Age-related changes in blood-brain barrier integrity in C57BL/6J mice

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Aging is the main risk factor for most chronic diseases, disabilities, and declining health. It has long been proposed that senescent cells—damaged cells that have lost the ability to divide—drive the deterioration that underlies aging and age-related diseases. However, definitive evidence for this relationship has been lacking. The use of a progeroid mouse model (which expresses low amounts of the mitotic checkpoint protein BubR1) has been instrumental in demonstrating that p16-positive senescent cells drive age-related pathologies and that selective elimination of these cells can prevent or delay age-related deterioration. Experiment designed to dissect how senescent cells develop in BubR1 progeroid mice and how they contribute to aging in this model will be presented. Senescent cells have become attractive therapeutic targets for the treatment of aging and age-related diseases. The potential applications and implications of such intervention strategies will be discussed.
Global Epigenetic Changes and Derepression of Transposable Elements in Replicative Senescence

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Replicative cellular senescence is an important tumor suppression mechanism and also contributes to aging. Progression of both cancer and aging include significant epigenetic components, but the chromatin changes that take place during cellular senescence are only beginning to emerge. We used high throughput methods to map the chromatin landscape in senescence. We report that senescent human cells exhibit widespread DNA hypomethylation and “chromatin opening”, but also more focal DNA hypermethylation and “chromatin closing”. Hypomethylation and chromatin opening occurs preferentially at gene-poor, late-replicating lamin-associated domains and is linked to mislocalization of the maintenance DNA methyltransferase (DNMT1) in cells approaching senescence. Low-level gains of methylation and chromatin closing are enriched in CpG islands and promoters. Paradoxically, global hypomethylation and hypermethylation of CpG islands and associated chromatin changes are also reported to be features of cancer cells. Hypermethylation of CpG islands promotes silencing of tumor suppressor genes. Global hypomethylation of cancer cells is thought to promote genome instability and de-repression of repeat elements, including retrotransposons. In turn, retrotransposition can also perturb genome integrity, activate and inactivate oncogenes and tumor suppressors, respectively. Remarkably, in senescent cells, the chromatin of major retrotransposon classes, Alu, SVA and L1, becomes hypomethylated and relatively more open, leading to an increase in their transcription and ultimately transposition. Constitutive heterochromatin in centromeric and pericentromeric regions also becomes relatively more open, and the transcription of satellite sequences increases. Our results suggest a fundamental reappraisal of the relationship between senescence, aging and cancer. While senescent cells do contribute to tumor suppression, at the epigenome level this tumor suppression appears to be flawed. Features of the senescent epigenome, such as activation of retroelements and methylation of CpG islands, are predicted to promote malignancy. Hence, accumulation of senescent cells with age equates to an accumulation of cells at risk of malignant progression, perhaps contributing to the age-associated increase in incidence of many cancers.

Notes:
Mechanisms of Immunity Loss in the Skin of Older Adults

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The overarching goal of our collaborative project was to both further a productive transatlantic collaboration and to enhance our understanding of age-related immune defects occurring in the skin and other less well examined sites of local immune responses. Another goal was to critically examine to what extent the popular and widely used rodent aging model (laboratory mice) can provide accurate and insightful information on immune aging in humans. We will report our progress to date with rich collaborative studies in the humans; the ongoing experiments to understand immune skin changes with aging; and the few useful parallels and many profound differences between skin immunity in old mice and elderly humans. We will describe the numerous experiments to mimic the human delayed-type hypersensitivity (DTH) response with proteins, adjuvants, peptides, live and killed microorganisms. We found that old mice, similarly to the old humans, exhibit depressed DTH in response to a large foreign protein, keyhole limpet hemocyanin (KLH), but that few other stimuli could elicit reproducible DTH in adult or old mice. We further found that numbers and activity of T regulatory (Treg) in the mouse skin do not correspond to human Treg changes and kinetics following DTH induction and recall. These results are prepared for publication to serve as a roadmap for future interventions and manipulations of the aging immune system.

Notes:
Environmental factors play crucial roles in ageing; in particular nutrition and temperature exert a strong influence on lifespan. In many species, optimal dietary restriction (DR) extends lifespan, and further reduction in food availability decreases lifespan. We found that as bacterial food levels are reduced over 10 orders of magnitude, C. elegans lifespan initially increases, then decreases and subsequently increases again. This DR effect occurs at 15°C, 20°C and 25°C, with food and temperature showing approximately additive effects.

We discovered that this complex effect of DR is blunted bidirectionally in a double mutant of daf-7/TGF-beta and tph-1 tryptophan hydroxylase (the rate-limiting enzyme in serotonin synthesis). daf-7 tph-1 double mutants live longer at food levels that decrease wild type lifespan, and conversely live shorter at food levels that increase wild type lifespan. Attenuation of the wild type food response implicate these signalling pathways in nutrient sensing, consistent with the expression of daf-7 and tph-1 in sensory neurons. Using microfluidics-enabled high-throughput imaging, we automated the analysis of daf-7 and tph-1 transcriptional reporter expression in single sensory neurons under diverse food and temperature conditions. daf-7 and tph-1 expressions are both food and temperature sensitive, enabling the animal to integrate these environmental inputs and respond holistically to modulate lifespan. This response system acts in a graded manner. Genetic and molecular epistasis experiments reveal that daf-7 and tph-1 signal in parallel and regulate each other via cross talk and feedback; the regulatory interactions suggest a mechanism for generating graded outputs. Together, our results show how representation and integration of environmental inputs at the level of sensory neurons by gene expression leads to functional changes in lifespan.

Notes:
Aging-associated thymic involution is independent of androgen signaling

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Immunosenescence, or the decrease in immune system function with aging, is a major contributor to the general decrease in the quality of human health associated with aging. A primary cause of aging-related immunodeficiency is the postnatal involution of the thymus with age, which results in decreased production of naïve T cells. Identification of the mechanisms by which this process occurs is of clear clinical relevance, and may identify new therapeutic targets for inducing thymic rebound and immune system regeneration. Current models suggest that the primary mechanism controlling initial aging-related thymic involution is the increase in sex steroid signaling, and in particular androgen signaling, associated with puberty – since surgical or chemical castration in humans or mice induces transient thymic recovery and increased T cell production, and Androgen Receptor (AR) deletion causes increased thymus size. Indeed, based on this model, sex steroid ablation has already been proposed as a therapeutic approach to ameliorate or reverse the effects of thymic involution with age. We have tested the hypothesis that AR signaling regulates aging-related thymic involution, via genetic analyses in mice. Our data show that AR null mice (Ar<sup>tfm</sup>) exhibit larger maximal thymus size, but initiated thymic involution with normal timing and hallmarks of involution, and that these functions require Ar expression in thymic epithelial cells (TECs). Inducing Ar deletion postnatailly also increased thymus size, but did not prevent re-involution. Ar expression in TECs was however required for castration-induced thymic rebound. We further show that the thymus can rebound repeatedly after repeated sex steroid ablation (SSA) with luprolide, although subsequent rebounds are not as effective. Finally, lupron injection after surgical castration does not affect thymus size. Collectively, our data establish that while AR signaling in TECs controls thymus size and is necessary and sufficient for sex steroid ablation (SSA)-induced rebound, the mechanisms controlling thymic involution are androgen-independent.

