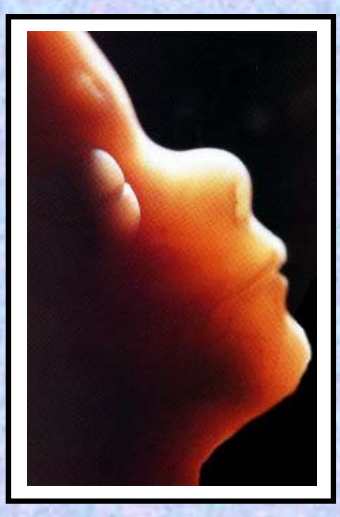
International Exchange Course

Developmental and Perinatal Biology



18th-22nd August, 2014
University of Toronto

Program

A Joint Course between:

University of Toronto & Karolinska Institute

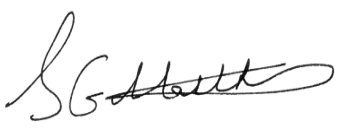
Welcome

Welcome to **Developmental & Perinatal Biology 2014**. This is the **18th Annual Exchange** in developmental and perinatal biology between The University of Toronto and The Karolinska Institute. The research course has been developed to provide a broad based interdisciplinary training for graduate students, research fellows, clinical fellows and residents in the area of developmental biology from both basic science and clinical perspectives. The workshop combines a lecture/seminar program with an active research component. The course is also offered as a component of a Graduate course (PSL1080H) at the University of Toronto and as a Graduate course at the Karolinska Institute.

In 2013, the course was held at the Karolinska Institute in Sweden from August 18-24, organized by Dr. Ola Hermanson with assistance from Aileen Gracias for the social program. It was attended by 5 faculty and 15 trainees from the University of Toronto. From the attendance and success in previous years, it has been clear that there is great interest in this type of summer course. This year we have experienced similar enthusiasm, with 4 faculty and 11 trainees attending from Sweden along with 21 trainees from The University of Toronto. In addition, a large number of Faculty from across the University of Toronto will contribute to the course.

The organization of this type of course requires a considerable input of energy. Therefore, we would like to take this opportunity to thank those on the organizing committee for helping to put the exciting course program together, and to Dr. Ola Hermanson for co-ordinating the Swedish side of the exchange. We would also like to thank Bev Bessey, Jenny Katsoulakos, Nicole Smith, Ursula Nosi and Vasilis Moisiadis who have provided invaluable organizational support. Finally, we would like to express our gratitude to our sponsors, many of whom have provided continuous support over the last 18 years, and who have made **Developmental & Perinatal Biology 2014** possible.

Please accept our warmest welcome to what we hope will be an exciting academic and social experience.



Dr. S. G. Matthews	Dr. R. Jankov	Dr. S. J. Lye
Professor	Associate Professor	Professor
Physiology, Ob/Gyn & Medicine	Paediatrics & Physiology	Ob/Gyn, Physiology & Medicine

Local Organizing Committee:

Stephen Matthews (Chair)
Lee Adamson
Bev Bessey
Brian Cox
Robert Jankov
Jenny Katsoulakos
Steve Lye
Vasilis Moisiadis
Ursula Nosi
Janet Rossant
Nicole Smith
Neil Sweezey

Co-Sponsors:

Hospital for Sick Children, Research Training Centre
Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital
Department of Obstetrics & Gynaecology, University of Toronto
Fraser Mustard Institute for Human Development, University of Toronto
Division of Neonatology, Hospital for Sick Children
Department of Physiology, University of Toronto
Faculty of Medicine, University of Toronto
Institute of Medical Science, University of Toronto

Location:

Registration: **18th August** – Outside Room 106, Health Sciences Building, University of Toronto

Lectures: **18th – 22nd August** – Room 106, Health Sciences Building, University of Toronto

Practical Workshops: Laboratories located in the Medical Sciences Building, University of Toronto, Lunenfeld-Tanenbaum Research Institute of Mount Sinai Hospital and Hospital for Sick Children Research Institute; NICU at Mount Sinai Hospital

Social Activities:
18th August - Informal Evening (Walking Tour/Dinner)
19th August - Opening Reception – The Faculty Club, University of Toronto
20th August - Picnic on Toronto Island / Dinner
21st August - Faculty Dinner
22nd August - Night Out on the Town
23rd August - Day Trip to Niagara Falls

FACULTY:

University of Toronto:

	Department	**Location**
Dr. Lee Adamson	Ob/Gyn	Lunenfeld-Tanenbaum Research Inst.
Dr. Harvey Anderson	Nutritional Sciences/Physiology	University of Toronto
Dr. Gary Bader	Molecular Genetics	University of Toronto
Dr. Steffen-Sebastian Bolz	Physiology	University of Toronto
Dr. Brian Cox	Physiology	University of Toronto
Dr. Yenge Diambomba	Paediatric	Mount Sinai Hospital
Dr. Denis Gallagher	PDF – Miller Lab	Hospital for Sick Children
Dr. Robert Jankov	Paediatrics & Physiology	Hospital for Sick Children
Dr. Andrea Jurisicova	Physiology	Lunenfeld-Tanenbaum Research Inst.
Dr. Gideon Koren	Paediatrics	Hospital for Sick Children
Dr. Robert Levitan	Psychiatry	Centre for Addiction and Mental Health
Dr. Stephen Lye	Ob/Gyn & Physiology	Lunenfeld-Tanenbaum Research Inst.
Dr. Stephen Matthews	Physiology	University of Toronto
Dr. Patrick McGowan	Biological Sciences	University of Toronto at Scarborough
Dr. Patrick McNamara	Paediatrics & Physiology	Hospital for Sick Children
Dr. Gaspard Montandon	Medicine & Physiology	University of Toronto
Dr. Anne-Maude Morency	Clinical Fellow - MFM	Mount Sinai Hospital
Dr. Andras Nagy	Ob/Gyn & IMS	University of Toronto
Dr. Lucy Osborne	Molecular Genetics	University of Toronto
Dr. Zdenka Pausova	Physiology	Hospital for Sick Children
Dr. Martin Post	Paediatrics & Physiology	Hospital for Sick Children
Dr. Norm Rosenblum	Dev. & Stem Cell Biology	Hospital for Sick Children
Dr. Janet Rossant	Molecular Genetics & Ob/Gyn	Hospital for Sick Children
Dr. John G. Sled	Medical Biophysics	Hospital for Sick Children
Dr. Marla Sokolowski	Ecology & Evolutionary Biology	University of Toronto
Dr. Peter Tonge	PDF – Nagy Lab	Lunenfeld-Tanenbaum Research Inst.
Dr. Susan Varmuza	Cell and Systems Biology	University of Toronto
Dr. Behzad Yeganeh	PDF – Post Lab	Hospital for Sick Children

Karolinska Hospital/Institute:

Professor Fanie Barnabé-Heider	Neuroscience
Professor Klas Blomgren	Women's and Children's Health
Professor Ola Hermanson	Neuroscience
Professor Stephen Strom	Department of Laboratory Medicine

Mats Sundin Fellows :

Dr. Sophie Petropoulos (PDF)	Karolinska Institute
Dr. Jessica Weidner (PDF)	University of Toronto

Monday, 18th August

8:30 Registration — Health Sciences Building, Outside Room 106

8:45 Welcome/Introduction — **Drs. Robert Jankov and Stephen Matthews**
International Exchange Program for Developmental and Perinatal Biology, University of Toronto

Stem Cells & Embryonic Development

(Health Sciences Building, Room **106**)
Co-ordinator: Dr. Janet Rossant

9:00 ***Stem cells and early embryo development***
Dr. Janet Rossant

9:40 ***Reprogramming to pluripotency***
Dr. Peter Tonge

10:20 Coffee

10:40 ***Neurogenesis and neural stem cells***
Dr. Denis Gallagher

11:20 ***The role of chromatin modifying factors in programming and reprogramming of stem cells***
Dr. Ola Hermanson

<u>Trainee Presentations</u> (Orals 1-4)

12:00 ***Detection of human glioblastoma-derived stem-like cells using an oligothiophene derivative versus CD133 and CD44*** Aileen Gracias[1]

12:15 ***Role of glypicans in axon guidance***
Bomin Kim[2]

12:30 ***Heparan sulfate proteoglycans regulate directed differentiation of pluripotent cells to functional airway epithelial cells by decellularized lung scaffolds***
Sheri Shojaie[3]

12:45 ***Expandable stem-cell derived alveolar-like macrophages that engraft to airways and remain functional*** Michael Litvack[4]

13:00 Lunch in MSB Cafeteria

14:00-15:30 ***Research Workshops:***
1) Physiology of the Pulmonary Circulation
2) Analysis of High-Throughput Data

18:00 **Informal Evening (*Walking Tour/Dinner on the Harborfront*)**

Tuesday, 19th August

Placenta and Birth

(Health Sciences Building, Room **106**)
Co-ordinator: Dr. Brian Cox

9:00 ***Assessing molecular heterogeneity in placental pathologies***
Dr. Brian Cox

9:40 ***Epigenetic regulation of placenta development***
Dr. Susan Varmuza

10:20 Coffee

10:40 ***Patterning of the placental micro-circulation***
Dr. John G. Sled

11:20 ***Injury and repair in the developing brain***
Dr. Klas Blomgren

<u>Trainee Presentations</u> (Orals 5-7)

12:00 ***Feto-neonatal cardiovascular adaptation in preterm and term infants***
Ulf Schubert[5]

12:15 ***JMJD6 - a novel oxygen sensor in the human placenta***
Sruthi Alahari[6]

12:30 ***Studies of the molecular pathways behind dyslexia***
Andrea Bieder[7]

13:00 Lunch in MSB Cafeteria

14:00-15:30 ***Research Workshops:***
1) Epigenetics
2) Somatic Cell Reprogramming

19:30 **Opening Reception – The Faculty Club, University of Toronto (41 Willcocks Street)**

Wednesday, 20th August

Cardiopulmonary Physiology

(Health Sciences Building, Room **106**)
Co-ordinator: Dr. Robert Jankov

9:00 ***Fetal lung development***
Dr. Martin Post

9:40 ***The role of autophagy in lung development***
Dr. Behzad Yeganeh

10:20 Coffee

10:40 ***Development of the neural control of breathing***
Dr. Gaspard Montandon

11:20 ***Chronic neonatal lung injury***
Dr. Robert Jankov

12:00 ***GROUP PHOTO***
(please assemble outside on steps of MSB facing King's College Circle)

12:15 **Lunch and Poster Session** – Physiology Seminar Room (MSB 3227)

Presentation by Mats Sundin Fellows (Health Sciences Building, Rm. 106)

13:30 ***Determining the human embryonic transcriptional landscape and the impact of glucocorticoids***
Dr. Sophie Petropoulos

