence for gene-environment interactions and experience-dependent plasticity of relevance to the pathogenesis of schizophrenia. These and other findings may inform mechanisms mediating brain and cognitive reserve, and the development of novel therapeutic approaches for specific neurological and psychiatric disorders.

SYM-13-04

MOLECULAR MECHANISM OF FAMILIAL PARKINSON'S DISEASE

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Parkinson's disease (PD) is the second most common neurodegenerative disease characterized by movement disorders and dopaminergic neuronal loss. It mostly occurs sporadically, but also genetically by mutations in a number of genes including alpha-synuclein, LRRK2, parkin, PINK1, DJ-1 and ATP13A2. Previously, we have demonstrated that mitochondrial dysfunction is a critical event toward the pathology induced by parkin and PINK1 mutations in Drosophila. Furthermore, PINK1 and parkin are genetically linked in a linear pathway with parkin acting downstream of PINK1. In parallel with the Drosophila results, mitochondrial dysfunction induced by PINK1 siRNA knockdown was markedly rescued by parkin expression in the mammalian system, demonstrating the conservation of the PINK1-Parkin pathway between flies and mammals. This link between the two genetic factors has opened up a new avenue for the research of familial PD. However, major questions that should be addressed are the mechanism by which PINK1 regulates Parkin and of how they protect mitochondria. In our recent study, we therefore investigated the detailed relationship between PINK1 and Parkin and their function in the mitochondria. Surprisingly, PINK1 regulates the localization of Parkin to the mitochondria in its kinase activity-dependent manner. In detail, Parkin phosphorylation by PINK1 on its linker region (also called RING0 domain) promotes its mitochondrial translocation, and the RING1 domain of Parkin is critical for this occurrence. We also demonstrate that mitochondria-translocated Parkin induces mitochondrial aggregation. Collectively, our results demonstrate the genetic and biochemical relationship between PINK1, Parkin and the mitochondria, and thereby suggest the possible mechanism of PINK-Parkin-associated PD pathogenesis.

SYM-13-05

PROMOTING PLASTICITY AND RECOVERY AFTER STROKE

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Neuromodulation techniques that promote neural plasticity may have clinical application following stroke. Stroke is a leading cause of adult disability worldwide. While improvements have been made in the early treatment of stroke, there have been no significant advances in rehabilitative treatment in recent years. The beneficial effects of therapy largely depend on experience-dependent neural plasticity, leading to the restoration of function in partly damaged networks, or compensation by intact networks within both the ipsilesional and contralesional hemispheres. Our understanding of these processes has deepened with longitudinal studies of cortical activity in people recovering from stroke. This has led to the development of techniques to promote neural plasticity, and beneficial reorganisation of cortical function, to enhance recovery. These techniques include pharmacological agents, non-invasive brain stimulation and coordinated movement patterns. This presentation will provide an overview of the mechanisms of these techniques, their strengths and limitations, and remaining barriers to their widespread clinical application. The results of research conducted in Auckland, examining the effects of repetitive transcranial magnetic stimulation, and Active-Passive Bilateral Training, will be highlighted. We have compared the effects of priming the motor system with these techniques prior to motor practice with the affected upper limb, with those produced by motor practice alone. We found that priming before motor practice produces beneficial neurophysiological changes in motor cortex function. These are associated with greater, and more sustained, improvements in upper limb function. An algorithm developed to guide the individualised application of these techniques will also be described, providing a glimpse of the possible future of stroke rehabilitation.