Notes:
S6K and Ageing

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Mutations that increase lifespan in diverse organisms from yeast to mice have shown that the nutrient-sensitive mechanistic target of rapamycin (mTOR) signalling network is a key regulator of ageing. Furthermore, the mTOR/pathway is pharmacologically tractable for intervention. For example, late-life administration of rapamycin extends lifespan in mice, and delays cognitive decline and immunisation failure. Understanding the downstream effectors of mTOR signalling that mediate the beneficial effects on ageing and age-related disease is therefore key for the development of approaches to increase healthspan. Previously, we have shown that mice globally lacking rpS6K1, a major downstream effector of mTOR, are long-lived and resistant to a broad range of ageing-related conditions, including metabolic disease, impaired motor function, immune dysfunction, cardiovascular disease and loss of bone mass phenocopying many of the effects of dietary restriction. To further delineate the tissues and pathways involved in this effect we have generated and physiologically phenotyped mice with conditional deletion of rpS6K1 in a number of tissues. For comparison, we have generated mice which overexpress another downstream target of mTOR, 4EBP1, in a tissue-specific manner. Mice lacking rpS6K1 or with enhanced 4E-BP1 activity in adipose tissue or lacking rpS6K1 in pancreatic beta cells have minimal metabolic phenotypes and are surprisingly not resistant to the effects of high fat diet. Studies in mice lacking rpS6K1 either in the entire central nervous system are in progress but mice with deletion of rpS6K1 in specific hypothalamic neuronal populations demonstrate mild but divergent effects upon glucose homeostasis. In contrast, enhanced 4EBP1 activity in skeletal muscle provides protects against adverse metabolic effects, maintaining glucose tolerance and insulin sensitivity. Together these findings demonstrate unanticipated complexity in the role of rpS6K1 and 4EBP1 signalling in the regulation mammalian metabolism which is likely to impact upon our understanding of the role of mTOR signalling in ageing.

Notes:
Immune cells protect the mind: Implications for ageing and neurodegenerative diseases

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Over the past decades, it became clear from our work that the immune system has pivotal roles in central nervous system (CNS) function, including neurogenesis, cognitive capacity, maintenance and repair, and the ability to cope with stressful conditions and to fight off neurodegenerative diseases. However, given the fact that interactions of neurons or glial cells with T lymphocytes rarely occur within the healthy CNS parenchyma, the underlying mechanism of this protective response is still a mystery. The CNS is separated from the rest of the body by the blood-brain-barrier and the blood-cerebrospinal-fluid barrier, including the choroid plexus (CP) and the meninges. We suggested that the CP acts as a sentinel for danger signals coming from the CNS parenchyma and can translate these signals, through its dialogue with the residing immune cells, into a defense or support mechanism, depending on the requirements of the tissue. We found that that the CP is constitutively populated with CD4+ effector memory cells with a T-cell receptor repertoire specific to CNS antigens. We further observed that age-related dementia is strongly related to aging of the immune system. Thus, our working hypothesis is that aging and neurodegenerative diseases reflect loss of equilibrium between the immune system’s ability to maintain homeostasis and the emerging risk factors within the CNS. We found that during aging, the CNS specificity in this compartment is largely maintained, but the cytokine balance shifts, resulting in an overall reduction in the ability of the CP to support neurogenesis and cognition, on one hand, and trafficking and surveillance required for maintenance and repair, on the other. Partial restoration of cognitive ability in aged mice, by lymphopenia-induced homeostasis-driven proliferation of memory T cells, is correlated with restoration of the cytokine ratio at the CP, and modulates the expression of plasticity-related genes at the hippocampus. The cytokine milieu at the CP epithelium is affected by peripheral immunosenescence and immune malfunction, with detrimental consequences to the diseased or aged brain. Amenable to immunomodulation, this interface is a unique target for arresting age-related cognitive decline and neurodegenerative diseases by immunemodulating it for optimizing its proper functioning as a selective and educative gate the for recruitment of inflammation-resolving cells to the CNS parenchyma.

Notes:
The interaction between diet and the microbiota and its influence on health in the Elderly

Ian B. Jeffery - on behalf of the ELDERMET consortium

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Over the last four decades, the life expectancy of elderly men and women has increased dramatically worldwide. However, the number of years an individual can expect to live a healthy life has not kept pace. This has resulted in an increasing proportion of our society that are living with age related disabilities related to inflamm-aging, sarcopenia and depression. The study of the microbiota and the microbiome is an area that is gaining recognition due to the potential of the microbiome to influence healthy ageing. There is a growing acceptance of the influence of diet upon the microbiome and the importance of this on clinical measures.

The ELDERMET study investigated the microbiota composition of a sizable cohort of elderly individuals living in the community and in residential care. There was significant associations between the microbiota composition and the reported long-term diet of the individuals as well as to residence location. Within the residential care we see changes in the microbial composition of the subjects that has significant associations to the changes in health related variables of the individuals.

Although these changes in older subjects are associated with diet, it has not yet been shown if dietary modulation can restore microbial community. Therefore it is the goal of the European Commission funded NuAge project (www.nu-age.eu) to combine microbiota studies and high quality dietary information, as well as dietary interventions, to fully access the diet-microbiota-health relationships in a multinational longitudinal study. This study will record extensive physical and clinical measurements and will seek to correlate observed changes with alterations in the microbiome, inflammasome and peripheral blood lymphocyte epigenome in the subjects.

Notes:
Effects of aging on M-cell maturation and function in Peyer's patches

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The gastrointestinal tract is continuously exposed to large amounts of commensal and pathogenic microorganisms. As well as mounting an effective immune response against food-borne pathogens, the mucosal immune system must also recognise the harmless antigens (Ag) associated with food and commensals and generate immunological tolerance against them. A single layer of intestinal epithelial cells bound by tight-junctions limits the access of intestinal pathogens into host tissues. To mount an immune response, gut lumenal Ag must first be transported across the intestinal epithelium into the gut-associated lymphoid tissues (GALT) such as the Peyer’s patches, colonic patches and isolated lymphoid follicles. Located within the specialized epithelia overlying these GALT (the follicle-associated epithelium; FAE) are M cells. These unique epithelial cells are specialized for the transcytosis of lumenal particulate Ag and pathogens. M cells enable the GALT to sample the contents of the intestinal lumen to elicit an appropriate immune response. The mucosal immune response in the gastrointestinal tract is significantly compromised by aging and is associated with diminished Ag-specific IgA antibody titres in the intestinal lumen and a decreased ability to generate tolerance to harmless Ag. Little is known of the mechanisms underlying the decline in intestinal immune function. Furthermore, nothing was known of the effects of aging on M cells. We show that M-cell density in the FAE of aged mice was dramatically reduced. As a consequence, aged Peyer’s patches were significantly deficient in their ability to acquire gut lumenal Ag. Further analysis revealed that although M-cell differentiation was unaffected, aging specifically impaired their functional maturation. These data reveal an important ageing-related defect in the mucosal immune system’s ability to sample luminal antigens. Identification of the mechanisms that underpin the dramatic decline in M-cell status in aged mice will help identify novel approaches to improve mucosal immunity in the elderly.