14:00 ***Toxoplasma gondii - a parasite's passage across the placenta***
Dr. Jessica Weidner

15:00 **Picnic – Toronto Island / Dinner**

Thursday, 21st August

Neurodevelopment

(Health Sciences Building, Room **106**)
Co-ordinator: Dr. Stephen Matthews

9:00 ***Gene by environment interaction in nervous system development***
Dr. Marla Sokolowski

9:40 ***Regeneration potential of the CNS***
Dr. Fanie Barnabé-Heider

10:20 Coffee

10:40 ***Epigenetics, neurodevelopment and life-long mental health***
Dr. Patrick McGowan

11:20 ***Williams Syndrome: A window on cognition, behaviour and language***
Dr. Lucy Osborne

<u>Trainee Presentations</u> (Orals 8-10)

12:00 ***Determining the effect of increased HH signaling on metanephric mesenchyme cell fate***
Sepideh Sheybani Deloui[8]

12:15 ***Sphingosine-1-phosphate and regulation of drug transporter activity in the developing blood-brain barrier*** Samantha Kearney[9]

12:30 ***Effect of neonatal fluoxetine treatment on 5-HT signaling in cortical regions of transgenic mice*** Kristina Perit[10]

13:00 Lunch in MSB Cafeteria

14:00-15:30 ***Research Workshops*:**
1) Advanced Molecular Techniques: Gene & Protein Expression Analysis
2) MSH NICU Visit

16:00 **Free Evening**

Friday, 22nd August

Developmental Origins of Health and Disease

(Health Sciences Building, Room **106**)

Co-ordinator: Dr. Stephen Lye

9:00 ***Fetal alcohol syndrome: The silent epidemic***

Dr. Gideon Koren

9:40 ***Prenatal smoke exposure and risk for adolescent obesity: Role of genetic and epigenetic modifiers***

Dr. Zdenka Pausova

10:20 Coffee

10:40 ***Using a DOHAD approach for brain development and behavioural outcomes***

Dr. Robert Levitan

11:20 ***Stem cell correction of liver disease***

Dr. Stephen Strom

<u>Trainee Presentations</u> (Orals 11-14)

12:00 ***Left heart structure and function in 6-year-old children born extremely preterm***

Lilly-Ann Mohlkert[11]

12:15 ***Running-enhanced neurogenesis can lead to forgetting in adult mice***

Aijing Gao[12]

12:30 ***Neurogenesis-mediated forgetting of hippocampal memories***

Axel Guskjolen[13]

12:45 ***Identifying the interacting regions between GluN1 & ND2 in the Src-NMDAR pathway***

David Scanlon[14]

13:00 Lunch in MSB Cafeteria

14:00-15:30 ***Research Workshops:***

1) Lab-on-a-Chip
2) Fetal Therapy Education

16:00 **Night Out on the Town**

Practical Workshops:

Physiology of the Pulmonary Circulation

Dr. Patrick McNamara

Location: **Hospital for Sick Children, McMaster Building**
1st Floor LAS Physiology Room
(Meet in front of building at southeast corner of Elm and Elizabeth Streets)

Date/Time: **Monday, August 18, 2014 / 2:00 – 3:30 pm**

Participants will be provided with theoretical and technical insights into cardiovascular physiology through the demonstration of ultrasound-based assessments of pulmonary haemodynamics and cardiac function in small rodents. Two-dimensional echocardiography and Doppler ultrasound will be performed on normal and pulmonary hypertensive neonatal rats to familiarise participants with the measurements used to assess pulmonary vascular resistance and right-ventricular function. Differences in findings between the normal and pulmonary hypertensive state will be highlighted.

Analysis of High-Throughput Data

Dr. Brian Cox

Location: **Discovery Commons, Medical Sciences Building, Room 3173**
University of Toronto

Leaders: **Dr. Brian Cox**
Katherine Leavey

Date/Time: **Monday, August 18, 2014 / 2:00 – 3:30 pm**

Within this workshop attendees will be introduced to the R programming language (http://www.r-project.org/) and its application to analysis of large data sets (http://www.bioconductor.org/). An emphasis will be on microarray data, but other data types will be addressed. The workshop will consist of a short lecture and a computer lab where attendees will analyze example data sets, with the goal of understanding the do's and do not's of large scale analysis.

Epigenetics

Dr. Sophie Petropoulos

Location: **Lunenfeld-Tanenbaum Research Institute, 25 Orde Street**
Room 6-1021

Date/Time: **Tuesday, August 19, 2014 / 2:00 – 3:30 pm**

This workshop will introduce the field of epigenetics, with focus on DNA methylation. Common techniques used to assess both targeted genes of interest and global DNA methylation will be introduced, described, and compared. Techniques include: LUminometric methylation assay (LUMA), methylated DNA immunoprecipitation (MeDIP), chromatin Immunoprecipitation (ChIP), bisulfite sequencing, and pyrosequencing. Finally the workshop will describe the implications of altered DNA methylation with regards to disease/disorder development and highlight potential therapeutic targets.
The workshop will provide an overview of epigenetics and its implication in disease/disorder development, focusing on transgenerational effects. Techniques utilized to assess changes in the epigenome will be described.

Somatic Cell Reprogramming

Dr. Andras Nagy

Location: **Lunenfeld-Tanenbaum Research Institute, 25 Orde Street**
Meet in Room 5-1019, 5th Floor

Leaders: **Dr. Peter Tonge**

Date/Time: **Tuesday, August 19, 2014 / 2:00 – 3:30 pm**

Trainees will visually assess somatic cell reprogramming events and learn techniques for picking colonies to isolate clonal reprogrammed cell lines. The use of fluorescence will be used to demonstrate the ability to monitor cell state and transgene expression.

Advanced Molecular Techniques: Gene & Protein Expression Analysis

Dr. Stephen Lye

Location: **Lunenfeld-Tanenbaum Research Institute, 25 Orde Street**
6th Floor, Room 6-1021

Leaders: **Dr. Oksana Shynlova**
Dr. Caroline Dunk

Date/Time: **Thursday, August 21st, 2014 / 2:00 – 3:30 pm**

This workshop will discuss the different techniques to asses and quantify protein expression in normal and pathological samples. It will describe (1) protein techniques such as Western blotting, ELISA, Luminex and immune-labeling to identify changes in protein expression, localization or secretion, as well as Proximity Ligation Assay, a technique that visualizes directly interacting proteins in tissue samples; (2) gene expression techniques such as Northern Blotting, quantitative PCR (real-time PCR), micro array analysis and in situ hybridization. The new RNA- sequencing technologies that include transcript identification, splice variant analysis and differential expression of the entire RNA portion of the transcriptome will be described.The properties and limitations of these techniques will be demonstrated.

NICU Visit at Mount Sinai Hospital

Dr. Yenge Diambomba

Location: **Mount Sinai Hospital, 7th Floor, Room 775-A**
(use Murray Street Elevators)

Date/Time: **Thursday, August 21, 2014 / 2:00 – 3:30 pm**

Mount Sinai Hospital operates one of the busiest perinatal services in Canada with more than 7,000 deliveries annually, many of which are high-risk. Participants will receive a tour of the Neonatal Intensive Care Unit (NICU) at Mount Sinai, and will hear about and discuss common perinatal issues and clinical and ethical dilemmas faced on a regular basis by neonatal clinicians.

Lab-on-a-Chip

Dr. Steffen-Sebastian Bolz

Location: **Medical Sciences Building, Room 3326, University of Toronto**

Leaders: **Dr. Jeff Kroetsch**
Dr. Darcy Lidington

Date/Time: **Friday, August 22, 2014 / 2:00 – 3:30 pm**

Resistance arteries significantly influence blood pressure and organ perfusion and, consequently, represent a prime therapeutic target in cardiovascular disease. However, technical challenges associated with harvesting and functionally assessing resistance arteries have discouraged the expansion of this research field and it remains a largely untapped resource.

The workshop will provide an overview of new molecular concepts describing the myogenic response as the single most important mechanism governing resistance artery function. The myogenic response is an intrinsic mechanism that continuously matches vascular resistance to the prevalent pressure (autoregulation). In addition to contributing to tissue perfusion control, the myogenic response also (i) protects fragile capillary beds from high transmural pressure, (ii) maintains capillary hydrostatic pressure at levels that minimize edema formation, (iii) provides the partial constriction necessary for vasodilator responses and (iv) contributes to the generation of peripheral resistance, which builds and maintains the systemic blood pressure. Any modification to the myogenic mechanism necessarily has widespread effects; indeed, altered myogenic responsiveness is a hallmark of several vascular pathologies.

Rodent models are prevalent research tools; yet their human applicability is strikingly limited and knowledge translation often lags well-behind basic science advancements. In particular, the use of isolated human resistance arteries in pressure myography studies, the only means of assessing intact artery function in vitro, is disproportionately sparse. This is largely because tissue samples containing relevant vascular beds are difficult, if not impossible, to obtain. Innovative technologies to better understand the regulation of blood pressure are highly relevant, since the technical limitations of conventional myography platforms have prevented widespread microvascular research.

In addition to an overview of the myogenic response, this workshop will introduce the Artery-on-a-Chip platform (AoC), an innovative tool for microvascular research developed by scientists from the Depts. of Physiology and Mechanical Engineering at the University of Toronto. This microfluidic device allows for the assessment of the structural and functional status of small resistance arteries (which are the primary regulators of blood pressure). By applying microfluidic knowledge and design principles, the AoC overcomes these limitations and provides a scalable, inexpensive and high throughput-ready alternative to conventional approaches that can be used with minimal training efforts.

In preparation for this workshop, please refer to the articles: (1) "A microfluidic platform for probing small artery structure and function" (Lab Chip. 2010; 10:2341-9) and the corresponding YouTube video under http://www.youtube.com/watch?v=ATwEGKlHwK8; (2) "Capitalizing on diversity: an integrative approach towards the multiplicity of cellular mechanisms underlying myogenic responsiveness (*Cardiovasc Res*. 2013; 97(3):404-12); and (3) "The emerging role of Ca2+ sensitivity regulation in promoting myogenic vasoconstriction" Cardiovasc Res. 2008;77(1):8-18.

Fetal Therapy Education

Dr. Anne-Maude Morency

Location: **Lunenfeld-Tanenbaum Research Institute, 25 Orde Street, 6th Floor, Room 6-1021**

Date/Time: **Friday, August 22nd, 2014 / 2:00 – 3:30 pm**

Fetal Therapy (the active treatment of a fetus in-utero) is one of the youngest and more rapidly developing disciplines in clinical medicine. The Fetal Therapy program at the University of Toronto is one of the larger units in North America. In this workshop, we will briefly review the history and evolution of in-utero fetal therapy. The principles which guide the use of fetal therapy at the University of Toronto will then be explored and the applications of these principles to clinical practice will be reviewed. Both medical management (for example maternal medication for fetal cardiac dysrhythmia) and surgical management (for example, fetal transfusion, shunting, etc.) will be examined, using video clips from the procedures. Specific conditions to be discussed include fetal anemia, lower urinary tract obstruction, complicated monochorionic twin pregnancies, congenital diaphragmatic hernia and congenital heart disease. Finally, potential future developments will be considered – including new technologies for education and treatment, etc.

