SYMPOSIA

SYMPOSIUM 1 – COMPARTMENTALISATION OF SIGNALLING IN SPINES AND DENDRITES: IMPLICATIONS FOR INFORMATION STORAGE

Sponsored by the John Curtin School of Medical Research, Australian National University

SYM-01-01

EXPERIENCE-DEPENDENT CHANGES IN LOCAL DENDRITIC BRANCH EXCITABILITY

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The arrival of highly correlated input patterns causes hippocampal CA1 pyramidal cells to generate local dendritic spikes whose propagation strength varies among branches. Further, spike propagation strength is a plastic feature of dendrites, as it can be strengthened by associative input/output patterns. These results raise the possibility that dendritic plasticity might participate in hippocampal memory formation. We, thus, hypothesized that experience in a spatially and socially rich environment could induce changes in branch excitability and local spike propagation strength. To test this we compared the local spike strength distribution in apical dendrites of CA1 pyramidal cells in acute slices prepared from rats with experience in an enriched environment to control animals. Spikes generated in primary parent dendrites of control rats were mostly strong (strong 87%, weak: 11%, none: 2%, n=45). In contrast, significantly fewer primary branches expressed strong spikes in enriched rats (strong: 69%, weak: 20 %, no spike: 11 %, n=54). However, propagation strength in terminal dendrites originating from the strong primary dendrites was enhanced in enriched rats (control: 0.79 ± 0.05 V/s, n=67; enriched: 1.48 ± 0.15 V/s, n=49), and more terminal branches were coupled to their primary dendrite (control: 12/69, enriched: 18/52). In contrast, dV/dt decreased in terminal branches originating from weak parent dendrites (control: 0.69 ± 0.07 V/s, n=8; enriched: 0.52 ± 0.04 V/s, n=19). These data indicate that the excitability profile of dendritic branch families is altered during experience such that strong branch families are enhanced while weak families are further suppressed. Our results show that in vivo experience is capable of modifying individual dendritic branch excitability and support the hypothesis that branch strength plasticity plays a role in learning.

SYM-01-02

DO DENDRITIC SPINES COMPARTMENTALISE SYNAPTIC VOLTAGE?

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Most excitatory input onto neurons in the brain occurs onto dendritic spines. Although much is known about calcium dynamics in spines during backpropagating action potentials (bAPs) and synaptic excitation, the membrane potential changes underlying these responses are unclear. Compartmentalisation of voltage in spines was investigated using imaging with voltage-sensitive dyes (JPW 3028) to determine the relative voltage changes in spines and parent dendrites in basal dendrites of layer 5 pyramidal neurons. The voltage fluorescence signal in the spine and parent dendrite in response to somatically evoked bAPs did not significantly differ in amplitude when normalized to the response to steady-state hyperpolarization, illustrating that bAPs successfully invade dendritic spines without voltage attenuation. To determine the spine voltage during synaptic stimulation, we located active spines using calcium imaging and directly compared synaptic responses evoked by extracelluar stimulation with that generated by bAPs. The voltage in dendritic spines during synaptic input was on average ~8 mV in amplitude, and not significantly boosted by voltage-activated channels. We estimate spine neck resistance to range from 10 and 500 MΩ, with an average of ~200 MΩ. Whether receiving excitatory input onto the spine head opposed to directly onto the dendritic shaft serves as a mechanism to modulate synaptic strength was assessed by calculating the effect of spine neck resistance on the amplitude of the EPSP at the soma. The measured range of spine neck resistances reduces somatic EPSPs by less than 15%, indicating that the spine neck does not act as a physical device to significantly modulate synaptic strength.

SYM-01-03

FACTORS CONTROLLING CALCIUM COMPARTMENTALISATION IN DENDRITIC SPINES

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Purpose: The majority of connections between excitatory neurons are made on small, specialized compartments call spines. These structures are connected to the dendrite by a thin spine neck that restricts diffusion between the spine head and the dendrite. This arrangement enables in synaptically evoked signalling molecules, such as calcium, to be concentrated within individual spines, thus conferring synapse specificity to the changes these signalling molecules trigger. Synaptically evoked calcium signals are not always restricted to spines. In projection neurons in the basolateral amygdala (BLA), and other brain regions, activation of metabotropic receptors evokes a focal rise in free calcium in the dendrite that propagates as a wave along the dendrite and invades the soma and nucleus. Here we investigated whether calcium waves invade the spines as they propagate. **Methods:** Whole-cell patch-clamp recordings and high-speed calcium fluorescence images were made from BLA projection neurons in rat brain slices. Calcium waves were evoked by local tetanic stimulation in the presence of ionotropic glutamate receptor antagonists. **Results:** Calcium waves were found to invade a subset of spines. The extent to which calcium waves invaded the spine head was correlated with the diffusional coupling between the spine and its parent dendrite and inversely correlated with the spine neck length. Spine invasion was also correlated with the half-width of the dendritic calcium rise, suggesting that the spine neck acts as a low pass filter of dendritic calcium signals. **Conclusion:** These results indicate that the thin neck, which separates spines from their parent dendrite, not only maintains calcium within the spine head, but also differentially excludes dendritic calcium signals from the spine head.

SYM-01-04

COMPARTMENTALISED CALCIUM SIGNALING SUPPORTS MULTIPLE FORMS OF LONG-TERM POTENTIATION IN CA1 PYRAMIDAL NEURONS

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Long-term potentiation (LTP) of synaptic transmission is an important information storage mechanism in the brain. In area CA1 of hippocampus LTP is triggered by an increase in postsynaptic calcium levels. However, multiple forms of LTP co-exist at these synapses, raising the question how one signal can selectively and appropriately activate multiple, independent cellular processes. We previously showed that compartmentalisation of calcium signals could induce different forms LTP. Thus, short-lasting LTP (LTP 1) required activation of ryanodine receptors (RyRs), which selectively increased calcium in synaptic spines. LTP of intermediate duration (LTP 2) was dependent on activation of IP3 receptors (IP3Rs) and subsequent calcium release specifically in dendrites. Long-lasting LTP (LTP 3) was selectively dependent on L-type voltage-dependent calcium channels (L-VDCC), which generated somatic calcium influx. Given such remarkable spatial selectivity, we recently asked whether these forms of LTP also differ in their requirement for postsynaptic action potentials (APs). Inhibition of APs had no effect on the persistence of LTP 1, but reduced the persistence of more durable forms (LTP 2 & 3). Calcium imaging revealed different requirements for APs in generating calcium signals in spines, dendrites, and somata, consistent with their known roles in the induction of each form of LTP. Finally, a strong somatic calcium signal, generated by TBS-patterned APs alone, dramatically transformed short-lasting LTP into a more persistent form. Together these data show that compartmentalisation of calcium signalling supports the co-existence of discrete neuronal learning mechanisms in hippocampal area CA1.

SYM-02-01

EXTENSIVE TELENCEPHALIC NEUROGENESIS BEFORE KINDERGARTEN

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The primate postnatal subventricular zone (SVZ) lies under the ventrolateral borders of the lateral ventricles as a discrete region of cells with gliogenic and neurogenic capacity regulated by multiple growth factors including EGF, NRG1 and BDNF. However, the specific role of each in SVZ cell development in vivo remains unclear, particularly in the human brain. We will use molecular neuroanatomical techniques to describe the postnatal spatial and temporal expression profile of growth factor receptor subtypes in the normal human brain. This will include the mRNA for ErbB receptors (for EGF and NRG1) and trkB receptors (for BDNF) in the SVZ from neonates, infants, toddlers, school age subjects, adolescents, young adults and adults. SVZ transcript levels of ErbB1 and ErbB4 are highest in neonates and diminished with age. SVZ ErbB4 mRNA quantities significantly decreased by >85% to almost undetectable levels after the first year of life, while SVZ ErbB1 transcript levels displayed more gradual reductions, stabilizing to 30–40% of neonate levels after the aage of 5 years. In the neonate and infant SVZ, ErbB4 and truncated trkB mRNA was localized to cell clusters resembling migratory neuroblast aggregates whereas ErbB1 mRNA was expressed in cells along but not within these clusters. ErbB2and truncated trkB mRNA appeared to be constantly expressed in the human SVZ at all postnatal ages as opposed to full length trkB and ErbB3 transcripts, which were not detected in the human SVZ at any age following birth. In addition, ErbB1- and ErbB4-immunoreactive cells and fibers were extensive throughout the human infant SVZ, but did not appear to overlap with PSA-NCAM-immunopositive clusters. Abundant PSA-NCAM and doublecortin positive cells were found in the developing SVZ and can also be detected in the adult human and monkey brain. Our results suggest that robust postnatal neurogenesis in the human brain is most extensive within the first year after birth; however they also suggest that neuronal migration and differentiation occur in the cortex even in adult life.