SYM-13-06

NEURAL MECHANISMS OF REINFORCEMENT LEARNING

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Reinforcement learning is a computational framework for an adaptive agent, such as humans, animals, robots and programs, to learn appropriate behaviors through exploratory actions and reward feedback. Our lab in Okinawa is working on the two major questions, 1) how the computational steps of prediction of forthcoming rewards, selection of an according action, and update of prediction by the outcome can be efficiently realized, and 2) how the parameters that regulate learning, such as the time scale of prediction, can be tuned to match the environment and agent's needs, both in the brain and artificial systems. From the prior findings that the dopamine neurons carry the reward prediction error signal and that the cortico-striatal synapses have dopamine-dependent plasticity, we hypothesized that the neurons in the striatum learn the action value, expected reward by taking a candidate action. We tested this hypothesis by recoding neural activities in the striatum while animal performed a choice task with variable probabilities of reward. The result showed that about one third of the neurons in the dorsal striatum changed the firing before action selection depending on the value of a particular action. For regulation of the parameters of learning, we hypothesized that the serotonergic system is responsible for setting the temporal discounting parameter that specifies how delayed rewards are taken into evaluation. To test the hypothesis, we recorded the concentration of dopamine and serotonin in the brain while rats performed a food-water navigation task. We observed a higher level of serotonin specifically during the delayed reward condition than in the immediate reward condition. These results brought us better insights and further questions as to the network and neurochemical mechanisms of reinforcement learning.

SYM-14-01

WORKING MEMORY, OSCILLATIONS AND DISEASE

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In this talk, we discuss the relationship between inhibition, synchrony, and rhythmicity and various dynamical pathologies of the central nervous system. We first describe a new role for gamma frequency oscillations in working memory. We then show how disruption of inhibition affects the oscillations as well as how it affects the ability of the network to maintain memories and switch between them. Reduction of the spiking model to a firing rate model provides intuition about the effects.

SYM-14-02

ROLE OF THE AXON IN ACTION POTENTIAL BURST ENCODING

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Action potential (AP) bursts provide a unique temporal code in spike trains of central neurons. Under various behavioural and network states the layer 5 (L5) pyramidal neurons of the cerebral cortex generate intrinsic AP bursts of 2 – 4 spikes at >200 Hz. The prevailing view on the cellular mechanism foar burst firing is that single backpropagating Na^+ spikes spatiotemporally coincide with dendritic calcium depolarisation, leading to secondary or multiple Na^+ spikes. As single Na^+ spikes in these cells originate in the axon initial segment I hypothesize that axonal mechanisms might also contribute to AP bursts. In L5 neurons in brain slices somatic current injections and blocking M-channels induces low-threshold burst firing. Burst firing could be reverted into regular AP firing patterns by both local inactivation of axon initial segment persistent Na^+ channels (I_{NaP}) with riluzole (3 μM, n = 4) or by blocking apical dendritic Na^+ channels with TTX (1 μM, n = 5). To differentiate between the relative contribution of dendritic and axonal current sources in burst firing, spike trains were assessed in neurons with variable axon lengths in the slice. L5 neurons with intact apical dendritic trees but short axons (<80 μm path length, n = 4) were observed to fire only tonically whereas L5 neurons with similar dendrites but longer axons (>120 μm, n = 7) show intrinsic low-threshold bursts. These results suggest an unprecedented role of axonal voltage-gated I_{NaP} in the encoding of AP bursts. A mathematical description of experimentally determined I_{NaP} and M-currents is generated to explore the role of axonal currents in computational models of burst firing L5 neurons.

SYM-14-03

HOW DO AXONS DETECT MOLECULAR GRADIENTS?

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Before axons can communicate information between neurons they must grow and find their appropriate targets. Axons use a variety of molecular cues to navigate through the complex environment of the developing nervous system. Some of the most important such cues are concentration gradients, and we are interested in the computations that axons perform to detect and respond appropriately to such gradients. Here I will discuss recent experimental and theoretical work suggesting that (1) axons compute gradient direction using an optimal strategy based on Bayesian principles, (2) axons adjust their strategy for reading out a decision regarding gradient direction depending on the steepness of the gradient, and (3) the cyclic nucleotide dependent switch between axonal attraction and repulsion observed in steep gradients may not generalize to shallow gradients. Together these results reveal a previously unappreciated role for the quantitative parameters of gradient signals in determining how axons respond to these gradients.

