



VIVISECTION

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RESEARCH AND LEARNING
Sick Kids

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Part One: a sampling of SickKids research

Maternal-pup interaction disturbances induce long-lasting changes in the newborn rat pulmonary vasculature.¹

On fourteen neonatal mice, Gastric emptying is reduced in experimental NEC and correlates with the severity of intestinal damage.²

Different immune cells mediate mechanical pain hypersensitivity in male and female mice.³

There's a role for NT-3 in the hyperinnervation of neonatally wounded skin.⁴

We extended our observations to non-hibernating animals and demonstrated that SGK1-null mice developed muscle atrophy. These mice displayed an exaggerated response to immobilization and starvation.⁵

Sprague-Dawley female rats underwent partial bladder obstruction by ligation of a silk suture around the proximal urethra next to a 0.9-mm steel rod.⁶

¹ **Maternal-pup interaction disturbances induce long-lasting changes in the newborn rat pulmonary vasculature.** American Journal of Physiology: Lung Cellular and Molecular Physiology, 2015

² **Gastric emptying is reduced in experimental NEC and correlates with the severity of intestinal damage.** Journal of Pediatric Surgery, 2017

³ **Different immune cells mediate mechanical pain hypersensitivity in male and female mice.** Nature Neuroscience, 2015

⁴ **A role for NT-3 in the hyperinnervation of neonatally wounded skin.** Pain, 2012

⁵ **Maintaining skeletal muscle mass: lessons learned from hibernation.** Experimental Physiology, 2014

⁶ **DNA Methylation and the YAP/WWTR1 Pathway Prevent Pathologic Remodeling during Bladder Obstruction by Limiting Expression of BDNF.** American Journal of Pathology, 2018

Mice were housed under a 12:12-hour light-dark cycle and allowed standard laboratory chow and tap water *ad libitum*. The following mouse strains were used: *Ezh2^{fl/fl}*, and *Myh6-Cre*. Neonatal MI and apical resection were performed as previously described. Briefly, P1 or P7 pups were randomized and anaesthetized by hypothermia for 5 min prior to thoracotomy. To induce MI, the left anterior descending [LAD] coronary artery was ligated with a 6–0 non-absorbable polypropylene suture [Medtronic, Minneapolis, MN, USA]. For apical resection, the apex of the heart was resected using fine scissors. Mice were allowed to recover under a heat lamp before being returned to their mother. Mice were sacrificed 1 or 3 weeks after surgery by decapitation according to approved protocols and hearts were collected for analysis.⁷

The Animal Care Committee of the Hospital for Sick Children, Toronto, Canada gave their approval for all experiments, and we adhered to the Canadian Council on Animal Care guidelines for the care and use of animals. The animals used in our study were adult [aged nine to ten weeks], pathogen-free, drug- & test-naïve male Sprague-Dawley rats [Charles River Breeding Laboratories, St. Constance, QC, Canada] weighing 300-400 g. The animals were housed in rectangular polycarbonate cages [two animals per cage] in a temperature-controlled 12-hr light/dark cycle. They were provided with unlimited access to water and were fed a standard non-purified diet [Prolab RMH 1000 LabDiet, PMI Nutrition International, Brentwood, MO, USA]. After arrival at the animal lab facility, the animals were acclimatized for seven days before any experiments took place, and an animal laboratory technician assessed the animals daily. Experiments were carried out from 0800-1400 hr in the animal laboratory at the Hospital for Sick Children.

To determine the effect of acute intravenous L-carnitine administration on the threshold for bupivacaine-induced cardiotoxicity, we anesthetized and surgically prepared 20 rats as previously described.

The tail vein and carotid artery were cannulated using a 24G AngiocathTM catheter [Becton Dickinson, Franklin Lakes, NJ, USA].

⁷ **Ezh2 is not required for cardiac regeneration in neonatal mice.** PLoS One, 2018

Electrocardiography, arterial blood pressure, and rectal temperature were monitored continuously.

Bupivacaine 0.5% [Astra Zeneca, Mississauga, ON, Canada] was then infused intravenously at a rate of $2\text{mg_kg}^{-1}\text{min}^{-1}$ using an AlarisTM Syringe Module calibrated infusion pump [Cardinal Health, Vaughan, ON, Canada] until asystole, which was defined as the absence of electrocardiograph activity for ten seconds after the last systole. At this point, arterial blood was sampled to determine plasma bupivacaine and serum L-carnitine concentration. The primary outcome measure was the probability of survival, with asystole being the event of interest.⁸

In two groups of rats, the tibial nerve was transected and immediately repaired. Gastrocnemius muscles were implanted with intramuscular electrodes for sham or muscle stimulation. Muscles were stimulated daily, eliciting 600 contractions for one hour/day, repeated five days per week.⁹

Neonatal Yorkshire piglets underwent sham surgical banding [sham, n = 6], staged bilateral pulmonary vein banding of all pulmonary veins except the right middle pulmonary vein [banded, n = 6], and staged pulmonary vein banding with losartan treatment [losartan, 1 mg/kg/d, n = 7]. After 7 weeks, the hemodynamic data were obtained and the piglets killed.¹⁰

In this study, helper-dependent adenoviral [HD-Ad] vectors were delivered to mouse and pig airways via intranasal delivery, and direct bronchoscopic instillation, respectively. Vector transduction was assessed by immunostaining of lung tissue sections, which revealed that airway basal cells of mice and pigs can be targeted in vivo.¹¹

⁸ **L-carnitine reduces susceptibility to bupivacaine-induced cardiotoxicity: an experimental study in rats.** Canadian Journal of Anesthesia, 2017

⁹ **Electrical muscle stimulation elevates intramuscular BDNF and GDNF mRNA following peripheral nerve injury and repair in rats.** Neuroscience, 2016

¹⁰ **Losartan ameliorates "upstream" pulmonary vein vasculopathy in a piglet model of pulmonary vein stenosis.** The Journal of Thoracic Cardiovascular Surgery, 2014

¹¹ **Transducing Airway Basal Cells with a Helper-Dependent Adenoviral Vector for Lung Gene Therapy.** Human Gene Therapy, 2018

Rabbit knees are useful models of human arthritis, because they get arthritis when we antigen-induce it in them.¹²

We developed an optogenetic strategy that allowed us to permanently express channelrhodopsin-2 [ChR2] in neuronal ensembles that were activated during contextual fear encoding in infant mice.¹³ We interrogate a Brain-wide Fear Memory Network in Mice.¹⁴

We find that contextual fear conditioning is modulated by shock intensity, prevented by an established amnestic agent [MK-801], lasts at least 14 days, and exhibits extinction. Furthermore, fish of various background strains [AB, Tu, and TL] are able to acquire fear conditioning, but differ in fear extinction rates. Taken together, we find that contextual fear conditioning in zebrafish shares many similarities with the widely used contextual fear conditioning paradigm in rodents. Combined with the amenability of genetic manipulation in zebrafish, we anticipate that our paradigm will prove to be a useful complementary system in which to examine the molecular basis of vertebrate learning and memory.¹⁵

