FINAL REPORT

Understanding the function of glia in the healthy and diseased CNS

BACKGROUND DETAILS

Location: Manly Beach, Sydney, Australia
Date of meeting: August 19-22, 2015
Venue: Novotel Manly Pacific Hotel, Manly Beach, Sydney, Australia
Meeting website: http://www.sydneygliameeting.com
Organisers: Bernardo Castellano and Iain L. Campbell

By bringing together leading International and Australian researchers this conference aimed to advance our understanding of the role played by glial cells in the function of the healthy CNS as well as in the aging and diseased CNS. Topics included the origins of glial cells, the signals regulating glial cell behaviour and function in the normal as well as neuropathologic states, the nature and mechanisms of glial cell interaction with neurons and the role this plays in regulating neuronal function and the contributions of glia to protection and injury in different CNS diseases such as Alzheimer's and Parkinson's disease, multiple sclerosis and motor neuron disease. For detailed information about the meeting content, please see the attached final meeting book.

REGISTRATION

Registration categories	Early bird January 15 - March 14	Regular March 15 - June 12	Late/on site After June 12
Invited speaker	0	0	0
Participant	550	600	675
Student	400	475	525
Social guests	150*	150	150
*Social events including conference dinner only			
Day rate	320	330	350
Conference dinner#	25	25	25
# 3 course meal and all beverages			

Registration fees included:

- Welcome reception
- Lunch (3 days)
- Coffee breaks (2.5 days)
- Poster session catering (1 day)
- Conference dinner (1 day)
- Conference meeting book

Invited Speakers did not pay registration fees and, in addition, they were provided with free accommodation with breakfast included for a maximum of 3 nights (August 19-21).

PROGRAM:

The final program included 2 plenary lectures and 6 symposia with the participation of a total of 25 invited speakers. In addition there were two sessions of oral communications (14 in total) and a poster session with 14 poster exhibiters.

See the attached meeting book which includes full details of the meeting program. Please note that due to late withdrawals by 2 originally invited speakers, changes were made to the speaker schedule which are not shown in the abstract section of the program.

INVITED SPEAKERS:

Plenary lecturers:

Florent Ginhoux,	University of Singapore, Singapore
Michael Sofroniew,	University of California at Los Angeles, USA

Symposia speakers:

*Estefania Acaz	Cajal Institute, Spain
Burkhard Becher	University of Zurich, Switzerland
Tika Benveniste	University of Alabama, USA
Knut Biber	University of Freiburg, Germany

Iain Campbell	University of Sydney
*Mike Dragunow	University of Auckland, New Zealand
Gilles Guillemin	Macquarie University, Australia
Markus Hofer	University of Sydney, Australia
Elly Hol	University of Utrecht, Netherlands
*Helmut Kettenman	Max Delbrück Center for Molecular Medicine, Germany
Trevor Kilpatrick	University of Melbourne, Australia
Nick King	University of Sydney, Australia
Shane Liddelow	Stanford University, USA
Toby Merson	University of Melbourne, Australia
*Mami Noda	Kyushu University, Japan
Ross O'Shea	Latrobe University, Australia
Trevor Owens	University of Southern Denmark, Denmark
Rosa Paolicelli	University of Zurich, Switzerland
*Milos Pekny	University of Goththenburg, Sweden
*Jake Streit	University of Florida, USA
Ann Turnley	University of Melbourne, USA
Alexei Verkhratsky	University of Manchester, UK
*Rohan Walker	University of Newcastle, Australia

* ISN Travel Grant Recipients

PARTICIPANTS:

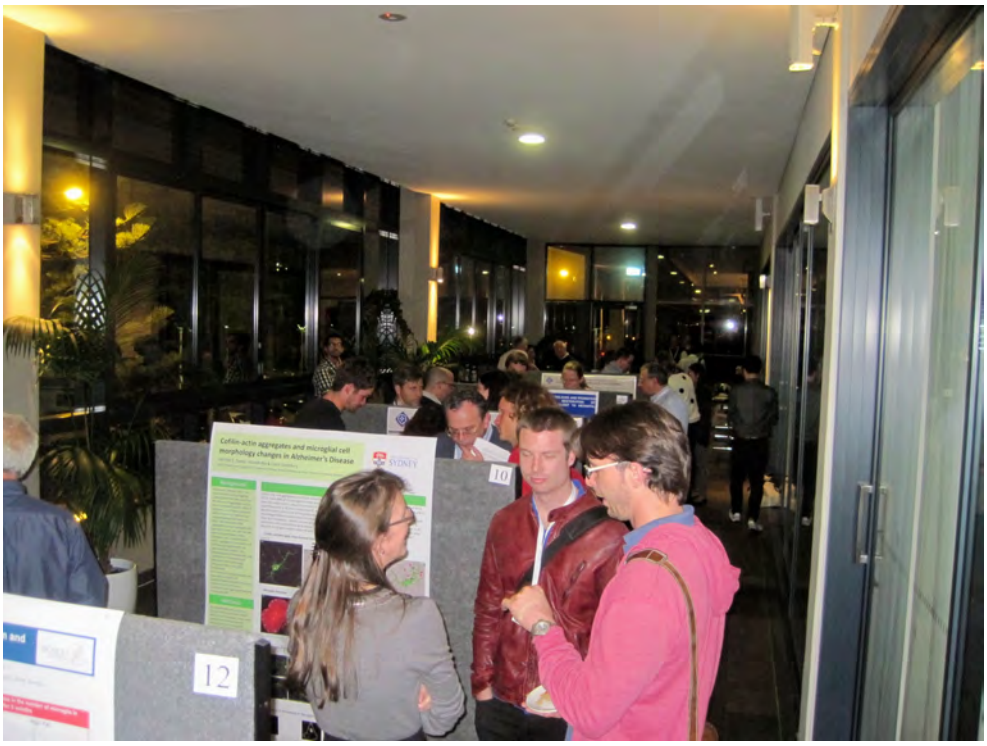
The meeting hosted a total of 77 scientific participants (including organizers and invited speakers) from around the world, bringing together a large number of specialists in the field. Around 25% of participants were postdocs and students. During the meeting, participants had the opportunity to interact and exchange knowledge over the course of the various scheduled scientific sessions as well as during meals and coffee breaks.

DOCUMENTS:

Prior to the meeting, participants received by email a copy of the final meeting book containing the program for the meeting and the meeting abstracts. On registering, the participants were provided with an identification badge and a meeting bag containing the meeting book as well as a pen and pad and sponsors leaflets.

SOCIAL EVENTS

Besides coffee breaks between scientific sessions, a welcome reception, three lunches, a poster session mixer and a closing dinner were offered to all participants. These events met the objective of promoting interaction among participants.



MEETING AWARDS FOR STUDENTS AND POSTDOCS

In order to support their costs associated with meeting registration and accommodation, a total of 12 students/postdocs were granted awards. All students/postdoc award recipients presented an oral communication or a poster. Two students from Spain were awarded grants from the Spanish Society for Neuroscience (SENC) to the value of 750 euros ($1,000.00) each for covering full registration and part of hotel accommodation. An additional 10 students/postdocs were awarded grants with funding (AU$3000) provided by the Institute of Neurosciences (INC), Autonomous University of Barcelona for covering part of registration. Finally, two poster prizes of AU$250 were made with the support of Miltneyi-Biotec. The names of the awarded students/postdocs were:

- Mirieia Recasens (ES) - SENC award
- Estefania Acaz (ES) - SENC award plus Miltenyi Biotec poster prize
- Chitban Chhatbar (GE) - INC award
- Mariano Guardia (US) - INC award
- Ilaria Prada (IT) - INC award
- Pavek Honsa (CR) - Miltenyi Biotec poster prize
- Jan Kriska (CR) - INC award
- David Dzamba (CR) - INC award
- Komal Kapoor (AU) - INC award
- Sofia Hassiotis (AU) - INC award
- Garry Niedermeier (AU) - INC award

TRAVEL SUBSIDIES FOR INVITED SPEAKERS

The following invited speakers and meeting organiser applied for and were granted a subsidy to assist with their travel costs:

Bernardo Castellano	$1,100
Michael Sofroniew,	$1,000
Burkhard Becher	$1,100
Tika Benveniste	$1,000
Knut Biber	$1,100
Elly Hol	$1,100
Shane Liddelow	$1,000
Toby Merson	$225
Ross O'Shea	$225
Trevor Owens	$1,100
Rosa Paolicelli	$1,100
Jake Streit	$425
Ann Turnley	$225
Alexei Verkhratsky	$1,100
Total awarded	$11,800

SPONSORSHIP

In addition to the USD$20,000 received from ISN, we attracted untied sponsorship from the University of Sydney (AU$8500), The NSW Government Department of Trade and Industry (AU$4500), Thermo Fisher (AU$1500) and Lonza (AU$1000). Sponsorship was acknowledged during the Meeting and in the meeting book (see attached).

BUDGET

EXPENSES	Number of persons/units	Per Person/unit	TOTAL
Delegate day charge	83	$232.00	$19,256.00
Accommodation	27	$717.00	$19,359.00
Dinner	61	$78.00	$4,758.00
Dinner beverages	61	$38.00	$2,318.00
Poster catering	80	$31.50	$2,520.00
AV	1	$1,534.00	$1,534.00
Miscellaneous	1	$500.00	$500.00
Miltenyi Poster Awards	2	$250.00	$500.00
INC website development	1	$3,000.00	$3,000.00
INC awards	1	$3,000.00	$3,000.00
SENC grants	2	$1,000.00	2,000.00
Invited speaker subsidies		$11,800.00	$11,800.00

TOTAL			$70,545.00

INCOME	Unit	$	TOTAL
ISN Grant	1	$20,000.00	$20,000.00
University of Sydney	1	$8,500.00	$8,500.00
NSW Trade	1	$4,500.00	$4,500.00
LONZA	1	$1,000.00	$1,000.00
Registration fees	1	$27,045.00	$27,045.00
Thermo Fisher	1	$1,500.00	$1,500.00
INC website development	1	$3,000.00	$3,000.00
INC awards	1	$3,000.00	$3,000.00
SENC grants	2	$1,000.00	2,000.00

TOTAL			$70,545.00

BALANCE			$0.00

SCIENTIFIC AND SOCIAL PROGRAM

Venue for all sessions: GILBERTS ROOM
NOVOTEL SYDNEY MANLY PACIFIC HOTEL

Wednesday, August 19

Arrival and check-in at hotel

16:00 - 18:00 Registration and poster set up

19:00 - 20:30 **Welcome reception**

Thursday, August 20

8:00 - 8:30 **Poster set up**

8:20 - 8:30 **Opening Introduction**

8:30 - 9:30 **Plenary Lecture (L1)**

Chair: Iain Campbell

- Florent Ginhoux (Singapore) - Ontogeny and differentiation of microglia

9:30 - 10:00 **Coffee Break**

10:00 - 12:00 **Symposium 1**: **Glial development in the CNS**

Chair: Trevor Kilpatrick

- S1.1. Ann Turnley (Australia) – Glial precursor cells: Response to traumatic brain injury (TBI) in the developing and adult brain
- S1.2. Michael Dragunow (New Zealand) - Role of PU.1 in human microglial biology
- S1.3. Tobias Merson (AUS) - Topographic organisation of oligodendrocytes in CNS white matter in health and disease.

12:00 - 13:30 **Lunch - Zalis Restaurant**

13:30 - 15:30 **Symposium 2: Mechanisms of altered glial cell phenotype**

Chair: Tika Benveniste

- S2.1. Shane Liddelow (USA) - Novel in vitro model systems to study "good" and "bad" reactive astrocytes
- S2.2. Helmut Kettenman (Germany) - Neurotransmitter/ - hormones control microglial functions
- S2.3. Estefania Acaz (Spain) - Role of estrogen receptors in the regulation of reactive gliosis
- S2.4. Jake Streit (USA) - Microglial phenotype in the ageing and diseased CNS

15:30 - 16:00 **Coffee break**

16:00 - 17:30 **Oral presentations - Session 1**

Chair: Rohan Walker

16:00 - 16:15 01. Genetic manipulation of astrocytes as a new cell target for CNS gene therapy**.**
Deborah Young (New Zealand)

16:15 - 16:30 O2. Control of neurogenic vs astrogliogenic fate in a restricted spinal cord progenitor domain.
Maria Sartoretti (Argentina)

16:30 - 16:45 O3. Viral encephalitis is controlled by IFNAR triggering of astrocytes and neurons that induce recruitment and proliferation of microglia.
Chintan Chhatbar (Germany)

16:45 - 17:00 O4. Glial microvesicles transfer miR-146a to neurons and modulate synaptotagmin I translation.
Ilaria Prada (Italy)

17:00 - 17:15 O5. Complement peptide C3a promotes astrocyte survival in response to ischemic stress
Marcela Pekna (Sweden)

17:00 - 17:15 O6. Astrocytes and microglia but not T lymphocytes are recruited in the spinal cord after intramuscular injections of adenovirus serotype 5.
Tushar Issar (Australia)

17:30 - 19:00 Poster session with drinks/snacks

P1. Withdrawn

P2. Crosstalk between neuronal CaMKIV and glial sigma-1 receptor signaling in depressive behaviors in mice.
Kohji Fukunaga (Japan)

P3. Differentiation potential of neonatal neural stem/progenitor cells is affected by Wnt signaling.
Jan Kriska (Czech Republic)

P4. Gene expression profiling of mouse astrocytes during ageing – advantages and limitations of single-cell approach.
David Dzamba (Czech Republic)

P5. ALK5 inhibition prevents astrogliosis and promotes myelination leading to restoration of sensorimotor function subsequent to neonatal hypoxia-ischemia.
Mariano Guardia Clausi (USA)

P6. Continual low-dose infusion of sulphamidase improves neuroinflammation in the MPS IIIA mouse brain.
Sofia Hassiotis (Australia)

P7. Metabolism of Tamoxifen in mouse brain: implications for fate-mapping studies using tamoxifen-inducible Cre/Lox system.
Martin Valny (Czech Republic)

P8. Differentiation potential of NG2 glia after ischemia is controlled by Sonic hedgehog.
Pavel Honsa (Czech Republic)