SYM-14-04

CHOLINERGIC NEUROMODULATION OF NEURAL EXCITABILITY

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Klaus M. Stiefel*, Yoe M. Uusisaari, Ben Torben-Nielsen & Boris S. Gutkin. * Presenting Author In this study, we explored the excitability of mouse cortical layer II/III pyramidal neurons (n = 20) under the influence of the neuromodulator acetylcholine. We investigated how two aspects of neural excitability are influenced by acetylcholine: phase reset curves and stochastic resonance. Phase reset curves (PRCs) are measures of excitability of a neuron. In such a curve, the spike time shift as a function of the phase of a perturbation to the voltage of a neuron is plotted. PRCs allow conclusions about the dynamics underlying spiking and the possible synchronization behavior of neurons. We measured PRCs in vitro and in simulations of varying levels of complexity (single- and multicompartmental). When applying the cholinergic agonist carbachol in vitro, this lead to a switch from a biphasic to a monophasic PRC in a significant number of neurons. This effect was reproduced in the simulations by reducing the K+ conductances responsible for the after-hyperpolarization. Stochastic resonance (SR) is the ability of information processing systems to function optimally under non-zero noise levels. It has been demonstrated in several neural systems. We investigated SR in vitro and in simulations, and found that the several of its features such as optimal noise levels are altered by cholinergic neuromodulation. These findings show that cholinergic neuromodulation can switch the basic type of neural excitability and that this switch alters the way neurons react to noise.

SYMPOSIUM 15 – PAIN, MOTONEURONES AND MOVEMENT: UNRAVELLING THE EFFECTS AND MECHANISMS

Sponsored by the Centre of Clinical Research Excellence in Spinal Pain, Injury and Health, University of Queensland

SYM-15-01

MOTOR CORTEX NEUROPLASTICITY AND ITS ROLE IN PAIN AND OTHER ALTERATIONS IN THE OROFACIAL REGION

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Many motor behaviours related to noxious stimuli involve segmental reflex circuits and mechanisms. For example, sustained jaw muscle activity induced by orofacial noxious stimuli depends on reflex circuits in and adjacent to the trigeminal brainstem sensory nuclear complex. However, supraspinal/suprabulbar areas play strategic roles in motor behaviour, and we have conducted a series of studies utilising intracortical microstimulation (ICMS) and single neurone recordings in the face primary motor cortex (MI) of monkeys and rats as well as transcranial magnetic stimulation (TMS) in humans to examine the possible role that face MI may play in behavioural adaptations to pain or other alterations in the orofacial region. Successful training of awake monkeys in a novel tongue-protrusion task is associated with significant increases in the proportions of MI tongue efferent zones (as revealed by ICMS) and of MI neurones with tongue protrusion-related activity and a tongue mechanoreceptive field, consistent with our TMS findings of significantly enhanced corticomotoneuronal excitability when humans learn the novel tongue-protrusion task. Moreover, intraoral pain in humans can interfere with the successful learning of the task and with the associated MI excitability changes, consistent with our ICMS findings in rats that intraoral noxious stimulation can reduce MI excitability; in addition, trimming or extraction of teeth or lingual nerve damage in rats results in significant changes in MI tongue or jaw muscle representations. These findings suggest the involvement of face MI neuroplasticity in orofacial motor skill acquisition and adaptation to pain, an altered dental occlusion or loss of lingual sensory function, and that it reflects dynamic and modifiable constructs that are modelled by behaviourally significant experiences and that may be critical to learning and adaptive processes.

SYM-15-02

IN-VIVO DECODING OF THE NEURAL DRIVE TO MUSCLES

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Each motor neuron active during a movement discharges action potentials as the result of synaptic input exceeding its threshold. Paradoxically, although we have detailed knowledge on the cellular and sub-cellular mechanisms associated to many motor neuron properties, we still do not have the relevant knowledge to associate these “micro” mechanisms to their functional effects on the “macro” motor output. This is due to a classic limitation in the investigation of motor neurons in vivo: only a few neurons can be tracked during natural movements out of hundreds of active neurons. In recent years, this limitation is however becoming less stringent. In this lecture, I will describe methods for interfacing in vivo human motor neurons with high-density systems of electrodes, either implanted into muscles or located over the skin. It will be shown that hundreds of channels of information and blind source separation techniques allow the full decoding of the discharge patterns of large populations of motor neurons in vivo. The importance in the assessment of large populations of motor neurons, in contrast to classic approaches, will be shown by the characterization of motor unit population behavior during fatigue and experimental muscle pain.