Mice were trained with a single foot shock [0.4 mA, Training], and 24 hrs later tested [Test 1]. Twenty-four hrs after the Test 1, the mice received systemic injections of MEM or vehicle [VEH] once a week for four weeks [MEM-4 or VEH group]. Another group received an injection of MEM only 24 hrs after Test 1 [MEM-1 group]. Contextual fear memory was assessed again four weeks after initial training [Test 2]. All groups displayed comparable and high freezing response levels during Test 1. In contrast, the MEM-1 and -4 groups showed reduced freezing compared to the VEH group in Test 2, although this reduction was only statistically significant in the MEM-4 group. These observations were consistent

¹² **Correlative BOLD MR imaging of stages of synovitis in a rabbit model of antigen-induced arthritis.** Pediatric Radiology, 2012

¹³ **Recovery of "Lost" Infant Memories in Mice.** Current Biology, 2018

¹⁴ **Chemogenetic Interrogation of a Brain-wide Fear Memory Network in Mice.** Neuron, 2017

¹⁵ **Contextual fear conditioning in zebrafish.** Learning & Memory, 2017

with previous findings, and indicated that post-training MEM treatment enhanced forgetting in a dose-dependent manner.¹⁶

We asked if two fear-conditioning events that occur closely in time are coallocated to overlapping populations of neurons, thereby functionally linking these memories. Mice received two events [event 1, event 2] featuring distinct auditory conditioned stimuli separated by varying inter-training intervals. Event 2 [CS2+footshock] was the same across groups, but event 1 content and timing differed.¹⁷

To probe for specific and redundant functions of vertebrate Itsn genes, we generated Itsn1, Itsn2, and double-mutant mice. While invertebrate mutants showed severe synaptic abnormalities, basal synaptic transmission and plasticity were unaffected at Schaffer CA1 synapses in mutant mice. Itsn1 mutant mice showed severe deficits in Morris water maze and contextual fear memory tasks, whereas mice lacking Itsn2 showed normal learning and memory.¹⁸

We're keen on investigation using techniques of isolated [Langendorff] rabbit heart, and immunized mouse models with fetal mice¹⁹

We have worked on several animal models of deafness including Meniere's disease, auditory neuropathy, ototoxic drug induced hearing loss, acoustic trauma, age-related hearing loss and cytomegalovirus [CMV] ear infection²⁰

¹⁶ **Hippocampal neurogenesis enhancers promote forgetting of remote fear memory after hippocampal reactivation by retrieval.** Elife, 2016

¹⁷ **Competition between engrams influences fear memory formation and recall.** Science, 2016

¹⁸ **Vertebrate intersectin1 is repurposed to facilitate cortical midline connectivity and higher order cognition.** Journal of Neuroscience, 2013

¹⁹ www.sickkids.ca/AboutSickKids/Directory/People/H/Robert-Hamilton.html

²⁰ www.sickkids.ca/AboutSickKids/Directory/People/H/Robert-Harrison.html

Here, we ask whether an enhanced neonatal acoustic environment can induce midbrain changes. Neonatal chinchillas were chronically exposed to a 70 dB S narrowband [2]±0.25 kHz stimulus for 4 weeks. In sound-exposed subjects, we find no change in the width of the 2 kHz hypothesis is not supported.²¹

ADA^{-/-} [FVB, 129-Adatm1Mw-TgN[PLADA]] mice and ADA^{+/-} littermate control mice were maintained in a pathogen-free environment, as previously described. ADA activity was assessed in fresh blood samples obtained from the tails of 7 days pp mice by conversion of [14C] Ado [Moravek biochemical, Brea, CA, USA] to inosine, followed by TLC separation, as previously reported. All animal procedures were approved by the Institute's Animal Care Committee and performed in accordance with the Canadian Council for Animal Care guidelines.

Mice received ERT with PEG-ADA [ADAGEN®; ENZON Pharmaceuticals Inc., Piscataway, NJ, USA, Sigma-Tau Pharmaceuticals, Inc.], or the equivalent volume of PBS ["untreated"] using 2 regimens.

Some ADA^{-/-} and ADA^{+/-} mice were transferred at 7 days pp to Oxycycler Exposure Chambers [Biospherix Ltd, Lacona, NY, USA] equipped with ProOx 110 controller [Biospherix], which provided 40% oxygen, as previously described. Oxygen concentrations were monitored continuously and maintained in the chamber at all times. The chambers were regulated with a 12 h light-dark cycle. Food and water were supplied *ad libitum*. Litter sizes were kept at no more than 6 pups in both the hyperoxia and room air groups. Oxygen saturation in the arterial blood of mice was analyzed using the ABL800 FLEX blood gas analyzer [Radiometer, Copenhagen, Denmark].

ABR recording was performed as described previously. Briefly, mice were anesthetized with i.p. injection of ketamine [100 mg/kg] and xylazine [10 mg/kg]. ABR was recorded with needle skin electrodes in a vertex/mastoid configuration. Signals were amplified [x1000], filtered [0.1–3.0 kHz] and averaged [512 sweeps] [Intelligent Hearing Systems Miami, FL, USA]. ABR in response to short gated tones at 8, 16,

²¹ **Changes to Neural Activation Patterns [c-fos Labeling] in Chinchilla Auditory Midbrain following Neonatal Exposure to an Enhanced Sound Environment.** Neural Plasticity, 2018

and 32 kHz was measured in each ear. Stimuli were presented using a high-frequency transducer connected in a closed system to the external ear canal. Stimuli were presented in the range of 90–0 dB sound pressure levels in 5 or 10 dB intensity steps.

Scanning electron microscopy [SEM] of the cochlea was carried out with the following steps: inner ear dissection, tissue fixation in 2.5% glutaraldehyde, specimen dehydration, cochlear micro-dissection to reveal the sensory epithelium, critical point drying, gold sputter coating, and scanning using the Hitachi S3400 electron microscope. The morphology of the sensory epithelium of the organ of Corti, particularly the integrity of hair cells in basal, middle, and apical cochlear turns was evaluated.²²

Cytomegalovirus [CMV] infection is one of the most common causes of congenital hearing loss in children. We have used a murine model of CMV infection to reveal functional and structural cochlear pathogenesis. The cerebral cortex of Balb/c mice [Mus musculus] was inoculated with 2000 pfu [plaque forming units] of murine CMV on postnatal day 3.²³

We examined Long Term Depression at the CA1 synapse in GluA2 KO mice by using several well-established inhibitory peptides known to block LTD in Wild Type rodents.²⁴

The standard mouse model of Down Syndrome is the Ts65Dn mutant mouse [Ts]. Using the Ts mouse, we have created an animal model of Infantile Spasms in Down Syndrome.

²² **Early Enzyme Replacement Therapy Improves Hearing and Immune Defects in Adenosine Deaminase Deficient-Mice.** *Frontiers in Immunology*, 2019

²³ **Cytomegalovirus [CMV] Infection Causes Degeneration of Cochlear Vasculature and Hearing Loss in a Mouse Model.** *Journal of the Association for Research in Otolaryngology*, 2017

²⁴ **Hippocampal Long-Term Depression in the Presence of Calcium-Permeable AMPA Receptors.** *Front Synaptic Neuroscience*, 2018

We used *Kcnj6* triploid mice, and compared the number of spasms via video analysis and EDR events via EEG to that of the Wild Type mice. Frozen embryos of *Kcnj6* trisomic mice were obtained from the European Mouse Mutant Archive, Germany.