SYM-02-02

MOLECULAR MEDIATORS, ENVIRONMENTAL MODULATORS AND BEHAVIOURAL CORRELATES OF ADULT NEUROGENESIS IN THE HEALTHY AND HUNTINGTON'S DISEASE BRAIN

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While much progress has been made in recent years in understanding how genetic factors and other molecular mediators contribute to various brain disorders, less is known about how levels of mental and physical activity, and associated experience-dependent plasticity, modulate pathogenesis. Huntington's disease (HD) is caused by a CAG trinucleotide repeat expansion encoding a polyglutamine tract in the huntingtin protein. HD patients exhibit cognitive deficits (culminating in dementia), psychiatric symptoms (the most common of which is depression) and motor abnormalities (e.g. chorea). In a transgenic mouse model of HD we have correlated early deficits of hippocampal neurogenesis with onset of cognitive and affective abnormalities, and identified potential molecular mechanisms mediating this 'pathological plasticity'. We have also demonstrated that altered sensory, cognitive and motor stimulation can dramatically modify the disease process in HD mice. A more specific component of environmental enrichment, voluntary physical exercise, was found to delay the onset of hippocampal-dependent cognitive and affective symptoms, in a similar fashion to chronic administration of selective serotonin reuptake inhibitors (SSRIs). Together with parallel BrdU labeling, immunohistochemistry and stereology, these findings suggest that the cellular mechanisms mediating this delayed onset of hippocampal-dependent behavioural deficits involve enhancement of adult neurogenesis. Our findings indicate that the modulatory effects of various environmental stimuli are mediated by experience-dependent changes in transcription of specific genes implicated in adult neurogenesis, thus identifying potential molecular targets for therapeutic intervention.

SYM-02-03

ACTIVATION OF ENDOGENOUS NEURAL STEM CELLS FOR THE PREVENTION OF AGE-RELATED COGNITIVE DECLINE

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Given that the role of tissue stem cells is essentially to maintain homeostasis, and most tissue stem cells undergo age-related alterations, we hypothesized that slowing the age-related decline in endogenous neural stem cells (NSCs) would slow or possibly prevent age-related cognitive decline. As little was known concerning the effects of ageing on NSCs, and to provide a benchmark of the effects of normal ageing on endogenous NSCs, we harvested the periventricular region (PVR) from serial vibratome sections through the entire brain(s) of juvenile (6-8 weeks), 6, 12, 18, and 24-month old mice, and cultured equal numbers of cells in the neural colony forming cell assay, which discriminates NSCs from more restricted progenitor cells. While age-related changes in NSC frequency varied along the neuraxis depending upon the rostral-caudal coordinate assayed, as a whole ageing resulted in a series of step-wise declines in NSC frequency starting with a ~40% decline at 6-months, and culminating in a ~90% decline by 24-months. In light of the positive benefits of physical exercise in man, and its ability to stimulate hippocampal neurogenesis (thereby augmenting learning and memory) in mice, we sought to determine whether exercise stimulates NSC proliferation, and whether this could slow or reverse age-related NSC decline. We now report for the first time, that running significantly increases NSC frequency in mice ≤18-months, but has the opposite effect in 24-month old mice. More importantly, we also report (by activating the growth hormone receptor) exercise also augments the regenerative capacity of aging mice, and can slow the age-related decline of endogenous stem cells. Current studies are not focused on determining whether this strategy is also effecting in slowing age-related cognitive decline in both normal mice, and models of neurodegeneration.

SYM-02-04

STEM CELLS, NEUROGENESIS AND DISEASES OF THE BASAL GANGLIA IN THE HUMAN BRAIN

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In the normal adult rodent brain, stem/progenitor cells in the subventricular zone (SVZ) of the lateral ventricle proliferate and migrate forming a neurogenic pathway, the 'Rostral Migratory Stream' (RMS), which extends from the SVZ to the olfactory bulb and provides for neuron replacement in the forebrain. **Purpose:** To investigate whether proliferating stem/progenitor cells and a RMS are present in the human brain and whether they provide for neuron replacement in the Huntington's diseased (HD) brain. **Methods and Results:** Our immunohistochemical studies on post-mortem human brain reveal the presence of proliferating progenitor cells and migratory neuroblasts in the SVZ and in a structure resembling the rodent RMS in the human forebrain. The human RMS begins at the SVZ, is closely associated with the rostral aspect of the caudate nucleus until it reaches the anterior olfactory cortex where it enters the olfactory tract en-route to the olfactory bulb. Also, we examined the SVZ in HD using immunohistochemical techniques to co-label cell cycle proteins, neuronal markers and astrocytic markers. Our results show that progenitor cell proliferation increases with pathological severity and 'CAG' repeat length in the HD gene and that stem cells in the SVZ form new neurons and glial cells in response to striatal cell death in HD. **Conclusion:** These results provide evidence of increased stem cell proliferation and neurogenesis in response to cell death in the diseased basal ganglia in the human brain and further indicate the plasticity and regenerative potential of the human brain.

SYM-03-01

PROCESSING OF COMPLEX SIGNALS IN THE AUDITORY SYSTEM STUDIED WITH IN-VIVO WHOLE CELL RECORDINGS

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Purpose: An important issue in neuroscience concerns the mechanisms that enable sensory neurons to respond selectively to certain stimuli and not to others. The feature we evaluate here is how the auditory system creates preferences for frequency modulations (FM) that sweep in one direction over those that sweep in the opposite direction. The generally accepted explanation for FM directionality is that it is created in the inferior colliculus (IC) through the linear processing of spectro-temporal asymmetries. The hypothesis assumes inputs to the IC are non-directional and thus both the preferred and non-preferred FM directions evoke the same degree of excitation and the same degree of inhibition. The different sweep directions, however, evoke different latencies such that excitation arrives first in response to the preferred direction whereas inhibition arrives first to the non-preferred direction. **Method**: We explore FM directional response properties of 25 IC neurons using whole-cell patch clamp recordings in awake bats, an animal that uses FM sweeps extensively in both its echolocation and communication signals. In 15 neurons we also calculated excitatory and inhibitory conductances evoked by upward and downward FM sweeps. **Results**-We found that contrary to the hypothesis of linear inputs and linear processing of spectro-temporal asymmetries, the inputs to all 15 IC cells were themselves directional and generated small directional differences in membrane potential that were amplified by thresholding into a far more pronounced discharge selectivity. **Conclusion:** The formation of selective discharge properties in sensory systems may not require major adjustments in connectivity but may instead be generated by minor adjustments of the strengths of existing inputs.

SYM-03-02

HONEYBEE LEARNING OF COMPLEX SCENTS: FROM MOLECULES TO MEMORY

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Purpose: Olfaction is a crucial sensory modality for all animals, but the mechanisms underlying processing and learning of complex scents are not well understood. Using the honeybee as a model, we investigated odour learning, formation and recall of scent memories. **Methods:** Using an associative learning paradigm, the Proboscis-Extension-Reflex Assay (PER), we trained three groups of honeybees (in each group, n=30) to three complex scent mixtures, each composed of 14 common floral odorants. After the bees had learnt the mixtures, we tested them with the individual odorants, to examine which ones they had learnt and which ones they ignored. We also examined innate preferences for the individual odorants. **Results:** Honeybees learnt a selection of 2 to 8 key odorants as representative for a scent mixture. Correlation analyses showed that neither physico-chemical properties of odorants, nor innate odour preferences play a role in key odorant selection, but concentration of an odorant in a mixture does, with odorants of high concentration being more likely to become a key odorant. The number and type of key odorants learnt was not fixed but depended on the composition of the scent mixture. The neural mechanisms underlying this process are likely to be found in the wiring of the first olfactory neuropil, the antennal lobes. **Conclusion:** Together with the bee's cognitive ability to generalize scents, the strategy of key odorant learning keeps flower recognition flexible: Bees are able to find and return to rewarding flowers within the constantly varying scent environment presented by flowering plants.

SYM-03-03

HEMISPHERIC SPECIALIZATION OF VISUAL AND AUDITORY FUNCTIONS

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Vertebrate species show surprising similarities in the functional specializations of the left and right hemispheres for processing visual information. The left hemisphere uses learnt programs or rules and focuses attention on the task being performed. The right hemisphere has broad attention to detect novel stimuli and it expresses intense reactions (e.g. agonistic behaviour and reaction to predators). Having a lateralized brain enhances the animal's ability to attend to two tasks simultaneously. Now we1 have also shown that attention to auditory stimuli in dogs is lateralized. **Methods** Dogs (n=14) were tested for lateralization of processing their species-typical vocalizations and the sounds of thunderstorms, using binaural playbacks. **Results** A significant preference was found to turn to the right (left hemisphere) to attend to vocalizations and left (right hemisphere) to attend to thunderstorm sounds. However, the specialization is not simply left for vocalizations and right for other sounds since dogs that were very fearful used the right hemisphere to attend to vocalizations, and there was a significant correlation between behavioural reactivity to the vocalizations and the lateralization index of head turning (r=0.87, p=0.00). **Conclusion** These findings, together with examples from other species, including primates, indicate that specialization of the left hemisphere for processing species-typical vocalizations and the right hemisphere for prosody pre-dates the evolution of human language and may have been an essential precursor of it. Specialization of the left hemisphere for routine processing of species-typical vocalizations that do not elicit fear is consistent with the idea that this hemisphere uses learnt rules. The right hemisphere is used when emergency responses may be required. 1. Siniscalchi, M., Quaranta, A. and Rogers, L.J. PLoS ONE, in press.

SYM-03-04

VOCAL LEARNING, MIMICRY AND BRAIN PLASTICITY IN SONGBIRDS AND THEIR RELEVANCE TO HUMAN SPEECH DEVELOPMENT

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The avian forebrain (pallium) does not have the layered structure of the mammalian cortex but, as is being discovered, it has many of the same functions. Importantly, birds share with humans (but few other orders and species) the ability to learn vocalisations. It has now been discovered (findings of 2008) that songbirds have mirror neurons: their High Vocal Centre x 'auditory-motor' neurons show a remarkable resemblance to 'visual-motor' mirror neurons in the frontoparietal cortex in primates. Evidence is mounting that, in humans, mirror neurons might have a role in language acquisition (as a gestural system), and this new discovery suggests that learned communication in birds might also involve activation of mirror-like neurons. Other researchers have shown that there are specialised forebrain circuits for vocal babbling in songbirds. Research results presented here address development and vocal learning of the Australian magpie (Gymnorhina tibicen). **Method** The vocalisations of juvenile magpies were recorded and analysed in specific timeframes of development (from 3-7 month of age), producing a large number of distinct vocal sequences for analysis and quantification. The example of the learning mimicry (a non-species-specific set of sounds) was used. **Results** The findings suggest that the acquisition of a vocal repertoire follows discreet stages of development and of phonetic play (babbling) and these roughly correspond to human language development. **Conclusion** These behavioural findings have a basis in brain mechanisms and suggest that songbirds may indeed offer a pertinent model for comparison to human language development.