SYM-15-03

MOTOR UNIT RECRUITMENT STRATEGY IS ALTERED WITH PAIN

Tucker K.J.
University of Queensland.

Movement is changed in pain. Despite intensive investigation, relatively simplistic mechanisms have been proposed to explain the neurophysiological basis for these changes. The widely accepted “pain adaptation” theory argues, very simply, that the nervous system adapts to pain by a uniform reduction in activity of muscles that generate painful force/movement, and a uniform increase in activity of muscles that oppose painful force/movement. Although this is supported by observation of muscle contraction behaviour during some specific movements, our recent data show changes in discharge of motor units (i.e. motoneurone and innervated muscle fibres) during pain that cannot be explained by this theory. Although discharge of some motor units reduce with pain (consistent with pain adaptation theory) other motor units increase discharge (both increase in discharge rate and/or newly recruited units during pain). Thus, excitability of motoneurones in painful muscles are not uniformly decreased. Instead there is a change in motor strategy. This observation questions the interpretation of a large body of research. Our new hypothesis is that rather than uniform inhibition of the motoneurone pool, there is reorganisation of the strategy of motor unit recruitment within muscles to reduce pain and/or protect the painful part. We have tested the robustness of our observation in a range of different muscles, when pain was induced in the test muscle, synergist muscles and non-muscular tissue. The changes in recruitment strategy are also observed when there is threat of pain with no nociceptive stimulation. The new motor unit recruitment strategy may be associated with a departure from orderly recruitment of motor units, and the direction of resultant muscle force.

SYM-15-04

OROFACIAL PAIN AND JAW MOTOR CONTROL

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Pain and limitation of movement are two of the cardinal signs of Temporomandibular Disorders, a common chronic orofacial pain condition. The relationship between pain and movement is clinically significant but controversial with two major theories having been proposed: the Vicious Cycle Theory and the Pain Adaptation Model. The Vicious Cycle Theory proposes a vicious cycle between pain and muscle activity. This Theory has little scientific basis but underpins many management strategies. The Pain Adaptation Model is more evidence-based and proposes that pain causes changes in muscle activity to limit movement and protect the sensory-motor system from further injury. The Pain Adaptation Model has many positive features but does not appear to explain the relation between pain and muscle activity in all situations. We propose that the relationship is influenced by the functional complexity of the sensory-motor system and the multidimensional nature of pain. This new Integrated Pain Adaptation Model states that pain results in a new recruitment strategy of motor units that is influenced by the multidimensional (i.e. biological and psychosocial) components of the pain experience. This new recruitment strategy aims to minimize pain and maintain homeostasis. This model emphasizes the individual reaction to pain and suggests a tailored approach towards management.

SYM-16-01

ACTIVITY-DEPENDENT REGULATION OF THE FUSION PORE AND MODE OF SECRETION FROM ADRENAL CHROMAFFIN CELLS

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Chromaffin cells of the adrenal medulla are a primary output of the sympathetic nervous system, releasing catecholamines and vasoactive peptide transmitters into the circulation. At basal sympathetic tone, chromaffin cells are stimulated at a low frequency by the innervating splanchnic nerve. Under this stimulation condition, chromaffin cells selectively release freely-soluble catecholamines through a transient kiss and run Ω-form fusion event. This exocytic mode is characterized by a narrow fusion pore between the granule lumen and the extracellular space, resembling the Greek letter "Ω" in profile. In contrast, under acute stress, enhanced sympathetic input drives chromaffin cells to increase secretory activity. Under these conditions the fusion pore dilates completely, leading to a full granule collapse into the cell surface, maximizing catecholamine release. Additionally, this collapse facilitates peptide transmitter exocytosis. Thus, chromaffin cells utilize two modes of exocytosis that differentially effect the release of catecholamine and peptide transmitter. It is our goal to understand the molecular mechanisms that are responsible for the transition from basal kiss and run exocytic behavior to the full collapse exocytosis observed under the sympathetic stress condition. We provide evidence for molecular regulation of the actin cytoarchitecture and a dynamin I-mediated signaling cascade as responsible for the activity-dependent dilation of the fusion pore and thus in the regulation of the adrenal acute stress response.