The embryos were implanted in surrogate FVB mothers. The FVB male offspring were mated with C57BL/6 μ nter to mitigate the recessive mutation of retinal degeneration seen in this strain. Both F1 males and females were used in this study. Mice were group housed [two to five mice per cage] on a 12-h light/dark cycle in an environment with controlled temperature [22 μ nter] °C humidity, with food and water *ad-libitum*.

Briefly, the mouse brains were quickly removed and sagittal 350 μ m slices were prepared.

Five to twelve-week-old *Kcnj6* Triploid mice and WT mice were sacrificed, and their brains were immediately removed from the skull. The cortex and thalamus were then dissected on ice. The samples were then homogenized in 2X RIPA lysis buffer using a bioruptor [Bioruptor™ UCD-200, Diagenode, Denville, NJ].

The drugs used were the same as indicated in Joshi et al, 2016. Gamma-butyrolactone [GBL] was obtained from Sigma-Aldrich, Co [St Louis, MO]. Ketamine was obtained from Bioniche [Bellville, ON]. Xylazine was obtained from Bayer Inc [Toronto, ON]. Anafen was obtained from Merial Canada Inc. All drugs were of the highest available purification. NBQX [HB0442] and D-AP5 [HB0225] were obtained from Hello Bio, Princeton, NJ and Picrotoxin [Cat # 1128] was obtained from Tocris Bioscience, Minneapolis, MN.

Behavioral extensor spasms were defined as bilateral simultaneous extension of the hindlimbs and forelimbs. Four to seven week-old mice were held gently by the back of the neck and suspended in the vertical position. Mice were injected with 100 μ g/

GBL-induced spasms were quantified by visual inspection. For the visual analysis, the forelimb and hindlimb movements were recorded by a video camera for a

duration of five minutes in three different interval periods: Min 20–25, Min 40–45 and Min 60–65, done as previously described.²⁵

Genetic manipulations in mice provide additional opportunities to understand the neurobiological mechanisms of infantile amnesia. Therefore, the primary goal of the current study was to characterize forgetting in infant mice in another hippocampus-dependent learning paradigm. Using a hidden platform version of the water maze task, we first assessed retention in mice that ranged in age from 15 to 150 days-old at the beginning of training. All groups exhibited spatial memory when tested one day after training. However, only mice that were 20 days or older at the time of training could remember one month later.

Mice were a cross between C57BL/6 [paternal] and 129Svev [maternal] strains [Taconic], which were bred in the Hospital for Sick Children animal facility. The day of birth was designated P0, and litter sizes ranged from 4 to 9 pups. After weaning [P21], mice were group-housed according to sex [2–5 per cage]. To control for potential litter-dependent effects on memory, each litter was split across experiments such that no more than 3 mice per litter was included in a single experimental condition. Females and males were assigned evenly across experimental conditions. All procedures were approved by the Animal Care Committee at The Hospital for Sick Children and Use Committee policies and conformed to both the Canadian Council on Animal Care [CCAC] and National Institutes of Health [NIH] Guidelines on the Care and Use of Laboratory Animals.

Basic training and test probes: Mice were trained in the hidden platform version of the water maze. A circular pool [120cm diameter, 50cm height] was filled with water [28°C] to a depth of 40cm. Water was made opaque by the addition of nontoxic paint. A circular escape platform [10cm diameter] was submerged approximately 0.5cm below the surface of the water in the centre of one of the pool quadrants. The pool was surrounded by a curtain painted with five large, distinct geometric shapes located 1-1.5m from the pool wall. In most experiments, mice received six training trials per day for three consecutive days. The trial ended when the mouse reached the hidden escape platform or after 60s had elapsed. If

²⁵ **Kcnj6(GIRK2) trisomy is not sufficient for conferring the susceptibility to infantile spasms seen in the Ts65Dn mouse model of Down syndrome.** Epilepsy Research, 2018

the mouse failed to locate the hidden platform, the experimenter's hand was placed over the platform [to serve as a visual cue] and the mouse was given an additional 15s to find the platform. If the mouse failed to do so, it was gently guided to the platform. The mouse stayed on the platform for 15s after which it was placed on a heated blanket for an additional 15s [total inter-trial interval of approximately 30s].

Memory was tested using a probe test. During the probe test, the escape platform was removed from the water and the mouse was allowed to swim freely for 60s. The mouse's behavior in the pool was recorded by an overhead video camera and tracked using automated software [Watermaze 3.0, Actimetrics]. During training, we analyzed escape latency, distance travelled, and swim speed. In the probe test, we quantified spatial memory by measuring amount of time mice spent searching in the target zone [20cm radius, corresponding to 11% of pool surface] versus average time spent in three other equivalent zones in other areas of pool²⁶

Justice is a pioneer in the field of mouse mutagenesis. Her research exploits that genes and whole chromosome regions are conserved between the mouse and human. Overall, her research aims to merge mouse modeling with clinical genetics to understand the basis for human diseases and to use mouse models to ameliorate disease states. Her internationally recognized program has produced hundreds of new mouse models of human disease, which have allowed for discoveries of gene functions in diverse areas such as cancer, reproduction, neurobiology, obesity and blood, heart and bone development²⁷

Twelve pigs were included in this study. Three control pigs were perfused with intravascular methyl methacrylate, and overlying tissue was corroded with potassium hydroxide and hydrochloric acid, leaving only a cast of vessels. Nine pigs underwent anterior costal cartilage graft laryngotracheoplasty and were survived

²⁶ **Age-dependent changes in spatial memory retention and flexibility in mice.** Neurobiology of Learning and Memory, 2017

²⁷ www.sickkids.ca/AboutSickKids/Directory/People/J/Monica-J-Justice-staff-profile.html

for various lengths of time [3 for 48 hours, 3 for 10 days, 3 for 3 weeks] prior to corrosion casting.²⁸

A randomized laboratory study was performed in anesthetized pigs. Lung injury was induced by surfactant lavage followed by 1 mechanical ventilation. Randomization [five pigs in each group] was to positive end-expiratory pressure [PEEP] alone or PEEP with continuous negative abdominal pressure [-5 cm H₂O] followed by 4 h of electrical impedance tomography were recorded, and injury determined by lung wet/dry weight ratio and interleukin-6 expression.²⁹

The original Webb and Tierney results were replicated in terms of lung/body weight ratio [45/0] and histology. In 45/0, P < pulmonary edema was overt and rapid, with survival less than 30 minutes. In 45/0 [but not 45/10], there was an increase in microvascular permeability, cyclical abolition of preload, and progressive dilation of the right ventricle.³⁰

To understand the biology behind autism - and to ask how many autisms are there - Dr. Lerch and his team set out to run a high throughput screen by using MRI to image as many mouse models as they could. With over 70 mouse models [and over 2000 individual mice] they find that heterogeneity is also the order of the day, yet a few brain areas are more consistently affected than others.³¹

We previously demonstrated that *Endoglin* heterozygous [*Eng*^{+/-}] mice subjected to dextran sulfate sodium [DSS] developed persistent gut inflammation and

²⁸ **Angiogenesis in costal cartilage graft laryngotracheoplasty: a corrosion casting study in piglets.**

