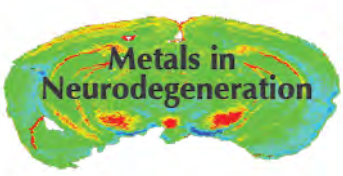
FINAL REPORT



Metals in Neurodegeneration
Satellite Meeting of the Joint ISN-APSN-ANS, Cairns, Australia
23-28th August 2015

Date: 28th August 2015

Venue: Rydges Tradewinds, Cairns (137 The Esplanade, Cairns, Australia)

Organisers:
Jeffrey Liddell (jliddell@unimelb.edu.au)
Scott Ayton (scott.ayton@unimelb.edu.au)

This meeting brought together world-leaders specializing on the study of metals in neurodegenerative diseases. This focused meeting was well attended and was a very successful one-day meeting.

Registration
Registration before 20th March 2015:
- Aus$50: Students
- Aus$100: All other registrants

Registration after 20th March 2015:
- Aus$150: All registrants

Registration fees included:
- Welcome reception
- Morning tea
- Lunch
- Afternoon tea
- Post-meeting mixer
- Conference Program and Abstract Book

Invited speakers did not have to pay Registration Fees.

Program
The program consisted of 17 oral presentations including 4 invited speakers, 3 early career presentations and 10 presentations selected from abstracts over 4 sessions throughout the day.
Invited speakers included:
- David Devos (France)
- Caroline Moreau (France)
- James Connor (USA)
- Ashley Bush (Australia)
- Joe Beckman (USA)

A poster session exhibited work from 8 posters.
See below for full program.

Participants
There were 28 registrants and 38 attendees, including 11 international delegates.

Prizes and Awards
2 Early Career Prizes were given for best student poster and best student presentation. Each received $300 to aid their attendance.

Best Student Presentation: James Hilton (Australia)
Best Student Poster: Nakisa Malakooti (Australia)

Social Event
A Meeting Dinner was held directly following end-of-meeting mixer.

Travel Awards to Invited Speakers
The Invited Speakers were granted the following amounts:

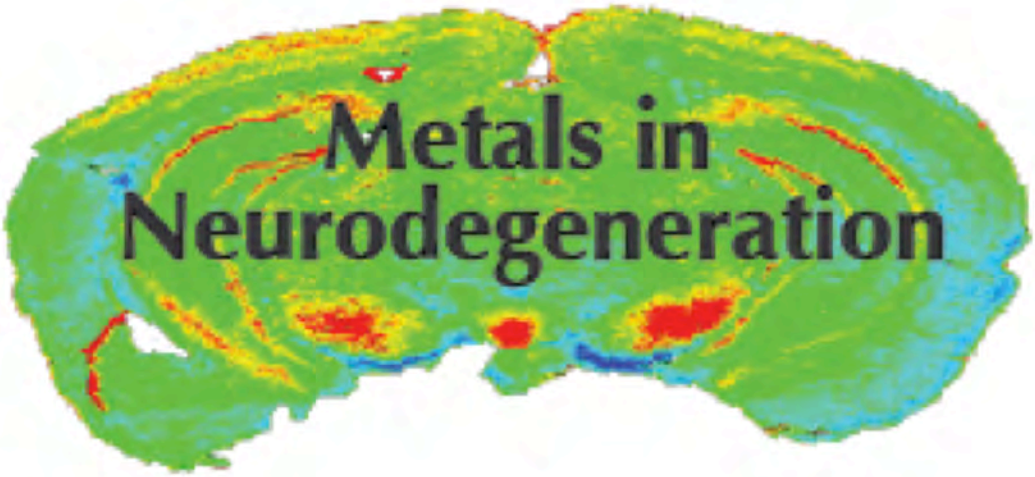
David Devos and Caroline Moreau	US$3000
James Connor	US$1500
Joe Beckman	US$1500

Sponsorship
This meeting was supported by a generous grant of US$7000 provided by the ISN.