SYM-04-01

UNDERSTANDING THE CONNECTIONS BETWEEN PAK1 KINASE AND FRAGILE X PROTEINS

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Loss of fragile-X-mental retardation protein associated FMR1 is the most common genetic cause of mental retardation in Man. FMR1 and the related FXR1 and FXR2 form part of a family that are well conserved in vertebrates. The Drosophila orthologue dFMR is genetically linked to the small GTPase dRac, and mouse FMR1 likewise linked to the Rac1 effector PAK1. I will describe the biochemical details underlying FXR1 as a direct binding partner for PAK1. Both FMR1 and FXR1 isoforms bind to PAK1 via their highly conserved central KH2 domain critical to the function of these RNA binding proteins. A mutant form of FXR1 with I304N substitution fails to bind PAK1 and is biologically inactive. PAK1 can phosphorylate FXR1 a regulatory site conserved in FMR1. A phospho-specific antibody directed to this site was used to show that cellular stress is accompanied by FMR1 and FXR1 phosphorylation at the conserved site, and by formation of stress granules containing phospho-FMR1 and active PAK. FXR1 is required for the proper development of muscles in zebrafish, with PAK1 phosphorylation and an ability to bind PAK1 being important, as tested by rescue experiments. We also show that chemical inhibition of PAK causes defects in zebrafish somite development, which is synthetic with respect to knock-down of FXR1 levels. This is the first clearly documented protein partner for FMR1 or FXR1 KH2 domain, the implications for signaling by fragile X proteins will be discussed.

SYM-04-02

DYNAMIN 1 INTERACTS WITH CALCINEURIN FOR SYNAPTIC VESICLE RETRIEVAL

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Purpose. Dynamin 1 (dyn1) is essential for synaptic vesicle endocytosis (SVE). Depolarisation of nerve terminals stimulates SVE by rapid dynI dephosphorylation mediated by the calcium-dependent phosphatase calcineurin (CaN). CaN also dephosphorylates at least 7 other key endocytic proteins. DynI has at least two splice variants producing short or long variants. It is dephosphorylated on four sites, two of which are only present in the long splice variant. Our aim was to determine the role of the two splice variants in dynI tail. **Methods.** Recombinant GST fusion proteins of dyn1-long and dyn1-short were expressed in bacteria and used to pull-down interacting proteins from rat brain nerve terminals. **Results.** While both dynI forms bound to the known partners syndapin and amphiphysin, dyn1-short exclusively bound to CaN. Binding was increased in the presence of calcium. Examination of the sequence of dyn1-short suggested the presence of a specific CaN binding motif that was not present in dynI-long. Site directed mutagenesis of the putative motif revealed CaN specifically binds to this sequence, which is not present in dynII or III. Peptide mimetics of the motif block CaN binding *in vitro* and inhibited depolarisation-dependent dynI dephosphorylation in synaptosomes. **Conclusions.** The results reveal the formation of an unexpected complex between dynI and its phosphatase in nerve terminals when nerve terminals are stimulated. This provides the first functional role for a dynI splice variant.

SYM-04-03

REGULATION OF CaMKII ACTIVITY

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CaMKII (Ca^{2+}/calmodulin stimulated protein kinase II) is a multifunctional Ser/Thr protein kinase that regulates normal neuronal function. CaMKII has also been implicated in epileptogenesis, the development of chronic pain syndromes and Parkinson's disease and post-ischaemia neuronal cell death and survival. CaMKII is regulated by multi-site phosphorylation and targeting to cellular microdomains through phosphorylation dependent interactions with specific proteins. Emerging evidence from several laboratories, including our own, suggests that these binding proteins can link CaMKII into multiple signalling pathways, and that varied functional outcomes occur following CaMKII phosphorylation depending on the identity and location of the binding partner. For most of the well characterised phosphorylation sites on all enzymes phosphorylation can occur wherever the enzyme is located in the cell (potentially to high stoichiometry), it changes the activity of the enzyme and often alters its interaction with binding proteins. This is also the case with CaMKII and its well characterised phosphorylation sites at Thr286 and Thr305/6. We have identified a site on CaMKII (Thr253) that may represent a new class of regulatory mechanisms controlled by phosphorylation. Thr253 is phosphorylated in vivo in response to physiological and pathological stimuli but it occurs only in a small proportion of the CaMKII molecules that are in specific cellular locations. Thr253 phosphorylation does not alter CaMKII activity per se but alters the interaction of CaMKII with binding proteins and therefore may target CaMKII activity. This type of regulatory mechanism can produced functional responses that are restricted to specific subcellular locations and /or certain cell types controlled by the specific binding proteins expressed in the cell at that location.

SYM-04-04

IDENTIFICATION OF NOVEL CDK5 SUBSTRATES INVOLVED IN ALZHEIMER'S DISEASE

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Purpose: Cdk5 is an important kinase that regulates cytoskeletal dynamics and neurite outgrowth/synapse formation in the brain. Deregulation of Cdk5 activity is known to be involved in several neurological disorders, including Alzheimer's disease (AD). Therefore, identification of its targets is crucial for understanding the defects in cytoskeletal dynamics observed in AD. **Methods:** Bioinformatics was used to identify proteins containing primary amino acid sequences that conform to a Cdk5 phosphorylation consensus sequence (S/TPXK/R). Genes of interest were cloned, expressed in cultured cells and purified via an N-terminal Flag-tag. Purified proteins were subjected to treatment without or with phosphatase, followed by incubation with Cdk5 and radiolabelled ATP in an *in vitro* kinase assay. Increased phosphorylation of phosphatase-treated protein compared to untreated protein indicates a physiological phosphorylation site. **Results:** Three novel substrates of Cdk5 were identified using this approach. Each is involved in the regulation of the actin cytoskeleton in the brain. Interestingly, the Cdk5 phosphosites are relatively resistant to dephosphorylation by phosphatases. This unusual phenomenon was previously observed for Cdk5 phosphosites on tau and CRMP2 that are hyperphosphorylated in AD. Phosphospecific antibodies are being generated to determine if these sites are also hyperphosphorylated in AD. **Conclusion:** Three new brain-enriched substrates of Cdk5 have been identified that display relative resistance to phosphatases and might be hyperphosphorylated in AD, as found for tau and CRMP2. If so, hyperphosphorylation of these proteins will be of great interest, since defects associated with the actin cytoskeleton occur early in the development of AD and may underlie initial memory and cognitive dysfunction in AD patients.

SYMPOSIUM 5 – THE AXON: ADVANCES IN CELLULAR PHYSIOLOGY AND PATHOPHYSIOLOGY

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SYM-05-01

AXONAL KV7 CHANNELS CONTROL MEMBRANE POTENTIAL STABILITY

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The axon initial segment (AIS) plays a crucial role in the initiation and modulation of action potentials propagating orthodromically into the axon and antidromically into the dendrites. In most central neurons the AIS and nodes of Ranvier express voltage-gated Kv7 channels at a high density, with weak expression in dendrites. **Purpose**: Whereas the gating properties of Kv7 channels are well described their functional roles in axons and nodes or Ranvier are not well understood. **Methods**: We used visually targeted subcellular inactivation of Kv7 channels with the selective blocker XE-991 (10 µM) combined with somatic and/or dual somatic-axonal whole-cell recordings of layer 5 pyramidal neurons in rat cortical slices. **Results**: Local inactivation of apical dendritic Kv7 channels changed the depolarisation-induced firing pattern from regular to high-frequency bursts (≥ 200 Hz) with only marginal changes in the subthreshold resting membrane potential and input resistance (2%, n = 5). In contrast, selective block of Kv7 channels in the AIS (or the first node) induced a significant depolarisation in resting membrane potential and increase in resting membrane resistance (13% and 64%, respectively, n = 13, $P < 0.01$). The inactivation of Kv7 channels in the AIS, but not at the first node, led to spontaneous recurrent episodes of spontaneous firing, resembling up- and down-states (n = 9). Selective inactivation of dendritic, but not axonal, Na^+ channels reversed the appearance of depolarisation-induced burst firing when XE-991 was bath applied (n = 8), supporting the idea that dendritic Kv7-channels gate burst firing. **Conclusion**: These data show that proximal axonal Kv7 channels are critical to maintain resting membrane stability and keep the axon initial segment below firing threshold.

SYM-05-02

DYNAMIC PROPERTIES OF CORTICAL AXONS AND SYNAPTIC TRANSMISSION

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Local intracortical communication depends upon transmission along the unmyelinated axons that connect nearby cortical cells to one and another. The properties of these axons and action potential generation may play an important role in the dynamical properties of the recurrent local cortical networks. Importantly, we observed that not only do cortical axons generate trains of action potentials at the axon initial segment in response to depolarization, but also that the depolarization itself propagates rapidly throughout the local axonal arbor. This depolarization may influence the propability of release at nearby synapses. This occurs in part through voltage dependent changes in action potential duration. The main K+ current that repolarizes action potentials in cortical axons is the rapidly activating, slowly inactivating D-current. Through inactivation of the D-current, depolarization appears to be capable of facilitating synaptic transmission between cortical neurons by broadening axonal action potentials. In addition, depolarization causes basal changes in resting Ca2+ concentrations in synaptic terminals that are within approximately 200 microns of the soma. These basal changes in synaptic Ca2+ levels may also influence synaptic transmission. These results indicate that the flexible electrophysiological properties of axons contribute significantly to intracortical processing and that the cortex may use a combination of “digital” and “analogue” processing in local networks.