Laryngoscope, 2014

²⁹ **Continuous Negative Abdominal Pressure Reduces Ventilator-induced Lung Injury in a Porcine Model.**

Anesthesiology, 2018

³⁰ **Adverse Heart-Lung Interactions in Ventilator-induced Lung Injury.** American Journal of Respiratory and Critical Care Medicine, 2017

³¹ www.sickkids.ca/AboutSickKids/Directory/People/L/jason-lerch-staff-profile.html

pathological angiogenesis. This time, mice drank water supplemented with 3% DSS [DSS, m.w. 36,000–50,000; MP Biomedicals, Solon, OH] for 5 days and were then returned to normal water. Body weight, water intake, activity, and diarrhea scores were measured daily for up to 23 days. At time of sacrifice, mice were anesthetized with ketamine [100 mg/kg intraperitoneally, i.p.] and perfused with phosphate-buffered saline through the left ventricle prior to organ harvesting. For gross histology, the distal colon was fixed in 4% paraformaldehyde, embedded in paraffin, sectioned longitudinally, and stained with hematoxylin and eosin. Representative images were obtained using the Olympus BX60 microscope [Center Valley, PA] at 100x magnification.³²

Pups received daily intraperitoneal bleomycin or saline from Postnatal Days 1 through 14 with or without Y-27632, a ROCK inhibitor. Bleomycin-induced lung injury is characterized in the neonatal rat by inflammation dominated by neutrophils and macrophages, inhibited distal airway and vascular development, and pulmonary hypertension, similar to human infants with severe bronchopulmonary dysplasia.³³

The high degree of similarity between the genes of humans and mice, the similar biology of these two species, and the ease with which the mouse genome can be experimentally manipulated, make the mouse an ideal model organism to study the genetics and biology of human disease.³⁴

Therefore, we examined *Lfng* expression by performing X-gal staining on mammary glands from six-week-old *Lfng*^{LacZ/+} virgin females. This result is consistent with studies on cells purified from the human mammary gland.³⁵

³² **Impaired resolution of inflammation in the Endoglin heterozygous mouse model of chronic colitis.** Mediators of Inflammation, 2014

³³ **Rho-kinase inhibitor prevents bleomycin-induced injury in neonatal rats independent of effects on lung inflammation.** American Journal of Respiratory Cell and Molecular Biology, 2014

³⁴ www.sickkids.ca/AboutSickKids/Directory/People/M/Colin-McKerlie.html

³⁵ **Lunatic Fringe Deficiency Cooperates with the Met/Caveolin Gene Amplicon to Induce Basal-like Breast Cancer.** Cancer Cell, 2012

Infected mice were treated with either water [control] or NEN via oral gavage 4 h post spore challenge and for 3 consecutive days after spore challenge. Typical symptoms of CDI in murine models include severe weight loss on days 2 and 3 post-challenge accompanied with diarrhea and high mortality rate in sham groups. All doses of NEN tested significantly protected mice from weight loss compared to control group. NEN protected mice from death in a dose-dependent manner, with all mice in the 50 mg p only 45% for control group. These results closely tracked the wet tail and diarrhea scores, which were significantly lower in NEN-treated groups.³⁶

Mice on a mixed C57BL/6J and 129 background bearing the *Trp53^{tm1Brn}* targeted mutation [*p53^{fl/fl}*] were a gift from Dr Benjamin Alman's laboratory. Mice expressing Cre under control of the human-GFAP promoter were acquired from the Jackson Laboratory [*FVB-Tg(GFAP-cre)25Mes/J*, stock #4600].

Cranial irradiation leads to a cascade of consequences affecting brain homeostasis: blood-brain barrier disruption, cell death, chronic inflammation, demyelination, endothelial cell dysfunction, and impaired neurogenesis. p53 Knock-Out mice and p53 Wild-Type mice were irradiated with a whole-brain dose of 7 Gy [n = 30] or 0 Gy [n = 28] at 16 days of age. Alterations in neuroanatomy were detectable in WT mice and emerged through 2 stages: an early volume loss within 1 week and decreased growth through development.

Perfusion fixation was performed immediately after the final in vivo scan, as previously described. Briefly, this procedure consists of transcardiac perfusion with a phosphate-buffered saline [PBS], heparin, and ProHance [gadoteridol, Bracco Diagnostics, Princeton, NJ] solution followed by a PBS, Prohance, and methanol-free formaldehyde solution. Brain samples within the skull were soaked in the second solution for 24 hours before being transferred to a storage solution of PBS, ProHance, and NaN³. To acquire structural ex vivo scans, brain samples within the skull were imaged using a T2-weighted 3-dimensional fast spin echo sequence.³⁷

³⁶ **Host-targeted niclosamide inhibits *C. difficile* virulence and prevents disease in mice without disrupting the gut microbiota.** Nature Communications, 2018

³⁷ **Loss Mitigates Early Volume Deficits in the Brains of Irradiated Young Mice.** International Journal of Radiation Oncology: Biology, Physics, 2019

Neither route of delivery nor MnCl₂ dose adversely affected spatial learning and memory on the water maze. However, especially at higher doses, mice receiving MnCl₂ via osmotic pumps developed skin ulceration which limited the imaging window.³⁸

We've confirmed White and Gray Matter abnormalities after cranial radiation in children and mice. Nineteen radiation-treated patients were divided into groups of 7 years of age and younger and 8 years and older. C57BL6 mice were treated with radiation [n=52] or sham treated [n=52] between postnatal days 16 and 36 and then assessed with in vivo and/or ex vivo MRI. In both cases, measurements of WM and GM volume, cortical thickness, area and volume, and hippocampal volume were compared between groups.³⁹

Adult mice were subjected to spared nerve injury (SNI), which elicited persistent pain hypersensitivity as determined by reduction in their threshold for paw withdrawal. In the *pièce de résistance*, we investigated the role of SOM interneurons in the development of neuropathic pain by daily CNO injections in SOM hM3dq DREADD mice. DREADD mice have Designer Receptors Activated by Designer Drugs. Remarkably, those DREADD mice who received our Designer Drugs did not develop pain hypersensitivity over the entire 28-day observation period following SNI.