Budget (AUS$)

Income		
Registrations		$3,350
ISN grant	US$7000	$9333
Total		$12,683

Expenses	
Venue, inc. welcome reception, morning tea, lunch and afternoon tea	$2000
Venue on-costs (AV, poster boards, post-meeting mixer, etc)	$1050
Printing of Conference Program and Abstract Book	$245
Travel Awards [supported by ISN]	$8733
Student Awards [supported by ISN]	$600
Total	$12,628



Satellite Meeting in conjunction with the 25th ISN Biennial Meeting, Cairns 2015

Rydges Tradewinds,
137 The Esplanade, Cairns,
28th August, 2015

THE FLOREY

ISN International Society for Neurochemistry

THE UNIVERSITY OF MELBOURNE

PROGRAM

		Session theme
8:30	Welcome	
8:40	David Devos & Caroline Moreau *Targeting chelatable iron as a therapeutic option in the treatment of Parkinson's disease*	Metal-based drug therapies *Session Chair: Anthony White*
9:20	Marco Nunez *Novel 8-hydroxiquinoline- and 7-hydroxycoumarin-based iron chelators as potential therapeutic agents for Parkinson's disease.*	
9:40	Kevin Barnham *Modulating metals as a therapeutic strategy for neurodegenerative diseases*	
10:00	Morning tea	
10:30	Paul Rosenberg *Zinc is an endogenous suppressor of cell survival and axon regeneration*	Pathogenic mechanisms of zinc *Session Chair: Kevin Barnham*
11:00	Jorge Busciglio *Zinc, hyperexcitability and neurodegeneration in Alzheimer's Disease*	
11:20	Anthony White *Neuro-metals as new targets for anti-inflammatory therapeutics*	
11:40	Alexandra Grubman *Examination of metal homeostasis in visual dysfunction*	
12:00	Jin-Sung Park *Parkinsonism-associated human ATP13A2 (PARK9) is required for zinc homeostasis and cellular bioenergetics*	
12:20	Lunch	
13:20	James Connor *Impact of HFE genotype on the CNS*	Pathogenic mechanisms of iron *Session Chair: Des Richardson*
13:50	Des Richardson *The oxidative stress paradox: Identification of non-ferritin mitochondrial iron deposits in a mouse model of Friedreich's Ataxia*	
14:20	Michael Huang *Cardiomyopathy in a mouse cardiac model of Friedreich's Ataxia involves the activation of the integrated stress response*	
14:40	Blaine Roberts *Elevated iron in Alzheimer's disease brain can be attributed to increased levels of hemoglobin*	
15:00	Afternoon tea	
15:25	Joe Beckman *Protection by copper delivery in SOD-transgenic mice and the importance of the Copper Chaperone for SOD1 (CCS)*	Pathogenic mechanisms of copper *Session Chair: Joe Ciccotosto*
15:55	Kay Double *Copper pathology and cell vulnerability in Parkinson's disease*	
16:15	James Hilton *Cumulative functional copper deficiency in spinal cords of amyotrophic lateral sclerosis (ALS) model mice and sporadic ALS cases*	
16:35	Carlos Opazo *Cu^{+} is a cofactor for ubiquitin conjugation and cellular protein degradation*	
16:55	Ashley Bush *Metals in aging and neurodegeneration*	Perspective
17:25	Finish	

Session 1: Metal based drug therapies

Session Chair: Anthony White

David Devos & Caroline Moreau

Targeting chelatable iron as a therapeutic option in the treatment of Parkinson's disease

FAIRPARK-I

The present work provides the first clinical evidence about the neuroprotective potential of a therapeutically safe chelation treatment on early- stage Parkinson's patients that were already stabilized for dopamine and responded significantly to treatment in both brain iron deposits and indicators of disease progression. The novel treatment relied on oral administration of deferiprone that by chelation of labile iron it conferred upon oxidation-stressed animals increased striatal-dopamine levels and improved motor functions, while essentially sparing systemic iron. The paradigmatic modality of chelation with deferiprone in Parkinson's disease should prompt multi-center studies in various disorders of regional iron accumulation or misdistribution.

FAIRPARK-II

This project seeks to demonstrate that conservative iron chelation therapy (i.e. with iron chelation and redeployment) with moderate dose DFP (30 mg/kg/day) slows the progression of handicap in de novo PD patients while not affecting systemic parameters. The 9-month, parallel-group, randomized, placebo-controlled, multicentre trial will be followed by a one-month wash-out period. The primary efficacy criterion will be the change in motor and non-motor handicap scores on the Total Movement Disorders Society Unified Parkinson's Disease Rating Scale between baseline and 36 weeks (i.e. in order to identify disease-modifying and symptomatic effects). The secondary efficacy criterion will be the change in score between baseline and 40 weeks (i.e. probing the disease-modifying effect only). Potential surrogate biomarkers will be assessed. The study results might prompt academic and industrial research on iron chelation as a disease-modifying treatment in neurodegenerative diseases.