Notes:
Changes in gut immunity during ageing

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Neuro-glial regulation of gut barrier functions during life

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The enteric nervous system (ENS) is fully integrated within the gastrointestinal tract (GI). Composed of neurons and enteric glial cells (astrocyte-like cells), the ENS is a key regulator of GI functions independently of the central nervous system. Besides controlling GI motility, increasing evidence has also identified a central role of the ENS in the control of key intestinal epithelial functions such as gut barrier (paracellular and transcellular permeability), intestinal epithelial cell proliferation and barrier repair processes. During this talk, I will present data showing changes of ENS functions and phenotype during life (post-natal and ageing) and its consequences upon its control upon gut barrier functions. Furthermore, being at the interface between our body and the gut luminal content, the ENS is sensitive to changes in gut nutritional or microbiota derived factors which ultimately will impact upon its control of gut barrier functions.

Notes:
You’re only as old as the microbes you feel: ageing and the gut-brain axis

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Elie Metchnikoff suggested that ingestion of certain beneficial bacteria can prolong life, and experimental animal studies may support this idea. While there is no specific consensus on exactly how bacterial species change in old age, there is decreased gut microbial diversity. One likely consequence of this is constipation.

Aged mice have decreased frequency of propulsive contractions for both colon and small intestine. Organ-bath motility measurements were used to test mechanisms and treatments related to decreased frequency of migrating colonic motor complexes and constipation. The myenteric plexus programmes propulsion and we propose that myenteric chemosensory intrinsic primary afferent neurons (IPANs) are dysfunctional in ageing and also provide a potential therapeutic target for constipation-relieving probiotics.

IPANs either decrease in number or undergo degenerative changes in old age. Patch clamp recording from mouse myenteric IPANs allowed comparisons between function for young versus old animals. Aged mice have a prolonged refractory period following IPAN action potentials, decreasing excitability. Additionally, absence of the microbiome in young germ-free mimicked the effects of ageing on IPANs. A possible molecular correlate of IPAN dysfunction was suggested by data showing that increased IKCa channel opening prolongs the refractory period, which could be shortened by luminal beneficial bacteria L. rhamnosus JB-1 bacteria. We have shown that IPANs can sense luminal JB-1 within 10 seconds of application and then relay the signals not only to enteric motor neurons but toward the brain. Brain effects of JB-1 were mediated by afferent vagal signalling, two thirds of which was relayed by a newly discovered gut intramural nicotinic sensory synapse that may gate health promoting effects of probiotics.

Since ageing as a life event is reported to be programmed centrally by gonadotropin-releasing-hormone in the medial-basal hypothalamus, our results and the projections of vagal afferents suggest a possible enteric sensory anti-ageing pathway that may underlie Metchnikoff’s life prolongation theories.

Notes:
The role of the immune system is to protect from infections as well as cancer and to achieve this without harming the host. There is now overwhelming evidence that the immune system is remodelled with ageing and that its basic functions are compromised, increasing susceptibility to infection, cancer and autoimmunity and reducing protective immune responses to vaccinations. The mechanisms underlying these changes are now beginning to be understood, raising the possibility of developing interventions to improve immunity in old age.

Drivers for remodelling of the adaptive immune system include the atrophy of the thymus, which reduces the input of new T cells, which in turn requires expansion of the peripheral pool of T cells to maintain homeostasis. Over time the peripheral T cells become senescent, with shortened telomeres and loss of key co-stimulatory molecules required for their function. In addition these senescent cells being to take on the phenotype and character of cytotoxic cells and are pre-disposed to responding to self antigens. The regulatory cells that would normally prevent autoimmune reactions are reduced in function in older adults, thus predisposing the older adult to autoimmune disease. For innate immunity the mechanisms driving the decline in function of cells such as monocytes and neutrophils are less well understood. However one concept that is emerging is that these cells become insensitive to activatory signals, due to constitutive signalling through membrane receptors. The latter may be due to the presence of low level inflammation (inflammaging).

Finally, opportunities to correct the age-related changes in immunity are now being developed. These range from approaches to reverse thymic atrophy, to modulation of receptor signalling (inhibition of PI3 kinase signalling) in innate immune cells to restore their function.

Notes:
MicroRNAs in ageing

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Gene expression analysis in *C. elegans* and other species has revealed dynamic microRNA (miRNA) expression changes during aging. Recent work has demonstrated that mutations in certain miRNA genes significantly affect both the lifespan of *C. elegans* and the expression levels of known aging-associated genes. These observations show that miRNAs function in pathways that impact life span. We also showed that miRNA expression patterns are useful biomarkers of *C. elegans* aging. Given the high conservation of miRNAs across species, it is likely that insights uncovered by this research will have high relevance towards our understanding of aging in higher organisms and humans. Given this, we have begun to examine whether miRNAs are also biomarkers of human aging.

Notes:
Specific *E. coli* genes that modulate *C. elegans* longevity discovered in a large-scale screen

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Ageing is an intriguing and extremely complex process, which could be influenced by factors including genetics, nutrition and gut microbiota. The gut microbial genome is 150 times the size of the host genome. However, there is limited understanding of the function of many microbial genes and how they might affect mammalian longevity. The nematode *Caenorhabditis elegans* is a genetically tractable model organism used to study ageing. Our previous studies have shown that inhibition of folate synthesis in *E. coli* by gene deletion or drug treatment extends *C. elegans* lifespan without slowing bacterial growth and worm fecundity (Virk et al. 2012, BMC Biology 10, 67). To identify novel microbial genes and gene function to extend animal lifespan, we performed a systematic screen of around 1100 *E. coli* single gene deletions using stringent criteria. Only 9 mutants robustly increased *C. elegans* lifespan. In contrast to previous hypotheses, our results demonstrate there is no correlation between bacterial proliferation and *C. elegans* longevity. However, disruption of specific microbial metabolic pathways increases host longevity. We identified genes that disrupt synthesis of a folate precursor through mutation of *pabA* and *pabB*. Disrupting the folate cycle needed for biosynthesis does not extend lifespan, suggesting that excess folate rather than excessive biosynthesis is important. The other genes found to increase lifespan when deleted include cellular transporters, and regulators of gene expression. Most of the identified genes are conserved in a wide range of gram-negative bacterial species, and have been linked to bacterial pathogenesis. The current work provides the prospect of characterizing gut microbial genes as pharmaceutical targets to improve longevity.