SYM-05-03

DETERMINATION OF ACTION POTENTIAL INITIATION SITES IN UNMYELINATED SENSORY NERVE TERMINALS

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Little is known about the process of sensory activation of thinly myelinated (Aδ) and unmyelinated (C) sensory neurones. Both these types of neurone form unmyelinated nerve terminal arbors with many naked nerve endings. We have used extracellular techniques to record action potential (AP) activity directly from sensory nerve terminals in guinea-pig cornea *in vitro*. In the cornea, spike shape analysis and pharmacological blockade of Na^+ channels indicates that sensory terminals are able to support regenerative APs. This finding raises the possibility that APs are initiated in the portion of axon where sensory signals are transduced into a receptor potential. We investigated this possibility in cold sensory receptors where there is an ongoing discharge of APs. Mapping AP initiation sites by collision of ongoing APs with electrically evoked antidromic APs reveals that, in most receptors, APs are initiated at a site located close to the nerve terminal. In about 90% of recordings, nerve terminal impulses (NTIs) are diphasic (positive-negative), with the initial positive phase reflecting the discharge of membrane capacitance as the AP invades the nerve terminal. Therefore most NTIs are generated by APs that are initiated at a point from which they propagate antidromically to site of recording. However, in about 10% of recordings, ongoing NTIs had an initial negative component generated by influx of Na^+ at the site of recording, indicating that APs initiated very close the nerve terminal. The findings demonstrate that the process of sensory signal transduction and the regenerative process producing APs can exist in parallel with one another in cold sensory nerve terminals.

SYM-05-04

FUNCTION AND DYSFUNCTION OF HUMAN AXONAL ION CHANNELS IN NEUROLOGICAL DISEASE

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While routine nerve conduction studies can document the presence of a neuropathy, they do not provide further insight into pathophysiology. Measurement of nerve excitability by threshold tracking provides complementary information to conventional nerve conduction studies and may be used to infer the activity of a variety of ion channels, energy-dependent pumps and ion exchange processes activated during the process of impulse conduction. Following the validation of new testing protocols, clinical excitability techniques are now being adopted to complement diagnostic nerve conduction studies. A number of recent clinical excitability studies will be highlighted which have (i) established a mechanism for the neuropathy associated with renal failure; (ii) provided insight into the mechanisms of neurotoxicity associated with oxaliplatin chemotherapy; (iii) documented the acute effects of tetrodotoxin-induced blockade of axonal Na+ channels in patients following puffer-fish poisoning. While clinical nerve excitability studies are still in their infancy, there is growing evidence of their utility to provide novel insights into the pathophysiological mechanisms involved in a variety of neuropathic disease processes.

SYM-06-01

MECHANISMS OF RECEPTOR ENDOCYTOSIS

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The endosomal/lysosomal system of mammalian cells is a highly-dynamic organelle and the trafficking pathways within the endosomal system are fundamental for a wide variety of key cellular processes. My group is developing cellular and computational approaches to identify novel mammalian proteins associated with the endosomal system. Recently we have focused on a regulated form of endocytosis, termed macropinocytosis. Macropinocytosis is the engulfment of bulk quantities of extracellular fluid via the dynamic invagination of membrane ruffles. Its role in the acquired immune response, cell signalling and as a point of entry for a variety of intracellular pathogens make a greater understanding of macropinocytosis a priority. Despite this, the molecular mechanisms that coordinate the formation and maturation of the macropinosome are poorly understood. Exploitation of the macropinosome as a visual model of endosome maturation in living cells has enabled us to systematically examine the discrete roles played by the 3-Phosphoinositides and effector proteins in macropinocytosis.

SYM-06-02

SORTILIN - LIVE OR LET DIE?

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Purpose: Neurotrophins (NT) are neuronal growth factors essential for development and maintenance of the nervous system. They are released in two forms with opposing biological functions. The pro-forms induce apoptosis by engaging a death signaling complex comprising the p75NTR neurotrophin receptor and Sortilin. By contrast, their mature counterparts stimulate neuronal survival and differentiation by binding to p75NTR in association with one of the tyrosine receptor-kinases TrkA, -B, and -C, respectively. The dual role of p75NTR as a regulator of both death and survival has been extensively studied in knockout mouse models. We recently generated a Sortilin knockout mouse and showed that the receptor is essential for p75NTR to induce death of neurons during certain stages of development and aging, and upon brain injury. We have now asked the question if Sortilin, like p75NTR, may also facilitate neuronal survival. **Methods:** We have generated mice deficient for both Sortilin and p75NTR, characterized their phenotype and studied the underlying mechanisms at the molecular level. **Results:** We find that the TrkA, -B and -C phenotypes present in the p75NTR null mouse are severely aggravated on the Sortilin knockout background. **Conclusions:** Our findings demonstrate a novel and unexpected role of Sortilin as a positive modulator of neuronal survival. The presentation will put forward a model that describes how Sortilin may dissociate the dichotomous events of neuronal survival and death.

SYM-06-03

THE ROLE OF SORLA IN AMYLOID PRECURSOR PROTEIN METABOLISM

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SorLA is a membrane protein highly expressed in neurons of the brain and genetically associated with late-onset Alzheimer's disease (AD).Its homology to intracellular sorting receptors that shuttle proteins between endosomes and Golgi suggests a related function of sorLA in neuronal trafficking processes. We have shown that sorLA interacts with the amyloid precursor protein (APP) in vitro and in cells, and that both proteins co-localize in Golgi and endosomal compartments. In accordance, sorLA controls APP transport in the biosynthetic pathway and protects APP from proteolytic breakdown to the amyloid β-peptide (Aβ). In addition, we have identified sorting signals within the cytoplasmic region of sorLA that interact with adaptor proteins and regulate receptor sorting. Mutations of the sorting motifs showed that mislocalization of sorLA greatly affects APP processing, and can either lead to an increase or decrease in Aβ production dependent on the subcellular distribution of the receptor mutants. Finally, ablation of sorLA expression in PDAPP transgenic mice results in increased APP processing, higher Aβ levels and a dramatic increase in amyloid plaque formation. Since sorLA-deficient mice also exhibit other hallmarks of late-onset AD and patients with the disease are characterized by a selective loss of sorLA expression, our data collectively suggest that sorLA dysfunction is causally linked to the development of sporadic AD.

SYM-06-04

REGULATION OF P75 NEUROTROPHIN RECEPTOR CLEAVAGE BY SORTILIN ?

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The p75 neurotrophin receptor mediates neuronal cell death during both the development of the nervous system and in adult neurodegenerative conditions. In order to further understand the mechanism by which p75 causes neuronal degeneration we have been characterising its death-signalling pathway. We are also interested in how p75 signals initiated at axonal terminals are transduced to promote cell death. We have shown that p75 death signaling is mediated following extracellular cleavage of p75 to generate a c-terminal fragment (CTF), as inhibition of this cleavage process prevents p75-mediated neuronal death. The CTF can then be further processed by gamma-secretase, generating an intracellular domain fragment that can regulate gene transcription. As yet, the factors that regulate the cleavage process and down-stream signal transduction remain uncertain. We hypothesise that increased levels of p75 in the plasma membrane would contribute to increased generation of the CTF, and that retention of the CTF within recycling endosomes following cleavage would, in turn, contribute to cell death signaling. We have therefore been investigating the regulation of p75's cellular "lifecycle". In addition to regulation of cleavage by trkA-mediated signals and palmitoylation of p75, we have found a crucial role for the extracellular domain of p75 for trafficking to the plasma membrane. Another p75 co-receptor, sortalin, which is required for p75 to promote cell death, enhances the cell surface expression of p75, and may regulate p75 cleavage and hence death signaling in this way. The potential outcomes of directing p75 fragments into different endocytotic compartments will also be discussed.

SYM-07-01

MAGNETOENCEPHALOGRAPHIC NEUROIMAGING REVEALS TIMING OF HUMAN PRIMARY VISUAL CORTEX ACTIVITY DURING DYNAMIC MENTAL IMAGERY

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Purpose: The notion that mental imagery and perception may employ common neural mechanisms is still strongly debated. In this study we used magnetoencephalography to assess the role of primary visual cortex in visual mental imagery. Methods: Healthy adults (n = 11) performed a visual mental rotation task that required them to make laterality judgements about pictures of human hands at varying degrees of misorientation from an upright position. A control task used identical visual stimuli but did not require mental rotation. MEG was measured with a 160 channel, full cortex KIT-Macquarie MEG system. Beamformer spatial filtering was used to image the anatomical sources of neuromagnetic brain oscillations elicited during the experimental tasks. Results: High frequency gamma band (60-80 Hz) oscillations were sustained through the entire stimulus presentation. This gamma response was localised to primary visual cortex in all subjects. The power of the V1 gamma response increased significantly during mental rotation compared to the control condition during a window of 400-800 ms after stimulus onset. Conclusion: Using new spatial filtering techniques, the MEG is able to image activation of V1 during visual mental imagery. Our results agree with previous haemodynamic imaging studies and support the conclusion that that visual mental imagery engages neural machinery used in relatively early stages of visual perception. The greater temporal resolution of MEG provides new insight into the timing of activity in V1 during mental imagery. Our results show that the imagery-specific response has a relatively late timing, consistent with top-down activation by cognitive processes rather than bottom-up activation by perceptual processes.