Marco T. Núñez

Novel 8-hydroxiquinoline- and 7-hydroxycoumarin-based iron chelators as potential therapeutic agents for Parkinson's disease

Abundant evidence indicates that mitochondrial dysfunction and iron accumulation are common features in neurodegenerative disorders of the central nervous system that include Alzheimer's disease, Friedreich's ataxia and Parkinson's disease. Causality between mitochondrial dysfunction and iron accumulation is apparent, since inhibition of mitochondrial complex I results in decreased Fe-S cluster synthesis and an increased mitochondrial labile iron pool (mLIP). In this work, we will relate the characteristics and sub-cellular locus of action of two novel series of chelators, with either 8-hydroxiquinoline (8-OHQ) or 7-hydroxycoumarine (7-HC) bases on cell and animal models of Parkinson's disease. In cell culture systems, we found that nanomolar concentrations of chelators 8-OHQ chelators Q1 and Q2 targeted mitochondrial iron and were extremely effective in protection against oxidative modifications (ROS, mitochondrial LIP, HNE and 8-OHG) induced by complex I inhibition. Sub-nanomolar concentrations of 7-OHC-based chelators prevented against cell death induced by complex I inhibition. Mitochondria-targeted Q1, given orally to mice, protected substantia nigra pars compacta neurons against oxidative damage and MPTP-induced neuronal death. Animal studies on 7-OHC chelators are in progress. Financed by grants FONDECYT 1030068 PIA-ACT1114 from CONICYT, Chile.

Kevin J. Barnham

Modulating metals as a therapeutic strategy for neurodegenerative diseases

Transition metals such as Zn and Cu play an important role in modulating neuronal/synaptic function. The metabolism of these metals is tightly regulated because of the potential for the metals to carry out "off-pathway" pathological reactions. Unfortunately, these regulatory mechanisms breakdown as a function of age, which is the number risk factor for many neurodegenerative disease such as Alzheimer's and Parkinson's disease. It is likely that aberrant metal metabolism plays an important role in age related neurodegenerative diseases.

We have developed therapeutic strategies to inhibit these pathological reactions. These include the MPAC technology designed to inhibit Aβ metal interactions, with the lead compound (PBT2) currently in clinical development. Mechanistic studies show that in addition to inhibiting Aβ/metal interactions PBT2 is able to chaperone the metal into cells thereby activating protective signaling cascades. This discovery prompted us to investigate the potential of metal based therapeutic agents for the neurodegenerative diseases. We identified the metal bis(thiosemicarbazonato) complexes as ideal scaffolds whose structures can be manipulated to fundamentally change the properties these compounds possess. With some forms of these complexes acting as metal delivery agents initiating neuroprotective signaling pathways, while other complexes deactivate reactive nitrogen species and show beneficial therapeutic activity in multiple animal models of PD and amyotrophic lateral sclerosis.

Session 2: Pathogenic mechanisms of zinc

Session Chair: Kevin Barnham

Paul A. Rosenberg

Zinc is an endogenous suppressor of cell survival and axon regeneration

A major problem in promoting repair in the CNS is first, to limit or prevent neuronal death, and second, to stimulate regeneration of injured axons. Like other CNS pathways, the optic nerve cannot regenerate if injured, causing lifelong losses in vision. Retinal ganglion cells (RGCs) can be induced to regenerate axons all the way from the eye to the brain through a combination of intraocular inflammation, elevating cAMP, and deleting the *pten* gene. Under these conditions, about 3% of the RGCs reinnervate appropriate target areas and restore simple visual responses. However, most RGCs continue to die and only 10% of surviving RGCs regenerate their axons. Zinc has been strongly implicated in neurodegeneration in response to ischemia as well as oxidative stress, and we queried whether zinc might have a role in the neuronal death following optic nerve injury. Using autometallography or the fluorescent zinc sensor ZinPyr1, we found that free Zn^{2+} becomes evident in the synapses that amacrine cells make onto RGC dendrites within one hour after optic nerve injury. Zn^{2+} subsequently accumulates in RGCs over a period of hours-to-days. Presynaptic Zn^{2+} accumulation requires nitric oxide production by NOS1 and the vesicular zinc transporter protein, ZnT3. Chelating Zn^{2+} using either TPEN or ZX1 reduces Zn^{2+} in amacrine cell terminals and promotes enduring RGC survival. Unexpectedly, zinc chelation also promotes extensive axon regeneration. Thus, Zn^{2+} is a major suppressor of RGC survival and axon regeneration after optic nerve injury. It will be important to investigate the mechanisms by which free zinc accumulation induces neuronal death and blocks axon regeneration in the optic nerve injury model, which is a uniquely accessible *in vivo* model of neurodegeneration. In addition, we are interested in applying insights gained from these studies to understand and promote recovery after other types of CNS damage.