Research Workshop Assignments:

MONDAY

Physiology of the Pulmonary Circulation

Leonardo Ermini
Issaka Yougbare
Lilly-Ann Mohlkert
Joyce Lee
Michael Litvack
Tacito Bessa
Gisela Reinfeldt Engberg
Ulf Schubert
Sheri Shojaie
Bomin Kim

Analysis of High-Throughput Data

Andrea Bieder
Giulia Zanni
Michael Vu
Fatima Lakha
Nir Melamed
Ahmed Saeed
Frances Wong
Sruthi Alahari
Kristina Perit
Sepideh Sheybani Deloui
David Scanlon
Aijing Gao
Vasilis Moisiadis
Ursula Nosi
Samantha Kearney

TUESDAY

Epigenetics

Aileen Gracias
Yiqiao Wang
Giulia Zanni
Sruthi Alahari
Leonardo Ermini
David Scanlon
Ahmed Saeed
Michael Litvack
Issaka Yougbare
Fabiha Rahman
Ursula Nosi

Somatic Cell Reprogramming (ONLY 10)

Aijing Gao
Lidi Xu
Sepideh Sheybani Deloui
Sheri Shojaie
Bradley Smither
Andrea Bieder
Xiaofei Li
Bomin Kim
Karin Salehi
Axel Guskjolen

THURSDAY

Advanced Molecular Techniques: Gene & Protein Expression Analysis

David Scanlon
Frances Wong
Axel Guskjolen
Aileen Gracias
Xiaofei Li
Yiqiao Wang
Nir Melamed
Bradley Smither
Leonardo Ermini
Giulia Zanni
Aijing Gao
Vasilis Moisiadis

NICU Visit at Mount Sinai Hospital

Gisela Reinfeldt Engberg
Sruthi Alahari
Tacito Bessa
Samantha Kearney
Bomin Kim
Joyce Lee
Kristina Perit
Fabiha Rahman
Lilly-Ann Mohlkert
Karin Salehi
Michael Vu
Ulf Schubert
Lidi Xu
Fatima Lakha
Xiaofei Li
Ursula Nosi

FRIDAY

Lab-on-a-Chip

Lidi Xu
Andrea Bieder
Ulf Schubert
Michael Vu
Joyce Lee
Lilly-Ann Mohlkert
Bradley Smither
Frances Wong
Axel Guskjolen
Nir Melamed

Fetal Therapy Education

Michael Litvack
Ahmed Saeed
Karin Salehi
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Tacito Bessa
Samantha Kearney
Kristina Perit
Fabiha Rahman
Sepideh Sheybani Deloui
Issaka Yougbare
Yiqiao Wang
Aileen Gracias
Sheri Shojaie
Fatima Lakha
Vasilis Moisiadis

ABSTRACTS

- Orals -

O1

Aileen Gracias, E. Kavanagh, M. Bäck, P. Nilsson, B. Joseph and O. Hermanson

Detection of human glioblastoma-derived stem-like cells using an oligothiophene derivative versus CD133 and CD44.

O2

Bomin Kim, N. Tassew and P. Monnier

Role of glypicans in axon guidance.

O3

Sheri Shojaie, L. Ermini, C. Ackerley, M. Bilodeau, J. Wang, I. Rogers, J. Rossant, C. Bear and M. Post

Heparan sulfate proteoglycans regulate directed differentiation of pluripotent cells to functional airway epithelial cells by decellularized lung scaffolds.

O4

Michael L. Litvack, C.A. Ackerley and M. Post

Expandable stem-cell derived alveolar-like macrophages that engraft to airways and remain functional.

O5

Ulf Schubert, M. Müller, H. Abdul-Khaliq, M. Norman and A.K. Edstedt Bonamy

Feto-neonatal cardiovascular adaptation in preterm and term infants.

O6

Sruthi Alahari and I. Caniggia

JMJD6 - a novel oxygen sensor in the human placenta.

O7

Andrea Bieder, H. Matsson, A. Falk, J. Kere and I. Tapia-Páez

Studies of the molecular pathways behind dyslexia.

O8

Sepideh Sheybani Deloui and N. Rosenblum

Determining the effect of increased HH signaling on metanephric mesenchyme cell fate.

O9

Samantha Kearney, W. Gibb and S.G. Matthews

Sphingosine-1-phosphate and regulation of drug transporter activity in the developing blood-brain barrier.

O10

Kristina Perit and E.K. Lambe

Effect of neonatal fluoxetine treatment on 5-HT signalling in cortical regions of transgenic mice.

O11

Lilly-Ann Mohlkert, O. Broberg, M. Hellström, C. Pegelow-Halvorsen, J. Hallberg, G. Sjöberg, A.K. Edstedt Bonamy, V. Fellman, M. Domellöf and M. Norman

Left heart structure and function in 6-year-old children born extremely preterm.

O12

Aijing Gao, P.W. Frankland and S.A. Josselyn

Running-enhanced neurogenesis can lead to forgetting in adult mice.

O13

Axel J. Guskjolen, J.R. Epp, S.A. Josselyn and P.W. Frankland

Neurogenesis-mediated forgetting of hippocampal memories.

O14

David P. Scanlon, W. Zhang, A. Bah, H.L. Leduc-Pessah, J.D. Forman-Kay and M.W. Salter

Identifying the interacting regions between GluN1 & ND2 in the Src-NMDAR pathway.

DETECTION OF HUMAN GLIOBLASTOMA-DERIVED STEM-LIKE CELLS USING AN OLIGOTHIOPHENE DERIVATIVE VERSUS CD133 AND CD44

Gracias, A.[1], Kavanagh, E.[2], Bäck, M.[3], Nilsson, P.[3], Joseph, B.[2] and Hermanson, O.[1]

[1]Department of Neuroscience and [2]Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden; [3]IFM, Linköping University, Linköping, Sweden

Glioblastoma multiforme is one of the most aggressive, malignant brain tumors, and is prone to relapse. One of the reasons of the relapse has been attributed to the existence of so called cancer stem cells in glioblastoma. These glioblastoma-derived stem-like cells (GSCs), which also have features of tumour-initiating cells (TICS), possess the ability to self renew and form tumors.

Previous studies from our labs have identified a Luminescent Conjugated oligothiophene (LCO) to be used as a molecular probe for the live detection of rat embryonic neural stem cells, but not differentiated cells, other stem cells such as ES cells, or various cancer cells. This LCO, referred to as p-HTMI, crosses the cell membrane freely, and within a few minutes illuminates autonomously in the cytoplasm of target cells. p-HTMI stained 1-2% of neural tumor cell cultures in a rat model of glioma (C6) but 100% of C6-derived stem-like cells. Here we report the use of this LCO to specifically detect the stem cell population in human glioma-derived stem like cells from three patients without triggering cell apoptosis or necrosis. The level of specificity was compared to other well-established stem cell markers such as CD133 and CD44. Preliminary results suggest that p-HTMI is a more specific yet more sensitive marker of GSCs than CD133 and significantly more selective than CD44. We propose that this LCO due to its unique optical property can be used for cell specification and potentially used for improved clinical therapy of glioblastoma.

ROLE OF GLYPICANS IN AXON GUIDANCE

Kim, B.[1,2], Tassew, N.[1,2] and Monnier, P.[1,2,3]

[1]Genetics and Development Division, Toronto Western Research Institute, Toronto, CA;
[2]Departments of Physiology and [3]Ophthalmology, Faculty of Medicine, University of Toronto, Toronto, CA

Development of the central nervous system (CNS) requires proper growth and integration of the developing neurons into their correct target sites. Chondroitin sulfate proteoglycans (CSPGs) is one class of molecules known to be abundantly expressed during development and inhibits outgrowth of neurites into incorrect targets through their receptor called RPTPsigma. However, the specific underlying mechanism of these molecules is yet to be discovered. Increasing evidence from our lab shows that the relocalization of receptors into plasma membrane microdomains rich in cholesterol known to compartmentalize cellular activities (lipid rafts) is required for the downstream signaling of these molecules. Here, I propose that the recruitment of RPTPsigma into lipid rafts is required to activate the downstream signaling pathway of CSPGs. Our preliminary data supports the idea that this recruitment is done by one or several of the GPI-anchored molecules that are present specifically in lipid rafts. Glypicans are a class of GPI-anchored heparan sulfate proteoglycans (HSPGs) that interact with RPTPsigma. To test this hypothesis, the chick visual system was used; retinal ganglion cells (RGCs) extend neurites that form the optic nerve, and forms synapses in the midbrain. First, we inhibited the formation of sugar chains attached to the protein core of HSPGs which are required for interaction with RPTPsigma in an in vitro culture of retinal explants. The result provided evidence that this interaction is required for the inhibitory effects of CSPGs on growing neurites. From here, several short hairpin RNAs against these glypicans will be used to selectively silence glypicans expressed in the developing RGCs to block glypican-RPTPsigma interaction. We believe that this will lead to enhanced outgrowth of RGCs cultured on CSPGs. The results from this project could provide insights into the development of therapeutic methods by targeting lipid rafts (cholesterol-reducing drugs) in cases where CSPGs are upregulated after CNS damage and promote nerve regeneration.

HEPARAN SULFATE PROTEOGLYCANS REGULATE DIRECTED DIFFERENTIATION OF PLURIPOTENT CELLS TO FUNCTIONAL AIRWAY EPITHELIAL CELLS BY DECELLULARIZED LUNG SCAFFOLDS

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Introduction & Objective: Efficient differentiation of pluripotent cells to proximal and distal lung epithelial cell populations remains a challenging task. The lung extracellular matrix (ECM) is a key component that regulates the interaction of secreted factors with cells during development and consequently plays a crucial role in directing lineage specific differentiation. Herein we demonstrate that decellularized lungs with intact matrix composition, devoid of all cellular components, can direct differentiation of embryonic stem cells (ESC) into airway epithelia, with morphological and functional similarities to native airways. **Methods:** Rat lungs were decellularized by sequential tracheal lavages and retrograde pulmonary arterial perfusion using a range of physical, chemical, and enzymatic treatments. Murine ESC were seeded onto scaffolds following endoderm induction using activin, and cultured at air-liquid interface, in serum free conditions, without addition of exogenous growth factors for the duration of three weeks. **Results:** Following seven days of culture, seeded cells express the earliest known lung lineage marker Nkx2-1 and adopt an epithelial-like tubular organization. Cells further differentiate to functional specialized epithelial cell types found in the trachea and bronchioles (ciliated, club, basal cells) with prolonged culture on scaffolds. Functional analysis revealed beating ciliated cells, club cells secreting Clara cells secretory protein, and polarized CFTR expression on epithelial sheets with channel functionality. Heparitinase I, but not chondroitinase ABC, treatment of scaffolds revealed that the differentiation achieved is dependent on heparan sulfate proteoglycans remaining on decellularized scaffolds. **Conclusion:** This work demonstrates that decellularized lung scaffolds effectively recapitulate the lung developmental milieu and are capable of directing differentiation of endoderm-induced ESC to functional lung epithelia, without the requirement of exogenous growth factors. Current work is focused on using decellularized scaffolds for directing differentiation to an alveolar lineage (type I and type II epithelial cells).