The Author declares no competing financial interests.⁴⁰ To facilitate the translation of his fundamental studies to the development of therapies, the Author is founding scientist and active in two startups [NoNO Inc and Afference Therapeutics].⁴¹ The Author was appointed Chief of Research in 2015 from his position as Associate Chief of Science Strategy and Commercialization. The Author is a Pfizer-awarded Pain Researcher, has been a Pfizer Travelling Fellow, and has recently mentored a Purdue-funded graduate student. The author holds 9 patents

³⁸ **Continuous manganese delivery via osmotic pumps for manganese-enhanced mouse MRI does not impair spatial learning but leads to skin ulceration.** NeuroImage, 2018

³⁹ **White and Gray Matter Abnormalities After Cranial Radiation in Children and Mice.** International Journal of Radiation Oncology: Biology, Physics, 2015

⁴⁰ **VIP cortical conductors set the tone for chronic pain.** Nature Neuroscience, 2017

⁴¹ www.sickkids.ca/AboutSickKids/Directory/People/S/Michael-Salter.html

and has consulted for Endo Pharmaceuticals, Pfizer, Avanir Pharmaceuticals, Abbott Pharmaceuticals, and PureTech Ventures.⁴²

Recently, Dr. Palaniyar was on a 6-month sabbatical at Shriners hospitals for Children [Boston] to develop a pulmonary NETosis model to study the effect of burn injury on immune suppression in the lungs. The current proposal focuses on this mouse model to further understand the effect of burn injury in pulmonary immunosuppression leading to sepsis.⁴³

Conflict of interest: L. Caldarone has nothing to disclose. Conflict of interest: A. Mariscal has nothing to disclose. Conflict of interest: S. Keshavjee is the co-founder and Chief Scientific Officer of Perfusix Canada Inc. and XOR Labs Toronto Inc. S. Keshavjee has received the following, outside the submitted work: grants from XVIVO Perfusion for research and providing clinical study support for other studies; and personal fees for acting as a consultant for Lung Bioengineering, United Therapeutics.⁴⁴

4CG pups were bilaterally gonadectomized at weaning [postnatal day 21] under isoflurane anaesthesia [1–4%]. Ovariectomies were performed via dorsal incisions. The ovaries were removed after cauterization of each uterine horn below the ovary and fallopian tubes. Male castrations were performed via an abdominal incision. Each testicle was removed after cauterization of the vas deferens. The inner skin layer was closed using absorbable sutures and the outer skin layer was closed with staples. After the surgery the mice were housed individually for one week to allow wound healing. 2 saline was injected subcutaneously on the day of surgery and for two additional days for analgesia and hydration.

The 8-arm radial arm maze [RAM] [MedAssociates, St. Albans, VT] consists of 8 identical, clear, plexiglass arms radiating from a central octagonal platform. Automatic pellet

⁴² Michael W. Salter, CV [Feb 10 2016]

⁴³ www.sickkids.ca/AboutSickKids/Directory/People/P/Nades-Palaniyar.html

⁴⁴ **Neutrophil extracellular traps in ex vivo lung perfusion perfusate predict the clinical outcome of lung transplant recipients.** European Respiratory Journal, 2019

dispensers were placed at the end of each arm, which were blocked with a clear plastic door. Noldus Ethovision software was used to track the mouse's movements and to automatically control access to the arms and release of food reward from the pellet dispensers.

Mice were trained for 10 trials per day for 5 days. In each trial, an individual mouse was placed in the centre of the maze with all arms closed. After a delay of 5 s, the doors to all arms opened, and the mouse was given 8 min to explore. Correct visits were defined as a mouse's first visit to a baited target arm. Once the mouse had visited all 4 baited arms or after 8 min, the mouse was returned to its home cage. After each trial, the maze was cleaned with Clidox to eliminate olfactory cues.

Mice were perfusion-fixed on postnatal day 65 as previously described. Briefly, mice were placed in a supine position and perfused via the left cardiac ventricle using 30 ml of phosphate buffered saline (PBS) [pH 7.4], 2 ml of ProHance[®] [gadoteridol, Bracco Diagnostics Inc., Princeton, NJ], and 1 ml of heparin [1000 USP units/ml] [Sandoz Canada Inc., Boucherville, QC] at room temperature [25 °C] at a rate of approximately 60 µl/h. After 4 min of perfusion, 4% paraformaldehyde (PFA) was subsequently infused at the same rate. After fixation, the heads were removed from the bodies, along with the skin, ears, and lower jaw. The skull was allowed to postfix in 4% PFA at 4 °C for 2 h in a solution of PBS, 2 ml of ProHance[®] [gadoteridol, Bracco Diagnostics Inc., Princeton, NJ], and 0.02% sodium azide [Fisher Scientific, Nepean, ON] until imaged 3–4 weeks postmortem.⁴⁵

Our ultimate goal is to uncover how the nervous system processes pain-related sensory information and how chronic pain arises from aberrations in that processing. Welcome new lab members! Welcome to Ket & Saikata, two new postdocs in the lab. Check out the People page to see what they will be working on.⁴⁶

⁴⁵ Impact of X/Y genes and sex hormones on mouse neuroanatomy. NeuroImage, 2018

⁴⁶ <http://www.prescottlab.ca>

Two lionfish were used for venom extraction. Briefly, the fish was euthanized by anesthetic overdose and venom extracted according to methods described below. TRPV1 knockout [KO] mice were obtained from Jackson Laboratories [Bar Harbour, ME, stock # 003770]. Male 8 to 12 weeks old C57BL/6 mice purchased from The Jackson Laboratory were used for all experiments except for some in vitro calcium imaging and electrophysiology. On the day of experiments, animals were transferred into the testing room from the animal facility and allowed to acclimate to the room in their cage for at least 45 minutes.

Mice were placed in a Plexiglas container on a glass surface for 15 minutes and recorded with a video camera to obtain a baseline of their grooming activity. They were then injected with 20 μ L of either saline, 44 μ g of venom, 4.4 μ g of venom, 0.44 μ g of venom, 0.044 μ g of venom, or 0.0044 μ g of venom into the intraplantar region of their left hind paw. Immediately after the injection, they were returned to their containers and filmed for 1 hour to measure their spontaneous pain behavior. Their spontaneous pain behavior was quantified as the amount of time the animal spent licking its left hind paw [injected paw], recorded in 5-minute bins for total of 12 bins over 1 hour. Punctate mechanosensitivity was measured as mechanical paw withdrawal thresholds, using von Frey monofilaments [0.04, 0.07, 0.16, 0.4, 0.6, 1, 1.4, 2, and 4 g] applied to the plantar surface of each hind paw. Monofilaments were applied 6 times to the plantar surface of each hind paw at 10-second intervals starting with the lowest force filament using the up-down method.

Animals were placed on a glass surface in Plexiglas containers. A thermal stimulus was pointed at the plantar surface of each hind paw through the glass using a Hargreaves apparatus [Stoelting, Wood Dale, IL], until the animal lifted its paw away from the heat source. The latency to their paw withdrawal was automatically recorded with the apparatus to the nearest 0.1 seconds; a cutoff latency of 20 seconds was used to avoid tissue damage. This was performed 5 times per paw with 10 minutes between each application; mean values per paw were calculated and used for statistical analysis.

Mice were placed on a glass surface [thickness: 6 mm] in Plexiglas containers; cold was applied by pressing a 10-mL syringe filled with ice against the glass directly under the plantar surface of each hind paw. The ice pellet was replaced between

each trial to compensate for the melting of the ice. Tests were performed on 3 separate days before venom injection to extract a preinjection baseline value, and then 2 hours, 1, 3, 5, 7, 10, and 14 days after injection.

Forty-four microgram doses of venom were boiled in a 100°C water bath for 1 hour before being injected into the intraplantar region of the left hind paw of the mice. Injections were followed by 1-hour video recordings to quantify their spontaneous pain behavior in 5-minute bins.