Jorge Busciglio

Zinc, hyperexcitability and neurodegeneration in Alzheimer's disease

Impaired zinc homeostasis has been implicated in Alzheimer's disease (AD), but the precise role of zinc in AD neuropathology is not well characterized. Zinc binds with high affinity to Aß and it is enriched in senile plaques. Previous results from our laboratory indicate that zinc released during excitatory neurotransmission increases oligomeric Aß formation and accumulation at synaptic contacts. In addition, zinc inhibits NMDAR channels and reduces neuronal hyperexcitability. Additional data shows that sequestration of zinc by AβO disrupts synaptic function. We sought to determine if synaptic zinc alters NMDAR response and affects neuronal hyperactivity using ZnT3KO transgenic mice, which lack vesicular zinc normally released during neurotransmission and have been found to have age-dependent memory deficits. We found that removal of synaptic zinc combined with blockade of NMDAR NR2A subunits increased activity-dependent activation of Erk1/2, suggesting that zinc inhibits NR2B-containing NMDARs, which trigger primarily neurodegenerative responses. A downstream effect of Erk1/2 activation, activity-dependent increase in *BDNF* mRNA, was markedly reduced in ZnT3KO hippocampus.
We also found a progressive and age-dependent increase in markers of seizure activity in ZnT3KO hippocampus, which closely correlated with the onset of cognitive impairment. Consequently, chronic treatment of ZnT3KO mice with anti-seizure medication for eleven weeks prevented the appearance of age-dependent memory deficits. Together, these results suggest that therapies directed to restore zinc homeostasis and normalize excitatory neurotransmission may prove valuable in managing neuronal hyperactivity and associated neurodegenerative changes in AD.

Anthony R. White

Neuro-metals as new targets for anti-inflammatory therapeutics.

Neurodegenerative diseases are caused by complex molecular pathways resulting in common neuropathological outcomes including protein aggregation, oxidative stress, neuroinflammation and neuronal cell degeneration. An increasingly well-recognized factor in these pathogenic changes is a loss of biological metal (biometal) homeostasis resulting in abnormal accumulation and function of copper, zinc, iron and additional key Neuro-Metals. We have demonstrated that changes to glial biometal metabolism has a critical role in early neurodegenerative disease processes, and is likely to be a major factor in subsequent neuroinflammatory responses. Our research has shown that *bis*(thiosemicarbazonato)-metal complexes offer an exciting new approach to treat neuroinflammation by inducing Nrf2-mediated anti-oxidant and anti-inflammatory responses in astrocytes and microglia. Ongoing research indicates that these compounds have the potential to shift neuroinflammatory responses from a cytotoxic M1 response to protective M2 response, potentially involving the mobilization of iron. The metal-complexes have generated robust anti-inflammatory and neuroprotective outcomes in multiple cell and animals of neurodegenerative diseases and are currently being developed for potential clinical application.

Alexandra Grubman

Examination of metal homeostasis in visual dysfunction

Neuronal ceroid lipofuscinoses (NCLs; or Batten diseases) are caused by mutations in one of 14 distinct Cln genes and are a group of fatal childhood neurodegenerative lysosomal storage diseases that present clinically with progressive loss of vision and motor decline. Our data in naturally occurring Cln6 mutant NCL mice, indicates that lipofuscin build up in the retinal pigment epithelium (RPE) induces visual loss prior to photoreceptor death. Cln6 mice also mimic other early signs of age-related macular degeneration (AMD), the leading cause of irreversible blindness in people aged over 50 years. Metal loss has previously been reported in the eyes of AMD patients, and zinc supplementation has been used as a treatment for some forms of AMD. Our recent studies also demonstrate metal dyshomeostasis in the brains of 6 different animal models of NCLs (CLN1,3,5,6 mice; CLN5,6 sheep) and in cerebellar cells isolated from Cln6 mice. We performed synchrotron X-ray fluorescence microscopy on retinal sections from control and Cln6 mice aged between 1 and 8 months. We observed age-dependent changes to the elemental profiles in control mice. Surprisingly, unlike the observed metal changes in the brain in Cln6 mice, there were no significant changes to the elemental concentrations in all retinal layers between control and Cln6 mice. Together, our data suggest that lipofuscin accumulation and buildup of debris in the retinal space may overwhelm nutrient delivery to photoreceptors, and contribute to blindness in these mice.