SYM-07-02

MECHANISMS OF COGNITIVE CONTROL IN LANGUAGE PRODUCTION: EVIDENCE FROM FMRI

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Purpose: Traditionally, theories of language production have tended to omit a role for central or executive mechanisms in controlling speech output. We investigated whether language production might involve activity in both medial and lateral regions of the frontal cortex implicated in central mechanisms of competition monitoring and cognitive control, respectively. **Methods:** A series of functional magnetic resonance imaging (fMRI) studies were conducted with picture naming tasks that paired target objects with distractor words in order to induce interference effects in speech output. All studies involved healthy, normal participants. **Results:** In addition to activity in left hemisphere perisylvian language related areas, the anterior cingulate cortex and left ventrolateral prefrontal cortex showed significant activity in association with interference effects in naming across studies. **Conclusion:** The results indicate that central mechanisms of competition monitoring and cognitive control are likely to operate during language production in order to select words that meet the current goal of speech.

SYM-07-03

WHAT CAN WE "INFER" FROM BRAIN DATA ABOUT THE MIND?

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Arguably, a common definition of the mind: "The collective aspects of intellect and consciousness and unconsciousness which are manifest in some combination of thought, perception, will, emotion, memory and imagination", is concordant with the idea that mental activity is a subset of all brain activity. Previous arguments that neuroimaging has yet to tell us anything about the mind, have been set around a very narrow, formal structure, particularly related to linguistic processing. In this paper, I will move from rejection of a simplistic "unitary" theory of brain function – that any mental acitvity engages the whole brain, to the rather more difficult issue of cognitive neuroscientific analogues of psychological constructs and how data from imaging modalities fMRI, PET, MEG and EEG have explained behaviour and suggested new theoretical approaches to understanding the mind and conscious behaviour.

SYM-07-04

ABSTRACT NOT AVAILABLE

SYM-08-01

THE APPENDIX: VESTIGIAL ORGAN OR IMMUNE MASTER CONTROLLER?

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Inflammatory bowel diseases are characterised by persistent and severe intestinal inflammation, resulting in significant morbidity in young populations. There are abundant data indicating mechanisms by which inflammation is perpetuated, but relatively little on homeostatic controls of immune-mediated inflammation. Appendicectomy at a young age protects against subsequent inflammatory bowel disease. Using a novel murine model of appendicitis which is associated with accumulation of appendiceal regulatory T cells (T_{reg}), we demonstrated that appendicitis and appendicectomy afforded significant antigen-dependent protection against subsequent TNBS colitis. This effect was restricted to juvenile mice with adult mice having no such protection. Appendiceal T_{reg} expressed the gut-homing chemokine receptors, CCR9 and CCR10, and the intestine-specific adhesion molecule, $\alpha_4\beta_7$. In adoptive transfer experiments using fluorescently-labelled appendiceal lymphocytes, appendiceal T_{regs} preferentially migrated to colon rather than to mesenteric lymph nodes or spleen. Analysis of colonic lamina propria lymphocytes revealed a substantial accumulation of T_{reg} in protected mice. These T_{reg} produced the immunoregulatory cytokine, IL-10. Neutralisation of IL-10 abrogated the protective effect of appendicitis. Our findings suggest that appendicitis results in an expansion of appendiceal T_{reg} which subsequently migrate to the colon and mediate protection against colitis by secreting IL-10. These observations highlight the critical homeostatic role played by immune cells of the appendix and their migratory pathways to the colon, and they suggest novel avenues for future novel anti-inflammatory therapies.

SYM-08-02

LOCAL AND DISTANT EFFECTS IN A CHEMICALLY INDUCED MODEL OF ILEITIS IN THE GUINEA-PIG

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It is well established that neuropathies of the enteric nervous system (ENS) underlie IBS and related conditions. The ENS is best characterised in guinea-pig. We have established an experimental model for ileitis in the guinea-pig induced by injection of TNBS into the lumen of the ileum. In addition to distinct phenotypic changes of various subclasses of guinea-pig ENS neurons after TNBS-induced ileitis, we found that neurons at a remote site, the celiac ganglion, become hyper-excitable. **Purpose:** Here we investigate cellular and molecular changes leading up to the altered neuronal phenotypes observed in the guinea-pig TNBS model. **Results:** Shortly after TNBS injection we observed transient morphological changes at the site of injection which were largely resolved after 7 days. Similar changes were observed at remote sites. We quantified the appearance of T cells and neutrophils in the ileum and in the celiac ganglion. At all locations analysed we observed a rapid transient increase of these cells. Interestingly, increased numbers of T cells are found at the SMP up to 28 days post-injection. Using qPCR we determined increases in the levels of Nav1.2 and 1.6 mRNA at the myenteric plexus, whereas levels of NaV1.2, 1.6 and 1.7 mRNA were up in the celiac ganglion after inflammation. To increase our understanding of ENS and celiac neuronal excitability we used combined in situ hybridisation and immunohistochemistry to map the expression of voltage-sensitive Ca^{2+} and Na^{+} channels. Studies aimed at detecting inflammation induced changes of these molecules in neuronal subtypes are in progress. **Conclusion:** We have quantified changes in ileum morphology, T cell and neutrophil infiltration and expression of Na^{+} channel mRNA after injection of TNBS, near and away from the site of injection. These observations contribute to the identification of therapeutic targets to resolve the neuropathies associated with IBS and related conditions.

SYM-08-03

VISCERAL HYPERSENSITIVITY INDUCED BY TNBS COLITIS: CONTRIBUTION OF MECHANOSENSITIVE AFFERENTS

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A subset of Irritable Bowel Syndrome (IBS) patients develop symptoms following gastrointestinal infection or inflammation. These patients, termed post infectious IBS (PI IBS), continue to suffer visceral hypersensitivity despite remission from the initial insult and histological recovery from structural lesions. We are currently following two separate lines of enquiry to examine the effects of inflammatory events on extrinsic colonic afferents. Firstly we demonstrated circulating peripheral blood mononuclear cell (PBMC) supernatants from patients suffering PI IBS have a raised proinflammatory cytokine profile compared to healthy controls (Liebregts et. al. Gastro. 2007), and these supernatants sensitise all classes of colonic extrinsic afferents to mechanical stimuli (Hughes et. al. Gut, in press). An interesting observation was the selective inhibition of pelvic afferents by PBMC supernatants from healthy subjects, likely mediated by an opiodergic mechanism. The second line of inquiry involves the characterisation of extrinsic colonic afferent mechanosensitivity in a murine model of PI IBS. Trinitrobenzenesulfonic acid (TNBS) administration induces acute architectural damage to the colon and an accompanied visceral hypersensitivity from which the animal recovers. A delayed hypersensitivity subsequently develops in the absence of overt colonic damage (Adam et. al. Pain. 2007). Our recent results indicate a mild inflammatory event induces acute mechanical hypersensitivity of lumbar splanchnic but not pelvic colonic afferents, while the delayed hypersensitivity evident one month following administration involves sensitisation of both afferent pathways. We are currently investigating potential mediators of both the acute and delayed mechanical hypersensitivity.

SYM-08-04

MECHANISMS OF SYMPATHETIC DYSFUNCTION DURING GASTROINTESTINAL INFLAMMATION

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Inflammation impairs the sympathetic regulation of affected tissues by altering neuronal excitability and neurotransmitter release. As Ca^{2+} influx through voltage-gated Ca^{2+} channels (VGCC) is a critical determinant of excitation-secretion coupling in nerve terminals, the aim of this study was to characterize the effects of inflammation on noradrenaline release and VGCC in dissociated sympathetic neurons. We compared H^3noradrenaline release and Ca^{2+} current (I_{Ca}) amplitude in superior mesenteric ganglion (SMG) neurons from control mice and mice with dextran sulphate sodium (DSS; 5% w/v)-induced colonic inflammation. Changes to I_{Ca} were investigated using electrophysiological, Ca^{2+} imaging and PCR techniques. Depolarization-induced noradrenaline release from the inflamed colon and uninflamed jejunum was significantly impaired during DSS colitis. Colitis reduced I_{Ca} of SMG neurons by inhibiting ω-conotoxin GVIA (300 nM)-sensitive N-type Ca^{2+} channels. Ca^{2+} influx in SMG neurons, as measured with Fura 2-AM, was also attenuated by colitis. This was accompanied by a decrease in mRNA encoding the Ca^{2+} channel alpha subunit (CaV 2.2) and a rightward shift in the voltage-dependence of activation of I_{Ca}. N-type channel blockade by ω-conotoxin GVIA inhibited the release of noradrenaline in the colon and the conotoxin-sensitive component of noradrenaline release was significantly smaller during colitis. These findings show that DSS-induced colitis attenuates noradrenaline release in the colon and jejunum and suggest that inhibition of N-type voltage-gated Ca^{2+} channels plays an important role. This may contribute to functional alterations in both inflamed and uninflamed regions of the GI tract during inflammation.