Acknowledgements: We thank the BBSRC for funding this project

Notes:
The DAF-16/FoxO transcription factor controls growth, metabolism and aging in *C. elegans*. The large number of genes that it regulates has been an obstacle to understanding its function. However, recent analysis of transcript and chromatin profiling implies that DAF-16 regulates relatively few genes directly, and that many of these encode other regulatory proteins. We have investigated the regulation by DAF-16 of genes encoding the AMP-activated protein kinase (AMPK), which has α, β and γ subunits. *C. elegans* has 5 genes encoding putative AMP-binding regulatory γ subunits, *aakg-1*-5. *aakg-4* and *aakg-5* are closely related, atypical isoforms, with orthologs throughout the Chromadorea class of nematodes. We report that ~75% of total γ subunit mRNA encodes these 2 divergent isoforms, which lack consensus AMP-binding residues, suggesting AMP-independent kinase activity. DAF-16 directly activates expression of *aakg-4*, reduction of which suppresses longevity in *daf-2* insulin/IGF-1 receptor mutants. This implies that an increase in the activity of AMPK containing the atypical AAKG-4 γ subunit caused by direct activation by DAF-16 slows aging in *daf-2* mutants. Knock down of *aakg-4* expression caused a transient decrease in activation of expression in multiple DAF-16 target genes. This, taken together with previous evidence that AMPK activates DAF-16 activity, implies the action of these two metabolic regulators in a positive feedback loop that accelerates the induction of DAF-16 target gene expression. The AMPK β subunit, *aakb-1*, also proved to be up-regulated by DAF-16, but had no effect on lifespan. These findings reveal key features of the architecture of the gene-regulatory network centered on DAF-16, and raise the possibility that activation of AMP-independent AMPK in nutritionally replete *daf-2* mutant adults slows aging in *C. elegans*. Evidence of activation of AMPK subunits in mammals suggests that such FoxO-AMPK interactions may be evolutionarily conserved.
mTOR-dependent mitochondrial content drives the senescent phenotype

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Cellular senescence is marked by a number of distinct phenotypic changes including increased Reactive Oxygen Species (ROS) generation, a persistent DNA damage response (DDR) and a senescent-associated secretory phenotype (SASP). Mitochondria, which play a central role in energy production, have been implicated in senescence and apoptosis. Maintenance of mitochondrial density has been shown to be essential for cellular homeostasis and a tightly regulated process controlled by various nuclear encoded genes; however, its role in ageing is not clear, with several studies reporting contradictory results.

Here, we show that induction of a DDR is invariably accompanied by an increase in mitochondrial density which helps maintain senescence. We demonstrate that enhanced or depleted mitochondrial content in senescent cells by either overexpression or knock-out of mitochondrial biogenesis regulator PGC-1α, depletion of mtDNA or activation of Parkin-mediated mitochondrial clearance impacts on ROS, DDR and ultimately on SASP. Mechanistically, we demonstrate that mitochondrial mass increase during senescence is dependent on nutrient-sensing pathway TOR (target-of-rapamycin) known to play a major role in ageing and cancer. Finally, we show that decreased mitochondrial content by either mTOR inhibition or PGC1-α deletion reduces age-dependent increase in telomere-associated damage in mouse liver. We propose that mitochondria are essential for senescence.

Notes:
Colorectal cancer is a major cause of morbidity and death throughout the world. However, there is very large variability in incidence, both across geographical boundaries, and over time in the populations of countries undergoing major economic and social change where an increased risk is strongly associated with economic development and prosperity. Such variations provide excellent evidence for the importance of environmental factors in the aetiology of the disease, though the precise mechanisms involved remain poorly understood. Colorectal cancer is known to develop primarily via the adenoma-carcinoma sequence. Although there is much variability in the profile of genetic abnormalities between tumours, the general principle is that transformation from normal mucosa to carcinoma is driven by the acquisition of somatic mutations. It is also increasingly recognised that epigenetic changes play an important role in carcinogenesis. Transcriptional silencing by the aberrant hypermethylation of CpG islands in the promoter regions of tumour suppressor genes is the most widely studied epigenetic event and, when artificially induced, has been shown to drive transformation. Although epigenetic states are relatively stable from one generation of cells to the next, they reflect dynamic patterns of enzyme activity that are potentially malleable in ways that genetic sequences are not. Thus they provide a mechanism whereby genomes can respond to physiological or environmental signals. This raises the possibility that diet and other environmental factors may act through such epigenetic mechanisms to influence the vulnerability of the normal colonic mucosa to cancer. I will discuss the evidence for the involvement of epigenetic mechanisms in the earliest stage of carcinogenesis in the colon and present recent data on the influence of ageing and selected nutritional and metabolic factors.

Notes:
High glucose decreases monocyte SIRT1 activity, increases p65 acetylation and modulates TNFα secretion

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Histone protein acetylation has a regulatory effect on gene expression; the presence of acetyl groups promotes gene expression whilst their removal by deacetylase proteins inhibits expression. The Sirtuin family of NAD+ dependent deacetylase proteins modulates the cellular inflammatory response by deacetylating histones associated with inflammatory gene promoter regions and also the acetylation status of p65 in the NF-κB complex. Lack of SIRT1 activity is associated with ageing. Since the deacetylase activity of the sirtuin family is regulated by energy availability a potential link exists between between ageing and inflammation associated with metabolic conditions. To investigate this further, THP1 monocytes were treated with increased concentrations of glucose (0-50mM) in order to measure its effects on deacetylase activity, P65 acetylation status and TNFα secretion. To confirm whether the in vitro effects of glucose mimicked ex vivo effects on primary monocytes, whole blood or monocytes isolated from whole blood taken from consenting healthy volunteers was supplemented with glucose (50mM) for 6 and 24hours and the effect on TNFα secretion in response to LPS measured by ELISA. THP1 monocytes treatment with 50mM glucose over a 24hour period resulted in decreased sirtuin1 activity, and increased P65 acetylation but TNF production in response to LPS was diminished by high glucose. Whole blood and isolated primary monocytes supplemented with 50mM glucose and LPS showed an increase in TNFα secretion in response to LPS compared to blood or primary monocytes supplemented with 5mM glucose. These data support the hypothesis that elevated blood glucose frequently seen during ageing may modulate inflammation and the ageing process through inhibition of SIRT1 activity.