Jin-Sung Park

Parkinsonism-associated human ATP13A2 (PARK9) is required for zinc homeostasis and cellular bioenergetics

Human ATP13A2 (PARK9) is a lysosomal P5-type ATPase with potential cation transporting capacity. Qualitative and quantitative ATP13A2 changes have been associated with the pathogenesis of Kufor-Rakeb syndrome (KRS), an autosomal recessive, juvenile-onset Parkinson's disease. Recently, we identified novel compound heterozygous ATP13A2 mutations (p.Leu1059Arg/p.Leu1085TrpfsX4) in two KRS patients and found evidence of mitochondrial dysfunction in patient-derived fibroblasts. To identify the cationic substrate of ATP13A2, we tested the toxicity of several metal ions on ATP13A2-deficient KRS patient-derived human olfactory neurosphere cells and found that the patient cells had an increased sensitivity to Zn^{2+}. Furthermore, we identified reduced intracellular free Zn^{2+} levels ($[Zn^{2+}]_i$), dysregulated expression of several ZnT/ZIP transporters and impaired Zn^{2+} sequestration into LC3-positive vesicles in the KRS patient-derived cells, indicating Zn^{2+} dyshomeostasis. Pharmacological treatments that increased $[Zn^{2+}]_i$ aggravated mitochondrial dysfunction as demonstrated by a further decrease in mitochondrial membrane potential in conjunction with an increase in intracellular ROS levels and mitochondrial network fragmentation, resulting in ATP depletion and eventual cell death. In addition, the patient cells displayed a reduction in several parameters of glycolytic function such as the maximal glycolytic capacity, pyruvate/lactate production and the NAD^+/NADH ratio. Increasing $[Zn^{2+}]_i$ exacerbated the glycolytic dysfunction as demonstrated by further reduction in the NAD+/NADH ratio. Conversely, Zn^{2+}-mediated toxicity in the KRS patient cells was efficiently blocked by wildtype ATP13A2 overexpression, antioxidant treatment, Zn^{2+} chelation, promotion of mitochondrial fusion and extracellular pyruvate supplementation.

Session 3: Pathogenic mechanisms of iron

Session Chair: Des Richardson

James Connor

Impact of HFE genotype on the CNS

Since we began investigating the prevalence of HFE gene variations in neurodegenerative diseases over 50 papers have appeared interrogating a number of neurodegenerative diseases. The most consistent data come from the motor neuron disease population were seven independent groups have reported that a specific polymorphism in the HFE gene (H63D HFE) is present in as many as 30% of amyotrophic lateral sclerosis (ALS) patients. We have made progress on understanding the role of H63D HFE using cell culture model and a H67D knock-in mouse model (homologous to H63D in humans). The presence of the H63D HFE gene variant is associated with activation of a number of disease mechanisms implicated in neurodegeneration such as iron accumulation, increased oxidative stress, abnormal glutamatergic secretion and endoplasmic reticulum (ER) stress and disruption of cholesterol metabolism. Performance on learning and memory tasks indicate that H67D-HFE mice have poorer cognitive function than WT-HFE mice. Transverse relaxation MRI parametrics were applied to H63D human carriers. Group based transverse proton parametric map analysis has revealed widespread decreases in relaxation rate (R2) in the H63D carriers specifically within white matter association fibers in the brain. Similar R2 decreases within white matter tracks were observed in the H67D mouse brain. Additional animal studies have revealed an accelerated disease profile in an animal model of MND carrying the HFE H67D gene variant. Moreover, exposure to cholesterol-lowering statins further accelerates the disease profile. There is clearly a substantial and significant impact of the HFE mutation on neurological function and therapeutic response. Because the HFE gene variants are the most prominent gene variants in the Caucasian population, the impact of this genotype on human neurodegenerative diseases and therapeutic intervention strategies should be considered a key player in the context of precision medicine.