EXPANDABLE STEM-CELL DERIVED ALVEOLAR-LIKE MACROPHAGES THAT ENGRAFT TO AIRWAYS AND REMAIN FUNCTIONAL

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Alveolar macrophages (AM) are the primary phagocytic cell of the lungs. They maintain clean airways under normal conditions to prevent acute or chronic lung injury caused by bacteria, viruses or apoptotic cells. Compromised clearance function attributed to acquired or genetic deficiencies of AMs represents critical components of chronic lung diseases, such as chronic obstructive pulmonary disease (COPD), asthma and cystic fibrosis (CF). A reliable source of competent cells that compliment resident populations and engraft to the airways is lacking. Pluripotent stem cells are a source of self-renewing and expanding cells that have the potential to become any type of tissue including myeloid macrophages. We therefore sought to generate alveolar-like macrophages from pluripotent stem cells and determine if these cells are functional *in vitro* and *in vivo*. We describe an *in vitro* differentiation protocol to generate alveolar-like macrophages from murine pluripotent stem cells that reflects the early developmental origins of AM. The cells we generate are functional and engulf bacteria and apoptotic cells both *in vitro* and *in vivo* and engraft to the healthy and compromised airways of mice. Furthemore, the cells are expandable and retain the characteristic AM ligand markers of F4/80, CD11c and SiglecF over extended periods of cell culture. Our study therefore provides a novel method for generating and expanding alveolar-like macrophages derived from pluripotent stem cells that can be used for therapeutic and investigative applications.

FETO-NEONATAL CARDIOVASCULAR ADAPTATION IN PRETERM AND TERM INFANTS

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Study 1
The primary aim of the first study is to compare aortic and carotid artery growths after very preterm birth with normal fetal and infant arterial growth. We hypothesize that the vascular growth arrest is not restricted to small vessels as in retinal arteries but a more general phenomenon affecting also large vessels. As blood flow to the placenta drives fetal aortic growth in utero, we also suggest that premature uncoupling of the placental circulation makes the aorta a particularly sensitive vessel for growth arrest after preterm birth. In addition, the missing physiological increase of IGF at the end of a normal pregnancy will contribute to this phenomena.

Study 2
In accordance to study 1, we will investigate both aortic and carotid artery intima-media thickness in a cohort of very preterm neonates appropriate for gestational age (AGA) and compare these to a control group of AGA infants born at term. We hypothesize that there might be a difference in IMT as the result of low IGF levels and an impaired relation between vessel wall and vessel diameter leading to altered hemodynamic situations in the vessel and for the heart.

Study 3
The aim of this study is to assess cardiac function in the transitional period from fetal to postnatal life by using the relatively new technique of speckle tracking and compare the data to conventionally performed echocardiography in a healthy population at a determined age. In addition to that, feasibility and reproducibility will be analyzed and critically evaluated concerning the clinical use of this new method.

Study 4
The final study will use the same technique as described in study 3 and compare cardiac function in preterm and term born infants. Actually, there is no data available in a premature cohort and it will be interesting how changes in vessel structure and diameter will interfere with cardiac function and secondly, how specific hemodynamic situations in the preterm group as the persistence of the ductus arteriosus will influence myocardial performance. We are planning to quantify systolic and diastolic right and left ventricular function and their contribution to global cardiac output. If there are differences in the study group and controls we will thoroughly describe them and try to find reasonable explanations and possible interventions for future trials.

JMJD6 - A NOVEL OXYGEN SENSOR IN THE HUMAN PLACENTA

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Human placental development is exquisitely regulated by changes in oxygen tension, a process that is crucial for a successful pregnancy. Persistent low oxygen is implicated in the pathogenesis of preeclampsia, a placental pathology characterized by hypoxia/oxidative stress. We have reported that elevated levels of HIF-1α (Hypoxia-inducible factor 1α) in preeclamptic placentae are primarily due to altered expression of the prolyl hydroxylase enzymes that regulate HIF-1α stability. Interestingly, emerging evidence implicates a family of Jumonji C (JmjC) domain containing histone demethylases as *novel oxygen sensors* and regulators of hypoxic gene expression. While JMJD1A and JMJD2B are transcriptional targets of HIF-1α, no information is available on JMJD6 and its interplay with HIF-1α. Hence, we aimed to characterize JMJD6 expression in the human placenta in normal and pathological conditions and delineate its relationship with HIF-1α.

Western blotting and immunohistochemical analyses of placentae from early gestation (5-14 weeks) and in preeclampsia revealed that JMJD6 expression *inversely* correlated with changes in oxygen tension in early gestation, and significant enrichment in preeclamptic placentae. *In vitro* exposure of JEG3 trophoblast cells to 3% O_2 or oxidative stress revealed not only an increase in JMJD6 protein but also enrichment within both the nucleoplasm and nucleoli relative to controls. HIF-1α knockdown by siRNA (cells) and anti-sense oligonucleotide (placental explants) strategies revealed that it is a positive regulator of JMJD6 mRNA. Alternatively, JMJD6 knockdown stabilized HIF-1α and this was associated with decreased expression of von Hippel Lindau protein, a negative regulator of HIF-1α stability. As well, JMJD6 overexpression resulted in enhanced VHL expression while destabilizing HIF-1α.

Our data signifies a novel role for JMJD6 as an oxygen sensor in the human placenta in physiological and pathological conditions. A better understanding of HIF-1α regulation in preeclampsia has implications for the development of diagnostic markers and therapeutic targets in this pathology.

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STUDIES OF THE MOLECULAR PATHWAYS BEHIND DYSLEXIA

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Dyslexia is a complex learning disability characterized by deficits in reading despite adequate intelligence, normal senses and proper socio-cultural opportunities. It is the most common reading disorder, affecting 5-10% of the population. Dyslexia has a strong genetic component and many susceptibility genes have been identified among which *DYX1C1*, *DCDC2* and *KIAA0319* are the most replicated. The molecular functions of these genes are little investigated, yet all the above have been implicated in neuronal migration and development. More recently, our group and others have shown a link between dyslexia candidate genes and cilia. We are investigating the role of dyslexia candidate genes in relation to cilia and centrosome. We are thus performing assays in a human ciliated cell line (RPE1, retinal pigment epithelial cells) and in tissues for sub-cellular localization by immunofluorescence (IF). In addition, we are looking for common molecular pathways of the candidate genes *DYX1C1*, *DCDC2* and *KIAA0319* by functional assays. We are using neuroepithelial-like stem cells (NES cells) derived from induced pluripotent stem cells (iPSCs) as a model to further dissect the molecular mechanisms involved in dyslexia. NES cells are self-renewing and can be differentiated along the neural and glial lineages. We have shown by qRT-PCR and IF that *DYX1C1*, *DCDC2* and *KIAA0319* gene expression is increased in NES cells during their differentiation to neurons and glia. In addition, they are growing cilia during differentiation. NES cells are thus a valid model system to study the function of dyslexia candidate genes. Using lentiviral expression constructs, we are overexpressing *DYX1C1*, *DCDC2* and *KIAA0319* in NES cells in order to study the downstream pathways of these genes.

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DETERMINING THE EFFECT OF INCREASED HH SIGNALING ON METANEPHRIC MESENCHYME CELL FATE

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Development of the permanent kidney begins at E10.5 in mice with reciprocal signaling between the intermediate mesoderm (IM) derived ureteric bud (UB) and metanephric mesenchyme (MM). The process of nephrogenesis in the MM gives rise to the complement of nephrons that serve as the functional filtration units within the kidney. Extra-metanephric stroma invades the MM to encompass the cortex of the kidney. The renal stroma is required for nephrogenesis and branching morphogenesis (Hatini, et al. 1996, Levinson, et al. 2005,Patterson, et al. 2001).

Cell-lineage tracing demonstrates that the stroma and MM derive from a common *Osr1+* population within the IM that subsequently expresses *Sall1*. However, the pathways that fate this population to either NM or stroma is unknown. Our lab has demonstrated that increased Hedgehog signaling (Hh) in the MM (*Rarb2-cre;Ptc1*$^{-/loxP}$) expands the stromal population while inhibiting nephrogenesis.

We hypothesize that Hh signaling controls the specification of NM and stroma in the IM. To test this, we will alter the state of Hh signaling in the IM at different embryological timepoints using two temporally (Tamoxifen-mediated) inducible mouse models: *Osr1Cre*^{Ert2}*;Ptc1*$^{-/loxP}$ and *Sall1Cre*^{ERT2}*;Ptc1*$^{-/loxP}$. The distribution of NM and stroma will then be assessed using fate-mapping.

We anticipate these results to enhance our understanding of Hh-dependent fate decisions in the developing metanephric kidney.

SPHINGOSINE-1-PHOSPHATE AND REGULATION OF DRUG TRANSPORTER ACTIVITY IN THE DEVELOPING BLOOD-BRAIN BARRIER

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P-glycoprotein (P-gp) is an efflux transporter located on brain endothelial cells (BECs). It is present early in fetal development and is important in protecting the developing brain. Sphingosine-1-phosphate (S1P) has been shown to modify P-gp function in the adult blood-brain barrier. S1P is formed by sphingosine kinases, and acts through 5 membrane receptors ($S1P_{1-5}$). The synthetic molecule FTY720 inhibits S1P production when administered at a high dose (K_i = 2μM). We hypothesized that a high dose of FTY720 would have age-dependent effects on P-gp function. BECs from gestational day 65 (GD65), postnatal day 14 (PND14) and adult guinea pigs were cultured and treated with FTY720 (5μM) for 15, 30, 60 or 120 minutes. P-gp function was assessed using the calcein-AM fluorescence accumulation assay. Treatment with high dose FTY720 reduced P-gp function in GD65 BECs at all time points, reduced P-gp function in PND14 BECs from 15-60 minutes and reduced cellular S1P content at 30 minutes ($P<0.05$). There was a tendency for FTY720 treatment to reduce P-gp function until 30 minutes in the adult. Treatment with a high dose of FTY720 rapidly reduced cellular levels of S1P and reduced P-gp function in BECs derived from the developing brain though the mechanisms of these effects are not known. Importantly, fetal and early postnatal BECs were more sensitive to the effects of FTY720 than were adult cells. These data suggest that if S1P levels are altered during development, as occurs during a number of pathologies, P-gp function may be compromised. Impaired P-gp function would increase the exposure of the developing brain to potentially harmful xenobioitics, drugs and other circulating factors.