SYM-09-01

SYNAPTIC PROPERTIES OF NEWBORN NEURONS

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In the dentate gyrus of the hippocampus new neurons are born from precursor cells throughout development and into adulthood. These newborn neurons hold significant potential for self-repair of brain damage caused by neurodegenerative disease. However, how newborn neurons integrate into the brain is far from understood due to a lack of knowledge of the molecular and functional characteristics of the synapses formed by newborn neurons. We have recently found that dissociated hippocampal cultures continue to produce new neurons at a surprisingly high rate. We have quantified the expression of synaptic proteins at newborn neuron synapses versus mature neuron synapses. We have found that the synapses formed by newborn neurons mature via the sequential recruitment of postsynaptic receptors. The metabotropic subtypes of glutamate receptors play an important role in the initial integration of newborn neurons, with the ionotropic receptors localising to synapses later on in newborn neuron maturation. We also observe a sequential recruitment of postsynaptic but not presynaptic scaffold proteins. GABAergic synapses were also found to mature faster than glutamatergic synapses. These data reveal the unique developmental profile of synapses of newly generated neurons, enabling us to determine how the function of newborn neuron synapses could contribute to restoring damaged neuronal networks.

SYM-09-02

NEUROMOTOR ADAPTATIONS WITH PHYSICAL ACTIVITY AND EXERCISE IN HUMANS

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There is growing evidence that physical activity and exercise produces adaptations within the central nervous system (CNS) that may be beneficial for motor function. To support this view, I will describe two separate examples of the neural mechanisms that mediate adaptations experienced by humans in response to physical activity. The first line of evidence suggests that regular physical activity may be beneficial for brain plasticity and learning. We have used a novel transcranial magnetic stimulation (TMS) paradigm (paired-associative stimulation, PAS) to examine the influence of regular physical activity on neuroplasticity in human primary motor cortex. In a group of physical active and sedentary young adults, we find that PAS induces larger changes in motor cortex excitability in the highly active subjects. This finding suggests that regular physical activity increases neuroplasticity in human motor cortex, which may be beneficial for the learning of new motor skills and for recovery from brain injury. The second line of evidence involves adaptations in the CNS after damage to the muscle with repetitive lengthening (eccentric) contractions. Single motor unit (MU) recordings were obtained from elbow flexor muscles to examine the behaviour of motoneurones after exercise-induced muscle damage. Substantial changes in MU activity were found immediately after eccentric exercise, which included an increase in MU recruitment and correlated MU discharge (synchronisation). These changes in MU activity remained elevated 24-hours after exercise, suggesting that they may contribute to the adaptation that occurs with a subsequent bout of eccentric exercise, where less muscle damage is known to occur. Together, these studies show that physical activity and exercise induce adaptations in the CNS that may be beneficial for motor function under some circumstances.

SYM-09-03

MAKING MAPS: A ROLE FOR TEN-M3 IN THE GENERATION OF VISUAL TOPOGRAPHY

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A key organising principle of the nervous system is its topography: the preservation of spatial relationships at multiple levels of neural processing. In the mammalian visual system this involves not only the topographic representation of each retina within visual areas, but also the alignment of crossed and uncrossed inputs from each eye to generate a single, cohesive map of visual space. Molecular guidance cues are known to play an important role in the generation of retinotopic maps, but little is known about how inputs from the 2 eyes are aligned with each other. We recently identified 3 members of the Ten-m/Odz family of transmembrane proteins as candidates for regulating visual connectivity, and have shown that Ten-m3 plays a key role in the generation of aligned retinal inputs. In Ten-m3 knockout (KO) mice, ipsilateral but not contralateral, inputs are dramatically mistargeted in the visual thalamus and midbrain. Connectivity between thalamus and cortex is largely normal. Thus, inputs arising from the two eyes are not aligned with each other in the visual cortex. This is associated with deficits in the performance of visually-mediated behavioural tasks. Remarkably, these deficits were rescued by acute monocular inactivation, suggesting they arise specifically as a consequence of the interocular mismatch. We hypothesise that the mismatch results in suppression which is relieved by monocular blockade. Ongoing electrophysiological studies and the pattern of c-fos staining following monocular or binocular viewing support this suggestion. We are also investigating the mechanisms by which Ten-m3 acts to regulate axonal guidance in an eye-specific manner in central targets. Current work indicates that other Ten-ms also play a role in regulating development of the visual pathway.

SYM-09-04

WHO DOES WHAT? UNRAVELLING THE CELLULAR COMPLEXITY OF THE BRAIN

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Understanding the role of individual brain cells in generating the specific behaviors that are produced by complex circuits is a fundamental goal in neuroscience. Our attempt to achieve this goal is based around dissecting the cellular complexity of the rostral ventrolateral medulla (RVLM). This region, located in the brainstem, provides essential tonic drive for the generation of sympathetic vasomotor nerve activity to the vasculature. Thus it plays a pivotal role in the regulation of blood pressure and appears to be involved in the development and maintenance of several cardiovascular diseases. In addition, most neural reflex circuits involved in the regulation of blood pressure exert their effects on sympathetic function via the RVLM. Work over the past two decades has demonstrated that there are two principal classes of output neurons that project from the RVLM to the sympathetic preganglionic neurons in the spinal cord. These can be differentiated, by their response to certain neurotransmitters and expression of enzymes responsible for the production of catecholamines, into C1 catecholamine and non-C1 cells. Whilst these two cell groups are the major output cells, emerging evidence points to important roles for both astrocytes and interneurons in determining the output from the RVLM. Using different combinations of replication-deficient different viruses and cell-specific promoters we have been able to target transgene expression to particular sub-groups of RVLM cells. Expression of excitatory and inhibitory proteins in the RVLM has enabled us to begin dissecting the functions of different RVLM cell groups and to understand their role in modulating blood pressure.

SYMPOSIUM 10 – EVOLUTION OF VERTEBRATE PHOTORECEPTORS AND RETINA

Sponsored by ARC Centre of Excellence in Vision Science

SYM-10-01

EVOLUTION OF INNER-RETINAL PHOTORECEPTION: THE VA AND MELANOPSIN PHOTOPIGMENTS

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In addition to its role as an image detector, the eye is responsible for a wide range of non-image forming behavioural and physiological responses to light - quite separate from vision. These differing sensory demands appear to have led to the evolution of two very different photoreceptor systems within the vertebrate eye. The discovery of the VA-opsin gene family in fish (Soni and Foster, 1997) led to the demonstration that a subset of retinal horizontal and ganglion cells are directly photoreceptive. This provided the first unambiguous evidence for a non-rod, non-cone photoreceptor in any vertebrate (Soni et al., 1998). Subsequent work combined electrophysiological, molecular and anatomical approaches to study the cell biology of these photoreceptors (Jenkins et al., 2003). A parallel line of investigation in mammals led to the discovery of another group of inner retinal photoreceptors. An interest in how mammalian circadian rhythms are regulated by light led to the development of a mouse model entirely lacking rods and cones (rd/rd cl). These mice retained a broad range of responses to light including circadian entrainment (Freedman et al., 1999), melatonin synthesis (Lucas et al., 1999) and pupil constriction (Lucas et al., 2001). These observations led to the identification of a small sub-set of photosensitive retinal ganglion cells (pRGCs) (Sekaran et al., 2003, Sekaran et al., 2005) that utilize the photopigment melanopsin (OPN4) (Hattar et al., 2003, Lucas et al., 2003). Recent studies have allowed the development of a working model of melanopsin phototransduction which shows a striking similarity to an invertebrate-like signalling pathway (Peirson et al., 2007). This presentation will examine and compare the evolution of the VA opsin and melanopsin photoreceptor systems of the vertebrates.

SYM-10-02

EVOLUTION OF THE VERTEBRATE EYECUP AND RETINA

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We present evidence that the vertebrate eye evolved through numerous subtle transitions, and we propose a scenario for the sequence of these successive changes. The great majority of the gradual transitions that occurred have not been preserved to the present time, either in the fossil record or in extant species, yet clear evidence of their occurrence remains. We describe the homologies that exist between the ciliary photoreceptors of chordate animals, consistent with a gradual emergence of the features of extant vertebrate photoreceptors. We discuss the remarkable “eye” of the hagfish, which has features intermediate between a simple light detector and an image-forming camera-like eye, and which we suggest represents a step in the evolution of our own eye that can be studied by modern methods. We also describe the important clues to the evolutionary origin of the vertebrate eye that can be found by studying the embryological development of the vertebrate eye, by examining the molecular genetic record preserved in our own genes and in the genes of other vertebrates, and through consideration of the imperfections (or evolutionary “scars”) in the construction of our eye. Taking these findings together, we suggest that the precursor of vertebrate eyes evolved as bilaterally paired organs similar to the pineal of non-mammalian vertebrates. Subsequently the neural processing power increased, by the evolution of retinal bipolar cells, and the optical apparatus also evolved, thereby providing image-forming vision.

SYM-10-03

EVOLUTION OF VERTEBRATE COLOUR VISION AND ROD VISION

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The duplex nature of the vertebrate retina was recognised over 200 years ago, where two relatively independent photoreceptor systems were hypothesized to operate over a wide range of light intensities; cones mediating photopic or bright light vision and rods mediating scotopic vision or dim light. With few exceptions, this functional dichotomy has been recognised in the retinae of representatives of all jawed vertebrate classes, although there has been intense selection pressure(s) to evolve photoreceptor subtypes within these two broad functional groupings in order to differentially sample the diverse light environments encountered both above and below the water. However, in the jawless vertebrates (lampreys and hagfishes), which have remained relatively unchanged for over 500 million years and represent the earliest stages in vertebrate evolution, the distinction between rods and cones is not well defined. Anatomical, spectral, molecular and functional studies on the complement of photoreceptor types in extant agnathans reveals that our vertebrate ancestors possessed at least four types of photoreceptors each with a different visual pigment, providing the capacity for colour discrimination and spectral tuning over a wide range of light intensities. Although the distinction between some photoreceptor types remains equivocal based on classical criteria, photopic vision appears to have preceded scotopic vision in vertebrate phylogeny, where the hybrid nature of agnathan photoreceptors may reflects an evolutionary transition in visual function. Changes in the expression of visual pigments and spectral tuning within different phases of the lifecycle, as well as marked differences in the complement of photoreceptor types in representatives of the three extant families of lampreys, all of which occupy different visual environments, suggest that light has been a critical driver in the evolution of vertebrate photoreception. Together with current multidisciplinary investigations of retinal photoreceptors in hagfishes, it is hoped that a clearer prediction of the evolution of both photopic and scotopic vision in vertebrates will soon become a reality.