P9. Withdawn

P10. Cofilin-actin aggregates and microglial cell morphology changes in Alzheimer's disease.
Claire Goldsbury (Australia)

P11. Ultrastructural study on spatiotemporal progression of microcalcification in the

hippocampal CA1 region following transient forebrain ischemia in rats.
Tae-Ryong Riew (Republic of Korea)

P12. Diet-induced reactive changes of the central nervous system and their role in neurodegeneration.
Barney Viengkhou (Australia)

P13. Early myelination and microstructural abnormalities in white matter in the YAC128 mouse model of Huntington disease.
Mahmoud A. Pouladi (Singapore)

P14. Strawberry notch homolog 2 is a novel inflammatory response factor expressed predominantly in astrocytes.
Taylor Syme (Australia)

P15. Oxidative stress a common link between stroke and Alzheimer's disease and curcumin can reduce this stress.
Kaneez Fatima-Shad (Australia)

P16. Notch signaling is regulated by IGF-1 in astrocytes**.**
Estefanía Acaz-Fonseca (Spain)

Evening free

Friday, August 21

8:00 - 10:00 **Symposium 3: Cytokine/growth factor regulation of glial cell function**

Chair: Ann Turnley

- S3.1. Trevor Kilpatrick (Australia) - Trophic factor regulation of oligodendrocytes in myelination and demyelination
- S3.2. Tika Benveniste (USA) - Efficacy of Jakinibs in Autoimmune and Neurodegenerative Diseases
- S3.3. Trevor Owens (Denmark) - The role of type I IFNs in the CNS
- S3.4. Iain Campbell (Australia) - IL-6/gp130 family cytokine signalling in astrocytes and microglia

10:00 - 10:30 **Coffee Break**

10:30 - 12:30 **Symposium 4: Microglia/macrophages and inflammation in the CNS**

Chair: Knut Biber

- S4.1. Markus Hofer (Australia) – The interferon regulatory factor 9 (IRF9) is a key regulator of cerebral interferon responses
- S4.2. Nicholas King (Australia) - Role of CNS myeloid cells in West Nile Virus encephalitis
- S4.3. Gilles Guillemin (Australia) - Kyneurenine pathway in glial cells and its involvement in neuroinflammatory diseases
- S4.4. Burkhard Becher (Switzerland) - What does GM-CSF do in Neuro-Inflammation: On microglia and tissue-invading myeloid cells

12:30 - 14:00 **Lunch - Zalis Restaurant**

14:00 - 16:00 Symposium 5: Microglial interactions with neurones

Chair: Jake Streit

- S5.1. Mami Noda (Japan) - Thyroid hormones in glioendocrine system in health and disease
- S5.2. Knut Biber (Germany) - Ramified microglia as neuroprotective cells in organotypic hippocampal brain slices
- S5.3. Rosa Paolicelli (Switzerland) - Microglia as critical players in pruning synapses and sculpting brain connectivity
- S5.4. Rohan Walker (Australia) - Stress induced modulation of microglia: a critical role in the regulation of mood state and cognitive function?

16:00 - 16:30 Coffee break

16:30 - 18:30 Oral presentations – Session 2

Chair: Trevor Owens

16:30 - 16:45 O7. Deletion of the type-1 interferon receptor in $APP_{SWE}/PS1_{\Delta E9}$ mice results in increased cognitive function and changed microglial phenotype**.**
Peter Crack (Australia)

16:45 - 17:00 O8. In vivo characterisation of dying neurons by microglia engulfment in the spinal cord.
Marco Morsch (Australia)

17:00 - 17:15 O9. Acute hypertension causes increased microglial contact with neuronal synapses in cardiovascular nuclei in rat brainstem.
Komal Kapoor, (Australia)

17:15 - 17:30 O10. Interferon regulatory factor (IRF) 8 regulates the microglial cell response to sterile nerve injury in the brain.
Rui-Dan Xie (Australia)

17:30 - 17:45 O11. STAT1 and STAT3 activational balance and transcriptional responses to gp130 cytokines in astrocytes versus microglia**.**
Meng-Ping Hsu (Australia)

17:45 - 18:00 O12. Intracellular copper delivery limits neuroinflammation
Alexandra Grubman (Australia)

18:00 - 18:15	O13. Gliaptic interactions: Targeted microglial-mediated phagoptosis in brain pathology**.** Carlos Barcia (Spain)
18:15 - 18:30	O14. Astrocyte-targeted IL10 production modifies expression of TREM2 and CD200R in activated microglia after perforant pathway transection. Mireia Recasens (Spain)
19:00 - 22:00	**Conference Dinner**

Saturday, August 22

8:30 - 10:30 **Session 6: Astroglial contributions to the healthy and diseased CNS**

Chair: Michael Sofroniew

- S6.1. Alexej Verkhratsky (UK) - Astroglia in ageing and neurodegeneration with an emphasis on Alzheimer disease
- S6.2. Milos Pekny (Sweden) - Astrocytes, neuroplasticity and CNS regeneration
- S6.3. Elly Hol (Netherlands) - Astrocytes in brain disease – molecular and functional changes
- S6.4. Ross O'Shea (Australia) - SOD1 mutations causing familial amyotrophic lateral sclerosis induce toxicity in astrocytes: evidence for bystander effects in a continuum of astrogliosis

10:30 – 11:00 **Coffee break**

11:00 - 12:00 **Plenary lecture (L2)**

Chair: Bernardo Castellano

Michael Sofroniew (USA) - Role of the astroglial response to injury

12:00 - 13:30 **Lunch - Zalis Restaurant**

13:30 **Meeting close**

THE ORGANISERS OF THE SYDNEY GLIA MEETING GRATEFULLY ACKNOWLEDGE THE SUPPORT OF THE FOLLOWING SPONSORS:

International Society for Neurochemistry
http://www.neurochemistry.org/

The University of Sydney
http://sydney.edu.au/science/molecular_bioscience/

NSW Government. Dept of Industry
http://www.industry.nsw.gov.au/

Institut of Neurosciences
Universitat Autònoma de Barcelona
http://inc.uab.cat/English/

Sociedad Española de Neurociencias
http://www.senc.es/

Life Technologies
http://www.lifetechnologies.com/au/en/home.html

Lonza
http://www.lonza.com/
about-lonza/media-center/press-kit.aspx


Understanding the Function of Glia in the Healthy and Diseased CNS
Satellite Meeting in conjunction with the 25th ISN Biennial Meeting - Cairns 2015 -
Chairs: Iain L. Campbell (USYD, Australia) & Bernardo Castellano (UAB, Spain)
Manly Beach, Sydney, August 19-22, 2015
www.SydneyGliaMeeting.com

ABSTRACTS

PLENARY LECTURE 1

L1. ONTOGENY AND DIFFERENTIATION OF MICROGLIA

Florent Ginhoux

*Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR), Singapore*

Microglia are the resident macrophage population of the central nervous system (CNS). Adequate microglial function is crucial for a healthy CNS; microglia are not only the first immune sentinels of infection and inflammation, but are also involved in the maintenance of brain homeostasis. Emerging data are showing new and fundamental roles for microglia in the control of neuronal proliferation and differentiation, as well as in the formation of synaptic connections. In parallel, recent studies on microglial origin indicate that these cells arise very early during development from progenitors in the embryonic yolk sac that produce cells able to persist in the CNS into adulthood. These unique immune cells are thus present at all stages of brain development, including the prenatal stage of neuronal circuit formation, which points to the intriguing possibility that microglia might be involved in development of the CNS. Here, we show that microglia participate to normal embryonic forebrain wiring regulating the progression of dopaminergic axons in the forebrain and the laminar positioning of subsets of interneurons in the neocortex. Our study reveals novel roles for microglia in the normal assembly of brain circuits and raises the possibility that dysregulated embryonic microglial function during pre-natal inflammation could impact forebrain connectivity and could contribute to the etiology of neuropsychiatric disorders.

Singapore Immunology Network (SIgN) core funding

SYMPOSIUM 1

S1.1. GLIAL PRECURSOR CELLS: RESPONSE TO TRAUMATIC BRAIN INJURY (TBI) IN THE DEVELOPING AND ADULT BRAIN

Turnley AM[1], Henderson S[1], Dent K[1], Turbic A[1], Cate H[1], Bye N[1].

[1]*Department of Anatomy and Neuroscience, The University of Melbourne, Parkville, Victoria, Australia*

Following TBI there is progressive demyelination and degeneration of axons within white matter and parenchymal damage. Little is known about neural and glial precursor cell responses to TBI and whether this differs in the myelinated adult brain versus the developing brain. We have examined focal lesions of sensorimotor cortex in adult mice and in neonatal (P7) mice in which myelination is commencing. EdU or BrdU were administered to label proliferative cells and immunohistochemical analyses performed 24hr-3 months post-injury.

In the adult, decreased immunoreactivity of the mature oligodendrocyte marker CC-1 was observed in external capsule adjacent to the site of injury from 2 days to 2 weeks post-TBI, compared to sham injured animals, with ongoing oligodendrocyte apoptosis after this time. Proliferation of oligodendrocyte precursor cells (labelled with the oligodendrocyte lineage marker Olig2) was observed at 48 hours post-TBI and significant numbers of these cells and their progeny survived and remained in the external capsule within the injured hemisphere until at least 3 months post-injury.

In the neonatal brain at 24hrs post-injury (P8), there were no differences between injured and control brains in the number of proliferative (EdU^+) cells, nor of $Olig2^+$, $CC\text{-}1^+$ or $GFAP^+$ cells, in the external capsule or cortex. In the external capsule, <15% of GFAP cells co-labelled with Olig2, while in the cortex ~80% of GFAP+ cells were Olig2+. By 72hrs, the number of GFAP+ cells in the injured cortex was increased and few (~5%) were co-labelled with Olig2. By 1 week there were increased numbers of newborn $Olig2^+/BrdU^+$ cells in the injured cortex compared to controls.

These findings demonstrate that in the adult, oligodendrocyte precursors respond to TBI by replacing damaged oligodendrocytes and this process occurs for months after injury. In the neonate, TBI produces changes to glial development, which we are currently elucidating.

NH&MRC of Australia and Stem Cells Australia

S1.2. ROLE OF PU.1 IN HUMAN MICROGLIAL BIOLOGY

Dragunow M and Smith A

Centre for Brain Research, The University of Auckland, Auckland, New Zealand

The transcription factor PU.1, is present under basal conditions in both rodent and human microglia. A number of studies have identified important regulatory roles for PU.1 in rodent microglia. We have focused our work on determining the functions of PU.1 in human microglia, derived from biopsy temporal lobe brain tissue from donors undergoing surgery for complex partial seizures. Using both pharmacological and genetic manipulations we have discovered that PU.1 knock-down is associated with impaired microglial phagocytosis. In contrast, M-CSF-mediated PU.1 (and CEBPβ) induction in microglia is associated with enhanced phagocytosis and increased microglial proliferation. To uncover the genes regulated by PU.1 in human microglia we undertook a transcriptome study of PU.1 knock-out human microglia. Expression of many genes was regulated by PU.1 in human microglia, some particularly relevant to microglial inflammation in Alzheimer's disease. Thus, PU.1 may be a pivotal regulator of microglial function, and a novel drug target for the development of treatments to reduce brain inflammation.

Health Research Council of New Zealand, Hugh Green Foundation

S1.3. TOPOGRAPHIC ORGANISATION OF OLIGODENDROCYTES IN CNS WHITE MATTER IN HEALTH AND DISEASE

Philipp T Röth[1], Stanislaw Mitew2, Yao L Xing[1], Jo Anne H Stratton[1], Bernard H Chuang[1], Richa B Tripathi[3], William D Richardson[3], Ben Emery[4], Trevor J Kilpatrick[2], Tobias D Merson[1]

[1]Florey Institute of Neuroscience and Mental Health, Multiple Sclerosis Division, Parkville, Australia; [2]The University of Melbourne, Department of Anatomy and Neuroscience, Parkville, Australia, [3]University College London, Wolfson Institute for Biomedical Research, London, United Kingdom, 4 Oregan Heath & Science University, Jungers Center for Neurosciences Research, Portland, OR.

Rapid electrical conduction in the vertebrate central nervous system (CNS) relies upon myelination of particular subsets of axons. However, the underlying mechanisms that orchestrate this process remain poorly defined. We have investigated how myelinating oligodendrocytes establish their unique topographical arrangement within white matter during early postnatal brain development. Linear arrays of glial cell somata occur in all white matter tracts throughout the CNS. These structures are characterised by the tandem alignment of glial cell bodies along the axonal axis. We demonstrate that linear arrays of cells in the corpus callosum appear before the onset of myelination of this white matter tract. Throughout postnatal ontogeny they continue to be generated and increase in size. Oligodendroglial lineage cells represent between 60-70% of all cells within linear arrays, irrespective of postnatal age, and the proportion of mature oligodendrocytes increases with age. By using Cre-lox fate mapping in transgenic mice, we demonstrate that different oligodendrocyte populations from the medial ganglionic eminence and the anterior entopeduncular area are responsible for forming intermixed as well as homogeneous linear arrays. Additional characterisation using hemizygous female mice carrying an X-linked β-galactosidase gene revealed that clonal expansion also contributes to the formation of linear arrays. Increasing the activity of subsets of transcallosal axons that project from the developing somatosensory cortex increased the generation of linear arrays. By contrast, cuprizone challenge in adult mice abolished linear arrays in the caudal corpus callosum and arrays were only partially regenerated by 7 weeks after cuprizone withdrawal. Collectively, our data provide new insights into the cellular dynamics underlying the generation of oligodendrocyte topography during normal development and ageing. Modulating electrical activity or inducing de/remyelination alters the juxtapositioning of oligodendrocyte cell bodies within white matter and could have important implications for how myelin internodes are organised in space.