Des Richardson

The oxidative stress paradox: Identification of non-ferritin mitochondrial iron deposits in a mouse model of Friedreich's Ataxia

There is no effective treatment for the cardiomyopathy of the most common autosomal recessive ataxia, Friedreich's ataxia (FA). This disease is due to decreased expression of the mitochondrial protein, frataxin, which leads to alterations in mitochondrial iron (Fe) metabolism. The identification of potentially toxic mitochondrial Fe deposits in FA suggests Fe plays a role in its pathogenesis. Studies using the muscle creatine kinase (MCK) conditional frataxin knockout mouse that mirrors the disease have demonstrated frataxin deletion alters cardiac Fe metabolism. Indeed, there are pronounced changes in Fe trafficking away from the cytosol to the mitochondrion, leading to a cytosolic Fe deficiency. Considering Fe deficiency can induce apoptosis and cell death, we examined the effect of dietary Fe supplementation, which led to body Fe loading and limited the cardiac hypertrophy in MCK mutants. Furthermore, this study indicates a unique effect of heart and skeletal muscle-specific frataxin deletion on systemic Fe metabolism. Namely, frataxin deletion induces a signaling mechanism to increase systemic Fe levels and Fe loading in tissues where frataxin expression is intact (i.e., liver, kidney, and spleen). Examining the mutant heart, native size-exclusion chromatography, transmission electron microscopy, Mössbauer spectroscopy, and magnetic susceptibility measurements demonstrated that in the absence of frataxin, mitochondria contained biomineral Fe aggregates, which were distinctly different from isolated mammalian ferritin molecules. These mitochondrial aggregates of Fe, phosphorus, and sulfur, probably contribute to the oxidative stress and pathology observed in the absence of frataxin.

Michael L. Huang

Cardiomyopathy in a mouse cardiac model of Friedreich's Ataxia involves the activation of the integrated stress response

Background: Friedreich's ataxia (FA) is a neuro- and cardio-degenerative disease due to frataxin-deficiency and leads to marked mitochondrial iron loading. The ensuing oxidative stress may contribute to degeneration of vital neuronal and cardiac cells, leading to death.
Methods: Using MCK conditional frataxin knockout (KO) mouse that develops severe FA cardiomyopathy, we examined the link between gene alteration and the observed progressive histological and functional changes. We examined KO mice from 3-weeks of age which are asymptomatic, up to 10-weeks when they die of the disease.
Results: Iron deposit was identified from 5-weeks in the KO mice, with markedly reduced cardiac function from 6-weeks. We identified early and marked upregulation of several stress-induced genes known to be activated by the integrated stress response (ISR), mediated by phosphorylation of the eukaryotic translation initiation factor 2alpha; (p-eIF2alpha;). In fact, p-eIF2alpha; levels wee markedly increased from 3-weeks in the KO mice. Importantly, chronic ISR potentiates heart failure via the downstream processes: autophagy and apoptosis. Indeed, the expression of a panel of autophagy and apoptosis markers was enhanced in the KO mice.
Conclusion: The pathogenesis of FA cardiomyopathy correlates with early and persistent ISR activation which precedes onset of autophagy and apoptosis. (Am J Pathol 2013;183:745)

Blaine Roberts

Elevated iron in Alzheimer's disease brain can be attributed to increased levels of hemoglobin

Iron is an essential element for cellular function. Although Fe is essential, it can also contribute to oxidative stress in cells when not properly managed. There is a debate in the literature around changes in Fe in AD brain. The data around this debate has exclusively involved bulk analysis of total Fe levels in post-mortem brain tissue. Bulk analysis is not able to yield the mechanistic detail about changes in Fe metabolism in AD brain. We have developed metalloproteomic techniques that allow us to investigate individual Fe proteins and metabolites directly from human brain tissue. Using these techniques we have been able to gain a detailed view about the metabolism of Fe and changes in Fe-proteins in AD. We find that there are significant elevations in the level of Fe proteins in AD brain. Haemoglobin was found to have the greatest elevation of the proteins investigated. Interestingly, we show that the haemoglobin is actually expressed by the neurons. We discuss the potential implications of these results for the pathogenesis of AD.

Session 3: Pathogenic mechanisms of copper

Session Chair: Joe Ciccotosto

Joe Beckman

Protection by copper delivery in SOD-transgenic mice and the importance of the Copper Chaperone for SOD1 (CCS)