EFFECT OF NEONATAL FLUOXETINE TREATMENT ON 5-HT SIGNALLING IN CORTICAL REGIONS OF TRANSGENIC MICE

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Background

In Canada, 15% of women will experience depression in their lifetime. Depression during pregnancy is common due to changes in biological and psychosocial elements, frequently described as 'baby blues'. Treatment for depression often includes selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine (FLX), which is frequently prescribed since it has relatively few side effects. There is growing evidence however that FLX treatment significantly affects the neural development of offspring both *in utero* and after birth. In cortical areas the morphology of neurons involved in serotonin (5-HT) signalling pathways is significantly altered by FLX. What is still unclear is how the activity of these neurons is altered. We propose to investigate the effects of neonatal FLX treatment on 5-HT signalling in offspring.

Outline of Proposed Research

Newborn mice will be given daily intraperitoneal injections of FLX (10 mg/kg) to mimic fetal exposure to FLX during the third trimester. We will use *in vitro* patch clamp electrophysiology to examine 5-HT signalling in layer V pyramidal neurons in the motor and somatosensory cortices of mice once they reach adolescence (P30). Neuronal responses to bath application of 5-HT will be recorded at rest and firing threshold. Pharmacological experiments will be performed to determine which 5-HT receptors are involved in responses. We will also examine how FLX treatment affects pyramidal neurons expressing the protein p11 which has been found to be critical to the response to FLX. We hypothesize that 5-HT signalling in the cortex will be disturbed by FLX treatment.

LEFT HEART STRUCTURE AND FUNCTION IN 6-YEAR-OLD CHILDREN BORN EXTREMELY PRETERM

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Background and Aim

Preterm birth has been associated with myocardial remodelling, arrested vascular growth, higher blood pressure and ventricular hypertrophy later in life. The aim of this study was to evaluate left heart structure and function in 6-year-old children born extremely preterm.

Method

Children born extremely preterm (EXP; <27 weeks of gestation) in Sweden 2004 to 2007 and matched controls born at term were included. Left ventricular mass index (LVMI), left ventricular end diastolic diameter (LVED) and fractional shortening (FS) were determined by echocardiography. Blood pressure, weight and height were also measured.

Results

EXP-children (n=88; mean GA 25.1 w; BW 817 g) were significantly shorter than controls (mean heights 117.8 and 122.8 cm, $p<0.001$). LVMI was 72.1 g/m^2 in EXP and 79.6 g/m^2 in controls ($p<0.01$). LVED was smaller (35.9 mm) in EXP than in controls (38.7 mm; $p<0.01$), also after adjusting for body surface area (BSA; $p<0.001$). FS was 36 % in EXP and 35 % in controls (n.s). Unadjusted systolic blood pressure was 3 mmHg lower in EXP compared to controls ($p<0.05$) but this difference disappeared after taking BSA into account.

Conclusion

Although the shape of the heart differed (smaller LVED in EXP), there was no left ventricular hypertrophy or other obvious signs of myocardial dysfunction in 6-year-old children born extremely preterm as compared to age-matched controls born at term. Further cardiac follow-up at older age is warranted and analyses of myocardial strain using two dimensional speckle tracking are underway.

RUNNING-ENHANCED NEUROGENESIS CAN LEAD TO FORGETTING IN ADULT MICE

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Forgetting is as important as remembering for healthy memory function, as it provides a mechanism for ridding the brain of unimportant information and allowing important new information to be more efficiently encoded. Yet, neurobiological studies of memory have focused almost exclusively on remembering, and little is known at a mechanistic level about forgetting. Recently, we have identified hippocampal neurogenesis as a key regulator of forgetting. In adult mice, we experimentally manipulated levels of neurogenesis after memory formation by running exercise, which is indicated to be able to increase neurogenesis and cell proliferation in dentate gyrus of hippocampus. We found that increasing neurogenesis promoted degradation of hippocampal memories formed through associative (contextual fear conditioning) and spatial (water maze) learning paradigms. The mechanism indicates that integration of new neurons likely induces forgetting by reconfiguring hippocampal circuits which would reduce the ability of a given set of cues to re-invoke the same pattern of activity, impeding memory recall. With time, Memories that are initially hippocampus-dependent may become less dependent on hippocampus but other brain regions. Consistently, we found that increasing neurogenesis immediately after training induced more forgetting of a contextual fear memory, whereas an equivalent increase after a 4 week delay had little effect on subsequent memory expression, which demonstrates that neurogenesis-mediated forgetting impact memories solely during their hippocampus-dependent phase.

NEUROGENESIS-MEDIATED FORGETTING OF HIPPOCAMPAL MEMORIES

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Hippocampal neurogenesis occurs throughout life. As these new neurons mature and form synaptic connections, they necessarily remodel existing hippocampal circuitry. Indeed, computational models predict that high levels of hippocampal neurogenesis should lead to degradation of information stored in already established circuits (1-3). Consistent with this, we have recently demonstrated that high levels of post-learning hippocampal neurogenesis both remodel hippocampal circuitry and lead to forgetting of previously stored information (4). Here we show that P17 infant mice (in which levels of hippocampal neurogenesis are high) can learn and remember the location of a hidden platform in the MWM for 1-14d, but show complete forgetting 1month following training. This infantile forgetting is not due to poor initial memory encoding, as overtraining infant mice does not overcome the forgetting phenotype. Interestingly, the memory is rescued by a 'reminder' trial, suggesting that infantile forgetting of spatial information stems from a deficit in memory retrieval rather than memory storage. One benefit of this forgetting phenotype is increased cognitive flexibility, as revealed by the infant groups superior reversal learning in later life. To determine the mechanism underlying hippocampal neurogenesis induced forgetting, we developed a mouse in which the Rho GTPase Rac1 can be selectively deleted from neural progenitors using a cre-loxP strategy. As a consequence, these newly generated neurons show reduced synaptic integration, as indicated by decreased dendritic growth, arborization, and spine maturation (5). Consistent with the hypothesis that forgetting is mediated by circuit remodeling caused by the synaptic integration of recently generated hippocampal neurons, inhibiting Rac1 expression in neural progenitors blocked the neurogenesis-induced forgetting effect. Together, these experiments further our understanding of the mechanisms underlying hippocampal neurogenesis induced forgetting.

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IDENTIFYING THE INTERACTING REGIONS BETWEEN GLUN1 & ND2 IN THE SRC-NMDAR PATHWAY

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Upregulation of NMDA receptors (NMDARs) by the tyrosine kinase Src is critical for chronic pain hypersensitivity in the spinal cord & hippocampal LTP. Src is anchored in the NMDAR complex by an adaptor protein, ND2, (NADH dehydrogenase subunit 2). The primary sequence requirements for the interaction between Src & ND2 have been determined, but the interacting regions between ND2 & the NMDAR complex have remained elusive until the present study. To elucidate the basis for this interaction, we transfected HEK293 cells with GFP-tagged ND2 or with one of a systematically generated series of GFP-tagged fragments of ND2, with NMDAR subunits or receptor controls. The NMDAR subunits/controls were fluorescently labeled & confocal images captured. Thresholded Pearson's Correlation Coefficient (PCC) was used as measure of colocalization. GFP-ND2 differentially colocalized with both GluN1 alone (0.61 ± 0.03) & a GluN1-C-terminal & Amino Terminal Domain (ATD) deletion mutant (0.61 ± 0.03), but the additional deletion of the TM4 region led to a loss of colocalization (0.09±0.04). Swapping in a GluN2A TM4 region into GluN1 recapitulated this colocalization (0.78±0.06), but neither the GluN1 nor the GluN2A TM4 alone colocalized with ND2, illustrating that TM4 is necessary but not sufficient for this interaction. The ND2 fragment 151-223 (0.71±0.02), also colocalized with GluN1 alone, but smaller ND2 fragments did not. Thus, we have determined the minimal interacting region of ND2 required in the ND2–NMDAR complex, & identified GluN1 TM4 as a critical GluN1-ND2 interacting region.

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THE EFFECTS OF PRENATAL SYNTHETIC GLUCOCORTICOID TREATMENT ON THE EXPRESSION OF DRUG TRANSPORTERS

Bessa, T.L.B.[1], Moisiadis, V.G.[1], Kostaki, A.[1] and Matthews, S.G.[1,2,3]

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Background: Preterm birth occurs in approximately 10% of pregnancies. The majority of women at risk for delivering prematurely receive treatment with synthetic glucocorticoids (sGC) to reduce the risk of respiratory distress syndrome and neonatal death. Throughout gestation, fetal organs are protected from exposure to potentially harmful substances by efflux drug transporters. The expression and function of two of these transporters, P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), have been shown to be altered in the fetal brain following acute exposure to sGC. However, little is known regarding the long-term effects of prenatal sGC exposure on these drug transporters. We hypothesize that prenatal treatment with multiple courses of sGC will result in increased expression of P-gp and BCRP in the brains, livers and adrenal glands of juvenile first (F_1) and second (F_2) generation guinea pigs.

Method: Pregnant guinea pigs were treated with the sGC betamethasone (Beta; 1mg/kg; n=6) or saline (C; n=6) on gestational days 40/41, 50/51 and 60/61 (term ~69 days). Adult F_1 females (Beta; n=6 & C; n=6) were mated with control males to produce F_2 offspring. F_1 and F_2 juvenile offspring were euthanized on postnatal day 40, and the cerebellum, liver and adrenal gland were collected. qRT-PCR and western blot will be utilized to measure the levels of P-gp and BCRP mRNA and protein .

Significance: Millions of children have received treatment with sGC *in utero*, yet little is known regarding how this treatment may affect their long-term health. The proposed study will provide insight into how prenatal treatment with sGC affects the chemical barriers protecting organs. The results of this study will demonstrate the effects of sGC on the barriers in young offspring, and will investigate whether there are lasting effects transmitted to the next generation.

EVIDENCE FOR ALTERED SPHINGOLIPID CONTENT IN MEMBRANE MICRODOMAINS IN PREECLAMPSIA

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Objective: Sphingolipids and cholesterol are essential elements of lipid rafts and caveole - dynamic membrane microdomains that cluster proteins and regulate a variety of cellular processes including signal transduction, endocytosis and membrane trafficking. We have recently found altered sphingolipid metabolism in preeclampsia, a serious disorder of pregnancy. The objective of this study was to examine the sphingolipid composition of microdomains in placentae from preeclampsia and normotensive control pregnancies.