SYMPOSIUM 2

S2.1. NOVEL IN VITRO MODEL SYSTEMS TO STUDY "GOOD" AND "BAD" REACTIVE ASTROCYTES

Liddelow SA[1,2], Munch A[1], Clarke LE[1], Barres BA[1]

[1]*Department of Neurobiology, Stanford University, CA., USA*
[2]*Department of Pharmacology and Therapeutics, The University of Melbourne, Victoria, Australia*

Astrocytes undergo profound changes in morphology and gene expression in response to brain injury and disease. But whether reactive astrocytes are harmful or helpful has been unclear. We recently found that the genes induced in reactive astrocytes depends on the nature of the inducing injury. After ischemia, reactive astrocytes upregulate neurotrophic factors suggesting they may be beneficial, whereas after systemic injection of lipopolysaccharide (LPS) they strongly upregulate multiple complement cascade components needed to drive synapse destruction suggesting they may be detrimental. These findings suggest that, like macrophages which exist on a spectrum from bad (M1) to good (M2) states, reactive astrocytes also exist in bad (A1) and good (A2) states. Here we show that LPS-induced M1 microglia are sufficient to induce A1 reactive astrocytes. M1 microglia do this by releasing IL1α, TNFα and C1q, which together are sufficient to induce A1 (bad) reactivity in purified astrocytes within 24h and are all required for M1 microglia to induce the A1 state. Using IL1α, TNFα and C1q together, allowed us to create the first defined serum-free cultures of pure A1 reactive astrocytes enabling us to investigate their function. By directly comparing the function of normal astrocytes with A1 astrocytes in vitro, we found that A1 astrocytes are unable to promote neuronal survival, axon outgrowth, synapse formation or synapse function, and have lost the ability to phagocytose synaptosomes and myelin debris. In addition to loss of their normal functions, A1 reactive astrocytes gained a powerfully neurotoxic function, releasing a toxic protein that specifically induces apoptosis of neurons and oligodendrocytes. Drugs that prevent the formation of A1 reactive astrocytes or inhibit this toxic protein may have great potential to treat neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Multiple Sclerosis.

Christopher and Dana Reeve Foundation

Novartis Institute for Biomedical Research

NHMRC (Australia)

S2.2. NEUROTRANSMITTER/ - HORMONES CONTROL MICROGLIAL FUNCTIONS

Kettenmann H

Cellular Neurosciences,
Max Delbrück Center for Molecular Medicine in the Helmholtz Association,
Berlin, Germany

Neurotransmitters and –hormones are the communication signals by which neurons interact. There are two known forms of intercellular communication in the central nervous system called wiring transmission and volume transmission. The latter assumes that neurotransmitter/-hormones more broadly activate extrasynaptic receptors also on non-neuronal cells including microglia. Over the past decade we and others identified a variety of functional neurohormone/neurotransmitter receptors on microglia. We have identified for instance receptors for dopamin, serotonin, angiotensin, endothelin, substance P or GABA. We found that only a subpopulation of microglial cells expresses functional receptors for a given neurotransmitter/neurohormone. The transmitter sensitive population can change in pathology; the population of microglia expressing functional muscarinic acetylcholine receptors increases in mouse models of stroke. In cultured microglia the sensitive population can be altered by activation. These receptors control a variety of microglial functions. We found for instance that muscarinic acetylcholine receptors control migration or serotonin receptors the ability of microglia to respond to injury. Thus, the receptor systems enable microglial cells to sense the activity of their neuronal environment.

Pannell et al. (2014) Brain Struct Funct DOI 10.1007/s00429-014-0962

Pannell et al. (2014) GLIA 62:667-679

Deutsche Forschungsgemeinschaft

S2.3. ROLE OF ESTROGEN RECEPTORS IN THE REGULATION OF REACTIVE GLIOSIS

Garcia-Segura LM[1], Azcoitia I[2], Arevalo MA[1], Acaz-Fonseca E[1].

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[2]*Department of Cell Biology, Faculty of Biology, Universidad Complutense, Madrid, Spain*

Estradiol is a neuroprotective molecule. It is both a hormone and a local factor produced in the brain. Although estradiol may directly act on neurons to promote neuroprotection in vitro, the participation of other cell types in the mechanisms of action of estradiol is also necessary to maintain global tissue homeostasis in vivo. In different brain injury models estradiol regulates reactive gliosis. Glial cells express estrogen receptors (ERs), including ERα, ERβ and G protein-coupled estrogen receptor-1 (GPER). Brain injury enhances the expression of ERs in reactive astrocytes and induces de novo the expression of aromatase, the enzyme that synthesizes estradiol, in these cells. Estradiol then acts on ERs in reactive astrocytes to stimulate the expression of neuroprotective molecules such as insulin-like growth factor-1, transforming growth factor β and neuroglobin. In addition, both ERα and ERβ are involved in the anti-inflammatory and anti-gliotic actions of estradiol on microglia and astrocytes. The anti-inflammatory action of ERα is exerted through the activation of the PI3K pathway, which in turns blocks nuclear factor κB activation and translocation to the cell nucleus. ERβ also plays an essential role in the regulation of the neuroinflammatory response of astrocytes. This effect is in part mediated by the upregulation of neuroglobin, a hemoprotein with partial sequence identity with vertebrate hemoglobin and myoglobin, which protects neurons from a variety of insults, such as hypoxia, glucose deprivation, oxidative stress, β-amyloid toxicity and experimental stroke. The regulation of reactive gliosis by locally synthesized estradiol not only plays a role on neuroprotection, but also in neuronal information processing as shown by the inhibition of aromatase in the spinal cord, which results in increased gliosis and increased pain perception.

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S2.4. MICROGLIAL PHENOTYPE IN THE AGEING AND DISEASED CNS

Streit WJ

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The primary function of microglia in the normal CNS is to provide neuronal protection and immune surveillance. With human ageing, microglia undergo a number of characteristic morphological changes that result in the presentation of a dystrophic phenotype likely reflecting the development of cell senescence. I believe that microglial senescence involves a diminution of microglial neuroprotective functions and accordingly have hypothesized that Alzheimer-type neurofibrillary degeneration (tau pathology) occurs as a consequence of an ageing-related decline in microglial ability to support neurons. To this end, we have studied microglial phenotypes specifically in those areas of the human cerebral cortex known to undergo primary neurofibrillary degeneration, i.e. the mesial temporal lobe. Our findings show that the appearance of tau-positive structures (neurofibrillary tangles, neuropil threads, senile plaques) in the entorhinal cortex and surrounding temporal gyri is accompanied and preceded by the development of widespread microglial dystrophy. The extent of microglial senescent degeneration correlates positively with the development of neurodegeneration in both Alzheimer and Down syndrome brain, and we therefore take these findings to support our hypothesis of microglial dysfunction. At the same time, our findings which failed to show the presence of activated microglia in the human temporal cortex provide evidence against the longstanding idea that neuronal degeneration is a result of overly activated, out-of-control microglia believed to produce a variety of detrimental neurotoxins. Thus, we believe that the brain's innate immune system (similar to the systemic immune system) is subject to an ageing-related decline in function due to the senescent deterioration of microglia. The implications for potential therapeutic approaches are profound: instead of trying to suppress microglia with anti-inflammatory drugs to prevent neurodegeneration, it may be more effective to develop agents that can stimulate microglial cell activity.

National Institues of Health, Cooper familiy of Indialantic, FL

ORAL PRESENTATIONS, AUGUST 20

O1. GENETIC MANIPULATION OF ASTROCYTES AS A NEW CELL TARGET FOR CNS GENE THERAPY

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To date, gene therapy strategies for treatment of neurological disorders have largely focused on the manipulation of neuronal physiology. Attention is now turning to astrocytes to determine whether this prevalent cell population could be an attractive alternative cell target for gene therapy. Our lab has a long-standing interest in the development and application of adeno-associated viral (AAV) vectors for mediating delivery of genes-of-interest to brain cells. We have shown that transgenes as well as artificial micro-RNA sequences that mediate gene silencing through RNA interference-based mechanisms can be expressed in astrocytes when these elements are placed under the control of the glial fibrillary-acidic protein (GFAP) promoter, and delivered in the context of different AAV vector serotypes to have a functional effect (Young et al., 2014, Gene Ther., 21(12):1029-40). Our recent work has focused on determining the neuroprotective potential of nuclear receptor related 1 (NURR1) a transcription factor important in maintenance of dopamine neurons and regulation of inflammatory mediators in astrocytes. AAV serotype 5 vectors expressing NURR1 or a control destabilized yellow reporter gene or empty vector were injected unilaterally into the rat substantia nigra before intrastriatal infusion of the neurotoxin 6-hydroxydopamine 3 weeks post vector injection. At 8 weeks, stereological quantification of tyrosine hydroxylase (TH)-positive dopamine neurons revealed NURR1 overexpression afforded greater protection of TH-positive cells and a recovery from motor deficits as assessed by contralateral forepaw usage compared to the control vector treatments. Lower immunoreactivity to astrocyte and microglia markers, GFAP and Iba1 respectively, in HA-NURR1 brains indicated reduced neuro-inflammation. Future work will be needed to investigate how AAV-mediated transduction efficiency and transgene expression is affected when vectors are introduced into the pathological milieu.

Royal Society of NZ Marsden Fund

O2. CONTROL OF NEUROGENIC VS ASTROGLIOGENIC FATE IN A RESTRICTED SPINAL CORD PROGENITOR DOMAIN

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Despite of the high heterogeneity in morphology and function of astrocytes in central nervous system, there is still an incomplete understanding about the cellular and molecular control of their development. By using mouse genetics, we studied a small group of ventral progenitors of the embryonic spinal cord -called p0-, which expresses the transcription factor Dbx1. After the production of V0 Interneurons (V0-IN), this progenitor pool is later committed to generate a subset of astrocytes. We named it as vA0. Their precursors radially migrate to populate a defined region of the lateral spinal cord. Mosaic fluorescent labelling showed that vA0 population is composed by both protoplasmic and fibrous astrocytes, demonstrating that a single progenitor domain produces astrocytes with different morphological features. We found that Dbx1 controls specification of these astrocytes, as in its absence vA0 is augmented at the expense of V0-INs. Notch signalling plays key roles in binary fate-cell decisions and glial determination. We evaluated if this pathway modulates early decision between producing V0 neurons and vA0 astrocytes, and whether differential Notch activity could be involved in Dbx1 function. Presenilin1 mutants, that have severely attenuated Notch signalling, exhibited diminished p0-derived glial cells, while V0-INs numbers were increased. Impairing Notch pathway with Ly411575 only at neurogenic stages, but not later, showed similar results. Additionally, gliogenic precursors are increased in Dbx1 mutants and are reduced in Psen1 mutants after the neurogenic stage. This prompted us to analyze key players of Notch signalling pathways in Dbx1 wt and mutant neural tubes before the initiation of gliogenesis. Surprisingly, while wild type p0 domain expresses Delta1 ligand, this domain is converted into Jagged1 positive in the absence of Dbx1. Our results suggest that the type of Notch ligand directed by Dbx1 transcription factor controls the differentiation of p0 precursors by biasing neurogenic vs astrogliogenic fate.

Agencia, Conicet

O3. VIRAL ENCEPHALITIS IS CONTROLLED BY IFNAR TRIGGERING OF ASTROCYTES AND NEURONS THAT INDUCE RECRUITMENT AND PROLIFERATION OF MICROGLIA

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Previously we found that during central nervous system (CNS) virus infection, type I IFN signaling of CNS resident cells is essential for survival and for clearance of the virus from CNS. Since astrocytes and neurons together make the highest cell mass of CNS, in this study we investigated the contribution of astrocytes and neurons to the clearance of VSV CNS infection. Using mice that lack type I IFN signaling on astrocytes (GFAPCre+/-IFNARfl/fl) or neurons (Syn1Cre+/-IFNARfl/fl), we found that type I IFN signaling of both astrocytes and neurons was essential to control VSV spread in the CNS.

Since microglia are CNS resident innate immune cells, we also looked at microglial phenotype in these animals. In the VSV infected wild type animals, six days post infection microglia were activated as evident by MAC-3 staining and an increase in their numbers in glomerular regions of olfactory bulb was also observed. Many microglia were Ki67 positive suggesting microglial proliferation.

Interestingly in VSV infected animals lacking type I IFN signaling in astrocytes or neurons, microglial activation was unaffected as seen by MAC-3 staining. However, microglial numbers were reduced compared with wild type animals. Further, in wild type animals, microglia had large cell body, thick and short processes and globular shape while in animals lacking IFN signaling in astrocytes or neurons, microglia were rod shaped with long processes suggesting different state of activation.

Taken together our results suggest that type I IFN signaling of astrocytes and neurons trigger microglial recruitment and proliferation which in turn contributes to viral control within the CNS.

This study was supported by the Niedersachsen-Research Network on Neuroinfectiology (N-RENNT) of the Ministry of Science and Culture of Lower Saxony, Germany (to M.S. and U.K.) and by a bilateral project of the German Centre for Neurodegenerative Diseases and the Helmholtz Centre for Infection Research (to M.S. and U.K.).

O4. GLIAL MICROVESICLES TRANSFER MIR-146A TO NEURONS AND MODULATE SYNAPTOTAGMIN I TRANSLATION.

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Beyond the classical secretory mechanism through which glial cells influence brain activity, astrocytes and microglia, release circular membrane fragments, the extracellular vesicles (EVs). EVs contain several components of the donor cell (RNAs/proteins/lipids) and transfer their cargo to recipient cells, functioning as an efficient intercellular delivery mechanism. Aim of this study was to investigate whether glial cells may regulate neuronal gene expressions through EV secretion. Using miRNA real-time-PCR panels, we identified a set of miRNAs differentially expressed in EVs produced by pro-inflammatory compared to pro-regenerative microglia. Among them we found a glia-enriched microRNA, the miR-146a, which is altered in brain disorders and targets neuron-specific genes. To investigate possible glia-to-neuron shuttling of miR-146a, we performed a Renilla/Luciferase-based assay transfecting hippocampal neurons with a miR-146a-specific sensor, and exposing them to glial-EVs for 24h. Exposure to glial EVs caused an increase in neuronal miR-146a levels, with a consequent immunoreactivity decrease of a validated miR-146a target, the synaptic vesicle protein synaptotagminI. Transfection of donor glial cells with an anti-miR-146a inhibitor or clocking phosphatidyl-serine residues on glial EVs, a determinant for EV recognition on neurons, resulted in unchanged mir-146a concentration in target neurons. Taken together, these data show that glia-derived EVs transfer biologically active mir-146a to neurons, highlighting the capability of glia to modulate neuronal gene expression. To investigate how the transfer of miRNA cargo takes place, we combined optical manipulation with live imaging. EVs positioned on the cell body make a quite stable interaction with neurons, remaining attached to the neuronal surface up to 1h. Together with confocal analysis of fixed neurons exposed to EVs for different time points, this observation rules out the possibility that EVs undergo rapid internalization or full fusion with cell membrane. Further investigation is ongoing to clarify whether EVs can open a transient pore to transfer their cargo to neurons.