Notes:
Ageing and osteoarthritis: the role of chondrocyte circadian clock in controlling cartilage homeostasis

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Objective: Osteoarthritis (OA) is the most common joint disorder for which age is a major risk factor. Symptoms (pain, stiffness and joint swelling) of OA show clear time-of-day effects. This study aims to characterize the circadian clock in cartilage tissue and identify tissue-specific clock target genes, and to investigate if the circadian clock changes during ageing and in an experimental mouse model of osteoarthritis.

Methods: Cartilage explants were taken from aged and young adult circadian clock reporter mice (PER2::luc) and real-time bioluminescence recordings were used to characterize the clock properties. Time-series microarrays were performed to identify rhythmic cartilage genes. Rhythmic genes were confirmed by qRT-PCR using mouse tissue, primary chondrocytes and a human chondrocyte cell line. Experimental OA was induced in mice by destabilization of the medial meniscus (DMM). Articular cartilage was microdissected and subjected to microarray analysis.

Results: We demonstrate that mouse cartilage tissue and a human chondrocyte cell-line contain intrinsic molecular circadian clocks. The cartilage clock is reset by temperature signals, while circadian period is temperature compensated. Cartilage from aged mice demonstrated significantly lower amplitude oscillations in PER2::luc bioluminescence. We identified the first circadian transcriptome in cartilage, revealing ~3.9% (615 genes) of expressed genes had a circadian pattern of expression. This included genes involved in cartilage homeostasis and survival, and with potential importance in the pathogenesis of osteoarthritis. Several clock genes were disrupted in the early stages of degeneration in a DMM osteoarthritis model.

Conclusion Our studies reveal an autonomous circadian clock in chondrocytes, implicated in key aspects of cartilage biology and pathology. Consequently, circadian disruption (e.g. during aging) may compromise tissue homeostasis and increase susceptibility to joint damage or disease.

Notes:
The effect of chronic sleep deprivation on immunity in older adults

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Sleep duration has declined over the last century and loss of sleep has been related to various adverse health outcomes. Several epidemiological studies demonstrated that short (<6.5 hours per night) and long (>7.5 hours per night) sleep duration are associated with increased risk of morbidity and mortality. Physiological ageing is characterized by perturbations to normal sleep and by a functional decline of the immune system (immunosenescence). We hypothesize that among older adults, physiological lack of sleep could contribute to immunosenescence. To date, 67 older adults participated in the study. Sleep duration and other qualitative parameters (efficiency, latency, wake after sleep onset (WASO) and average sleep bout) were assessed by actigraphy, a non-invasive method of monitoring rest/activity cycles. Blood samples were taken to evaluate a variety of immune functions. Data obtained so far show that sleep does not affect immune function but several sleep variables are associated with changes in immune cell populations. As analyzed by multiple linear regressions controlled for age, sex and body mass index (BMI), the numbers of white blood cells (WBC), granulocytes, monocytes and the granulocyte to lymphocyte ratio, a biomarker of ageing, are all negatively associated with both sleep duration and efficiency (p<0.05). In addition, WASO and average sleep bout, two indicators of sleep fragmentation, also correlate with the granulocyte to lymphocyte ratio (p<0.05). Overall, these data indicate that sleep influences the distribution of immune cells in the blood. In particular, short sleep duration may promote a pro-inflammatory status, as suggested by the changes in WBC, monocytes and granulocytes, and bad sleep quality could worsen the skewing towards myelopoiesis, which is typical of ageing.

Notes:
How stem cells speak with immune cells

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The recent advances in stem cell biology have raised great expectations that diseases and injuries of the central nervous system (CNS) may be ameliorated by the development of non-haematopoietic stem cell medicines. Yet, the application of stem cells as CNS therapeutics is very challenging and the interpretation of some of the outcomes is not completely unambiguous. In fact, the initial idea that stem cell transplants work only via structural cell replacement has been significantly challenged by the observation of consistent cellular signalling between the graft and the host. Cellular signalling is the foundation of coordinated actions and flexible responses and it is well established that this signalling takes place through different pathways involving networks of interacting molecules, which in turn transmit patterns of information between cells. Sustained stem cell graft-to-host exchange of signals has led to remarkable trophic effects on endogenous brain cells and beneficial modulatory actions on innate and adaptive immune responses that ultimately promote the healing of the injured CNS. Among a number of promising candidate stem cell sources, mesenchymal/stromal stem cells (MSCs) and neural stem/precursor cells (NPCs) are being extensively investigated for their capacities to signal to the immune system upon transplantation in experimental CNS diseases.

Here I will focus on the main cellular signalling pathways that grafted stem cells use to establish a therapeutically relevant cross talk with immune cells, examined the potential role of local inflammation in these communications, and finally reflect on the forthcoming challenges related to the translation of these exciting experimental proofs into ready-to-use clinical medicines for inflammatory CNS diseases.

Notes:
POSTER ABSTRACTS

1. The Effect of ageing and chronic stress on regulatory CD19$^{+ve}$CD24$^{hi}$CD38$^{hi}$B cells

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B cells can play both protective and pathogenic roles in the same pathological setting. In recent years, subsets of B cells with immunoregulatory properties have been identified in murine models of autoimmune disorders and these cells down regulate immune responses via secretion of IL10. In humans, immature transitional B cells with a CD19$^{+ve}$CD24$^{hi}$CD38$^{hi}$ phenotype have been reported to regulate immune responses via IL10 production. We found the frequency and numbers of CD19$^{+ve}$CD24$^{hi}$CD38$^{hi}$ B$_{regs}$ were reduced in the PBMC pool with age. IL10 production following activation via either CD40, or Toll-like receptors was also impaired in B$_{regs}$ of healthy older donors. When investigating the mechanisms involved we found that CD19$^{+ve}$CD24$^{hi}$CD38$^{hi}$ B cell function was compromised by age-related effects on both T cells and B cells: specifically CD40 ligand expression was lower in CD4 T cells from older donors following CD3 stimulation and signaling through CD40 was impaired in CD19$^{+ve}$CD24$^{hi}$CD38$^{hi}$ B cells of older donors as evidenced by reduced phosphorylation (Y705) and activation of STAT3. Additionally, we also measured IL10 secretion by B cells after stimulation through CD40, or Toll-like receptors and reported a significant reduction in IL10 secretion by B cells with age. Further, a negative correlation has been observed between B$_{regs}$ IL10 production and serum autoantibody (Rheumatoid factor) levels in older adults. We therefore propose that an age-related numerical and functional deficit in CD19$^{+ve}$CD24$^{hi}$CD38$^{hi}$ B cells may contribute towards the increased autoimmunity and reduced immune tolerance seen with aging. Finally, on examining the synergistic effect of physical distress of a hip fracture and psychological distress of development of depressive symptoms on B$_{regs}$, we have reported a decline in frequency as well as a significant decline in IL10 producing CD19$^{+ve}$CD24$^{hi}$CD38$^{hi}$ B cells post stimulation in hip fracture patients with depressive symptoms compared to healthy older donors.
2. The older adult cell membrane fatty acid profile favours a pro-inflammatory phenotype

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During ageing, there is a loss of bone mineral density and lean mass with a concomitant increase in body fat mass. In turn, increased body fat mass is associated with a redistribution of adipose tissue, a decrease in lower body subcutaneous fat storage and an increase in the plasma non-esterified fatty acids (NEFA). We have investigated the hypothesis that there is an age-associated increase in NEFA in favour of saturated fatty acids which drive an inflammatory phenotype in monocytes during ageing.