Methods: Since lipid microdomains are resistant to solubilisation by non-ionic detergents, detergent-resistant membrane (DRM) fractions were isolated from placentae of preeclamptic (PE) and age-matched control pregnancies (PTC). The DRMs were characterized using both raft and caveole markers, i.e. caveolin-1, placental alkaline phosphatase (PALP), and lipid ganglioside (GM1). Lipids were subsequently extracted from DRMs and analyzed by tandem mass spectrometry (MS/MS).

Results: As anticipated detergent-insoluble membrane fractions were enriched in cholesterol, caveolin-1, PALP and GM1. Lipidomic analysis revealed no changes in cholesterol amount in PE placentae vs. controls. In contrast, the content and distribution of ceramides and sphingomyelins with various fatty acyl-CoA lengths were altered in microdomains from PE placentae vs. PTC. In particular, CER18 and SM18 were increased in PE-derived lipid microdomains. As studies have reported the presence of endoglin, a key player in the genesis of PE, in the apical trophoblast membrane, we next examined its presence in lipid microdomains. Western Blot analysis showed the presence of endoglin in lipid microdomains where it associated with MMP14, the matrix metalloproteinase responsible for its cleavage. Interestingly, immunoprecipitation experiments showed that endoglin associates with lipid raft, but not with caveolae, domains.

Conclusion: Increased levels of SM18 and CER18 in lipid microdomains found in preeclampsia may lead to changes in microdomain functions thereby contributing to altered trophoblast turnover typical of this disease (Supported by CIHR).

TAWANA MAA (HEALTHY MOTHER): OPTIMIZING MATERNAL NUTRITION THROUGH EMPOWERING COMMUNITY RESOLUTION OF BASIC FOOD QUALITY

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Many aspects of the mother's health and life-style before pregnancy have been shown to affect her subsequent pregnancies with potential to impact the health of her children, but one area of particular concern is the mother's weight before pregnancy. A mother who is underweight prior to becoming pregnant also puts her baby at higher risk for complications, mainly because of the association between underweight status and malnutrition (Ehren-berg et al., 2003). Therefore, malnutrition needs to be addressed to maximize positive outcomes for both mother and baby.

Imagine that you have no way of checking whether the product you manage to get is of the same quality you are used to. This is the food insecurity faced by many mothers in the developing world. Our idea is to imagine a way to reduce that food insecurity by showing how inexpensive, open and accessible technology can be organized in a way that allows community-based tracking and evaluation of the nutritional quality of food ingredients consumed locally.

Our partner will be a local NGO's. Together we will create a social network centered on the quality of home-made flatbread that accounts for more than 60% of the calories consumed by people living in those communities. Our project will be focused on the nutritional assessment and ensuring that pregnant and nursing mothers get adequate nutrition in a sustainable and teachable way.

THE EFFECT OF MECHANICAL VENTILATION ON CERAMIDE PRODUCTION AND LUNG DEVELOPMENT

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Introduction & Objective: Bronchopulmonary dysplasia (BPD) is the most common chronic lung disease of neonates. Very premature infants that are subjected to mechanical ventilation and oxygen supplementation due to respiratory failure are prone to lung injury that may result in chronic lung disease, such as BPD, with lifelong consequences. The lungs of BPD patients are characterized by a simplification of normal lung complexity with fewer and larger alveoli. Various molecules are implicated in lung injury including sphingolipids. In particular, ceramides play a role in the regulation of apoptosis and autophagy. Both processes are critical for development and share many key regulators. Long chain ceramides have been recognized as a pro-apoptotic molecule that also switches autophagy towards cell death. Here, we investigated whether mechanical strain (mimicking ventilation) of immature (fetal) lung epithelial cells affects ceramide production, as well as cell death, thereby negatively affecting normal lung development.

Methods: Rat lung epithelial cells were isolated at E19 of gestation (term = 22 days), which represents the late canalicular/early saccular stage of lung development; similar to the lung developmental stage of a very premature (24-27 wks) infant. The cells were subjected to mechanical strain at continuous high (20%) cyclic stretch. The cells were then collected for protein and immunohistochemical analyses. Ceramide intermediates and ceramide levels were measured by mass spectral analysis using tandem mass spectrometry (LC MS/MS).

Results: Ceramide levels and cell death increased with duration (>12 hrs) of stretch. Minimal changes in levels of LC3B-II (typical marker for autophagy) were observed while, in contrast, cleaved-caspase 3 (typical marker of apoptosis) levels were increased.

Conclusions: The data suggests that fetal rat lung epithelial cells subjected to stretch produce more ceramides, leading to increased apoptosis but not autophagy.

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DEVELOPMENT AND POTENTIAL OF SPINAL CORD STEM CELLS

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The discovery of stem cells has raised great hope for regenerative medicine. It has been speculated that stem cells transplantations can promote functional recovery in several diseases including spinal cord injury (SCI). However several limitations are associated with transplantation-based approaches for clinical use. Therefore recruitment of endogenous cells represents an attractive and non-invasive alternative. Recently, we have shown that stem cell potential is confined to ependymal cells in the adult spinal cord. However, the knowledge about these cells during development is poorly understood. Using non-inducible transgenic mice line, we have studied ependymal cells genesis during development. We found, based on the expression of FoxJ1, that the first ependymal cells appeared at E17 and that all ependymal cells are generated around the central canal at birth time. Unexpectedly, we found that FoxJ1 is transiently expressed in V1 neurons (from E10) and in two different subpopulations of astrocytes in the dorsal funiculus (from E15) and in the lateral white matter (from E17). We then studied the effect of age on the stem cell potential of ependymal cells. Using neurospheres cultures, we showed that self-renewal capacity of ependymal cells declined over time from P5 to P21 to adult mice but can be reactivated after SCI. Indeed, P21 neurospheres after SCI mimicked the self-renewal capacity of P5 neurospheres. Altogether, we here define the origin of ependymal cells during development and characterize new transiently Foxj1^{+} cell populations. We also demonstrate that the stem cell potential of ependymal cells is higher in juvenile animals suggesting that these cells could be functionally targeted in order to improve regeneration upon SCI in young human adult.

ANALYSIS OF GENE-SPECIFIC AND WHOLE GENOME METHYLATION IN ART AND NATURALLY CONCEIVED PREGNANCIES

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BACKGROUND: There is strong evidence that the normal epigenetic reprogramming after fertilization is vulnerable to environmental stresses. This provided a rationale for the concerns that ART-related interventions might result in epigenetic mutations in the offsprings of ART pregnancies. Indeed, we have recently investigated in our lab the DNA methylation patterns in cord blood samples from ART and control pregnancies using the Illumina Infinium HumanMethylation27 microarray which provides information on DNA methylation in 27,578 CpG sites throughout the genome. Using this technology we found an association between ART and methylation changes that occurred at unique CpG site locations (e.g., non-CpG island sites).

OBJECTIVE: In view of these results, we now aim to compare DNA methylation patterns in cord-blood and placenta samples from ART and control pregnancies using the newer Illumina Infinium HumanMethylation450K platform which provides information on more than 480,000 CpG sites covering 99% of RefSeq genes throughout the genome. We hypothesize that using this even larger scale approach we will be able to more comprehensively characterize the CpG sites/genes that are differentially methylated in ART pregnancies.

METHODS: Placenta and cord-blood samples from couples with ART and naturally-conceived pregnancies have been obtained from the bio-bank of Mount Sinai Hospital. Multifetal gestations and pregnancies complicated by major fetal anomalies were excluded from both groups. Genome-wide and site-specific analysis of DNA methylation will be done using the new Illumina HumanMethylation450K arrays. Whole-genome and site/gene specific methylation patterns will be compared between the ART and control groups and will be further stratified by factors such as type and location of genes/CpG sites and clinical factors. Gene/CpG site specific methylation changes will be confirmed by pyrosequencing and will be correlated with gene expression levels. Based on the magnitude of differences in mean methylation levels and the variance of methylation level from our previous analyses, a sample size of about 10 samples in each group will be required in order to detect an inter-group difference in mean methylation level of 5% as significant with a power of 80% and a conservative type-I error of 1%.

MULTIPLE COURSE ANTENATAL GLUCOCORTICOID HAS TRANSGENERATIONAL EFFECTS ON BEHAVIOR AND ENDOCRINE FUNCTION

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Antenatal synthetic glucocorticoid (sGC) exposure can permanently 'program' metabolic, cardiovascular and neurologic function in offspring. Data are emerging that demonstrate transmission of the effects of sGC to the subsequent (F_2) generation of offspring. However, no study has examined paternal transmission of the effects of antenatal sGC treatment on offspring behaviors or HPA function. We hypothesized that sGC would result in increased activity, reduced attention and reduced HPA function in first, second and third generation offspring (F_1, F_2, F_3).

Pregnant guinea pigs were treated with three courses of betamethasone (Beta; 1 mg/kg) or saline (Veh) (Veh N=40, Beta N=43). Animals were allowed to deliver naturally (term ~ 69 days). In each generation, offspring underwent behavioral and endocrine testing as juveniles (days 19-35). Another group of male offspring was mated with naïve females in adulthood to create F_2 offspring; this paradigm was repeated to produce F_3 offspring.

Beta resulted in increased locomotor activity in F_1 juvenile males and females, and F3 females ($P<0.05$). There was no effect of Beta on locomotor activity in F_2 offspring. F_1 Beta offspring produced greater cortisol responses to stress than did Veh offspring ($P<0.05$). F_2 Beta females also produced a more rapid and greater cortisol response to stress ($P<0.05$), and there was a trend for reduced production of cortisol in Beta males ($P=0.07$). Attention was reduced in F_2 Beta females ($P<0.05$), but not affected in another group.

This study has identified very long-term effects of antenatal treatment with sGC. Subsequent molecular analyses will further our understanding of the mechanisms by which prenatal exposure to excess glucocorticoid programs the development of the neuroendocrine system (specifically the HPA axis), as well as illuminate the mechanisms leading to programming across generations. The findings will have an important impact on the indications of usage of synthetic glucocorticoids in clinical settings.

ANTENATAL GLUCOCORTICOID TREATMENT AND PROGRAMMING OF THE JUVENILE PITUITARY

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Objective: Approximately 10% of pregnant women are at risk of preterm delivery and receive treatment with synthetic glucocorticoids (sGCs) to reduce the risk of respiratory distress syndrome in the newborn. This treatment is effective at reducing neonatal morbidity and mortality, however, little is known about the consequences of this treatment on hypothalamic-pituitary-adrenal (HPA) function in the juvenile period. We have previously shown that HPA reactivity to stress is elevated in juvenile offspring exposed to sGCs in late gestation. In the present study, we investigated the hypothesis that prenatal exposure to sGCs results in altered molecular regulation of HPA-regulatory genes in the juvenile anterior pituitary.