Mice were deeply anesthetized with ketamine and xylazine before being transcardially perfused with 10 mL of phosphate-buffered saline [PBS] followed by 25 to 30 mL of 4% paraformaldehyde 90 minutes after receiving an intraplantar injection of either a dose of venom [44, 4.4, 0.44, 0.044, 0.0044 mg] or saline. Their spinal cords were dissected and post-fixed in 4% paraformaldehyde for 1 hour, followed by cryoprotection in PBS containing 30% sucrose for 72 hours. The spinal cords were then embedded in optimal cutting temperature compound and cut into 25-mm transverse sections with a cryostat [Thermo Scientific, Waltham, MA, Microm HM525 NX Cryostat].⁴⁷

Three-dimensional reconstruction created by MRI imaging of the WT and mutant kidneys demonstrates an expanded renal pelvis and no pelvis exit. Measurement of the pelvis size demonstrates a nine-fold increase in mutants [$n=10$ mutants and six WT; $P<0.05$] [I]. [J and K] Dye injected to the renal pelvis of the left control kidney passes through the ureter and accumulates in the bladder, but it does not traverse the UPJ [arrowhead] and accumulates in the pelvis in the mutants.⁴⁸

Our primary objective was to determine whether the use of MRgFUS in this eloquent brain region could be performed without histological injury and functional deficits. Physiological assessment was performed by monitoring of heart

⁴⁷ **Lionfish venom elicits pain predominantly through the activation of nonpeptidergic nociceptors.** Pain, 2018

⁴⁸ **Activated Hedgehog-Gli Signaling Causes Congenital Ureteropelvic Junction Obstruction.** Journal of the American Society of Nephrology, 2018

and respiratory rates. Motor function and co-ordination were evaluated by Rotarod and grip strength testing.⁴⁹

The small size of the mouse placenta enables ex vivo three-dimensional imaging of the entire, intact uteroplacental tree via microcomputed tomography [micro-CT]. CD-1 mice were purchased from Charles River Laboratories. Images of the nonpregnant uterine vasculature were obtained from 8- to 10-wk-old virgin females in estrus, which was initiated via priming the cage with male urine and confirmed through vaginal appearance. Males were mated in-house with 8- to 14-wk-old virgin females, and the morning that a vaginal copulation plug was detected was designated Embryonic Day 0.5 [E0.5]. Uteroplacental vasculature was studied at E5.5, E8.5, E9.5, E11.5, E13.5, E15.5, and E17.5 of gestation. Infusion was stopped when the bright yellow color of the contrast agent was seen entering the uterine microvasculature [in nonpregnant females] or entering the uteroplacental microvasculature of the exposed pregnant uterus, thus generating samples in which only the arterial vasculature and microvessels contained the contrast agent. After tying off the inferior vena cava, the system was pressurized to 20 mm Hg [i.e., microvascular pressure] while the compound polymerized. The uterus was then removed and immersed in formalin.

Our study reveals some strong similarities between mouse and human uteroplacental arterial expansion and the resulting fall in vascular resistance. Both species deliver blood into relatively open blood spaces in the exchange region via highly coiled spiral arteries and funnel-like channels, anatomy that is characteristic of the hemochorial placenta. Indeed, trophoblast-lined, distal dilations of the spiral arteries in humans augment the diameter by 5- to 6-fold in a seemingly analogous way to the trophoblast-lined, funnel-like maternal canals and canal branches of the mouse, which we show similarly augment the terminal diameter of vessels feeding the exchange region by 4.5-fold. In addition to these similarities in vascular expansion, the calculated 47% decrease in vascular resistance in the mouse from E9.5 to E17.5 is strikingly similar to the 50% decrease in vascular resistance in the human uterine arterial circulation from mid gestation to term. Similarities in uteroplacental structure, growth, and resistance in mice and humans during

⁴⁹ **Brainstem blood brain barrier disruption using focused ultrasound: A demonstration of feasibility and enhanced doxorubicin delivery.** *Journal of Controlled Release*, 2018

normal pregnancy supports the use of mouse models to discover mechanisms controlling normal uteroplacental vascular expansion and why it fails in some pathological human pregnancies.⁵⁰

A substantial proportion of pregnancies in Africa are at risk of malaria in pregnancy [MIP] however the impact of *in utero* exposure to MIP on fetal neurodevelopment is unknown. We show that malaria-exposed offspring have persistent neurocognitive deficits in memory and affective-like behaviour compared to unexposed controls.

Female BALB/c mice [wild-type or *C5ar*^{-/-}] between 6–8 weeks of age were mated with male BALB/c [wild-type or *C5ar*^{-/-}] mice [8–9 weeks] obtained from Jackson Laboratories [Bar Harbor, ME]. *C5ar*^{-/-} females were mated with *C5ar*^{-/-} males, therefore all offspring were also *C5ar*^{-/-}. Naturally mated pregnant mice were infected on G13 with 10⁵ *P. berghei* ANKA-infected erythrocytes via injection into the lateral tail vein. A lower dose of inoculum was used in this study to eliminate a low birth weight phenotype and increase the number of live births. Control pregnant females were injected on G13 with RPMI media alone. Thin blood smears were taken daily and stained with Giemsa stain [Protocol Hema3 Stain Set, Sigma, Oakville, ON] to monitor parasitemia. For pharmacological blockade experiments polyclonal rabbit antiserum raised against rat C5a or pre-immune control rabbit antiserum [Sigma G9023] was administered via tail vein injection 2 hours prior to malaria infection [0.25mL] and 72 hours post infection [G16] [0.25mL]. Immediately following delivery all pups were given to surrogate [BALB/c wild-type] dams. All mice were weighed weekly beginning at one week of age. All litters were weaned at 3 weeks of age.

On the test day, each animal was exposed for 10 minutes to a LEGO construct [LEGO Group, Billund, Denmark] and a Hot Wheels car [Mattel, Inc., El Segundo, CA, USA]. The objects were previously determined to be of matched saliency for mice. All tests were video recorded using ANYMAZE software. Time spent exploring both objects was recorded. Exploration was scored when the mouse touched an object with its forepaws or snout, bit, licked or sniffed the objects from a distance of no more

⁵⁰ Quantification of Gestational Changes in the Uteroplacental Vascular Tree Reveals Vessel Specific Hemodynamic Roles During Pregnancy in Mice. *Biology of Reproduction*, 2016

than 1.5cm. Following exploration mice were returned to their home cage. Three hours after the initial exposure, mice were returned to the test cage and were exposed for 5 minutes to one object from the original test pair and to a novel object. All animals that explored objects for less than 10 seconds were removed from analysis.

The Tail Suspension Test [TST] is a well-validated murine model of affective behavior. Each mouse was suspended by a small piece of masking tape on the tail for a 6-minute duration. All tests were video recorded using ANYMAZE software. The freezing [immobility] and mobility were coded during testing.

The Contextual Fear Conditioning [CFC] test is used to assess learning and memory dependent upon hippocampal [spatial learning] and amygdala [emotional, cued learning] function. All testing was conducted using a computer-controlled fear conditioning system [TSE, Bad Homburg, Germany]. Fear conditioning took place in a plexiglass chamber [20 cm x 20 cm x 36 cm] within a fear-conditioning box that was under constant illumination. During the testing, mice were single-housed and were brought into the testing room individually. The conditioning trial [Day 1] consisted of a single trial in which the mouse was placed in the test chamber [conditioning context] for 180 sec, after which a 30 sec tone was played [10 kHz, 75 dB SPL]. Termination of the tone coincided with the onset of a 2 sec shock [0.7 mA, constant current] delivered through a stainless steel grid floor. The mouse was left in the chamber for a further 30 sec, so that handling upon removal from the testing chamber would not be associated with shock. Contextual memory was assessed 24 h after the conditioning trial [Day 2]. Mice were returned to the chamber and left for 210 sec. Conditioned memory was assessed 48 h after completion of the conditioning trial [Day 3]. Mice were again returned to the testing chamber. The context was altered by covering the stainless steel rods on the floor with smooth plastic and covering the chamber walls with paper towel. After 180 sec in the chamber, the tone was played for 180 sec. Across days 1–3, total freezing [as measured by total number of light beam breaks] was recorded by the fear conditioning system.