Peripheral blood was taken from consenting healthy male volunteers from a young (18-30 years old) and midlife (>50 years old) cohort (n=14 per group) and the plasma NEFA profile and red blood cell fatty acid profile was examined for saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. Monocyte phenotype was examined by CD14, CD11b and CD36 expression and plasma cytokines (IL-6, IL-10 and TNF-α).

In older adults, circulating SFA, MUFA and PUFA were significantly elevated in older adults and membrane fatty acids were enriched in SFA, particularly palmitate C16:0 and lignoceric C24:0 acids, with age suggesting a reduced ability to store NEFA and a biosynthetic switch towards SFA accumulation in membranes with age. Palmitate is an important precursor for de novo ceramide, which may play an important role in driving inflammation (Gao et al, 2012). The circulating TNF and IL-6 concentrations were significantly higher and IL-10 concentrations were significantly lower in the plasma from older men than in healthy younger men. In addition, the mean CD14, CD36 and CD11b expression levels were increased in older compared to younger PBMC. Together these findings support the hypothesis that a change in lipid handling with ageing may predispose accumulation of membrane lipids which promote an inflammatory phenotype.

Reference
3. Age-related changes to lumbosacral spinal cord motoneurons that modulate bladder and bowel functions in male C57BL/6 mice.

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Incontinence and sexual dysfunction are often increased in the aged human population. In rats and mice the pattern of micturition and faecal clearance also changes with ageing and is suggestive of bladder and bowel dysfunction. In mammals, bladder and terminal bowel functions are regulated by co-ordinated activities of the autonomic and somatic nervous systems. Several spinal nuclei contain somatic motoneurons that innervate muscles involved in sphincter closure and copulation. These lumbosacral nuclei include the dorsolateral nucleus (DLN) and the spinal nucleus of the bulbocavernosus (SNB), homologs to the Onuf’s nucleus in humans. Another group of neurons in the sacral parasympathetic nucleus (SPN) regulate bladder emptying. Motoneurons in these nuclei are apposed by serotonin (5-HT), substance P (SP), vesicular glutamate transporter 2 (VGlut2), and corticotropin-releasing factor (CRF) inputs. In rats the number of these inputs is altered by ageing. Despite the increasing use of mice as a model, equivalent data in this species is lacking. We are therefore investigating if there are age-related changes to the inputs onto male C57BL/6 mouse spinal motoneurons involved in regulation of bladder and terminal bowel function. The first stage of our study has been to characterise the nature and density of inputs onto these motoneurons in young (3-4m) mice, and to compare these with inputs in aged (28-30m) animals. Inputs are quantified using ImagePro software in order to generate data on statistically significant differences between young and aged groups. To demonstrate that these inputs represent synaptic terminals, we have visualised them using transmission electron microscopy (TEM). Ultrastructural morphology of the motoneurons from the two age groups is also revealed by TEM. Data on the age-related morphological changes to these cells and to their inputs from other regions of the brain will provide important insights into how the development of bladder/bowel dysfunction in the elderly may be neuronally derived.

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4. Systems biology of ageing in haematopoiesis and the immune system

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Haematopoiesis is differentially compromised during the normal ageing process. B cell development in the bone marrow is severely affected while erythroid differentiation appears relatively unaffected. We have embarked on an ambitious project to determine the underlying mechanisms behind this apparent difference.

The combinatorial recombination of variable (V), diversity (D) and joining (J) gene segments within developing B lymphocytes contributes to the enormous variation in the binding specificities of mammalian antibodies. This V(D)J recombination results in genes that encode immunoglobulin molecules with different variable regions and thus different binding specificities.

In ageing pro-B cells, the primary immunoglobulin heavy chain antibody repertoire is restricted, resulting in impaired response to infection, but both the overall defect and the underlying mechanisms are not known. The mouse immunoglobulin heavy chain (Igh) locus contains 142 V genes that are able to recombine throughout a 2.5 Mb region of DNA. For a particular V gene to be used in recombination it must be brought into close spatial proximity to a DJ by looping of the locus and it must also be in a chromatin environment that is accessible to the recombination machinery. The exact epigenetic states that determine the likelihood of recombination for a particular V gene are poorly understood.

We have developed an unbiased next generation sequencing-based assay to determine the individual V, D and J genes used in each recombined allele within a B lymphocyte population. This V(D)J-seq assay enables the interrogation of antibody repertoires at unprecedented depth and resolution, and we are using this assay to understand the normal antibody repertoire and the nature of defects in the repertoire during ageing.
5. microRNAs – new promise in defeating muscle wasting

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As the ageing population increases, it is essential to determine the mechanisms involved in ageing of the musculoskeletal system. A common characteristic of ageing is loss of skeletal muscle (sarcopenia) leading to a decreased life quality. There is currently little data available showing involvement of epigenetic mechanisms in musculoskeletal ageing although such mechanisms are undoubtedly involved in the ageing process (1). microRNAs (miRNAs) are novel regulators of posttranscriptional gene expression. microRNAs control myogenesis, regeneration, ageing and cellular programming (2, 3, 4). Cell fate decisions take place during muscle regeneration. To unravel the role of microRNAs during ageing, we are studying the role of the most promising microRNA in this context. Our preliminary data shows differential expression of miRNAs during muscle ageing, atrophy and regeneration. We validated genes related to muscle biology and chromatin remodelling factors as miRNA targets in vitro. This could lead to design of novel therapeutics for individuals affected by age-related musculoskeletal dysfunction, effectively improving their lifestyle.

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6. Impaired neutrophil extracellular trap (NET) formation: a novel defect in the innate immune system of aged individuals.


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Neutrophils provide immediate frontline protection against rapidly dividing bacteria and fungi. To contain and eliminate these pathogens, neutrophils deploy several anti-microbial mechanisms, which include phagocytosis, generation of reactive oxygen species (ROS), degranulation and the recently described formation of extracellular traps. Consisting of a DNA backbone decorated with granule-derived peptides and enzymes, neutrophil extracellular traps (NETs) are ejected into the extracellular space, where they assist in pathogen entrapment and neutralisation.

