

## Research Awardees: 2010

### Basic Research Grant Program:

Regular Research Grants

Post-Doctoral Fellowships

Ad hoc Awards

### Translational Research Grant Program:

HeART Awards

ANGEL Awards

### Regular Research Grants

Bérubé, Nathalie G.

Bissonnette, John and Paton, Julian FR

Gan, Wen Biao

Monteggia, Lisa

Ruan, Yijun

Shatz, Carla J.

Sweatt, David J.

Zhou, Zhaolan

Bérubé, Nathalie G., University of Western Ontario, 2 years, \$99,990

Title: Epigenetic regulation of gene expression by MeCP2 in the mouse brain

Lay Summary: RTT syndrome is a disease that affects the normal development of the brain. The MECP2 gene has been identified as the most commonly mutated gene in children diagnosed with RTT syndrome. The protein that is produced by the normal form of the gene is able to bind DNA and regulate the activity of other genes in brain cells. However, we still don't fully understand how this protein works and what role it plays. Another protein called ATRX was recently demonstrated to bind directly to MeCP2, suggesting that perhaps they work together to regulate brain genes. ATRX is a

protein that also binds DNA, is mutated in some forms of mental retardation syndromes, and is another important regulator of brain development. In this study, we will examine the relationship between MeCP2 and ATRX using cultured cells and genetically engineered mice. We will determine whether MeCP2 and ATRX bind and regulate common genes in brain cells and will study how this could relate to abnormal packaging of DNA and altered brain function. The proposed studies will help us understand how MeCP2 works normally in brain cells and this knowledge will provide avenues to design new therapies to alleviate or reverse brain dysfunction in RTT syndrome patients.

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Bissonnette, John M., Oregon Health and Science University and Paton, Julian FR, University of Bristol, 2 years, \$100,000

Title: Pharmacological treatment of respiratory disorders in a mouse model of Rett syndrome

Lay Summary: Breathing abnormalities consisting of rapid deep respiration followed by cessation of breathing and an irregular interval between breaths are a common and distressing feature of Rett syndrome (RTT). Using a mouse model we have shown that injections of a compound that boosts the brain concentration of the inhibitory neurotransmitter <sup>3</sup> amino-butyric acid (GABA) markedly reduces the incidence of these respiratory disturbances in heterozygous females. Drugs of this type are not available for children. We, however, also showed that an activator of serotonin at 1a receptors improves respiration to a similar degree. To date the treatments have involved all organs and tissues in the animals. In order to better understand the basis of the respiratory problems in RTT, this proposal will examine the effects of activating serotonin receptors in very localized areas of the brain so as to define the population of neurons that are causing the breathing problems. We will also establish that the serotonin activist works by enhancing a specific potassium ion channel. Establishing that serotonin acts by activation a specific type of potassium channel will pave the way for treatments that target these potassium channels directly. We will further strengthen our hypothesis by anatomical studies that count the number of connections that GABA neurons make with expiratory neurons. Taken together these studies may lead to new treatments for the respiratory disorders in Rett syndrome.

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Gan, Wen Biao, New York University, School of Medicine, 2 years, \$100,000

Title: Disruption of experience-dependent dendritic spine plasticity in MeCP2 mutant mice

Lay Summary: Rett Syndrome (RTT) is a developmental brain disorder primarily caused by mutations in a gene encoding methyl-CpG-binding protein 2 (MeCP2). Many studies have suggested that MeCP2 mutations disrupt synapse development and neural network functions in RTT. MeCP2 mutant mice have provided one of the best experimental systems to study the pathogenic mechanisms of Rett Syndrome. The goal of this proposal is to investigate the impact of MeCP2 mutations on synapse development and plasticity in the cortex of living MeCP2 mutant mice. These studies will reveal how loss of MeCP2 gene function leads to abnormal development of neuronal circuits and causes behavioral deficits in Rett Syndrome. Our studies may also establish an important assay for testing the effectiveness of treatment for Rett Syndrome.

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Monteggia, Lisa, University of Texas Southwestern Medical Center, \$99,998

Title: Elucidation of Epigenetic Mechanisms in Rett Syndrome

Lay Summary: The underlying mechanisms that contribute to autism spectrum disorders are unknown. Recent work has established DNA methylation as an important regulator of long-term synaptic function. Importantly, alterations in DNA methylation have been suggested to underlie neurodevelopmental disorders such as Fragile X and Rett Syndrome, both diseases with an autism component. In this application, we propose a comprehensive strategy to address the role of DNA methyltransferases DNMT1 and DNMT3a, key enzymes that methylate DNA, in neuronal function and behavior using a combination of genetic, behavioral, electrophysiological and optical imaging techniques. Our laboratory possesses all the necessary expertise and tools to accomplish the goals set in this proposal. Our aim is to evaluate the role of irregularities in DNA methylation and resulting imbalanced excitation and inhibition as the common denominator linking neurodevelopmental disorders. Given the importance of elucidating molecular components that may underlie neurodevelopmental disorders, as well as the recent interest in epigenetic mechanisms that may contribute to long-term alterations in brain function, we believe this proposal examining alterations in DNA methylation on synaptic function and complex behavior has important implications for neurodevelopmental disorders including autism.