At 8 weeks of age animals were anesthetized with a ketamine [150mg/kg]/xylazine [10mg/kg] mix and perfused transcardially with 30 mL of solution A [1xPBS + 2mM ProHance + 1µL/mL Heparin] and then with 30 mL of solution B [1xPBS + 4%

Paraformaldehyde + 2mM ProHance]. Animals were then decapitated and skin, cartilage and lower jaw was removed. Tissue was left at 4°C for 24 hours in 10 mL of solution B and then transferred into 10 mL of solution C [1xPBS + 0.02% sodium azide + 2mM ProHance] for storage prior to scanning. Tissue was left in solution C for a maximum of 6 months prior to scanning.⁵¹

Mice were treated systemically with a single chemotherapy agent at an infant equivalent age, then allowed to age to early adulthood [9 weeks]. Vincristine, doxorubicin, and methotrexate were observed to produce the greatest deficiencies in brain development as determined by volumes measured on MRI, whereas doxorubicin, methotrexate, and l-asparaginase altered heart structure or function. Longitudinal studies of vincristine revealed widespread volume loss immediately following treatment and impaired growth over time in several brain regions.⁵²

⁵¹ **Experimental Malaria in Pregnancy Induces Neurocognitive Injury in Uninfected Offspring via a C5a-C5a Receptor Dependent Pathway.** PLoS Pathogens, 2015

⁵² **Brain Development and Heart Function after Systemic Single-Agent Chemotherapy in a Mouse Model of Childhood Leukemia Treatment.** Clinical Cancer Research, 2018

Part Two: **Press Releases**

P.Release 1 [Aug 30 2018] [maternal deprivation experiments,](#)
[force-starvation experiments, at Sick Kids](#)

August 30, 2018
Toronto

It's a side of Sick Kids they don't want you see: a massive vivisection centre, where thousands of animals - including many neonatal mammals - are subject to cruel and painful experiments.

Pain is indeed the point of the experiments run by the current Chief of Research, Michael Salter. Salter is a pain expert - an expertise acquired through liberal use of cats, rats, and mice over thirty-plus years.

Salter and his vivisectioning colleagues operate out of the Peter Gilgan Centre: a twenty-story building, at Bay & Elm, connected to the hospital campus. Their victims include piglets, mouse pups & rat pups. They've induced cardiac arrest & neuro-degeneration in their animal subjects. They've taken pups from mothers to track the effect of this "stressor". They've taken pups from their mothers and fed them infant formula designed to kill them with severe intestinal damage. They've damaged rats' facial nerves. They've immobilized & starved mice to compare their muscle degeneration to hibernating squirrels.

The authors of these studies include the Hospital's Anaesthesiologist-in-Chief [Mark Crawford] and the Hospital's Paediatrician-in-Chief [Ronald Cohn].

From what Bali can tell, all the animal labs are on floors 4 thru 20 at the Peter Gilgan Centre. On the afternoon of August 21, Bali was removed by three security guards from the Centre's third floor when he approached the Office of Research Operations with basic questions, like: how many, and what kinds, of animals do

they kill every year at Sick Kids? Do they use gas chambers to kill them, or decapitators? Bali made a short video essay documenting his initial inquiry, here:

<https://www.youtube.com/watch?v=C6E0CoQGc7o&t=5s>

Bali has since submitted an FOI [Freedom of Information] request for this info. He's sceptical they'll give him anything. His previous attempts have been denied by Ryerson University, whose vivisectors are affiliated with MARS and with the Li Ka Shing Knowledge Institute at St. Michael's Hospital. They cited an exception in the FOI rules that allows them to withhold info if they believe its release could "endanger life or physical safety of a person" or "endanger security of a building".

Bali covered his experience with Ryerson / St. Mike's, here:

<https://philpapers.org/rec/PAUMIR>

his attempt was recently covered by Sarah Krichel at The Eyeopener, here:

<https://theeyeopener.com/2018/04/what-ryerson-isnt-telling-you-about-their-animal-testing/>

Then again, local activists have had some success with Freedom of Info requests. Animal Alliance of Canada got a disturbing look into the military research on pigs, rats, and rabbits at DRDC Downsview, in Downsview Park:

<https://philpapers.org/rec/BALARA-4>

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SickKids a demonic death-cult

September 6, 2018

Toronto

SickKids, a demonic death-cult. an early-modern brick & limestone, hung with pics of plucky, made-up urchins - while high in the new glass tower out back, they sacrifice kids for profit & power.



scientist in focus: Paul Delgado-Olguin

from his recent work on **cardiac injury in neonatal mice**:

Briefly, P1 or P7 pups were randomized and anaesthetized by hypothermia for 5 min prior to thoracotomy. To induce MI, the left anterior descending (LAD) coronary artery was ligated with a 6–0 non-absorbable polypropylene suture (Medtronic, Minneapolis, MN, USA). For apical resection, the apex of the heart was resected using fine scissors. ^[1]

Nature.com has pics of the popular procedure, slicing baby mice hearts:

<https://www.nature.com/articles/nprot.2014.021/figures/1>

anaesthetized by hypothermia likely means these babies were placed on ice before their chest was sliced. they "were allowed to recover under a heat lamp before being returned to their mother." they "were sacrificed 1 or 3 weeks after surgery by decapitation according to approved protocols and hearts were collected for analysis."

Ethical Statement: all Gilgan Centre animal use is according to the standards set by the Canadian Council on Animal Care, and in harmony with FDA and AVA guidelines on cervical dislocation of juveniles with our hands, over a garbage can.

in another recent study, "Sprague-Dawley female rats underwent partial bladder obstruction by ligation of a silk suture around the proximal urethra next to a 0.9-mm steel rod." [2]



scientist in focus: Paul Frankland

it's just a wee electro-shock. enough to induce paralyzing fear in his subjects, but he's not doing Blanchard-style **predator exposures** up there.

Mice were trained with a single foot shock (0.4 mA, Training), and 24hrs later tested (Test 1). Twenty-four hrs after the Test 1, the mice received systemic injections of MEM (50 mg/kg body weight (bw)) or vehicle (VEH) once a week for four weeks (MEM-4 or VEH group). Another group received an injection of MEM only 24hr after Test 1 (MEM-1 group). Contextual fear memory was assessed again four weeks after initial training (Test 2). All groups displayed comparable and high freezing response levels during Test 1. In contrast, the MEM-1 and -4 groups showed reduced freezing compared to the VEH group in Test 2 (Figure 1A), although this reduction was only statistically significant in the MEM-4 group. These observations were consistent with previous findings (Akers et al., 2014), and indicated that post-training MEM treatment enhanced forgetting in a dose-dependent manner. [3]

no archaic **predator exposure** - unless, say, the Conditioning predator is the large, lumbering man who electrocutes them then opens their heads.