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O5. COMPLEMENT PEPTIDE C3A PROMOTES ASTROCYTE SURVIVAL IN RESPONSE TO ISCHEMIC STRESS

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Astrocytes are the most numerous cells in the central nervous system with a range of homeostatic and regulatory functions. Under normal conditions as well as after ischemia, astrocytes promote neuronal survival. We have previously reported that the complement-derived peptide C3a stimulates neuronal differentiation of neural progenitor cells and protects the immature brain tissue against hypoxic-ischemic injury. Here, we studied the effects of C3a on the response of mouse cortical astrocytes to ischemia. We have found that chemical ischemia, induced by combined inhibition of oxidative phosphorylation and glycolysis, up-regulates the expression of C3a receptor in cultured primary astrocytes. C3a treatment protected wild-type but not C3a receptor deficient astrocytes from cell death induced by chemical ischemia or oxygen-glucose deprivation by reducing ERK signaling and caspase-3 activation. C3a attenuated ischemia-induced upregulation of glial fibrillary acidic protein, however the protective effects of C3a were not dependent on the presence of the astrocyte intermediate filament system. Pre-treatment of astrocytes with C3a during recovery abrogated the ischemia-induced neuroprotective phenotype of astrocytes. Jointly, these results provide the first evidence that the complement peptide C3a modulates the response of astrocytes to ischemia and increases their ability to cope with ischemic stress.

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O6. ASTROCYTES AND MICROGLIA BUT NOT T LYMPHOCYTES ARE RECRUITED IN THE SPINAL CORD AFTER INTRAMUSCULAR INJECTIONS OF ADENOVIRUS SEROTYPE 5

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Background: Adenovirus is an ideal vector for the transient expression of therapeutic transgenes to treat spinal cord injury (SCI). However, central injections of adenovirus have been shown to trigger an immune response that nullifies its therapeutic potential. We have developed a gene therapy scenario whereby transgenes are shuttled to spinal cord motor neurons via intramuscular injections. However, the immune response in the spinal cord after such delivery has yet to be characterised.

Objective: To characterise the immune response in the spinal cord and the targeted muscle after intramuscular injections of adenovirus.

Methods: Wild-type C57Bl6 mice received a series of injections of adenovirus (serotype 5) encoding eGFP (Ad.eGFP) along the full length of the motor end plates of triceps brachii. One week later, spinal cord tissue and triceps brachii were processed histologically and subjected to Iba1, GFAP and Cd3 immunohistochemistry to decipher whether macrophage and microglia, astrocytes and T lymphocytes are recruited by the adenovirus. Spinal cord tissue and muscle were imaged under epifluorescence microscopy and analysed with ImageJ software.

Results: In naïve animals, T lymphocytes and macrophages are absent from triceps brachii. Microglia and astrocytes are present in the naïve spinal cord but at baseline levels, and no T lymphocytes can be observed. In Ad.eGFP-injected animals, a strong recruitment of T lymphocytes and macrophages is observed in triceps brachii. In these mice, the presence of microglia and astrocytes was dramatically increased in the spinal cord, however, no T lymphocyte recruitment was observed.

Conclusion: These results are in sharp contrast with previous reports of the presence of T lymphocytes in the CNS after direct injections of adenovirus. This indicates that intramuscular injections to deliver transgenes to the spinal cord motor neurons is not only a minimally invasive but also a clinically relevant way to shuttle transgenes to the spinal cord.

National Health and Medical Research Council (NHMRC), Australia

POSTERS

P1. WITHDRAWN

P2. CROSSTALK BETWEEN NEURONAL CAMKIV AND GLIAL SIGMA-1 RECEPTOR SIGNALING IN DEPRESSIVE BEHAVIORS IN MICE

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Ca^{2+}/calmodulin-dependent protein kinase IV (CaMKIV) is expressed in both mature and immature granular cells in mouse dentate gyrus (DG). Recently Song et al reported that CaMKIV null mice exhibit depressive behaviors and impair neurogenesis as assessed by bromodeoxyuridine (BrdU) incorporation into newborn cells of the hippocampal DG (Int J Neuropsychopharmacol 2012;16:803) and that the typical SSRI, fluoxetine fails to enhance the DG neurogenesis and to improve depressive behaviors in CaMKIV null mice. On the other hand, sigma-1 receptor (Sig-1R) is highly expressed in astrocytes in the DG. Here, we addressed whether Sig-1R agonist (SA4503) and SSRI (fluvoxamine) with affinity for Sig-1R rescue the depressive behaviors in CaMKIV null mice. Treatment with SA4503 or fluvoxamine for 14 days completely rescued the depressive behaviors in CaMKIV null mice. By contrast, treatment with paroxetine, which lacks affinity for Sig-1R, did not improve these behaviors. Reduced numbers of BrdU-positive cells and decreased BDNF mRNA expression seen in the DG of CaMKIV null mice were significantly restored by chronic Sig-1R stimulation with SA4503 or fluvoxamine. We previously reported that Sig-1R stimulation with SA4503 and fluvoxamine promotes ATP production through increased mitochondrial calcium transport (Tagashira et al., BBA2013;1830:3082). As expected, the reduced ATP production in the DG of CaMKIV null mice was improved by chronic Sig-1R stimulation. Taken together, Sig-1R stimulation rescues the SSRI-resistant depressive behaviors observed in CaMKIV null mice, suggesting attractive therapeutics for SSRI-resistant depressive patients.

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P3. DIFFERENTIATION POTENTIAL OF NEONATAL NEURAL STEM/PROGENITOR CELLS IS AFFECTED BY WNT SIGNALING

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The canonical Wnt signaling pathway has an important role in embryonic development and the establishment of neurogenic niches. Here we aimed to elucidate the effect of Wnt signaling on the differentiation potential of neonatal neural stem/progenitor cells (nNS/PCs), isolated from the frontal lobe of the mouse brain, *in vitro*. To manipulate Wnt signaling at different cellular levels, three transgenic mouse strains, enabling tamoxifen triggered activation/inhibition of Wnt signaling, were used. To follow the fate of nNS/PCs, immunocytochemical staining, the patch-clamp technique and calcium imaging were employed. We identified three distinct current profiles among differentiated nNS/PCs. Flat-shaped $GFAP^+$ cells displayed passive time- and voltage-independent K^+ currents, while round DCX^+/$MAP2^+$ cells expressed fast activating outwardly rectifying K^+ currents (K_A) and delayed outwardly rectifying K^+ currents (K_{DR}). Star-like $NG2^+$/$PDGFR\alpha^+$ cells with a complex current pattern were characterized by inwardly rectifying K^+ currents (K_{IR}), in addition to K_{DR} and K_A. Wnt signaling pathway inhibition caused a marked decrease in the incidence of the cells displaying an outwardly rectifying current pattern (neuron-like cells), while the number of the cells with a passive and complex current pattern increased. In neuron-like cells, we also detected increased K_{DR} and K_{IR} current densities, decreased incidence of cells expressing voltage-dependent Na^+ channels and lowered Ca^{2+} responses to glutamate application. On the other hand, activation of Wnt signaling led to opposite effects in the incidence of the three distinct cell types, current density of K_A in the cells showing a complex current pattern, and in the current density of voltage-dependent Na^+ channels. Taken together, our data indicate that the canonical Wnt signaling pathway promotes nNS/PCs differentiation into cells with neuronal characteristics.

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P4. GENE EXPRESSION PROFILING OF MOUSE ASTROCYTES DURING AGEING – ADVANTAGES AND LIMITATIONS OF SINGLE-CELL APPROACH

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The single-cell RT-qPCR technique brought new dimension to gene expression profiling. In contrast to bulk samples, it enables to identify rare cells or particular subpopulations within the population of studied cells and together with single-cell gene expression correlations leads to better biological interpretations. The aim of this study was to compare RT-qPCR results obtained from single-cells and bulk samples containing thousands of cells, to describe this relationship and characterize advantages and limitations of single-cell approach. For this purpose, we used FACS-sorted cortical astrocytes isolated from 1-22 months-old GFAP-EGFP mice. First, we described mutual dependence of these two types of results in a mathematical formulation (sigmoid function) and set the technical criteria for obtaining high quality data. We further analyzed the possibilities of single-cell data normalization according to the reference genes, which is a common practice in bulk but not in single-cell samples. We identified the most suitable reference genes (*ACTB, GAPDH* and *PPIA*) and the minimal amount of cells needed for such normalization. In contrast to bulk samples, results obtained from single-cells enable to calculate correlations in expression of particular genes and to perform principal component analysis which identifies potential cell subpopulations. However, the detection limit of single-cell samples does not allow analysis of genes with low expression. Having data from both, single-cell and bulk samples, provided additional information helping us to better understand changes in gene expression which we demonstrate in particular examples of genes expressed in mouse cortical astrocytes during ageing. The conclusion of our study is that the best picture of gene expression is reached in the combination of results obtained from single-cells and bulk samples.

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P5. ALK5 INHIBITION PREVENTS ASTROGLIOSIS AND PROMOTES MYELINATION LEADING TO RESTORATION OF SENSORIMOTOR FUNCTION SUBSEQUENT TO NEONATAL HYPOXIA-ISCHEMIA

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Our previous studies have shown that during recovery from H-I there is aberrant production of astrocytes and there is evidence that reactive astrocytes inhibit the differentiation of oligodendrocyte progenitors. We have identified TGFß1 as a cytokine that is produced subsequent to H-I that collaborates with other cytokines to stimulate the production of astrocytes from SVZ glial progenitors. The specific goal of this study was to evaluate gliogenesis and sensorimotor function after H-I when the type 1 TGFß1 receptor ALK5 is antagonized in the Vanucci P6 H-I rat model. Osmotic minipumps loaded with SB505124 were implanted 2 days after H-I and two intra peritoneal injections of BrdU were administered at 7 days after H-I. At 21 days after H-I, different sensorimotor functional tests were performed. The following day, the animals were sacrificed and their brains processed for Immunofluorescence. Sections were stained using markers for astrocytes and cells at different stages in the oligodendroglial lineage in combination with BrdU. During recovery from H-I there was a 4-fold increase in the number of GFAP+/BrdU+ cells in the affected neocortex, striatum and corpus callosum accompanied by 50% decrease in the numbers of Olig2+/BrdU+ cells. These neuropathological alterations were correlated with functional impairments in the behavioral tests. All of these parameters were brought back towards normal levels with SB-505124 treatment. Our results indicate that SB-505124, an inhibitor of ALK5 signaling, decreases astrogliosis, promotes myelination and restore sensorimotor function after neonatal H-I.

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P6. CONTINUAL LOW-DOSE INFUSION OF SULPHAMIDASE IMPROVES NEUROINFLAMMATION IN THE MPS IIIA MOUSE BRAIN

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MPS IIIA is a neurodegenerative and neuroinflammatory paediatric lysosomal storage disorder characterised by a deficiency in sulphamidase enzyme, which primarily affects the central nervous system, leading to an increase in heparan sulphate and neurodegeneration. Currently there is no marketed effective treatment.

Six-week old MPS IIIA mice received equivalent amounts of sulphamidase enzyme via either repeated intra-cisternal bolus injections or slow pump-based delivery over a period of one month. Age-matched untreated MPS IIIA/unaffected mice were used as controls. Sulphamidase distribution, lysosomal storage and neuroinflammation were examined in the 10-week old mouse brains, via immunohistochemistry.

Enzyme assays indicated that the activity of sulphamidase in the pumps was retained over the 4-week period. Immunohistochemistry revealed that slow pump-based delivery of sulphamidase enabled widespread distribution in the MPS IIIA mouse brain, with more limited distribution seen in mice receiving repeated intra-cisternal bolus injections.

Slow pump-based delivery of sulphamidase to MPS IIIA mice initiated statistically significant decreases in both lysosomal engorgment and neuroinflammation; in particular microgliosis. Whilst MPS IIIA mice receiving repeated intra-cisternal bolus injections exhibited reductions in lysosomal substrate storage, there was little impact on neuroinflammation.

Although longer-term evaluations are required, this proof-of-principle study indicates that pump delivery of sulphamidase is comparable to or better than repeated intra-cisternal bolus injections in MPS IIIA mice, highlighting the potential of using pump delivery of enzyme as a therapeutic option to treat MPS IIIA patients.

This study was funded by the Australian National Health and Medical Research Council (Grant #565074 to JJH and KMH) and Shire Human Genetics Therapies.

P7. METABOLISM OF TAMOXIFEN IN MOUSE BRAIN: IMPLICATIONS FOR FATE-MAPPING STUDIES USING TAMOXIFEN-INDUCIBLE CRE/LOX SYSTEM

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Tamoxifen-inducible Cre/Lox system is widely used in fate-mapping studies for visualization or functional modification of specific cell-type population at desired time-points. In experiments, in which the cell fate is influenced by any treatment, such as surgery or drug administration, the time window between tamoxifen injection and the treatment should be large enough to allow complete degradation of tamoxifen and its metabolites. Otherwise, they might promote recombination in cells that start express the Cre recombinase as a result of the treatment, which can lead to data misinterpretation. We aimed to define the optimal time window allowing the complete degradation of tamoxifen and its metabolites, such as 4-hydroxytamoxifen, N-desmethyltamoxifen, endoxifen and norendoxifen, in mouse brain after injection of 400 mg/kg tamoxifen i.p. Therefore,we determined EC50 of all substances and employed liquid chromatography coupled with mass spectroscopy to determine their degradation kinetics. Our results revealed that tamoxifen and its metabolites were completely degraded within 10 days in young adult C57BL/6 mice (male), while the age-matched FVB male mice displayed faster degradation kinetics and metabolized all substances within 6 days. Moreover, 18 months old C57BL/6 mice were unable to metabolize all substances within 14 days. Interestingly, disruption of blood-brain barrier by middle cerebral artery occlusion 14 days after tamoxifen injection caused a sudden increase in 4-hydroxytamoxifen concentration in injured hemisphere exceeding its EC50 value. Taken together, we show that tamoxifen metabolism in mouse brain is age- and strain-dependent, and is affected by the disruption of blood-brain barrier following ischemic injury. These findings indicate the possible weaknesses of fate-mapping of neural cells using tamoxifen-inducible Cre/Lox systems.