SYM-10-04

THE EVOLUTION OF VIOLET- AND UV-SENSITIVE VISUAL PIGMENTS IN BIRDS AND MAMMALS

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Purpose: A rod and four cone classes of visual pigments are found in birds but this is reduced to a rod and only two cones classes in mammals. In both groups, UV-sensitivity is present in some species but not in others. UV-sensitivity is conferred by the shortwave-sensitive (SWS1) class of cone pigments. The ancestral SWS1 pigment was UV-sensitive (UVS) but many birds and mammals have red-shifted violet-sensitive (VS) pigments. In mammals, these VS pigments have evolved on many occasions but generally involve a common mechanism whereby the phenylalanine residue at site 86 is replaced, generally with a polar residue. An exception to this is found in prosimian primates where site 86 either retains phenylalanine as in the Aye-aye or is occupied by different residues. In contrast to the mammals, UVS pigments in birds have evolved secondarily from an avian VS pigment. The molecular mechanism also differs, with the key change being the acquisition of cysteine at site 90. The purpose of the study was to determine the molecular basis for these red-shifted SWS1 pigments. **Methods:** Native and mutant SWS1 coding sequences were cloned from various species and expressed *in vitro* to obtain absorbance peaks. **Results:** *In vitro* spectral analysis of recombinant SWS1 pigments demonstrate that (1) Ser86 is the key change in the evolution of avian VS pigments, (2) the retention of Phe86 in the Aye-aye does not result in a UVS pigment, and (3) other prosimian pigments with different residues at site 86 are VS. **Conclusions:** The evolution of VS pigments in birds arose from a Phe86Ser substitution and in primates utilized a different molecular mechanism to that seen in other species.

SYM-11-01

REGULATION OF CNS MYELINATION BY INTEGRINS AND GROWTH FACTORS

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The signals triggered by the interaction of newly-formed oligodendrocytes and axons in the CNS are interesting in that they promote oligodendrocyte survival and important in that their failure appears to underlie the lack of repair in many patients with MS. We have used transgenic mice and myelinating co-cultures to examine the molecular mechanisms that underlie this interaction. We have shown previously that integrins regulate the oligodendrocyte survival enabled by axon contact by binding laminins expressed on and around the axons and by subsequent amplification of growth factor signaling. We find that the immunoglobulin superfamily molecule contactin is associated in oligodendrocytes with these laminin binding integrins and that amplification is inhibited by siRNA-mediated knockdown of contactin in oligodendrocytes. We further demonstrate that the signals from contactin and integrin were integrated by differential phosphorylation of the Src-family kinase Fyn. The combined effect of these two molecules is an enhanced activity of Fyn. We conclude, therefore, that a novel integrin/contactin complex integrates signals from extracellular laminins and axonal L1 to regulate oligodendrocyte survival. Turning to the next step, myelination, we find that mice expressing a dominant negative β1 integrin in myelinating oligodendrocytes require a larger axon diameter to initiate timely myelination. Myelinated axons, however, display normal myelin thickness. We conclude that in addition to any role in survival signaling β1 integrin also plays a key role in the axoglial interactions that sense axon size and initiate myelination, but not in those that determine myelin sheath thickness. Funded by the Wellcome Trust, the NMSS, the UK MS Society, the Gulbenkian PhD Program in Biomedicine and the Danish Medical Research Council.

SYM-11-02

GAS6 DEFICIENCY INCREASES OLIGODENDROCYTE LOSS AND MICROGLIAL ACTIVATION IN RESPONSE TO CUPRIZONE-INDUCED DEMYELINATION

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Oligodendrocyte death and microglial activation are common features of central demyelinating disease. It is now appreciated that a limited repertoire of cytokines can regulate these events. In particular, we have found that the cytokine, Gas6 and its receptors Tyro3, Axl and Mer (TAM) regulate oligodendrocyte survival and microglial activation in vitro. In cuprizone-induced demyelination, the expression of Axl, Mer and Gas6 mRNA was increased in the corpus callosum in a temporal profile correlating with the increased microglial/macrophage activation in this model. In contrast, expression of Tyro3 decreased, correlating with the damage and loss of oligodendrocytes. In Gas6-/- mice subjected to cuprizone-induced demyelination, the degree of demyelination was greater than in control mice, notably in the more rostral regions of the corpus callosum (myelination reduced by 36%) as assessed by luxol fast blue staining and ultrastructural analysis. Loss of myelin coincided with an increased loss of oligodendrocytes in Gas6-/- mice, throughout the corpus callosum. Microglial marker expression (IBA1) was also increased in Gas6-/- mice but this was restricted to the rostral segment of the corpus callosum. Together, these results suggest that TAM receptor activation and regulation can independently influence both oligodendrocyte survival and microglial activation after CNS damage and provide insights into potentially novel therapeutic strategies for central demyelinating disease.

SYM-11-03

ABSTRACT NOT AVAILABLE

SYM-11-04

MECHANISMS OF MYELINATION BY OLFACTORY ENSHEATHING CELLS

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Purpose: A crucial aspect of CNS repair is the remyelination of axons resulting in restoration of functional conductivity. Can olfactory ensheathing cells (OEC) provide this role in the damaged CNS and what determines their success? **Methods:** We have used dorsal root ganglion (DRG) and retinal explant co-cultures (RGC) and spinal cord demyelination models to ascertain OEC myelination potential and mechanisms. Immunohistochemistry, western blots (WBs), quantitative PCR, RT-PCR, lentiviral vectors and electron microscopy were used to ascertain OEC myelination potential. **Results:** Protein levels for protein zero (PO), myelin basic protein (MBP), myelin associated glycoprotein (MAG) and 2'3'-cyclic nucleotide 3' phosphodiesterase (CNP) showed spatial distribution within the olfactory bulb layers (n=6). Purified adult OEC (n=16) showed negative and positive regulation of protein and mRNA levels for the 4 myelin genes when compared directly to SCs (n=12). This was pronounced in medias containing cAMP, serum and NRGβ1. Adult OEC were unable to form compact myelin sheaths in the presence of NGF and/or BDNF in RGC (n=54) or DRG (n=22) co-cultures whereas SC and oligodendrocyte co-cultures had MBP and PO profiles. Spinal cord transplants of adult, postnatal and embryonic (GFP^+) OECs (n=30) showed the presence of PO myelin following an ethidium bromide lesion. **Conclusion:** OEC potential and ability to form myelin *in vitro* and *in vivo* is dependent on the age of the OEC not the calibre of the axon. Results indicate OEC have non SC-like myelin gene expression characteristics *in vitro* and do not follow the classic myelin formation seen by Schwann cells. These differences suggest separate regulatory mechanisms of myelin expression and may indicate an inability of OEG to form compact myelin in some situations.

SYM-12-01

MECHANISMS AND FACTORS GUIDING CORTICAL INTERNEURON MIGRATION

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Tan, S-S. Brain Development Laboratory, Florey Neurosciences Institute, TheUniversity of Melbourne, Parkville, 3010, Australia How cortical interneurons find their long-range destinations are of great interest in improving our understanding cortical neuron assembly. **Purpose**: To investigate the patterns of interneuron migration by a number of complementary approaches, including gene perturbation, analysis of mutant mice and direct imaging of live interneurons in cortical slice cultures. **Method**: The migratory environment of migrating interneurons was examined for the presence of specific molecules and surface receptors. Confocal microscopy to track movements of single migrating neurons was peformed at periodic intervals, on wild-type and mutant mice. A number of molecular pathways was studied, including Reelin-signaling and purinergic-receptor signaling. **Results and Conclusion:** Although cortical interneurons share similar goals with projection neurons in trying to reach the cortical plate, they employ vastly dissimilar strategies to get there. In this talk, I will demonstrate some key points of difference in their cellular and molecular strategies.

SYM-12-02

PATTERNING OF THE NEOCORTEX BY FGF-SIGNALING

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I will discuss our work on the role of FGF-signaling in controlling the size and nature of the rostral neocortex. We have evidence that Fgf8 has a general role in patterning rostral parts of the cortex, whereas other Fgf genes, such as Fgf17, have a selective role in regulating development of the frontal cortex subdivisions. For instance, Fgf17 mutants have hypoplasia of the dorsomedial frontal cortex, whereas the orbital frontal cortex appears normal. FGF-signaling represses expression of COUP-TFI and Emx2, transcription factors that promotes rostroventral and rostrodorsal cortical fate, respectively. On other hand, Fgf15 appears to have an opposite function – Fgf15-/- mutants have reduced COUP-TFI expression. Emx2/Fgf17 and COUP-TFI/Fgf8 compound mutant analysis shows that these transcription factors have opposing roles with FGF8 and 17 in frontal cortex patterning. We have evidence that COUP-TFI represses FGF-signaling - thus, COUP-TFI can attenuate rostral signals to modulate telencephalic regionalization and to promote differentiation at the expense of proliferation.