Mutant SODs confer a toxic gain of function that leads to motor neuron degeneration in humans, dogs, mice, rats, zebrafish and *Drosophila*. This gain-of-function involves partially unfolded intermediates of SOD that lack at least one of the two metal atoms critical for stabilizing SOD. Over-expression of mutant human superoxide dismutase (SOD^{G93A}) in transgenic mice induces many features of ALS and has become the most widely used model for any neurodegenerative disease. However, after 20 years of searching, no pharmaceutical treatment has reproducibly extended the 130-day lifespan of high-expressing SOD^{G93A} mice by more than 10-20%. We instead began exploring what processes accelerate the development of ALS in SOD mice. The most rapid acceleration of ALS results when the human Copper-Chaperone-for-SOD (CCS) is coexpressed with human SOD, which results from an acute copper deficiency in the CNS. We found that the copper carrier CuATSM is extremely protective in these mice. Most of the treated mice continue to survive for nearly two years with minor symptoms. We sought to determine how CuATSM protects $CCSxSOD^{G93A}$ mice from developing ALS and understand what happens to reinitiate the disease when the drug is withdrawn. Randomized and blinded trials of $CCSxSOD^{G93A}$ mice were conducted with CuATSM withdrawn to initiate disease and restarted after mice developed symptoms. SOD was measured in spinal cords of mice by mass spectrometry and cytochrome *c* oxidase by spectrometric assays. CuATSM given to high-expressing Gurney SOD^{G93A} mice coexpressing the human Copper Chaperone for SOD (CCS) protected against ALS for 20 months with many mice still surviving or being censured for unrelated issues. When CuATSM treatment was stopped, the SOD^{G93A}xCCS mice developed severe motor dysfunction in two months and died in the next month. Progression could be stopped by resuming treatment with CuATSM. These rescued mice largely recovered and survived for an additional 6-12 months. Mass spectrometry was used to measure native SOD directly in ventral spinal cord, and showed that CuATSM treatment completed maturation to the fully functional Cu,Zn SOD in SOD^{G93A}xCCS mice and that SOD protein doubled in concentration compared to end-stage SOD^{G93A} mice. CuATSM protects in SOD^{G93A}xCCS mice by at least two mechanisms -- supplying copper to cytochrome c oxidase and completing the maturation of SOD to its mature form containing copper and zinc. All humans ALS patients likely express CCS, making this model closer to the human condition than the standard ALS model. CuATSM is remarkably nontoxic and is in use in humans now.

Kay Double

Copper pathology and cell vulnerability in Parkinson's disease

Increased iron in vulnerable regions of the Parkinson's disease brain is believed to represent a useful target for novel therapies for this disorder. We have quantified intraneuronal metal changes using synchrotron X-ray microfluorescence in regions of varying vulnerability in the Parkinson's disease brain. As expected, iron levels in the Parkinson's disease substantia nigra were significantly increased but we also identified a 65% decrease in intraneuronal copper in this vulnerable brain region. This decrease in copper was associated with a marked reduction in immunoreactivity for the neuronal copper transport protein 1. Parallel changes in cellular copper pathways in the substantia nigra in cases of incidental Lewy body disease suggest these changes occur early in the disease process. A reduction in copper was not observed in non-degenerating regions of the Parkinson's disease brain independent of the expression of α-synuclein pathology. At the functional level, reduced cellular copper was associated with altered activity of the cuproprotein, superoxide dismutase 1 (SOD1) in the substantia nigra. More recently, we have identified aggregation of SOD1 protein in a pattern reflecting cellular loss, but independent of α-synuclein pathology, in the Parkinson's disease brain. Aggregation of SOD1 is linked to neurodegeneration in familial and sporadic Amyotrophic Lateral Sclerosis, suggesting that aggregation of this protein in the Parkinson's disease brain may also contribute to degenerative processes. Given the regional and temporal pattern of changes in copper-dependent pathways in the Parkinson's disease brain we suggest that copper pathology may represent an additional mechanism by which vulnerable neurons are damaged in this disorder.

James Hilton

Cumulative functional copper deficiency in spinal cords of amyotrophic lateral sclerosis (ALS) model mice and sporadic ALS cases

Objectives: We recently reported that increasing copper bioavailability both therapeutically and genetically acted to protect spinal cord motor neurons, whilst concomitantly improving locomotor function and survival of ALS model mice. These outcomes indicate that copper deficiency contributes to the pathogenesis of ALS. For the present study we directly assessed the extent of copper deficiency in ALS. Methods: We assessed levels and copper-dependent activity of the antioxidant SOD1, the ferroxidase ceruoplasmin and mitochondrial cytochrome c oxidase in the spinal cords of SOD1G37R ALS model mice from pre-symptom through to late symptom stages of disease progression. We also assessed post-mortem spinal cord samples from human cases of sporadic ALS. For mouse tissue analyses, non-transgenic littermate and wild-type SOD1 overexpressing mouse spinal cords, and non-disease affected livers were used as controls. Spinal cords from healthy controls were included in the human tissue analyses. Results: All analyses revealed a strong disparity between protein levels measured and their copper-dependent activity, consistent with a broad functional copper deficiency in ALS. This disparity was evident at an early age in the ALS model mice and was cumulative as disease progressed. Conclusions: These results indicate the cause of ALS phenotype in mutant SOD1 mice is not restricted to mutant SOD1 toxicity and may be driven in part by broader consequences of functional copper deficiency. The fact that the human ALS tissue we examined included only sporadic cases of the disease indicates the role of functional copper deficiency in ALS extends beyond mutant ALS cases.