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P8. DIFFERENTIATION POTENTIAL OF NG2 GLIA AFTER ISCHEMIA IS CONTROLLED BY SONIC HEDGEHOG

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NG2 glia constitute a fourth glial cell type in the adult mammalian central nervous system that is distinct from other neural cell types. Several studies have shown that these cells display wide differentiation potential under pathological conditions in vivo, where they give rise predominantly to reactive astrocytes. The aim of this study was to identify the growth factors that influence the NG2 glia differentiation after cerebral ischemia.

We used transgenic Cspg4-cre/Esr1/ROSA26Sortm14(CAG-tdTomato) mice, in which tamoxifen administration triggers the expression of red fluorescent protein (tomato) specifically in NG2 glia. Differentiation potential (in vitro and in vivo) of tomato-positive (tomato+) NG2 cells from control or post-ischemic brains was determined using the patch-clamp method and immunohistochemistry in the presence of drugs, which activate or inhibit Sonic hedgehog (Shh) signaling pathway. The ischemic injury was induced by middle cerebral artery occlusion, a model of focal cerebral ischemia (FCI).

Tomato+ cells isolated from control brains and cultivated in vitro without Shh signaling manipulation displayed membrane and immunohistochemical properties corresponding to several cell phenotypes. They predominantly comprised NG2 glia (45.0 ± 3.9%), astrocytes (22.0 ± 4.1%), oligodendrocytes (18.7 ± 5.0%) and pericytes (13.3 ± 3.8%). On the other hand, NG2 glia isolated from the post-ischemic cortex differentiated in vitro preferentially into astrocytes (74.1 ± 5.4%) at the expense of NG2 glia. The activation of Shh signaling in NG2 glia isolated from control brain significantly increased differentiation of NG2 glia into astrocytes, while the inhibition of Shh signaling such differentiation diminished.

We also manipulated Shh signaling in vivo after FCI. After Shh signaling activation the number of astrocytes derived from NG2 glia increased in the gliotic scar, while Shh signaling inhibition their number decreased.

Taken together, our data indicate that Shh is an important factor that controls the composition of gliotic scar formed around ischemic injury.

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P9. WITHDRAWN

P10. COFILIN-ACTIN AGGREGATES AND MICROGLIAL CELL MORPHOLOGY CHANGES IN ALZHEIMER'S DISEASE

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Background: Alzheimer's disease (AD) is the most common form of ageing-related dementia. We have found that aggregates of the actin-cytoskeletal associated protein cofilin in AD brain parenchyma correlate with the extent of hallmark tau neuropathology and are observed in and around microglial cells. An innate inflammatory-like response involving brain resident microglia is characteristic of AD. The spatiotemporal characteristics and significance of this response are not fully understood. A better understanding of how microglial responses and/or their dysfunction exacerbate AD is needed so that these may provide targets for disease-modification and the development of new therapies. Methods: Microglia have polymorphic morphologies including cells with multiple long thin processes, short thicker processes, retracted processes and processes with redistribution of Iba1 protein causing a beaded or fragmented appearance. We quantified these microglial cell morphologies within AD temporal and cingulate cortex compared with aged-matched control cases, younger control cases and other neurodegenerative disease. Results: There was no change in the total density (about 150 cells per mm^2 in all groups) and apparent viability of microglial cells in the AD brain. Cell densities also remained consistent with ageing. By contrast, there were statistically significant changes in microglial morphology specifically associated with neurodegenerative disease and not with general ageing. These included in AD, a higher proportion of microglia with redistribution of Iba1 protein along their processes into bead-like or fragmented clusters. Conclusions and outlook: Our results are in line with previous reports that have described beaded and fragmented microglial processes in the AD brain. The significance of these observations to microglial function and AD remains incompletely understood. Further investigation is needed to determine whether these changes reflect a primary event early in the disease course and/or if they represent a disease-exacerbating response to a neurodegenerative process or earlier synapse loss.

NHMRC

The Rebecca Cooper Medical Research Foundation

P11. ULTRASTRUCTURAL STUDY ON SPATIOTEMPORAL PROGRESSION OF MICROCALCIFICATION IN THE HIPPOCAMPAL CA1 REGION FOLLOWING TRANSIENT FOREBRAIN ISCHEMIA IN RATS

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Calcification on areas of neuronal degeneration is commonly found in neuropathological disorders including ischemic injury, and neuronal calcium precipitation could be regarded as a part of compensatory mechanism to reduce further brain damage. However, the onset and spatiotemporal progression of calcification in the ischemic hippocampus have not been characterized in detail. The present study examined spatiotemporal profile of the calcification in the CA1 hippocampal region, an area undergoing delayed neuronal death after transient forebrain ischemia. No specific staining for alizarin red S was detected in the hippocampus of sham-operated and ischemic rats by 7 days after reperfusion. At 14 days, however, amorphous to granule-like alizarin red staining was evident in the CA1 strata oriens and radiatum, but not in the pyramidal cell layer(PCL), and there was a further increase at 28 days, although weaker in the pyramidal cell layer. By the osmium/potassium-bichormate method and electron probe microanalysis, we found in the CA1 dendritic subfield that electron-dense precipitation was abundantly observed within the mitochondria in degenerating dendrites that were still contacted by uncalcifying neurites by 7 days after reperfusion. At 14 days, calcium deposits appeared to spread beyond mitochondria and extend over the whole neurite and these calcifying neurites were frequently aggregated and fused together. Most of these calcifying dendrites were surrounded by astrocytes showing dense bundles of intermediate filaments. In degenerated soma in the CA1 PCL, however, calcium deposition was noted within, but not beyond, mitochondria, although most of cell organelles disappeared and only amorphous substances remained. These calcification patterns were maintained by 28 days after reperfusion, but large folded, or multilobulated calcifying deposits of variable form and size were frequently observed throughout the CA1 hippocampus. Our data suggest that microcalcification occurred within mitochondria of degenerating dendrites may serve as a nidus for further calcium precipitation in the ischemic hippocampus.

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P12. DIET-INDUCED REACTIVE CHANGES OF THE CENTRAL NERVOUS SYSTEM AND THEIR ROLE IN NEURODEGENERATION

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Obesity has reached epidemic proportions worldwide and is strongly associated with several comorbidities including type 2 diabetes, hypertension and stroke, collectively summarised as `metabolic syndrome´. Furthermore, there is also evidence for a relationship between over-nutrition and neurodegenerative diseases such as Alzheimer´s disease and Parkinson´s disease. Importantly, obesity-associated changes are not restricted to peripheral tissues, but are also observed in the brain. For example in the High Fat Diet (HFD) mouse model of obesity, pathological changes include inflammatory and reactive changes in the hypothalamus that potentially exacerbate disturbance to physiological regulation of feeding and metabolism.

Our aim was to characterise the chronic morphological and molecular changes of microglia and astrocytes in the brain of mice fed on different macronutrient imbalanced diets. Mice (male, C57Bl/6) were fed on a standard chow (SCD), HFD or high sucrose diet (HSD) for three or six months. Mice on a HFD showed an increase in the number of hypothalamic microglia and astrocytes compared with those on the SCD and HSD. Morphological changes in microglia of HFD fed mice suggested an activation of these cells. In addition to the changes in the brain, mice on a HFD showed abnormal glucose tolerance, increased levels of inflammatory markers in the serum and increased vacuolation in the liver.

Our preliminary results suggest that in mice, consumption of a HFD results in morphological and molecular changes in the hypothalamus, an area crucial for energy homeostasis. Furthermore, our whole body approach provides novel insights into the molecular basis of diet-induced pathology in the brain and peripheral organs and how these changes are interconnected.

The University of Sydney

P13. EARLY MYELINATION AND MICROSTRUCTURAL ABNORMALITIES IN WHITE MATTER IN THE YAC128 MOUSE MODEL OF HUNTINGTON DISEASE

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Numerous imaging and post-mortem studies have shown that white matter (WM) atrophy occurs in pre-symptomatic Huntington disease (HD) patients, and worsens upon disease onset and progression. However, the early pathological manifestations of this atrophy on the molecular and microstructural levels remain poorly defined. In this study, we used electron microscopy and transcriptional analyses to demonstrate that myelination deficits in the YAC128 full-length mouse model of HD mice are a very early pathological observation, appearing before the manifestation of behavioural deficits. We also applied diffusion tensor imaging in the YAC128 HD mice and demonstrate that there are early and progressive microstructural WM abnormalites in YAC128 mice. The WM deficits in diffusion and molecular measures long before neuronal loss points to an early and potentially primary pathogenic role for white matter pathology in HD.

We are indebted to the Biomedical Research Council and the National University of Singapore for financial support.

P14. STRAWBERRY NOTCH HOMOLOG 2 IS A NOVEL INFLAMMATORY RESPONSE FACTOR EXPRESSED PREDOMINANTLY IN ASTROCYTES

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We have identified a novel putative transcriptional regulator with little known function called strawberry notch homolog 2 (Sbno2) as a prominent IL-6 stimulated gene in astrocytes. The aim of this study was to: (i) examine the

regulation of Sbno2 in astrocytes *in vitro* and *in vivo*, and (ii) establish lentiviral transduction of target cells with a Sbno2 transgene for the functional characterization of Sbno2. In murine and human cultured astrocytes *Sbno2* gene expression was significantly up-regulated in a dose- and time-dependent fashion following treatment with hyper-IL-6 (IL-6 + soluble IL-6 receptor). These changes were reflected by corresponding alterations in the level of the SBNO2 protein. The level of Sbno2 mRNA was also upregulated significantly in murine astrocytes by other glycoprotein130 cytokine-family members and the pro-inflammatory cytokines IL-1β and TNF-α. Cycloheximide treatment resulted in markedly higher Sbno2 mRNA and did not abolish the up-regulation of Sbno2 mRNA mediated by hyper-IL-6. Actinomycin D treatment resulted in a rapid reduction in hyper-IL-6-induced Sbno2 mRNA. Following intra-peritoneal LPS injection in mice, Sbno2 mRNA levels in the brain increased significantly. Cellular localization studies revealed that this increase in Sbno2 mRNA occurred predominantly in astrocytes and in the choroid plexus, and in some microglia, endothelial cells, and neurons. Characterisation of transduced NIH-3T3 fibroblasts revealed transgenic SBNO2 localised exclusively to the nucleus but not the nucleoli. Our findings indicate that the *Sbno2* gene is broadly regulated in astrocytes and likely encodes a nuclear factor involved in the innate host response. A mouse model for the conditional deletion of the *Sbno2* gene in astrocytes developed by us recently will provide an invaluable tool for evaluating further the function of Sbno2 in mammalian cells.

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P15. OXIDATIVE STRESS A COMMON LINK BETWEEN STROKE AND ALZHEIMER'S DISEASE AND CURCUMIN CAN REDUCE THIS STRESS.

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Literature (Mauro Silvestrini et al 2006) indicates that Alzheimer's disease (AD) and Stroke not only coexists but interacts too. Clinical investigations also highlighted that there is a critical relationship between vascular events and AD pathology such that they can influence each other. Signs of inflammation due to significant oxidative damage are considered to be important hallmarks for both AD and Stroke. Epidemiological studies also suggest reduced AD and stroke risk with long term use of nonsteroidal and anti-inflammatory drugs (NSAIDs). Long term use of NSAIDs can cause liver and kidney toxicity. One alternative of NSAID is a polyphenolic antioxidant curcumin. We tested a low 170 ppm and high dose 5500 ppm of curcumin on the scopolamine HBr (1.2 mg kg s.c.)-induced impairment of spontaneous alternation behavior in the rectangular-maze test, an index of short-term memory in mice as well as in the middle cerebral artery occlusion (MCAO) mice model of stroke. Our initial finding indicates that in both AD and Stroke model reactive oxygen species (ROS) plays an important role and curcumin can act as a scavenger of ROS. In addition as literature (Yamamotoa et al 1997 and E Skrzypczak-Jankun et al 2000) indicated that curcumin is also reported to inhibit phospholipase and lipoxygenase, which could contribute to overall NSAID neuroprotective function. We concluded that curcumin can be a promising anti-inflammatory agent for controlled clinical trials to establish its safety and efficacy for prevention and treatment of inflammatory diseases such as AD and Stroke.

P16. NOTCH SIGNALING IS REGULATED BY IGF-1 IN ASTROCYTES

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Notch signaling pathway is crucial for cell fate specification and cell proliferation during healthy central nervous system (CNS) development. Notch is also involved in the growth of different cancer cell types, mainly under the regulation of estrogens. Notch receptors, its ligands (Jag, Dlk) and effectors (Hes) are still expressed in the adult CNS, but their roles are not fully understood yet. In recent years, it has been demonstrated that Notch pathway is involved in microglia activation against harmful stimuli.

Estradiol, progesterone and insulin-like growth factor 1 (IGF-1) are endogenous neuro-hormones that exert strong anti-inflammatory properties in the brain, including the control of reactive gliosis. Using an in vitro model of reactive astrogliosis induced by lipopolysaccharide (LPS) in primary cultures of cortical or hypothalamic astrocytes, we assessed the regulation of Notch signaling during astrocytic reactivity, and whether the anti-inflammatory properties of estradiol, progesterone or IGF-1 were mediated by the regulation of Notch signaling.