*Equity Statement: the Egan lab is a gender-inclusive lab where we grow tumors in the breasts of **six-week-old $Lfng^{LacZ/+}$ virgin females**, then sacrifice them.*

To define where and when Notch is activated in the developing mammary gland, we used LacZ knock-in mice for various Notch pathway genes. Typically, boundaries between Fringe-expressing cells and nonexpressing cells are sites of consequential Notch signaling (Irvine, 1999). Therefore, we examined *Lfng* expression by performing X-gal staining on mammary glands from six-week-old *Lfng^{LacZ/+}* virgin females (Zhang and Gridley, 1998). Interestingly, *Lfng* expression was restricted to basal cells, in particular to cap cells of terminal end buds (TEBs) (Figure 1A), which have MaSC activity (Bai and Rohrschneider, 2010). This result is consistent with studies on cells purified from the human mammary gland, which show that *LFNG* expression is > 20-fold enriched in stem and/or bipotent progenitor cells as compared with luminal restricted progenitors (Raouf et al., 2008).^[4]

some legal questions

Gilgan Centre research raises neat Criminal Code issues, like: are they violating Section 445.1, that prohibits "willful infliction of unnecessary pain, suffering or injury to an animal or a bird" ?

judgement turns on our reading of the **necessity**. agent-necessity is relative to some goal, and in Salter et al's defense, Gilgan research *is* necessary - if you want to inflict fear, pain, maternal deprivation, and starvation on your weakers.

Gilgan research is necessary for Salter et al to meet the mandate of their generous sponsors, and keep their kids in private school.

legal issues, like: should Gilgan Centre vivisectionists be executed by a re-instated death penalty? should they merely be stripped of their credentials, then shamed until they repent?

a death penalty, re-instated, couldn't apply, by current law, retroactively. yet **a meta-law that would allow retroactive laws** could be passed, first.

better, Gilgan Centre researchers could be tried by the higher Law we all know in our hearts - tried e.g. for Crimes Against Life, in a future Nuremberg.

the Tower & its builders

how liable are Diamond & Schmitt for building this death centre?

did they sub-contract the caging & the kill-rooms?

In a physical sense, Diamond Schmitt Architects' design aims to demystify science by inviting the public into the building's lobby. (A pass card is needed to get through the doors leading to the elevator bank and up to the research floors.)

"If you're a passerby on the street, you get a sense of what's going on in this building," said Mr. Schmitt, nodding to the glass walls. "We're trying to reveal the mystery of research, which endlessly confounds people, with a sense of transparency."

The welcoming lobby features warm wood, floor-to-ceiling glass panes and pillars clad in polished stainless steel. It includes a multi-flight staircase leading to a terraced, wood seating area – not unlike the immensely popular City Room at the Four Seasons Centre, where Diamond Schmitt set out to demystify opera.

"Exactly the same principle is at work here," Mr. Schmitt said. ^[5]

the animal labs are spread somehow from floors 4 thru 21. on the afternoon of August 21, Bali was removed from the building by three security guards when he approached the Office of Research Operations [on floor 3] with basic questions: how many & what kinds of animals do they kill each year? do they gas them? decapitate them?

Bali made a short video essay documenting his visit, here:

<https://www.youtube.com/watch?v=C6E0CoQGc7o&t=5s>

he awaits a decision on his Freedom of Information request for the hospital's 2016-2017 animal-use data. whatever SickKids decides, Bali encourages all SickKids staff - from senior scientist to security & custodial worker - to use their cameras, and publish pics & vid.

notes

- [1] **Ezh2 is not required for cardiac regeneration in neonatal mice.** [PLoS One](#). 2018 Feb 21.
- [2] **DNA Methylation and the YAP/WWTR1 Pathway Prevent Pathologic Remodeling during Bladder Obstruction by Limiting Expression of BDNF.** [Am J Pathol](#). 2018 Aug 17.
- [3] **Hippocampal neurogenesis enhancers promote forgetting of remote fear memory after hippocampal reactivation by retrieval.** [Elife](#). 2016 Sep 26.
- [4] **Lunatic Fringe Deficiency Cooperates with the Met/Caveolin Gene Amplicon to Induce Basal-like Breast Cancer.** [Cancer Cell](#). 2012 May 15.
- [5] **Sick Kids gets a Bay Street tower of its own.** [The Globe and Mail](#). August 26, 2013. updated May 11 2018.

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btw, if the end-time Hypothetical came to pass & i could soothe a livid skygod into mercy on you Murderers by giving up myself - i'd do it, perhaps.

SickKids call police on alleged harasser

September 7, 2018

Toronto

A local activist, Paul Bali, has been asked this morning by Toronto Police to not contact Sick Kids staff. Sick Kids will consider any further contact from Bali to be Criminal Harassment. Police have also notified Bali he's to stay off Sick Kids property, or he'll be charged with Trespassing and investigated for Harassment.

Bali recently published two Press Releases on vivisection at The Peter Gilgan Centre, the hospital's 21-story research facility at Bay & Elm. On his first Release he bcc'd all the Centre's scientists, who are also affiliated with U of T. He simply disclosed a fraction of Sick Kids' research, compiled from public sources.

His second Release was sent out Wednesday to media & area bioethicists - which included two bioethicists affiliated with Sick Kids. It's likely this second Release that led Sick Kids to contact police. It's an invective in the theologic register, laced with bitter irony, and includes debate on what the appropriate punishment for vivisection should be - including the death penalty. Bali can appreciate why such a communication would cause anxiety for some researchers. But he reserves the right, as an ethicist, to engage in hypothetical jurisprudence, and to condemn mass murder in his strongest language.

Gilgan Centre researchers have conducted maternal deprivation experiments, starvation experiments, chronic pain experiments, and fear experiments on mammals. They have induced heart attacks in neonatal mice and breast tumours in juveniles. They decapitate their animal subjects when done with them. Other common killing methods at vivisection centres include gas chambers & induced heart attack. Bali has asked Sick Kids to disclose all their killing methods, and their annual animal-use stats, in an FOI request he submitted last week.

Bali first approached the Gilgan Centre in late August, seeking overview data on animal use at Sick Kids. he was escorted from the facility by three security officers. He posted a short video of his initial inquiry **here**.

Since the second Release, Sick Kids has contacted Bali's employer, Ryerson University. Bali has been a full-time instructor in Philosophy there since 2005. The Dean of Arts yesterday instructed Bali to [i] cease from identifying himself with the university in his "outside activities"; and [ii] ensure that all his communications comply with University civility policy. To the first demand, Bali maintains that Animal Ethics has been his central Philosophic interest for more than four years, and he is hired in part for his Philosophic "Currency", which means staying active in his areas of specialty. He has incorporated Animal Ethics into most of his courses & writing. To the second demand, Bali concedes that he has likely violated rules of academic civility, in his second missive to area professors, and regrets this. Yet he questions the coherence of Civility Requirements in a modern Academy that allows, indeed rewards, the cruelty described in his Releases.

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RESEARCH AND LEARNING