Compared to younger subjects, older adults report an increased incidence of bacterial and fungal infection, suggesting that the protective nature of neutrophils wanes with age. Indeed, many groups have reported an age-related reduction in phagocytosis, ROS production and degranulation by human neutrophils. However, only one study has examined the impact of age on NET generation, in which it was shown that after Staphylococcus aureus challenge, neutrophils from aged mice exhibited impaired NET formation. Based on this observation and the increased incidence of bacterial and fungal infection reported by older adults, we investigated whether a reduction in NET formation is also a feature of human ageing.

Here, we show that following tumor necrosis factor-alpha (TNF-α)-priming, neutrophils from aged donors generate significantly fewer NETs when challenged with either interleukin-8 (IL-8) or lipopolysaccharide (LPS), and that this is accompanied by an age-associated decline in ROS generation. Interestingly, when treated with phorbol 12-myristate 13-acetate (PMA), which activates neutrophils independently of surface receptors, no age-related impairment was found in NET formation or ROS generation, suggesting that aberrant intracellular signalling proximal to the cell membrane is responsible for the age-related decline in NET formation and ROS production. This aberration however does not lie within the mitogen-activated protein kinase signalling pathway as we found phosphorylation of P38 and ERK1/2 following TNF-α or IL-8 stimulation respectively to be comparable in neutrophils isolated from young and old donors.
7. Impact of gamma delta T cells on murine intestinal mucus properties

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Mucus is the first point of contact of the gut microbiota with the host and thus a key component of the innate immune system. A second crucial line of defence relies on the rapid detection and killing of bacteria that penetrate through the mucus layers. \(\gamma\delta\) T cells, a subset of intraepithelial lymphocytes (IEL), have recently been proposed to form an essential component of the hierarchy of immune defences by responding to the presence of invading bacteria through cross-talk with neighbouring epithelial cells. Changes in mucus glycosylation and decreases in mucus-secreting cells, as well as decreases in \(\gamma\delta\) T cell numbers occur with increasing age. To study the impact of IELs in modulating mucus secretion and mucin expression and glycosylation, we used a \(\gamma\delta\) T cell deficient (TCR\(\delta^{-/-}\)) mouse model. Our data showed significant differences in the average goblet cell number per crypt in the small intestine and distal colon of TCR\(\delta^{-/-}\) mice compared to C57BL/6 wild-type mice, which positively correlate with changes in crypt lengths. No significant changes in mucus thickness between the two groups of mice were observed as measured \textit{in vivo}. However there were significant differences in mucin glycosylation between the TCR\(\delta^{-/-}\) and C57BL/6 wild-type mice. We confirmed that TCR\(\delta^{-/-}\) mice were more susceptible to DSS-induced colitis. Our mucin and glycan gene microarray analysis showed differential expression in several immune and inflammatory genes in the small intestine and colon of the two groups of mice, as supported by qRT-PCR. Together these findings suggest a role of \(\gamma\delta\) T cells in modulating mucin glycosylation either directly or indirectly, which may explain the increased susceptibility of TCR\(\delta^{-/-}\) mice to experimentally induced colitis. An \textit{ex vivo} model of mucus-producing intestinal crypts (organoids) has been established in TCR\(\delta^{-/-}\) mice to gain more insights into the molecular mechanisms underlying the observed changes.

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8. Enhancing axonal transport in ageing mice by nerve regeneration

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During normal ageing, we lose axons at an alarming rate, far exceeding that of neuronal cell bodies. Axon loss occurs throughout adult life, not just in the elderly, but the consequences become greatest in old age. Cumulative losses reach 45% of brain white matter and 40% of sensory endings in skin. Normal ageing is the biggest single risk factor for Alzheimer’s disease, Parkinson’s disease, motor neuron disease, glaucoma and many other disorders. Losses in normal ageing and disease are additive, so age-related axon loss should contribute to the increase in risk and severity of symptoms. Thus, understanding the mechanisms of age-related axon loss is important both for normal ageing and age-related disease.

Using MitoS mice, we have previously confirmed that with age the mitochondrial transport in individual axons declines in both directions. The number of moving particles decreases, while the velocity of individual particles remains unaltered. We propose that the decline in axonal transport contributes to vulnerability particularly in the most distal axon regions.

In the present study, we test the hypothesis that peripheral nerves in ageing mice are intrinsically capable of restoring axonal transport to levels found in young adults for a sustained period. Our preliminary results show that axon degeneration and regeneration after sciatic nerve crush can enhance axonal transport of mitochondria in two-year-old MitoS mice. Our data show a 75% enhancement in the number of anterogradely moving mitochondria relative to the contralateral, unoperated nerve and a 50% increase in the retrograde direction. This indicates that the peripheral nervous system has the ability to traffic more material to and from axons but somehow fails to do this as we age. Further investigation is needed to understand why this happens and whether transport could be enhanced in other ways.

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Metabolic regulation of cellular ageing in obese/diabetic subjects

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Metabolic disorders such as obesity and type 2 diabetes are increasing worldwide and there is growing evidence that these disorders may lead to accelerated ageing. The mechanisms by which metabolic disorders regulate the ageing process are poorly understood. More specifically, it is unclear what role if any muscle mass and fat storage have. Recently, a novel hormone released by muscle cells was described that had the ability to induce mitochondrial biogenesis in adipose tissue. This hormone, irisin, might therefore be able to induce a calorie restriction-like phenotype through increased energy expenditure. Our hypothesis for the present study is that body composition and circulating irisin levels could influence telomere length. In this cross-sectional study of non-obese individuals, a total of 82 participants (44 males and 38 females, age 18-83 years) with a mean body mass index of 24 were studied. Fasting blood samples were collected along with anthropometric measurements using a segmental body composition analyser. DNA was isolated from whole blood samples and relative telomere length determined using real-time PCR. Anthropometric measures were explored individually using Pearson’s bivariate correlations. Multiple regression was used to explore all the significant predictors of telomere length. Chronological age was a strong negative predictor of telomere length (-0.552, p = <0.001) suggesting that it is a sound marker of age in this cohort. In the absence of chronological age, visceral fat levels were the strongest predictor of telomere length (SE 0.0408, p = <0.001) with global muscle mass (SE 0.0135, p=0.003) and irisin (SE 0.0046, p = 0.027) positively predicting telomere length with an adjusted R² of 26.8. These data reinforce the notion that a healthy lifestyle, with appropriate diet and exercise, can enable healthy ageing and suggests that irisin has a novel role as a regulator of the ageing process.
10. Ultrastructural analysis of changes in neurons of the mouse internal anal sphincter during ageing