Notch signaling pathway was down-regulated in astrocytes after LPS stimulation by two different mechanisms: repressing Notch-1 transcription (and hence, decreasing the trans-activation of the route) and by inducing the overexpression of Jag-1 (generating cis-inhibition of Notch). These inhibitory effects of LPS on Notch pathway were observed in both cortical and hypothalamic astrocytes.

Under physiological conditions, IGF-1 increased Hes-5 transcription by inducing Notch-1 cleavage, in a MAPK pathway-dependent manner. Besides, IGF-1 completely reverted the LPS-induced downregulation of Notch

pathway. However, estrogens and progesterone did not regulate Notch pathway under physiological conditions, and only reverted part of the LPS effects on Notch pathway.

Currently we are studying the involvement of Notch in cytokine synthesis, cell cycle progression and stellation, in order to decipher the exact functionality of Notch pathway during astrogliosis. All together, our findings suggest that Notch signaling pathway plays a role in reactive astrogliosis and that it is regulated by IGF-1.

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SYMPOSIUM 3

S3.1. TROPHIC FACTOR REGULATION OF OLIGODENDROCYTES IN MYELINATION AND DEMYELINATION: A ROLE FOR TAM RECEPTORS

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Several families of growth factors and cytokines are known to regulate the differentiation and myelination of oligodendrocytes. However, many of these molecules have pleiotropic activities and would therefore be expected to have off-target effects if applied therapeutically. We have identified a family of receptor tyrosine kinases, known as the TAMs (Tyro3, Axl, Mertk) that have more limited expression profiles and which could prove to be useful therapeutic targets to enhance remyelination. In prior work, we have identified that mice deficient in Gas6 one of the cognate ligands of the TAM receptors, exhibit a greater loss of oligodendrocytes, enhanced microglial activity and potentiated myelin loss in comparison with control mice in an animal model of central demyelinating disease. The three TAM receptors have complementary expression profiles, with Mertk and Axl predominantly expressed by mononuclear cells including microglia whereas Tyro3 is expressed by oligodendrocytes in the healthy quiescent state and in the context of remyelination. We have identified that in Tyro3 knockout mice the onset of myelination in post-natal optic nerves is delayed. Absence of Tyro3 does not potentiate demyelination but in ex vivo neuronal-ologodendrocyte co-cultures absence of Tyro3 severely abrogates myelination potential. These results raise the possibility of selectively targeting Tyro3 for therapeutic benefit with restricted off-target effects.

S3.2. EFFICACY OF JAKINIBS IN AUTOIMMUNE AND NEURODEGENERATIVE DISEASES

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Multiple Sclerosis (MS) is a chronic inflammatory and neurodegenerative disease of the CNS, characterized by inflammation, demyelination and axonal damage. Cells of the innate (macrophages, microglia, neutrophils) and adaptive (T-cells, B-cells) immune system contribute to both the pathology and repair of MS. Parkinson's Disease (PD) is a neurodegenerative disease characterized by the deterioration of motor activities that are controlled by the nigrostriatal system. The motor symptoms of PD result from the progressive and selective loss of dopaminergic (DA) neurons in the midbrain substantia nigra pars compacta. Additionally, the PD brain is characterized by cytoplasmic and neuritic fibrillar α-synuclein (α-syn) inclusions (known as Lewy bodies (LB) and Lewy neurites (LN), respectively). Recently, an impressive number of publications have implicated inflammation as a major pathogenic factor in PD, with involvement of both innate and adaptive immune cells. MS and PD are characterized by abundant production of cytokines that activate and regulate the functions of immune and glial cells. The Janus Kinase (JAK)/Signal Transducer and Activator of Transcription (STAT) pathway is the major signaling system utilized by cytokines, and is critical for development, regulation and termination of innate and adaptive responses. Dysregulation of the JAK/STAT pathway has pathological implications for a number of autoimmune and neuroinflammatory diseases. We have documented aberrant activation of the JAK/STAT pathway in pre-clinical models of both MS and PD. We will discuss the efficacy of inhibitors of the JAK pathway, known as Jakinibs, in these pre-clinical models, and describe the mechanisms that underlie their beneficial effects on neuroinflammation and neurodegenerative responses.

National Institutes of Health and the Michael J. Fox Foundation

S3.3. THE ROLE OF TYPE I IFNS IN THE CNS

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Immunoregulatory cytokines play an important role in controlling neuroinflammation and are of interest as therapeutic targets in diseases such as Multiple sclerosis (MS). Type I interferons (IFN) are soluble secreted cytokines that comprise the multi-gene alpha family and a single gene beta molecule. They signal through a shared IFNAR receptor and are implicated in antiviral responses. IFNβ is used as a first-line therapy for MS. Type I IFN are induced within the central nervous system (CNS) during inflammation or by experimental application of ligands for innate receptors. Cell sources include microglia as well as extraparenchymal myeloid cells. Responding cells include microglia and astrocytes. Effects of Type I IFN induction within the CNS include suppression of EAE in mice. The mechanism for this suppression may include induction of regulatory cytokines. Unlike MS, the antibody-mediated demyelinating disease Neuromyelitis optica (NMO) is refractory to IFNβ treatment. Correspondingly, NMO-like pathology is reduced in mice that lack the IFNAR receptor. Transgenic expression of IFNα1 in astrocytes induced inflammation and neurodegeneration (Akwa, Campbell, 1998). We are pursuing the possibility that differential IFNα versus IFNβ signaling contributes to discordant effects in the CNS.

Danish Council for Health Research, Lundbeckfonden, Danish Multiple Sclerosis Society.

S3.4. IL-6/GP130 FAMILY CYTOKINE SIGNALLING IN ASTROCYTES AND MICROGLIA

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The IL-6/gp130 family of cytokines includes IL-6, IL-11, leukaemia inhibitory factor (LIF) and oncostatin M (OSM). These cytokines have significant roles in the central nervous system (CNS) including regulating the function of astroglia and microglia in a broad range of processes that span neurodevelopment to neuroinflammation. Although astroglia and microglia are major producers as well as targets of this family of cytokines little is known about the relative roles that these glial cells have in mediating the actions of the different IL-6/gp130 cytokines and if and how there is biological specificity in the response of these cells to these cytokines. Microarray studies by us revealed that in addition to overlapping responses, these glial cells can exhibit unique responses to the same cytokine despite sharing common signal transduction pathways. The binding of the IL-6/gp130 cytokines to their cognate receptors triggers signal pathway activation via gp130 and phosphorylation of STAT1 and STAT3 as well as the ERK/MAPK pathway. We have found that multiple molecular mechanisms underlie the responses of astroglia and microglia to IL-6/gp130 cytokines that contribute to biological specificity. These include, differential expression of specific receptors (e.g. microglia have IL-6Rα but astroglia do not, while the opposite occurs for OSMRβ) and varying levels of activation of STAT1, STAT3 and ERK depending on the cytokine involved. The neurobiology of IL-6 is complicated further by the fact that while astroglia lack IL-6Rα these cells still respond to the combination of IL-6 plus the soluble IL-6R in a process know as trans-signaling. Using a transgenic mouse model with the production of IL-6 targeted to astrocytes we found that trans-signaling is responsible for many of the detrimental actions of this cytokine in the CNS.

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SYMPOSIUM 4

S4.1. THE INTERFERON REGULATORY FACTOR 9 (IRF9) IS A KEY REGULATOR OF CEREBRAL INTERFERON RESPONSES

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The type I interferon (IFN-I) family of cytokines contains multiple members including several IFN-αs and IFN-β. IFN-Is are critical for protecting the host from infection with viruses and microorganisms. However, inadequate production is linked to several neurological diseases, also known as cerebral type I interferonopathies. The interferon regulatory factor 9 (IRF9) plays an essential role in mediating the effects of IFN-I. It does this by forming a complex with the signal transducers and activators of transcription (STAT) 1 and STAT2.

A major focus of our work is to clarify the role of IRF9 in neuroinflammation. Our findings demonstrate that IRF9 deficiency can be either detrimental or beneficial for the host, depending on the underlying pathogenetic mechanism. In mice, transgenic expression of IFN-α in astrocytes results in a spontaneous neurological disease that recapitulates key features of human cerebral type I interferonopathies. Our results reveal that IFN-I responses can occur in the CNS in the absence of IRF9 and that, in vivo, IRF9 protects against the pathophysiological actions of IFN-I in the CNS. Furthermore, our results also show that in astrocytes and microglia, IFN-I induces an IFN-γ-like response in the absence of IRF9 but not STAT1 or STAT2. By contrast, IRF9 is required for the lethal neurological disease of wild type (WT) mice following infection with lymphocytic choriomeningitis virus (LCMV). In contrast to WT mice, LCMV infection of IRF9-deficient mice caused a transient non-fatal disease with virus spread and virus persistence. Importantly, IRF9 deficiency resulted in an incapacitated antiviral CD8+ T cell response. Our findings also demonstrate that systemic IRF9 deficiency, rather than an intrinsic T cell defect, mediates this abnormal host response. In summary, these results reveal that IRF9 is a key regulator of IFN-I-mediated inflammatory and immune responses in central nervous system (CNS).

The work presented was in part funded by the Deutsche Forschungsgemeinschaft (DFG; Ho3298-2) and start-up funding provided by the School of Molecular Bioscience and The University of Sydney

S4.2. ROLE OF CNS MYELOID CELLS IN WEST NILE VIRUS ENCEPHALITIS

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Infection of the central nervous system (CNS) by the neurotropic flavivirus, West Nile, (WNV) involves neuronal infection only, but a major microglial activation response, including the formation of microglial nodules, and a rapid massive influx of bone marrow (BM)-derived monocytes/macrophages, may ultimately induce fatal pathology in WNV encephalitis.

We have shown that a variety of critically-timed interventions changes the lethal outcome in a murine model of WNV encephalitis from 100% mortality to up to 80% survival, by diminishing, but not abrogating, infiltrating myeloid populations, diverting them to the spleen, where they are sequestered. Importantly, this is accompanied by robust, sterilizing long-term immunity. This abrogation may be specific for some myeloid subsets, while earlier subsets may be protective in the CNS.

Whilst these late, lethal myeloid immigrants are certainly derived from the bone marrow, little is known about the the kinetics and function of other possible myeloid subsets earlier in this immigration, or the precipitating events that mobilise BM monocytes and their progenitors in response to CNS infection. In addition, what influences the final phenotype and fate of immigrant myeloid cells remaining in the brain and how they interact with native microglia is unclear, but this may have significant implications, not only for resolution of the virus infection, but also for post infective sequelae in recovering individuals. Bone marrow chimaeric and adoptive transfer experiments in WNV- and dengue-infected mice indicate the brain milieu is crucial for the differentiation of inflammatory monocyte immigrants, and are shedding more light on the bone marrow response, the ontogeny, the migratory requirements and the downstream phenotype and functions of these cells in flavivirus infection.

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S4.3. KYNEURENINE PAHWAY IN GLIAL CELLS AND ITS INVOLVEMENT IN NEUROINFLAMMATORY DISEASES

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The kynurenine pathway (KP) of tryptophan metabolism is one of the major regulatory mechanisms of the immune response. Activation of the KP is implicated in the pathogenesis of a wide range of neuroinflammatory diseases. Several pro-inflammatory mediators can activate indoleamine 2,3 dioxygenase (IDO-1) one of the first and regulatory enzymes of the KP. A prolonged activation of the KP leads to production and accumulation of several neuroactive metabolites including the potent excitotoxin quinolinic acid (QUIN). Every brain cell types appear to express differently the KP enzymes and producing different KP metabolites. Neurons, astrocytes, oligodendrocytes and brain microvascular endothelial cells produce neuroprotective compounds whereas activated microglia, pericytes, infiltrating macrophages synthesize and release neurotoxic KP metabolites. We have shown that QUIN levels correlated with the total scores on Suicide Intent Scale indicating that changes in glutamatergic neurotransmission is specifically linked to suicidality. This dynamic and complex interplay between KP metabolites from different brain and immune cells is directly involved in the global and progressive inflammatory response involved in the neuropathogenesis of several major neurodegenerative diseases and psychiatric disorders including schizophrenia, autism, depression, suicide.

NHMRC, ARC, MSRA.

S4.4. WHAT DOES GM-CSF DO IN NEURO-INFLAMMATION: ON MICROGLIA AND TISSUE-INVADING MYELOID CELLS

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The granulocyte-macrophage colony stimulating factor (GM-CSF or CSF-2) has emerged as a crucial cytokine produced by auto-reactive helper T (TH) cells to initiate neuro-inflammation. Multiple cell types can sense GM-CSF, but the nature of the pathogenic GM-CSF-responsive cells is a matter of debate. Using conditional gene targeting, we systematically deleted the GM-CSF receptor (Csf2rb) from specific subpopulations throughout the myeloid lineages. We conditionally deleted GM-CSF –reactivity specifically in monocytes, dendritic cell population as well as in microglia. I will discuss our findings and the surprising function of microglia upon sensing GM-CSF. Furthermore, I will discuss a new mouse model where deregulation of GM-CSF production by TH cells leads to Neuro-inflammation in an antigen-independent manner.

SYMPOSIUM 5

S5.1. THYROID HORMONES IN GLIOENDOCRINE SYSTEM IN HEALTH AND DISEASE

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There is a close relationship between endocrine system and the central nervous system (CNS). Among hormones closely related to the nervous system, thyroid hormones (THs) are critical for the development and function of the CNS; not only for neuronal cells but also for glial development and differentiation. Any impairment of THs supply to the developing CNS causes severe and irreversible changes in the overall architecture and function of human brain, leading to various neurological dysfunctions. In adult brain, impairment of THs, such as hypothyroidism and hyperthyroidism, can cause psychosis such as schizophrenia, bipolar disorder, anxiety and depression as well as high risk of Alzherimer's disease. Though hypothyroidism impairs synaptic transmission and plasticity, the effect on glial cells and cellular mechanisms are largely unknown. We have reported non-genomic effects of T3 (3, 3', 5–triiodothyronine), an active form of TH, on microglial functions and their signaling. Exposure to T3 increased migration, membrane ruffling and phagocytosis of primary cultured mouse microglia. Injection of T3 together with stab wound attracted more microglia to the lesion site in vivo. The T3-induced microglial migration or membrane ruffling was dependent on TH transporters and receptors (TRs), followed by various intracellular signaling. In addition, T3-indueced activation of glial cells in cortex and hippocampus were dependent on sex and age of the animals. These results may help to understand physiological and/or pathophysiological functions of T3 in the CNS.