Methods: Pregnant guinea pigs were subcutaneously injected with sGC, betamethasone (1 mg/kg), or saline on gestational days 40,41,50,51,60 and 61. Subsequently, we investigated the expression of key HPA regulatory genes in juvenile offspring pituitaries.

Results: In sGC-exposed juvenile female anterior pituitaries, there was a significant increase in proopiomelanocortin (POMC) protein expression ($p < 0.05$), strong trends for reduced arginine vasopressin receptor 1B (Avpr1b), corticotrophin releasing hormone receptor 1 (Crhr1) and weak trend for reduced glucocorticoid receptor (Gr) expression. In juvenile male offspring, though sGC treatment did not alter *Pomc* expression, there were strong trends for increased *Avpr1b* and *Crhr1* mRNA levels. Gr expression did not change in sGC-exposed male juvenile anterior pituitaries.

Conclusions: The increased POMC protein expression in anterior pituitaries of female juvenile sGC-exposed offspring suggests an increased anterior pituitary drive to the adrenal, and this is consistent with the increased HPA responsiveness to stress in this group. While there were no overall significant effects of sGC treatment on Avpr1b and Crhr1 expression (indicators of pituitary sensitivity to hypothalamic signals) in the juvenile animals, significant effects may emerge as the animal age. These studies have expanded our understanding of the mechanisms underlying prenatal sGC exposure on the programming of neuroendocrine function in juvenile animals.

TRANSPLANTATION OF AUTOLOGOUS MINCED BLADDER MUCOSA FOR A ONE-STEP RECONSTRUCTION OF A TISSUE ENGINEERED BLADDER CONDUIT

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Surgical intervention is sometimes needed to create a conduit from the abdominal wall to the bladder for self-catheterization. We developed a method for tissue engineering a conduit for bladder emptying without *in vitro* cell culturing as a one-step procedure. In a porcine animal model bladder, wall tissue was excised and the mucosa was minced to small particles. The particles were attached to a tube in a 1 : 3 expansion rate with fibrin glue and transplanted back by attaching the tube to the bladder and through the abdominal wall. Sham served as controls. After 4-5 weeks, conduits were assessed in respect to macroscopic and microscopic appearance in 6 pigs. Two pigs underwent radiology before termination. Gross examination revealed a patent conduit with an opening to the bladder. Histology and immunostaining showed a multilayered transitional uroepithelium in all cases. Up to 89% of the luminal surface area was neoepithelialized but with a loose attachment to the submucosa. No epithelium was found in control animals.
CT imaging revealed a patent channel that could be used for filling and emptying the bladder. Animals that experienced surgical complications did not form conduits. Minced autologous bladder mucosa can be transplanted around a tubular mold to create a conduit to the urinary bladder without *in vitro* culturing.

EFFECTS OF A DISRUPTED BLOOD-BRAIN BARRIER ON CHOLESTEROL HOMEOSTASIS IN THE BRAIN

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Presence of the blood-brain barrier (BBB) is critical for cholesterol metabolism in the brain, preventing uptake of lipoprotein-bound cholesterol from the circulation. The metabolic consequences of a leaking BBB for cholesterol metabolism has not been studied previously. Here we used a pericyte-deficient mouse model, *pdgfb*$^{ret/ret}$, shown to have increased permeability of the BBB to a range of low-molecular mass and high-molecular mass tracers.

There was a significant accumulation of plant sterols in the brain of the *pdgfb*$^{ret/ret}$ mice. By dietary treatment with 0.3% deuterium labeled cholesterol we could demonstrate a significant flux of cholesterol from the circulation into the brain of the mutant mice roughly corresponding to about half of the measured turnover of cholesterol in the brain. We expected the cholesterol flux into the brain to cause a downregulation of cholesterol synthesis. Instead cholesterol synthesis was increased by about 60%. The levels of 24S-hydroxycholesterol (24S-OHC) were significantly reduced in the brain of the pericyte deficient mice but increased in the circulation. After treatment with 1% cholesterol in diet the difference in cholesterol synthesis between mutants and controls disappeared. The findings are consistent with increased leakage of 24S-OHC from the brain into the circulation in the pericyte-deficient mice. This oxysterol is an efficient suppressor of cholesterol synthesis and the results are consistent with a regulatory role of 24S-OHC in the brain. To our knowledge this is the first demonstration that a defect BBB may lead to increased flux of a lipophilic compound out from the brain. The relevance of the findings for the human situation is discussed.

MOLECULAR AND CLINICAL STUDIES IN INTESTINAL MALROTATION

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Rationale

Objectives

Elucidate the molecular background to intestinal malrotation taking advantage of inherited cases and new technical developments in genetics and verify candidate genes by functional studies in zebra fish.

Study the clinical course of the disease using own case files from at least 180 identified paediatric cases since 1993 or older.

Determine the risk for associated disorders, subsequent abdominal surgery and the relative risk for relatives using national population based registries.

Studies Planned

Study 1: Description of clinical data before surgery and a postoperative follow-up.

Study 2: Molecular studies

Study 3: Analyse a target array for ciliopathies in DNA from patients with malrotation and test any postitive result by using a zebra fish model.

Study 4: An epidemiologic register-based study of patients operated for intestinal malrotation with or without volvulus

Progress

I am now in the process of analyzing clinical data from medical charts, performing semi-structured interviews with patients for follow-up and collection of blood samples.

STIMULATION OF QUIESCENT STEM CELLS BY GLUCAGON-LIKE PEPTIDE-2 (GLP-2) IN THE MURINE SMALL INTESTINE

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Glucagon-like peptide-2 (GLP-2) is a proglucagon-derived peptide that is secreted by enteroendocrine L-cells. This hormone has profound physiological and pharmacological effects including promoting intestinal growth. A degradation-resistant GLP-2 analogue, h(Gly^2)-GLP-2, has been cleared for human use as the drug, teduglutide. GLP-2-induced crypt cell proliferation has been shown to require an insulin-like growth factor-1 (IGF-1) – intestinal epithelial-IGF-1 receptor (IE-IGF-1R) signalling pathway. However, the intestinal stem cells involved in this pathway to stimulate intestinal growth, is not known. One intestinal stem cell pool of interest is the quiescent, radiation-resistant, B lymphoma Mo-MLV insertion region 1 homolog (Bmi-1)-positive stem cells at the +4 position of the crypt. It has been shown that teduglutide increases survival of stem cells after radiation and that GLP-2 treatment increases intestinal stem cell number in this model. Therefore, we hypothesize that GLP-2 stimulates the proliferation of +Bmi-1 stem cells in the murine small intestine and that this occurs in an IE-IGF-1R-dependent fashion. A mouse model with enhanced green fluorescent protein (GFP) reporter knocked into the Bmi-1 gene to label +4 position stem cells was used to determine if GLP-2 acts on this stem cell pool. These mice were injected with 0.1μg/g of h(Gly^2)-GLP-2 or phosphate buffered saline (PBS) daily for 11 days and jejunal segments were collected. To confirm GLP-2's proliferative effects in this mouse model by functional analysis, haematoxylin & eosin-stained jejunal sections were measured along the crypt-villus axis. Crypt-villus height increased from 375±8μm to 490±12μm in Bmi-$1^{+/+}$ males (n=6, $P<0.001$) and increased from 383±11μm to 467±21μm in Bmi-$1^{GFP/+}$ females (n=7-8, $P<0.01$) injected with h(GLY^2)-GLP-2 vs PBS. This suggests that the Bmi-$1^{GFP/+}$ mouse model is appropriate for studying the proliferative effects of GLP-2 on the crypt. Preliminary proliferative analyses and mRNA expression levels have been examined to start to assess if GLP-2 stimulates +Bmi-1 stem cell proliferation.

A ROLE FOR TASK CHANNELS ON BASAL FOREBRAIN CHOLINERGIC NEURONS IN MODULATING SLEEP AND WAKEFULNESS

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Introduction and Objective: Cholinergic basal forebrain neurons have widespread projections to the cortex, and modulate brain arousal state and consciousness. Basal forebrain cholinergic neurons are innervated by monoaminergic histamine projections from the tuberomamillary nucleus, which have their highest activity in wakefulness and lowest activity in states of sleep and anesthesia. However, the mechanism by which histamine modulates basal forebrain cholinergic neurons is unclear. TWIK-like acid sensitive K+ (TASK) leak channels are activated by general anesthetics (causing neuronal inhibition) and inhibited by monoamines (causing neuronal excitation). Here we test the hypothesis that histamine at basal forebrain cholinergic neurons modulates cortical activation via TASK channels.

Methods: Wild-type mice and transgenic (TASK f/f) mice that lack both TASK-1 and TASK-3 channel subunits in cholinergic neurons were chronically instrumented to record the electroencephalogram (EEG) and neck electromyogram (EMG). Histamine (1mM) dissolved in artificial cerebrospinal fluid, or vehicle alone, was then locally microperfused into the basal forebrain using reverse microdialysis.

Results: Preliminary data (n=8 wild-type and n=6 TASK f/f mice) show that during non-rapid eye movement (NREM) sleep, histamine microperfusion into the basal forebrain significantly ($P<0.05$) decreased EEG power in the 1-4Hz band in wild-type but not in TASK f/f mice. Histamine also increased EEG power in the 30-50Hz band; and this increase was significantly attenuated in the TASK f/f mice. During wakefulness, histamine decreased EEG power in the 1-4Hz and 4-8Hz bands and increased EEG power in the 8-12Hz, 12-30Hz and 30-50Hz bands; however there was no significant difference between wild-type and TASK f/f mice.

Conclusion: These results demonstrate that TASK channels partially mediate the shift in EEG towards heightened arousal induced by histaminergic activation of basal forebrain cholinergic neurons. Future studies will test the hypothesis that basal forebrain cholinergic neurons modulate cortical deactivation via TASK channels, using custom-designed methodology to locally deliver volatile anesthetics to the basal forebrain.

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MOLECULAR NETWORK CONTROLLING PROPRIOCEPTIVE NEURONS IDENTITY AND THEIR CONNECTIONS

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The coordination of movement depends essentially on sensory inputs provided by proprioceptive afferents that are located in the dorsal root ganglia (DRG) positioned along the spinal cord and relay sensory information from stretch receptors located in the skeletal muscles (muscle spindles) and their tendons (Golgi tendon organs). The generation of these proprioceptive afferents follows a systematic developmental sequence of events, from neuron birth, target innervation to spinal cord ingrowth. These specification steps are thought to be controlled by a tight interplay between dedicated programs of genes and environmental cues that together orchestrate the formation of the spinal circuits. These processes have been shown to depend essentially on the molecular triad NT3-ER81-Runx3. However, their direct biological activities as well as the identity of the factors regulating their expression are still unclear. Here, we aim at providing an integrated view of the mechanisms that control the multiple steps of proprioceptive neuron development, from birth to central afferentation, and at understanding if, when and how a limited set of molecular factors orchestrate their different stages of development.