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Gastrointestinal disorders, including chronic constipation, faecal impaction and incontinence, are a major cause of morbidity in the elderly. Movement of contents along the gut and defaecation occur as a result of smooth muscle contraction and relaxation, which are regulated by the co-ordinated activity of many cell types. These include neurons of the enteric nervous system, particularly myenteric neurons, located in small ganglia embedded within the intestinal smooth muscle. Previous studies using light microscopy and immunolabelling techniques have provided evidence for neurodegenerative changes in the myenteric plexus during ageing in several species. Age-related changes in enteric neurons at the ultrastructural level however, have been very little studied. We are therefore characterising the changes that occur in myenteric neurons in the mouse internal anal sphincter (IAS) during ageing by conventional transmission electron microscopy. Here we describe the results of a semi-quantitative ultrastructural analysis of enteric neurons and glia in myenteric ganglia of the IAS of C57BL/6 mice at 3-4 and 24-25 months of age. An increase in the number of autophagic vacuoles, lipofuscin, inclusions with the appearance of autolysosomes, other inclusions and abnormal mitochondria were detected in myenteric neurons, but not glial cells, of old animals. These changes support previous evidence suggesting that there may be age-related neurodegeneration of enteric neurons, and indicate that a breakdown in cellular housekeeping processes may occur in ageing enteric neurons. Such changes could result in impaired neuronal function in the IAS and so contribute to disruption of normal defecation.

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11. A New Defined Synthetic (Holidic) Medium For Drosophila Studies On Ageing

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Drosophila melanogaster has been a laboratory model for over 100 years. Normally, a diet mainly consisting of sucrose and yeast is used to support fly growth, egg-laying and lifespan in the laboratory. However, in different laboratories, a bewildering variety of food types are applied for fly maintenance that could lead to inconsistent experimental outcomes, both for classic nutritionally sensitive traits, such as growth, fecundity and lifespan, but also for metabolic and other traits that are proving sensitive to particular food constituents. To address these issues, we have developed a defined synthetic medium for Drosophila made entirely from purified ingredients that is adequate to support adult egg-laying and lifespan. In term of ageing studies, we found Drosophila lifespan on the defined medium is as long as on the yeast food and flies null for the insulin-like peptides (dilp2,3,5) exhibited equivalent longevity as on yeast food. This is the first holidic medium for Drosophila ageing research.

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12. **Beyond the resting state: Age differences in neural networks identified during naturalistic viewing**

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Previous work has shown age differences in the expression of neural networks identified during both active task performance and wakeful resting state. In the present study, we asked whether age differences in network expression persist even when these networks are driven by a relatively unconstrained, naturalistic task, such as movie viewing. A large, population-representative sample (ranging in age from 18 to 88) was scanned using functional magnetic resonance imaging (fMRI) while viewing Alfred Hitchcock’s “Bang! You’re Dead” for eight minutes. Functional data were analyzed using tensor-independent components analysis (tensor-ICA), a multivariate analysis technique that identifies patterns of neural activation that are shared across subjects over time. This method successfully identified a set of networks that were commonly activated by participants throughout the movie – including networks of regions previously associated with lower-level auditory and visual processing, as well as those commonly implicated in higher-level cognitive tasks (e.g., the multiple demand network). Relative to younger adults, older adults showed weaker expression of several of these networks and their timecourses of activation were more variable across participants, suggesting that individual reactions to the movie became more idiosyncratic with age. Moreover, the degree to which participants expressed some of these networks was associated with performance on a variety of cognitive measures taken outside the scanner, suggesting that age differences in neural network integrity may have important ramifications for cognitive health.

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13. Age-related change in resting state BOLD variability: vascular or neuronal?

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Current neurocognitive theories of ageing use evidence from functional magnetic resonance imaging (fMRI) studies in order to infer brain activity. However, the blood-oxygen level-dependent (BOLD) signal measured in fMRI is a composite of neural and vascular signals. Therefore, it is essential to account for age-related alterations in neurovascular coupling when investigating age-related neurocognitive functioning with fMRI. The amplitude variability in resting-state BOLD (rsBOLD) signal has been proposed as an index of the vascular reactivity and has been investigated with other calibrating techniques such as breathhold and hypercapnia. However, these techniques are often difficult to perform in elderly cohorts, and this variability can be more practically measured from rsBOLD maps as resting state fluctuation amplitude, RSFA, and amplitude of low frequency fluctuations, ALFF. The RSFA and ALFF are reliable candidates for use in adjusting for age-related non-neural vascular changes in task-specific fMRI-BOLD data. However, this application requires evidence that age-related differences in rsBOLD variability are purely vascular, i.e. do not reflect any differences in neural fluctuations at rest. The present work addresses these points by i) comparing rsBOLD variability maps to other resting state networks (RSNs) and ii) relating measures of rsBOLD variability to measures of cardiovascular health, i.e. heart rate (HR), heart rate variability (HRV) and body-mass index (BMI) using mediation analysis. We computed rsBOLD variability maps in a population-representative cohort (n = 250, aged 20-85) within the Cambridge Centre for Ageing and Neuroscience (Cam-CAN). Maps of rsBOLD variability were then decomposed using independent component analysis (ICA) into components with independent patterns of resting state amplitude variability across participants. ICA identified two cortical components of interest, one including primary sensory and motor areas, and the other including parietal and frontal secondary association areas. The distribution of these two components was unlike RSNs. In addition, HR, HRV and BMI all mediated age-related declines in both of these ICA components of rsBOLD variability. These results demonstrate that rsBOLD variability is modulated by measures of vascular health and is not driven solely by changes in variance of neural activity. We propose that rsBOLD variability can be used as an estimate of vascular reactivity for correction of task-specific fMRI-BOLD signal. This correction is particularly useful in large scale neuroimaging studies of ageing, or with frail subjects, where alternative measures of vascular reactivity can be impractical.
14. Age-related changes in blood-brain barrier integrity in C57BL/6J mice

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The blood-brain barrier (BBB) is formed by the endothelial cells of the brain microvasculature, which control the molecular traffic between the blood and brain to maintain the neural microenvironment. MicroRNAs (miRs) are endogenous non-coding small RNAs that have emerged as important regulators of gene expression. BBB leakage in cerebral cortex has been reported in normal ageing and age-related diseases in both humans and rodents (1-3). Our preliminary data and other studies suggest that the deregulation of miR levels, especially miR155, in cerebral endothelial cells (CECs) may be critical in BBB dysfunction. To date, information on the mechanisms underlying age-associated BBB dysfunction and the possible role of miRs in these processes, in particular in endothelial cells, is lacking. The prime goal of this project is therefore to determine the role of endothelial microRNAs in modulating BBB function in ageing mice. In this study, in addition to analysis of changes in miRs in ageing endothelial cells we are also investigating whether BBB permeability is increased in ageing mice. Here we describe initial analysis of BBB integrity in C57BL/6j mice at 3, 12, 18 and 24 months of age using labelled paracellular permeability tracers (Evans blue and/or different molecular sizes of fluorescein isothiocyanate (FITC)–dextran). This work will systematically characterise age-associated changes in BBB function in mice, which may contribute to understand the mechanism of normal ageing.

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