SYM-12-03

THE MULTIFUNCTIONAL RECEPTOR, NEOGENIN, IS REQUIRED FOR NEUROGENESIS AND NEURONAL MIGRATION IN THE EMBRYONIC MOUSE FOREBRAIN

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At the onset of neurogenesis, vigorous proliferation occurs within the ventricular zones of the embryonic forebrain. In the ventral forebrain, progenitors give rise to interneurons that migrate tangentially into the developing cortex in the dorsal brain. Abnormal neuronal migration is the underlying cause of several human disorders, including lissencephaly and epilepsy. Moreover, abnormal neurogenesis and migration are now being linked to other common disorders such as schizophrenia, dyslexia and autism. However, at present, little is understood about how extrinsic factors within the local environment influence progenitor division, cell fate determination and neuronal migration. We hypothesize that the multifunctional receptor, Neogenin, regulates neuronal differentiation and interneuron migration in the early forebrain. Neogenin has been identified as a receptor for members of the Repulsive Guidance Molecule (RGM) family and plays a pivotal role in the guidance of axonal projections during embryonic development. We have previously demonstrated that in the mouse at embryonic day 12 to 14, Neogenin is expressed on neural progenitors within the ventricular zones of the cortex and ganglionic eminences of the ventral forebrain. Furthermore, Neogenin is also present on young interneurons as they migrate from the ganglionic eminences into the cortical plate. Our preliminary examination of Neogenin null embryos has revealed perturbations in the development of both the early cortex and ventral forebrain. This phenotype may be explained, at least in part, by our recent studies in wildtype embryos demonstrating that Neogenin-RGMa interactions regulate both neurogenesis and interneuron migration in the embryonic mouse forebrain.

SYM-12-04

EXPRESSION OF MARKERS OF NEURONAL MIGRATION AND INTERNEURONS IN THE POST-NATAL HUMAN CORTEX

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In the adult primate brain it is undisputed that new neurons are produced by two neurogenic regions: the dentate gyrus of the hippocampus and the subventricular zone (SVZ), adjacent to the lateral ventricle from which new granule and periglomerular cells migrate to the olfactory bulb. However, it remains controversial as to whether the SVZ also gives rise to cortical neurons that migrate to the cortex during postnatal life. The human cortex quadruples in size during the first two years of life and, in particular, the cortical grey matter volume increases from birth to approximately 5 years of age and therefore recruitment of new neurons into the cortex may contribute to cortical growth. Measures of neuronal migration markers, DCX and NAV1, in the cortex of neonates to adults, indicate protracted neuronal migration well into postnatal life. Measures of markers of GABAergic interneurons in postnatal cortex from neonates to adults, displayed chronologically distinct and prolonged profiles of maturation. The prefrontal cortex becomes functionally mature after adolescence in primates and the morphological development of the prefrontal cortex grey and white matter correlates with the development of cognitive functions. This study provides molecular evidence for arrival of new neurons and protracted maturation of interneurons in postnatal life which may play a significant role in the prolonged postnatal development of the prefrontal cortex and consequently the behavioural and cognitive development of this region.

SYM-13-01

FRONTOTEMPORAL LOBAR DEGENERATION WITH TDP-43 PROTEINOPATHY

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Major discoveries have been made in the recent past in the genetics, biochemistry and neuropathology of frontotemporal lobar degeneration (FTLD), the second most frequent cause of early-onset dementia after Alzheimer's disease (AD). TAR DNA-binding protein of 43 kDa (TDP-43), encoded by the TARDBP gene, has been identified as the major pathological protein of FTLD with ubiquitin-immunoreactive inclusions (FTLD-U) with or without amyotrophic lateral sclerosis (ALS), and sporadic ALS. Recently, mutations in the TARDBP gene in familial and sporadic ALS with and without FTLD have been reported which demonstrate that abnormal TDP-43 alone is sufficient to cause neurodegeneration. Several familial cases of FTLD-U, however, are now known to have mutations in the progranulin (GRN) gene, but granulin is not a component of the TDP-43- and ubiquitin-immunoreactive inclusions. Further, TDP-43 is found to be a component of the inclusions of an increasing number of neurodegenerative diseases, including AD. Other FTLD with TDP-43 proteinopathy (FTLD-TDP) entities include: FTLD with valosin-containing protein (VCP) gene mutation and FTLD with ALS linked to chromosome 9p. Molecular, biochemical, and neuropathologic studies indicate a close association between genotype and neuropathologic phenotype. In summary, recent discoveries have generated new insights into the pathogenesis of a spectrum of disorders called TDP-43 proteinopathies including: FTLD-TDP, FTLD-TDP with ALS, ALS, and a broadening spectrum of other disorders. It is anticipated that these discoveries and a revised nosology of FTLD will contribute toward an accurate diagnosis, and facilitate the development of new diagnostic tests and therapeutics.

SYM-13-02

MUTATIONS IN TDP-43 UNDERLYING FAMILIAL AND SPORADIC FORMS OF MND/ALS

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Amyotrophic lateral sclerosis (ALS, otherwise known as motor neuron disease, MND) is a rapidly progressive neurodegenerative disorder that leads to the loss of motor neurons and death within 3 to 5 years of first symptoms. Around 10% of ALS cases are familial (FALS), while the remainder occur sporadically (SALS). Known ALS genes combined only account for about 20% of familial ALS cases, or around 2% of all ALS cases. Frontotemporal dementia (FTD) is estimated to be associated with between 3 and 22% of ALS cases and families with co-morbid ALS and FTD are increasingly being recognised. The pathogenesis of ALS involves multiple molecular mechanisms. To date, research of disease mechanisms has focused on SOD1, however SOD1 pathology is rare in ALS. A new disease mechanism involving the TAR DNA-binding protein (TDP-43) has recently been identified. Ubiquitin positive protein aggregates are a common pathological hallmark of ALS and FTD and misfolded TDP-43 is a principal component of these aggregates. We identified rare pathogenic mutations in TDP-43 among FALS and SALS cases. This strongly suggests that aberrant TDP-43 plays a direct pathogenic role in ALS as a whole. The discovery of mutant TDP-43 also offers an opportunity to develop cell and animal models to investigate the biological basis of ALS as well as test novel therapeutic strategies. The function of TDP-43 in the nervous system and its mechanistic role in neurodegeneration is unclear. We are using molecular genetic and cell biology strategies to investigate the functional consequences of mutations in TDP-43 and recapitulate pathological features of TDP-43 in ALS.

SYM-13-03

IDENTIFYING NEURODEGENERATIVE MECHANISMS WITH MODEL CELL SYSTEMS

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Alpha-synuclein has been identified as a central component of Parkinson's Disease (PD): gene duplication or mutant alleles of alpha-synuclein [SNCA/PARK1] result in familial PD while Lewy bodies, cytoplasmic inclusions consisting primarily of aggregated alpha-synuclein, are a defining feature of PD. Although alpha-synuclein can associate with synaptic vesicles, little is known about either its native function or the mechanism by which it contributes to PD. The yeast *Saccharomyces cerevisiae* is a powerful experimental system for studying such complex biological processes. Using yeast PD models, in which induction of alpha-synuclein recapitulates many PD features, several genetic screens identified genes that modify alpha-synuclein induced cytotoxicity. Among 5000 genes screened we found that overexpression of the yeast orthologue (YPK9) of PARK9, a predominantly neuronal P-type ATPase responsible for a recessive form of early-onset parkinsonism, ameliorated alpha-synuclein cytotoxicity. Yeast cells lacking YPK9 are sensitised to manganese while overexpression of YPK9 confers resistance to manganese, suggesting that PARK9 is a manganese pump; a significant finding as manganese is a risk factor for PD. A separate genome-wide screen identified genes (potential PD risk factors) whose loss sensitises cells to levels of alpha-synuclein that are non-toxic in wild-type cells. Clusters of these genes were associated with the mitochondria, an organelle closely linked to PD, membrane trafficking and specific cell signaling pathways. The success of using a model system will be expanded to identify modifers of cytoxic TDP-43, a protein closely linked with sporadic ALS, FTLD-U and recently associated with Lewy body disorders.

SYM-13-04

IDENTIFICATION OF A NOVEL GENE FOR FRONTOTEMPORAL LOBAR DEGENERATION – MOTOR NEURON DISEASE

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Frontotemporal lobar degeneration (FTLD) is the most common cause of dementia after Alzheimer's disease in people aged under 65. TAR DNA binding protein (TDP- 43) has been shown to pathologically define both FTLD and motor neuron disease (MND). The FTLD-MND locus on chromosome 9 is the only major outstanding autosomal dominant dementia locus for which the causal gene has not been identified. Here we identify this gene (*PD9*) by positional cloning in an Australian pedigree with familial FTLD-MND and show how mutations in *PD9* result in TDP-43 translocation to the cytoplasm, providing a common mechanism for this and related neurodegenerative disorders. The *PD9* mutation is located in the 3' untranslated region (UTR) and mutation screens in cohorts of familial FTLD or presenile dementia patients identified a further four mutations. Functional analyses of two 3' UTR mutations using chimaeric luciferase constructs revealed significantly increased gene expression. No overt pathologic staining of PD9 protein was observed in the brains of *PD9* mutation carriers. Over-expression of *PD9* cDNAs in neuronal cell lines shunted TDP-43 from the nucleus to the cytoplasm, re-capitulating the neuropathology of cytoplasmic inclusions of TDP-43. Treatment of cells with drugs known to affect PD9 protein mimicked the effect of *PD9* overexpression, resulting in the translocation of nuclear TDP-43 to the cytoplasm. Since PD9-acting drugs can modulate the cellular correlates of the pathological features of both FTLD and MND, our results suggest the therapeutic potential of the known PD9 drugs as clinical treatments for the TDP-43 proteinopathies.