Suported by Kyushu University, Research Support Center in Graduate School of Medical Sciences.

S5.2. RAMIFIED MICROGLIA AS NEUROPROTECTIVE CELLS IN ORGANOTYPIC HIPPOCAMPAL BRAIN SLICES

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Microglia are the resident CNS immune cells constituting the first-line of defense in brain-injury. They are characterized by a typical ramified morphology in the healthy brain, while in the diseased brain microglia show a more macrophage-like morphology having shortened and thickened processes and sometimes amoeboid shape. During the last decade it has been established that microglia are by no means inactive under so-called resting conditions instead they are highly active and constantly surveil their environment. However, uncovering the function of ramified microglia has just begun. The lack of knowledge about these cells under normal conditions is mostly due to the fact that microglia are experimentally very difficult to approach. They are very reactive cells that rapidly respond to any brain disease or injury; thus, culturing them inevitably causes activation and to functionally investigate ramified microglia ex vivo, in situ or in vitro was so far was very difficult, if not impossible.

Organotypic hippocampal slice cultures (OHSCs) are a well-accepted tool to study different neurobiological aspects including neuron-glia interactions. This culture model combines a very close in vivo situation with relatively easy possibilities of experimental manipulation to investigate numerous neurobiological questions. Using OHSCs we have established a technique to study ramified in vivo-like microglia in a cell culture setting. Removal and replenishment of microglia allows the generation of chimeric brain tissue. Using microglia replenished OHSCs (Mrep-OHSC) we here present a protective and beneficial function of ramified microglia in various models of neuronal death or neurodegenerative disease.

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S5.3. MICROGLIA AS CRITICAL PLAYERS IN PRUNING SYNAPSES AND SCULPTING BRAIN CONNECTIVITY

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Microglia are phagocytic cells of myeloid origin that infiltrate the brain during development and play an important surveillance and scavenging function. An established literature has documented how these immune cells of the brain promptly move toward the site of damage, engulf cellular debris, and rapidly act to resolve injuries in a pathological context. However, very little is known about their role in physiological conditions. Emerging data from the last few years are now depicting microglia as constantly active cells, scanning the surrounding environment and closely interacting with neurons, even in the absence of noxious stimuli, and new functions have been described in the developing brain.

In the hippocampal CA1 region, mice lacking the chemokine receptor Cx3cr1 show a transient reduction in the number of microglia, associated with an excess of dendritic spines during the early postnatal weeks, thereby suggesting an important role for microglia in pruning synapses. Interestingly, the resulting defects in synaptic connectivity observed in Cx3cr1 KO mice are associated with long-term impairments in social interaction, and increased repetitive behavior, hallmarks of autism spectrum disorders. These findings provide evidence for a critical microglia function in remodeling synapses and sculpting brain connectivity, and support the hypothesis that deficits in microglia-mediated synaptic pruning may contribute to long-lasting structural and behavioral features of some neurodevelopmental disorders.

S5.4. STRESS INDUCED MODULATION OF MICROGLIA: A CRITICAL ROLE IN THE REGULATION OF MOOD STATE AND COGNITIVE FUNCTION?

Walker, F.R.

University of Newcastle

The role of microglia in the regulation of mood state and cognitive function.

Several recent reports have identified that psychological stress can both structurally and functionally alter microglia, cells that are pivotal to the production and maintenance of a neuroinflammatory state in the brain. The ability of stress to modulate microglial activity is of interest for two main reasons (a) stress is major risk factor in the emergence of depression and (b) depression appears to be characterised by enhanced levels of neuroinflammation. These two facts have led to the hypothesis that psychological stress may elicit changes in mood state and cognitive function by driving microglial mediated neuroinflammatory events. In investigating this hypothesis our research group, using a variety of behavioural approaches, has previously found that chronic stress sufficient to induce an increase an anhedonic and a decline in cognitive performance co-occurred with an increase in microglial activation within mood regulatory forebrain nuclei (notably the medial prefrontal cortex and amygdala). We have now subsequently, established that targeting stress induced microglial activation with anti-inflammatory agents improves stress induced cognitive decline. Moreover, using a variety of techniques we have identified at a cellular level that microglial activity is intimately linked with neuronal activity. Interestingly, we have also observed that the stress induced changes in microglial activity are not clearly associated with signs of neurodegeneration. Indicating that the stress induced increase in microglial activation is occurring via a non classical mechanism. Currently, our group is now functionally characterizing, using a variety of ex-vivo techniques, the inflammatory status of microglia within the mood regulatory nuclei where we have observed differences following exposure chronic stress. Collectively, these findings may prove to be relevant in furthering our understanding of the neurobiology of depression.

NHRMC

ORAL PRESENTATIONS, AUGUST 21

O7. DELETION OF THE TYPE-1 INTERFERON RECEPTOR IN $APP_{SWE}/PS1_{\Delta E9}$ MICE RESULTS IN INCREASED COGNITIVE FUNCTION AND CHANGED MICROGLIAL PHENOTYPE

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Introduction: Neuro-inflammation has been implicated in Alzheimer's disease (AD) pathology. Critical to this inflammatory response in AD patients is the severe gliosis and enhanced pro-inflammatory cytokine load within the amyloid-beta (Aβ) plaque microenvironment. Type-1 interferons (IFNs) are master regulators of the innate immune response yet their role in AD progression is unclear. We have previously demonstrated that type-1 IFN production and signalling is up-regulated in human post-mortem AD brains and $APP_{SWE}/PS1_{\Delta E9}$ mice, and that primary cultured IFNAR1-/- neurons are protected from Aβ1-42 toxicity (Taylor et al., 2014). We have now generated 9-month old $APP_{SWE}/PS1_{\Delta E9}$ x IFNAR1-/- mice, lacking IFNAR1 and assessed the severity of disease.

Methods and results: Spatial learning and memory deficits were rescued in $APP_{SWE}/PS1_{\Delta E9}$ x IFNAR1-/- mice as assessed by the Morris water maze, showing reduced escape latency and path length and greater trial success compared to $APP_{SWE}/PS1_{\Delta E9}$ mice (n=14-18, $p<0.05$). Immunohistochemistry of serially sectioned $APP_{SWE}/PS1_{\Delta E9}$ x IFNAR1-/- mice revealed little change in amyloid plaque load, but western blot densitometry revealed a decrease in soluble monomeric Aβ compared to $APP_{SWE}/PS1_{\Delta E9}$ mice (n=6, $p<0.05$). QPCR identified attenuated IFNα, IRF7 and TNFα expression in APPSWE/PS1ΔE9 x IFNAR1-/- mice and western blot confirmed down-regulated Stat-3 phosphorylation compared to APPSWE/PS1ΔE9 mice (n=4-6, $p<0.05$). Interestingly, immunohistochemistry $APP_{SWE}/PS1_{\Delta E9}$ x IFNAR1-/- mice revealed enhanced cortical astrogliosis (GFAP) surrounding Aβ deposition; microgliosis (IBA-1), in these same mice, was attenuated not ablated (n=9, $p<0.05$). QPCR identified elevations in M1 markers iNOS, CD11b, CD32 and CD33 in $APP_{SWE}/PS1_{\Delta E9}$ mice and in comparison M2 markers YM1, ARG1 and TREM2 were up-regulated in $APP_{SWE}/PS1_{\Delta E9}$ x IFNAR1-/- mice (n=6, $p<0.05$).

Conclusions: These results confirm that type-1 IFNs contribute to the inflammatory pathology of $APP_{SWE}/PS1_{\Delta E9}$. Removing type-1 IFN signalling attenuates pro-inflammatory cytokine secretion, alters cortical gliosis, triggers a neuro-protective M2-like microglial polarisation and rescues cognitive deficits.

NHMRC of Australia, ARC

O8. IN VIVO CHARACTERISATION OF DYING NEURONS BY MICROGLIA ENGULFMENT IN THE SPINAL CORD

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Microglia are specialised phagocytes in the vertebrate central nervous system (CNS). As the resident immune cells of the CNS they play an important role in the removal of dying neurons during both development and in several neuronal pathologies. Microglia have been shown to prevent the diffusion of damaging degradation products of dying neurons by engulfment and ingestion. Here we describe a live imaging approach that uses UV laser ablation to selectively stress and kill spinal neurons and visualise the clearance of neuronal remnants by microglia in the zebrafish spinal cord. In vivo imaging confirmed the motile nature of microglia within the uninjured spinal cord, even in the absence of an 'activating' trigger. Importantly, selective ablation of an individual neuron triggered the rapid activation of microglia, leading to phagocytic uptake of neuronal debris within 20-30 minutes. This process of microglial engulfment is highly dynamic, involving the extension of processes towards the lesion site and consequently the ingestion of the dying neuron. 3D rendering analysis of our time-lapse recordings revealed the formation of phagosome-like structures in the activated microglia located at the site of neuronal ablation, establishing for the first time a powerful method to visualise this process directly. This real-time representation of microglial phagocytosis in the living zebrafish spinal cord provides novel insights into understanding the mechanisms of microglia-mediated neuronal clearance. Furthermore, the combined ablation and visualisation techniques offer new opportunities to investigate the molecular mechanisms regulating phagocytic microglial behaviour, and their essential role in maintaining cellular homeostasis.

This work was supported by a Discovery project grant from the Australian Research Council (DP140103233).

O9. ACUTE HYPERTENSION CAUSES INCREASED MICROGLIAL CONTACT WITH NEURONAL SYNAPSES IN CARDIOVASCULAR NUCLEI IN RAT BRAINSTEM

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Microglia play an important role in maintaining neuronal synaptic activity levels within their physiological working range during homeostasis. Rostral- and caudal-ventrolateral medullary regions (RVLM and CVLM, respectively) of the brainstem are the key nuclei involved in all aspects of the cardiovascular system. We hypothesize that microglia detect alterations in the electrical activity of cardiovascular neurons by sensing the synaptic levels of neurotransmitters and then execute the required action, in order to drive neurons towards a resting state. The number of synapses co-localising with microglial end-point processes were quantified following the induction of acute hypo- (hydralazine) and hyper- (phenylephrine) tension in 36 male Sprague-Dawley rats at 4 time points (n=3 each); 0.5, 2, 6 and 10 hrs.

Following induction of hypertension for 6 hours, microglial contact with RVLM neuronal synapses (increased γ-aminobutyric acid (GABA) levels), increased from 359 to 473 ($p \leq 0.001$, Chi-square test (CST)) and remained elevated at 10 hrs ($p \leq 0.001$, CST). In the CVLM, hypertension increased microglial process motility from 0.5 hrs onwards; the number of synapses (increased glutamate levels) contacted by microglia increased from 252 to 362 at 0.5 hrs ($p \leq 0.001$, CST) and remained elevated at all of the following time points. Induction of hypotension for 2 hrs, reduced the number of RVLM neuronal synapses (reduced GABA levels) in contact with microglia from 333 to 278 ($p \leq 0.05$, CST); an effect that persisted for 10 hrs. However, when subjected to hypotension, microglial contact with CVLM neuronal synapses (reduced glutamate levels), decreased from 387 to 252 from 6 hrs ($p \leq 0.001$, CST) onwards. No changes were induced in microglial morphology or phenotype.

We, therefore, conclude that microglial sampling of neuronal synapses, in the RVLM and CVLM region, is independent of the levels of neuronal activity and is directly proportional to the levels of constitutively expressed neurotransmitters such as GABA and glutamate.

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O10. INTERFERON REGULATORY FACTOR (IRF) 8 REGULATES THE MICROGLIAL CELL RESPONSE TO STERILE NERVE INJURY IN THE BRAIN

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The transcription factor interferon regulatory factor (IRF) 8 has a key role in the cellular response to IFN-γ and is involved in myeloid cell differentiation. We have shown IRF8 to be a constitutive, IFN-γ-stimulated nuclear factor that regulates the homeostatic properties of microglia. Here we determined the role of IRF8 in the microglial response to sterile neuronal injury. Nucleoside diphosphatase (NDPase) histochemistry revealed gross alterations in the morphology of IRF8 KO microglia, which were stunted and hypertrophied. After facial nerve axotomy (FNA) in WT mice, a progressive increase in microglial activation was observed in the lesioned facial nerve nucleus (FNN) peaking at day 7 and was accompanied by dense staining for Iba1, lectin, NDPase and CD11b. By contrast, in IRF8 KO mice, the microglial response was markedly attenuated with little staining for Iba1, while the density of staining for lectin, NDPase and CD11b was reduced significantly. The attenuated microglial response in IRF8 KO mice was paralleled by a significant decrease at day 3 post-FNA in proliferation as monitored by BrdU, phosphohistone 3 and Ki67-positive cells. Furthermore, the number of PU.1-positive cells was reduced in the FNN of IRF8 KO mice compared with WT at day 7 post-lesion. The wrapping of individual motor neuron cell bodies in the axotomised FNN by microglia involved in synaptic stripping and phagocytosis was incomplete in IRF8 KO mice. Astrogliosis, present at day 3 post-FNA, did not differ between WT and IRF8 KO axotomised mice. Finally, in IRF8 KO mice, the degeneration of axotomised motor neurons was significantly increased. These studies further highlight IRF8 as a key homeostatic transcription regulator of microglial cell function in the healthy brain, and moreover, demonstrate a crucial role for IRF8 in regulating the response of microglia but not astrocytes to neuronal injury.