CELLULAR SYSTEMS BIOLOGY OF EARLY HUMAN PLACENTA DEVELOPMENT

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The mammalian placenta is an essential organ of pregnancy, maintaining close communication between the mother and developing fetus by regulating transport, angiogenesis and immune tolerance, but we know little about early cellular development that generates the cell types that drive these processes. We hypothesize that the placenta will have different trophoblast p rogenitors and stem cells and that these will drive cell-cell interactions and signaling events that organize and direct placental development. To identify these progenitor cells we utilized a large screen of 370 cell surface markers (the CD antigens) using a high throughput screen on a flow cytometer. Analysis of cell surface marker expression between week 6 and week 10 revealed 22 downregulated and 2 upregulated proteins indicating the presences of dynamic cell populations during human placenta villi formation. We selected EpCAM and CDCP1 for more targeted ingestigation because they show decreasing expression with placenta development as we would expect for progenitor cells. To characterize these populations, we employed immunohistochemistry to show EpCAM positive cells localize to the villus cytotrophoblasts and discrete populations of cells within the stroma in early stages of placenta development. Flow cytometry analysis of cells from the week 5 placenta shows a large $EpCAM^{+}/EGFR^{+}$ population where as EpCAM and CDCP1 show single positive populations only. By week 8 of gestation the CDCP1 population is near absent, while EGFR and EpCAM continue to be expressed. These populations have been isolated for for genome wide gene expression analysis and future work will include use of cell culture based differentiation to further characterize the cells and their developmental potential.

WIG-1, A P53 INDUCED GENE ESSENTIAL FOR EARLY EMBRYOGENESIS

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Wig-1, WT p53 induced gene-1, encodes an unusual zinc finger protein that binds double stranded RNA. Like HuR, Wig-1 has shown to bind to and regulate several ARE-containing mRNAs, such as p53, Fas, p21, N-Myc and c-Myc. One interesting story about Wig-1 is that we tried to generate Wig-1 knockout mice, but failed to generate any Wig-1 KO offsprings or embryos at any stages before blastocyst. As we know both N-Myc and c-Myc are essential for embryonic development and KO of N-/C-Myc are lethal to embryo. Since I just mentioned Wig-1 regulates both N-Myc and c-Myc (in cancer cells), we speculate that Wig-1 KO mice die before blastocyst stage, possibly due to the simultaneous dyregulation of both c- and N-Myc. Indeed, we studies if Wig-1 regulates N-Myc and c-Myc in mouse embryonic stem cell (ESC), we found that knockdown of Wig-1 in ESC leads to decreased expression of c-Myc and N-Myc. In addition, several microarray studies support a role for Wig-1 in germ cells and ESCs. In this project, we aim to investigate how Wig-1 regulates c-Myc and N-Myc or any other targets, which then leads to embryonic lethality.

PATHOLOGY OF PLACENTA IN FETAL AND NEONATAL IMMUNE THROMBOCYTOPENIA: ROLES OF TH17 IMMUNE RESPONSES AND ANGIOGENIC FACTORS

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The maternal alloimmune response to platelet alloantigens causes fetal/neonatal immune thrombocytopenia (FNAIT). Intracranial hemorrhage, severe bleeding diathesis, and miscarriages are clinical futures of FNAIT with poor outcome. Whether miscarriage in FNAIT is caused by placental pathology is poorly understood. Pregnancy success is critically dependent on the type of maternal immune response to fetal allogenic cells, since inflammation affects placenta angiogenesis and growth. We hypothesize that a maternal Th17 pro-inflammatory immune response to fetal platelet antigens may target trophoblast allogenic cells and impair placental function. Results: immunized mice experienced miscarriages occurring around embryo day 14, while no fetal death was reported in the naïve control group. The Th17 related cytokines such as IL-23 and MCP-1 increased in plasma of immunized mice compared to control. Angiogenic inhibitors are upregulated and Flt-1 overexpression in the decidua affect plasma PlGF/sFlT-1 ratio which significantly decreased resulting in poor angiogenic signalling. The end of organogenesis at embryo day 14 is concomitant to trophoblast invasion and hypervascularization of the placenta. Interesting the Doppler ultrasound clearly demonstrated impaired blood flow in the placenta. Furthermore, blood vessel density by isolectin IB_4 immunostaining and hematoxylin-eosin staining of paraffin embed section confirmed impaired vessel development and decreased number of red blood cells in the placenta of affected fetuses.

Interestingly, we also demonstrated that intravenous immunoglobulin (IVIG), which induced anti-inflammatory responses, ameliorated placental function and increased the number of live fetuses born from immunized mothers.

Conclusions: We observed that reduced placental angiogenesis in FNAIT impaired placental growth and blood perfusion leading to fetal loss. IVIG rescued placental pathology by inhibiting the Th17 pro-inflammatory response.

CHARACTERIZATION OF THE EFFECTS OF LITHIUM IN THE IRRADIATED DEVELOPING BRAIN

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Radiotherapy is common in the treatment of brain tumors in children, but often causes deleterious, late appearing sequelae, including cognitive decline. Lithium has shown neuroprotective effects in animal models of ischemia and irradiation (IR). Our aim then was to investigate the effects of lithium after irradiation of the young developing brain.

C57BL6/J female mice were irradiated with 8Gy at PND21 and fed 2.4% Li_2CO_3 chow for 4 weeks. We investigated lithium distribution in the brain at different time points using secondary ion mass spectrometry (SIMS), the effects of lithium on proliferation (Ki67) and neurogenesis (DCX). We measured serum lithium levels and body weight. In addition we irradiated *in vitro* young hippocampal neural stem cells and treated them with 1 and 3 mM LiCl and then investigated apoptosis (AnnexinV), cell cyle analysis (PI) and DNA damage (γH2AX) using fluorescence activated cell sorting (FACS).

We found that lithium accumulates mainly in neurogenic brain regions but 4 weeks of treatment was not sufficient to rescue proliferation and neurogenesis *in vivo*. Our *in vitro* study showed that 3mM lithium was able to rescue from IR the proliferating cells as well as reducing DNA damage. Apoptosis was not reduced by lithium.

IR effects on proliferation and neurogenesis were not rescued by lithium treatment *in vivo*. However our *in vitro* results suggest that a higher dose is needed to protect the proliferating cells from IR damage.

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Developmental and Perinatal Biology 2014

Course Evaluation

Please complete, remove and return the evaluation. This is **important** because it will help us in the design of future courses. Thank you.

Trainee from: (please tick) Karolinska Institute _____ University of Toronto _____

Lecture Course: (please circle one)

1) Stem Cells and Embryonic Development:

Overall	*could be improved*	1	2	3	4	5	*excellent*
a) Dr. J. Rossant		1	2	3	4	5	
b) Dr. P. Tonge		1	2	3	4	5	
c) Dr. D. Gallagher		1	2	3	4	5	
d) Dr. O. Hermanson		1	2	3	4	5	

2) Placenta & Birth:

Overall	*could be improved*	1	2	3	4	5	*excellent*
a) Dr. B. Cox		1	2	3	4	5	
b) Dr. S. Varmuza		1	2	3	4	5	
c) Dr. J. G. Sled		1	2	3	4	5	
d) Dr. K. Blomgren		1	2	3	4	5	

3) Cardiopulmonary Physiology:

Overall	*could be improved*	1	2	3	4	5	*excellent*
a) Dr. M. Post		1	2	3	4	5	
b) Dr. B. Yeganeh		1	2	3	4	5	
c) Dr. G. Montandon		1	2	3	4	5	
d) Dr. R. Jankov		1	2	3	4	5	

*4) **Neurodevelopment:***

Overall	*could be improved*	1	2	3	4	5	*excellent*
a) Dr. M. Sokolowski		1	2	3	4	5	
b) Dr. F. Barnabé-Heider		1	2	3	4	5	
c) Dr. P. McGowan		1	2	3	4	5	
d) Dr. L. Osborne		1	2	3	4	5	

*5) **Developmental Origins of Health and Disease:***

Overall	*could be improved*	1	2	3	4	5	*excellent*
a) Dr. G. Koren		1	2	3	4	5	
b) Dr. Z. Pausova		1	2	3	4	5	
c) Dr. R. Levitan	1	2	3	4	5		
d) Dr. S. Strom		1	2	3	4	5	

Please outline how you feel the lecture course could be improved:

Practical Courses:

Please indicate which 3 practical courses you attended and evaluate each below:

1. Physiology of the Pulmonary Circulation _____
2. Analysis of High-Throughput Data _____
3. Epigenetics _____
4. Somatic Cell Reprogramming _____
5. Advanced Molecular Techniques: Gene & Protein Expression Analysis _____
6. NICU Visit at Mount Sinai Hospital _____
7. Lab-on-a-Chip _____
8. Fetal Therapy Education _____

1st Course (Physiology of the Pulmonary Circulation)

Course Content:

could be improved 1 2 3 4 5 *excellent*

Course Organization:

1 2 3 4 5

2nd Course (Analysis of High-Throughput Data)

Course Content:

could be improved 1 2 3 4 5 *excellent*

Course Organization:

1 2 3 4 5

3rd Course (Epigenetics)

Course Content:

could be improved 1 2 3 4 5 *excellent*

Course Organization:

1 2 3 4 5

4th Course (Somatic Cell Reprogramming)

Course Content:

could be improved 1 2 3 4 5 *excellent*

Course Organization:

1 2 3 4 5

5th Course (Advanced Molecular Techniques: Gene & Protein Expression Analysis)

Course Content:

could be improved 1 2 3 4 5 *excellent*

Course Organization:

1 2 3 4 5

6th Course (NICU Visit at Mount Sinai Hospital)

Course Content:

could be improved 1 2 3 4 5 *excellent*

Course Organization:

1 2 3 4 5

7th Course (Lab-on-a-Chip)

Course Content:

could be improved 1 2 3 4 5 *excellent*

Course Organization:

1 2 3 4 5

8th Course (Fetal Therapy Education)

Course Content:

could be improved 1 2 3 4 5 *excellent*

Course Organization:

1 2 3 4 5

How could the practical courses be improved:

Social Program:

could be improved 1 2 3 4 5 *excellent*

Could the social program/accommodations be improved:

Overall Course:

could be improved 1 2 3 4 5 *excellent*

How do you think the course could be improved next year:

Thank You

NOTES