SYM-16-02

AN UNBIASED APPROACH TO IDENTIFYING PROTEINS CRITICAL TO THE MECHANISM OF CALCIUM-TRIGGERED MEMBRANE FUSION

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Fast, calcium-triggered membrane fusion provides temporal and spatial control of exocytosis in many cells. Stage-specific preparations of calcium-sensitive, release-ready cortical vesicles (CV) enable the tight coupling of quantitative molecular and fusion assays necessary to dissect molecular mechanisms [1-4]. As an unbiased approach to identifying critical proteins, the effects of thiol-reactive reagents (TRR) on CV-CV fusion have been analysed - to date, these are known to inhibit fusion. We now show that iodoacetamide (IA) enhances the calcium-sensitivity and kinetics of fusion [5]. Potentiation was even greater if strontium was used; this substantial effect is highly indicative that IA promotes fusion via a thiol site that regulates a calcium-sensing step of triggered fusion. Considering the inhibitory roles of other TRR, this implicates at least two distinct thiol sites in the fusion process: one affecting fusion competency (the ability to fuse) and one that modulates fusion efficiency (calcium-sensitivity & kinetics). Capitalizing on the potentiating effect of IA, we have identified fluorescent TRR with similar effects: Lucifer yellow IA, monobromobimane and dibromobimane reduce the EC50 for calcium by ~50%. We are using these reagents to simultaneously enhance fusion and label proteins involved; as calcium-sensing proteins are likely situated in cholesterol-enriched membrane areas [2-4,6] we are narrowing the list of protein candidates by isolating these. 1) J Cell Sci 116, 2087 (2003); 2) J Cell Sci 118, 4833 (2005); 3) Biophys J 94, 3976 (2008); 4) J Cell Sci 119, 2688 (2006); 5) J Chem Biol 2, 27 (2009); 6) Biochem J 423, 1 (2009).

SYM-16-03

IDENTIFYING NOVEL ROLES IN CELL COMMUNICATION FOR DISEASE-RELATED PROTEINS

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Defective release of hormones and neurotransmitters can underlie facets of human disease. The focus of our research is in identifying previously unidentified roles of disease-related proteins in cell communication. To this end we have identified several genes associated with Down Syndrome, Alzheimer's Disease, Huntington's Disease and Diabetes that regulate cell function; in particular vesicle exocytosis, endocytosis and fusion pore kinetics. We have obtained evidence gained through the use of genetic and electrophysiological tools illustrating the role of these proteins in cell communication. This presentation will also highlight the possible implications of such a physiological role in the relevant disease settings.

SYM-16-04

ENDOCYTOSIS IN SECRETORY EPITHELIAL CELLS

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The process of regulated secretion involves fusion of granules with the cell membrane followed by endocytic recovery of membrane. Our recent work in epithelial cells shows that granule fusion can be complex and that there are multiple routes endocytosis. Mouse pancreas was excised and collagenase-digested to produce fragments of pancreatic tissue. The tissue fragments were bathed in extracellular fluorescent dyes and either imaged live with 2-photon microscopy or after paraformaldehyde fixation with confocal microscopy. Cell exocytic responses were stimulated with acetylcholine (0.1-1 μM) its action terminated by the application of Atropine (10 μM). Upon exocytosis the extracellular fluorescent dye enters and labels the granules. Using different dyes and different times of dye addition we have developed methods to enable positive identification of whether the fusion pores are open or closed. With a range of acetylcholine concentrations we now show that secretion is not only regulated by the control of the numbers of fusion events but also by the dynamics of granule fusion itself. In further experiments we have used the dynamin inhibitor, dynasore (80 μM) to investigate if dynamin is a regulator of endocytosis. In control experiments we prove this drug is effective in inhibiting transferrin uptake in cultured cells. Using dynasore as a tool in pancreatic acinar cells we show that the fusion pore is a site of regulation and is closed by dynamin (most likely dynamin 2). In live-cell experiments we are currently investigating whether dynamin inhibition affects endocytosis. Our data provide evidence for whole-granule recapture that is independent of dynamin. We thus conclude that there are dynamin dependent and dynamin independent mechanisms of endocytosis in secretory epithelial cells.