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O11. STAT1 AND STAT3 ACTIVATIONAL BALANCE AND TRANSCRIPTIONAL RESPONSES TO GP130 CYTOKINES IN ASTROCYTES VERSUS MICROGLIA

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The gp130 cytokines, including IL-6, IL-11, LIF and OSM, have important roles in neuroinflammation and neurodevelopment. However, the nature of any cell-specific responses of astrocytes and microglia to this family of cytokines and their molecular basis is not well understood. Here, the expression of the gp130 family cytokine receptors and subsequent signal pathway activation were examined in murine astrocytes and microglia in vitro. Astrocytes had high levels of OSMR mRNA and lower levels of IL-6R, LIFR and IL-11R mRNAs. In contrast, microglia expressed higher levels of IL-6R mRNA, similar levels of LIFR and IL-11R mRNAs, and no detectable OSMR mRNA. In astrocytes, OSM induced STAT1 phosphorylation to a greater extent than hyperIL-6 (IL-6 linked to the soluble IL-6 receptor) and LIF. However, STAT3 activation did not differ significantly between the three cytokine treatments. Conversely, hyperIL-6 and LIF but not OSM, induced phosphorylation of STAT3 and only minimal phosphorylation of STAT1 in microglia. Microarray analysis comparing the response by astrocytes to OSM, hyperIL-6 and LIF revealed up-regulation of many transcripts synonymous with IFN-regulated genes. However, this IFN-like response to OSM was largely abolished in STAT1 KO astrocytes. The gene expression profile of OSM and hyperIL-6 treated astrocytes was somewhat similar while that for LIF differed the most. Despite signalling via a common gp130 pathway, these findings illustrate intercellular and intracellular specificity in the action of the gp130 cytokines in astrocytes and microglia which is achieved through a number of mechanisms that include cell-specific localisation of receptors and differential levels and balance of activation of STAT1 and STAT3.

This project was funded by NHMRC project grant 632754. MPH was a recipient of an Australian Postgraduate Award.

O12. INTRACELLULAR COPPER DELIVERY LIMITS NEUROINFLAMMATION

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Our team has developed a family of neuroprotective bioavailable copper-delivery bisthiosemicarbazone (Cu-btsc) compounds. Prototype compounds improve cognition, motor functions and survival in mouse models of AD, ALS and Parkinson's disease, and reduce toxic Aβ trimers and tau phosphorylation. We demonstrated anti-inflammatory actions of Cu-btscs in murine and human cell models of neuroinflammation. IFN/TNF-stimulated primary murine astrocytes and microglia treated with Cu-btscs displayed reduced amoeboid morphology, and secreted less damaging factors (including pro-inflammatory chemokine MCP-1 and nitric oxide), while protective anti-oxidant metallothionein expression was enhanced. VCAM-MRI studies confirmed that Cu-btscs are anti-inflammatory following LPS challenge. These actions appear to involve copper delivery to inflamed microglia, as demonstrated by our in vitro Synchrotron X-ray fluorescence microscopy studies.

We are developing novel derivatives of neuroprotective Cu-complexes, differing in the ligand backbone to facilitate subtle but important variations in lipophilicity, cellular uptake, subcellular localisation and metal release, with the aim of identifying compounds with higher efficacy compared to our prototype compounds. Our preliminary analyses have identified compounds with greater anti-inflammatory potential than our prototype complexes and the anti-inflammatory compound, minocycline. An improved understanding of how Cu-complexes modulate inflammation will provide valuable insight into pathogenic and protective mechanisms in neurodegeneration.

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O13. GLIAPTIC INTERACTIONS: TARGETED MICROGLIAL-MEDIATED PHAGOPTOSIS IN BRAIN PATHOLOGY

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Microglial cells are able to phagocytose entire cells in healthy and pathological circumstances of the brain. However, it remains unclear whether microglia phagocytose just as scavengers or whether the process of phagocytosis requires specific cell death induction or phagoptosis. In the present work we show evidences that microglial cells are able to phagocyte entire cells in different scenarios of brain damage in vivo. This phagocytosis is preceded by the apposition of microglial cells to the target, forming the so-called phagocytic gliapse, which is characterized by the formation of a flat interface, accompanied by the polarization of organelles towards the intercellular space. In this process Rho/Cdc42 signaling is fundamental for the specific polarization of the cells, as well as the polarization of Iba-1 and F-actin in the conformation of lamellas and leading edges. The polarization of the Golgi apparatus to the interface and the one-to-one gliaptic-target suggest that gliaptic establishment may be critical in phagocytosis-dependent cell death induction in brain pathology.

This work was supported through grants from the Spanish Ministry of Economy and Competitiveness (RYC2010-06729, SAF2010-21274 and SAF2013-45178-P).

O14. ASTROCYTE-TARGETED IL10 PRODUCTION MODIFIES EXPRESSION OF TREM2 AND CD200R IN ACTIVATED MICROGLIA AFTER PERFORANT PATHWAY TRANSECTION.

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One of the endogenous mechanisms regulating inflammatory cell activation following brain injury is the expression of modulator and/or inhibitor membrane receptors. Recently, studies on endogenous regulatory receptor TREM2 demonstrate that TREM2-mediated phagocytic function of microglia/macrophages (MM) is required for debris clearance and inhibitory receptor CD200R-mediated deactivation of microglia is necessary for maintenance of Central Nervous System (CNS) homeostasis. The factors that control the expression of TREM2 after CNS injury are still unclear, although the influence of the microenvironment, especially the local production of cytokines may play a key role. Hence, the objective of this study was to characterize the effects of local production of the anti-inflammatory cytokine IL-10 on TREM2 and CD200R expression using an axonal anterograde degeneration model. For this purpose, unilateral perforant pathway transection (PPT) was performed in transgenic mice with astrocyte-targeted production of IL-10 (GFAP-IL10Tg) and their corresponding wild types (WT) littermates. Our results showed a basal low expression of TREM2 in non-lesioned (NL) GFAP-IL10Tg in contrast to WT where TREM2 was mostly absent. After PPT, in GFAP-IL10Tg an increase in TREM2+ MM was noted in the molecular layer of the dennervated dentate gyrus being always higher compared to WT. TREM2+ MM colocalized with DAP12 in both WT and GFAP-IL10Tg at all time-point studied. About CD200R, there was no expression in the NL WT; whereas a low level of CD200R was observed on NL GFAP-IL10Tg. After PPT, an increase of CD200R was observed in both animals but the peak of expression in GFAP-IL10Tg was delayed compared to WT. In conclusion, this study demonstrates that local production of IL-10 by astrocytes in the CNS may modify the microglial response associated with PPT by modulating endogenous regulatory receptors as TREM2 and CD200R. Future studies are concentrated on evaluating whether the differences of TREM2 and CD200R expression affects axonal sprouting.

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SYMPOSIUM 6

S6.1. ASTROGLIA IN AGEING AND NEURODEGENERATION WITH AN EMPHASIS ON ALZHEIMER DISEASE

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The common and prevailing point of view considers neurones as main substrates of pathological progression of neurological diseases. Today, however, we know that integration and information processing in the brain occurs though close interactions of two cellular circuits represented by neuronal networks embedded into internally connected astroglial syncytia. Our understanding of glial function changed dramatically over last two decades. This change concerns the whole concept of how the brain is organized, and how the development, life and death of neural circuits are controlled. There is compelling evidence demonstrating that these are the astrocytes that are creating the compartmentalisation in the CNS, and these are the astrocytes that are able to integrate neurones, synapses, and brain capillaries into individual and relatively independent units.

Neuroglial cells are intimately involved in all forms of neurological diseases and this are neuroglia, which, to a very large extent, determine the progression and outcome of neuropathological process. Astrocytes are specifically involved in various neurodegenerative diseases including Alzheimer's disease, Amyotrophic lateral sclerosis, Parkinson's disease and various forms of dementia. Recent evidence suggest that early stages of neurodegenerative processes are associated with atrophy of astroglia, which causes disruptions in synaptic connectivity, disbalance in neurotransmitter homeostasis and neuronal death through increased excitotoxicity. At the later stages astrocytes became activated and contribute to neuro-inflammatory component of neurodegeneration.

S6.2. ASTROCYTES, NEUROPLASTICITY AND CNS REGENERATION

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Reactive gliosis or reactive astrogliosis is a term used for the morphological and functional changes seen in astroglial cells/astrocytes responding to CNS injury or seen in many neurological diseases. This defensive reaction of astrocytes is likely aimed at handling the acute stress, limiting tissue damage and restoring homeostasis, it also seems to inhibit adaptive neural plasticity mechanisms underlying recovery of function. However, the knowledge about astrocyte functional diversity and its molecular basis is limited.

We used single-cell gene-expression profiling of freshly isolated astrocytes from uninjured mouse hippocampus and after deafferentation to test two hypotheses: that hippocampal astrocytes constitute subpopulations that respond differentially to trauma, and that injury induces a response in astrocytes in the contralesional hippocampus. Based on their gene expression profiles, we show that astrocytes in the uninjured hippocampus are highly heterogeneous. The identification of subpopulations of astrocytes in the injured hippocampus suggests differential response of astrocytes to injury. Injury-triggered changes in gene expression in individual astrocytes on the injured and contralesional side were surprisingly similar. The response in the contralesional hemisphere appears to be a part of the global response to injury and may play a role in determining the extent of functional recovery.

Understanding the roles of astrocytes in the healthy and diseased CNS can contribute to the development of treatment strategies that will, in a context-dependent manner and at appropriate time points, modulate reactive astrogliosis in order to promote CNS repair and reduce the neurological impairment.

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S6.3. ASTROCYTES IN BRAIN DISEASE – MOLECULAR AND FUNCTIONAL CHANGES

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Astrocytes are actively involved in neuronal communication. In age-related neurodegenerative diseases, such as Alzheimer's disease (AD), astrocytes become reactive. The research in my lab is focused on understanding the molecular and functional changes in reactive astrocytes, and the consequences for neuronal communication. Whole genome-wide analysis of acutely isolated astrocytes from aged and AD mice revealed that aged astrocytes have a more immune activated phenotype [1] and that the neuron-support function of AD astrocytes is declined [2]. We also observed that reactive glia in AD have an increased expression of the immunoproteasome subunits [2], resulting in an increase in immunoproteasome activity in AD brains [3]. In our current studies we are focusing on the functional changes in reactive astrocytes and their effect on neuronal communication in models for brain disease.

Reactive astrocytes have a more pronounced intermediate filament network, and the characteristic protein in this cytoskeletal network is Glial Fibrillary Acidic Protein (GFAP) [4]. Absence of GFAP in AD mouse astrocytes leads to a slightly more inflammatory phenotype [5]. Modulation of the GFAP network in an astrocytic cell line, results in significant changes in genes involved in cell-cell signaling and interacting with the extracellular matrix [6]. Taken together, our data show that a change in the GFAP intermediate filament network can change the transcriptome of astrocytes, thereby potentially adapting physiological processes in these cells.

In conclusion, our research shows molecular changes in astrocytes due to aging and degenerative processed. These changes might be partly induced by the modulation of the GFAP constitution of the astrocytic intermediate filament cytoskeleton.

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S6.4. SOD1 MUTATIONS CAUSING FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS INDUCE TOXICITY IN ASTROCYTES: EVIDENCE FOR BYSTANDER EFFECTS IN A CONTINUUM OF ASTROGLIOSIS

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Astrocytes contribute to the death of motor neurons via non-cell autonomous mechanisms of injury in amyotrophic lateral sclerosis (ALS). Since mutations in the gene encoding Cu/Zn superoxide dismutase (SOD1) underlie the neuropathology of some forms of familial ALS, we explored how expression of mutant SOD1 protein, A4V SOD1-EGFP, affected the biology of cultured murine astrocytes. 72 h after transfection, astrocytes expressing A4V SOD1-EGFP displayed decreased mitochondrial activity (~45%) and L-glutamate transport (~25%), relative to wild-type SOD1-EGFP. A4V SOD1-EGFP altered F-actin and Hoechst staining, indicative of cytoskeletal and nuclear changes, and altered GM130 labelling suggested fragmentation of Golgi apparatus. SOD1 inclusion formation shifted from discrete to "clumpy" over 72 h. A4V SOD1-EGFP more rapidly produced inclusions than G85R SOD1-EGFP and formed more "clumpy" aggregates. A4V, but not wild-type SOD1-EGFP, exerted a substantial, time-dependent effect on GFAP expression, and ~60% of astrocytes were stellated and hypertrophic at 72 h. Spreading toxicity was inferred since at 72 h ~80% of bystander cells exhibited hypertrophy and stellation. This evidence suggests that mutant SOD1-containing astrocytes release destructive species that alter the biology of adjacent astrocytes. This panoply of mutant SOD1-induced destructive events favours recruitment of astrocytes to non-cell autonomous injury in ALS.

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PLENARY LECTURE 2

L2. ROLE OF THE ASTROGLIAL RESPONSE TO INJURY

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Astrocyte responses to CNS damage and disease, astrogliosis, have been recognized structurally for over 125 years. Until recently, functional roles of these responses were sparsely studied and enigmatic. Negative effects were often assumed. Over the past 20 years, a complex picture of beneficial functions and potentially negative effects of astrogliosis has emerged from studies dissecting molecular mechanisms of astrogliosis in vivo using transgenic loss-of-function strategies and animal models of diverse CNS disorders. Rather than being a simple 'all-or-none' process, astrogliosis is being revealed as a broad spectrum of potential changes that astrocytes can undergo, ranging from subtle alterations in molecular expression, to substantial cellular hypertrophy without cell proliferation, to cell proliferation and scar formation, all of which are determined in a context specific manner by diverse signaling events that vary with the nature and severity of different CNS insults. Loss-of-function studies show that under normal circumstances, newly proliferated scar-forming astrocytes exert both pro- and anti-inflammatory functions and serve as critical barriers that restrict neurotoxic inflammation and thereby limit tissue damage and preserve tissue and function after CNS traumatic injury, stroke, infection, autoimmune attack and degenerative disease. Less is understood about functions of non-proliferative hypertrophic reactive astrocytes. The potential for dysfunctions of astrocytes and astrogliosis is also being recognized. A conceptual framework is emerging that encompasses both 'normal astrogliosis' critical for preservation of tissue and function after CNS insults, and 'dysfunctional astrogliosis' that exerts harmful effects due to genetic defects, genetic polymorphisms, autoimmune attack, or chronic exposure to inflammatory stimuli caused by secondary infections. Recognizing and understanding differences between beneficial and dysfunctioning astrogliosis, and the context dependent circumstances that distinguish benefit from harm, is likely to be fundamental to dissecting the cellular mechanisms underlying many CNS disorders.

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