Carlos M. Opazo

Cu^{+} is a cofactor for ubiquitin conjugation and cellular protein degradation

The initial steps of ubiquitination require ATP and inorganic cofactors, including Mg^{2+} and an unknown metal ion. Here we reveal this as Cu^{+}. Low micromolar Cu concentrations selectively and rapidly (3 h) induce the formation of ubiquitinated proteins in cell lines and primary neuronal cultures in the absence of cell damage, but do not inhibit the purified 20S proteasome. Cells overexpressing the CTR1 Cu-uptake transporter have greater copper uptake and higher ubiquitinated protein levels. By contrast, depletion of intracellular copper levels by diamsar, a high affinity Cu chelator, decreases both the endogenous levels of ubiquitinated proteins and the accumulation of ubiquitinated proteins induced by proteasome inhibition. Cu-induced ubiquitin protein were confirmed to be of a chemical nature similar to orthodox ubiquitin adducts, but are enriched with bound Cu. Pulse-chase experiments demonstrated that copper accelerates protein degradation in primary neuronal cell cultures. In addition, we found that Cu(I) is important for the degradation of a wide range of proteins in different cell lines and in primary neuronal cultures. In cell-free system, which mimics a reductive intracellular environment, Cu as Cu^{+} induces ubiquitination catalyzed by E1/E2/E3 ligases. In tissue culture, copper promoted degradation of p53, a substrate of UbcH5b E2 conjugating enzyme. We found that the cell-free activity of UbcH5b is regulated by Cu^{+}. These data indicate that Cu^{+} is the previously unidentified metal ion cofactor for enzymatic ubiquitination, and intracellular protein degradation. These findings have implications for proteinopathies with altered Cu homeostasis, such as Alzheimer's disease and Lewy Body Disease.

Ashley Bush

PERSPECTIVE: Metals in aging and neurodegeneration

Alzheimer's disease (AD) and Parkinson's disease (PD) are incurable and common neurodegenerative diseases that complicate aging. Therapeutic approaches that focus on the protein aggregates that typify these disorders have been disappointing in clinical trials, suggesting that the neurodegeneration is not merely due to proteinopathy. In both diseases, there is a severe dysregulation of metal homeostasis in affected brain tissue, with iron elevation reported in cortex (AD) and nigra (PD). This contributes to severe oxidative damage that characterizes both diseases. In addition, zinc accumulates in the amyloid proteopathy of AD, and copper levels fall in the affected tissues in both diseases. Iron also increases in the brain and other tissues with normal aging, changes that contribute to the aging phenotype in *C. elegans*.

We have determined that the major proteins implicated in AD and PD have important functions in metal transport, and particularly are components of an iron regulatory system that fails in aging. The amyloid protein precursor (APP), like ceruloplasmin (CP), facilitates the export of iron from cells by stabilizing cell surface ferroportin, and prevents dietary iron from accumulating in the brain. Tau impacts on iron export by trafficking APP to the cell surface. Elevated CSF ferritin levels have recently been reported to predict conversion of MCI to AD, and the major genetic risk factor for AD, Apolipoprotein E, has a striking relationship with CSF ferritin that implies a causal role for iron in AD. Knockout mice for both ceruloplasmin and APP develop iron-mediated PD pathology, remedied by iron chelators. The predisposition of the nigra to PD is explained by the enriched population of neurons that coenrich high concentrations of iron with dopamine. Small molecules that target iron accumulation have been effective in animal models of these diseases, and a recent phase 2 clinical trial of deferiprone in PD lowered nigral iron and improved clinical readouts. The neurodegenerative diseases of aging occur on a background of age-dependent fatigue of metal regulatory systems, and an inescapable build-up of brain iron. The involvement of all major proteins implicated in AD in metal trafficking raises the possibility that this major neurodegenerative disorder is caused by a collapse of metal homeostasis, and supports the adjustment of metal regulation as a priority pharmacological target. Trials of such drugs are at a very early stage, but warrant considerably more development.