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Ruan, Yijun, Genome Institute of Singapore, National University of Singapore, 2 years, \$100,000

Title: Using ChIA-PET to unravel high order chromatin regulatory functions of MeCP2

Lay Summary: Aberrant methyl-CpG binding protein2 gene (MeCP2) has been shown to lead to deficits in neuronal maturation, synaptogenesis, neural circuit connectivity, and cause a host of neuropsychiatric phenotypes. Extensive studies have shown that MeCP2 regulates the expression of a wide range of genes in the hypothalamus directly and indirectly, and can function both as an activator and as a repressor of transcription. However, the exact role of MeCP2 in gene regulation remains obscure. Like many transcription factors, MeCP2 binds mostly to non-proximal promoter regions, suggesting that long-range chromatin interactions may be involved as a mechanism for transcription regulation. In this study, we propose to use our recently developed whole genome approach for Chromatin Interaction Analysis using Paired-End-Tag sequencing (ChIA-PET) to unravel the high order chromatin regulatory functions of MeCP2.

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Shatz, Carla J., Stanford University, 2 years, \$99,885

Title: MHC Class I molecules and receptors as therapy for Rett Syndrome?

Lay Summary: Rett Syndrome is a neurological disorder affecting mainly girls, who exhibit normal development for the first 6-18 months of life, but then manifest a gradual loss of motor skills, social withdrawal, and mental retardation. The

cause of this devastating condition is believed to be mutations in a gene called MeCP2. Fascinating recent studies suggest that MeCP2 may control expression of crucial genes responsible for proper maturation of neuronal connections during developmental 'critical periods'. Critical periods are windows of time during infancy and childhood responsible for the fine-tuning of cognitive, motor and sensory systems. If appropriate input is not received at these times, refinement and maturation of neuronal connections do not occur properly. This experience-dependent fine-tuning is called "plasticity", and we hypothesize that Rett Syndrome patients may suffer from plasticity defects. Our lab has recently shown that a group of genes- MHC Class I- which function in the immune system, are crucial for controlling the extent of neuronal plasticity. Using a strain of mice that lack the MeCP2 gene and display Rett Syndrome symptoms, we will examine if plasticity during visual system critical periods is altered in these mice. Next, we plan to determine if MHC Class I levels are changed in these mutant mice due to the absence of MeCP2. If a modulation in level of MHC Class I is seen, we will attempt to reverse Rett Syndrome symptoms by restoring MHC Class I protein levels or signaling via MHC I receptors in mouse brains. These experiments should not only help to illuminate the etiology of Rett Syndrome, but they may also suggest an intriguing link between the neural and immune systems, as well as identify new and promising targets for therapy.

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Sweatt, David J., University of Alabama, Birmingham, 2 years, \$100,000

Title: MeCP2 in Cognitive Function in the Adult Nervous System

Lay Summary: Rett Syndrome is a neurodevelopmental disorder, the underlying genetic basis of which is mutation/deletion of the MeCP2 gene and resultant disruption of normal MeCP2 function. The mutated gene product is present throughout development but is also present in the fully developed adult CNS. It is unclear if Rett Syndrome is caused exclusively by disruption of MeCP2 function during development, or whether loss of MeCP2 in the mature CNS might also contribute to neurobehavioral and cognitive dysfunction in Rett patients. Indeed, recent data from Adrian Bird's group has suggested that loss of normal MeCP2 function in the adult nervous system contributes to neurobehavioral dysfunction in Rett Syndrome. Addressing this question is critically important because of the implications for developing potential new treatments for Rett Syndrome. If MeCP2 functions to control cognition in the mature CNS, cognitive dysfunction in Rett Syndrome might in significant part be due to disruption of MeCP2's actions in the fully developed CNS. A new understanding of the role of MeCP2 in the adult CNS might allow the development of new therapeutic approaches to Rett treatment based on restoration or augmentation of MeCP2 function after CNS development is largely finished.

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Zhou, Zhaolan, University of Pennsylvania, School of Medicine, 2 years, \$100,000

Title: The study of Rett Syndrome with Mecp2 T158A knockin mice

Lay Summary: Rett Syndrome (RTT) is caused by mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2). Genetically modified mice with loss of MeCP2 function mimic human RTT in several aspects. Despite the advances in genetic studies of RTT, the pathogenic mechanisms by which dysfunction of MeCP2 lead to neurological symptoms remain poorly understood, and thus hindering the development of therapeutics. Among all the mutations that are associated with RTT, mutation of Threonine 158 is one of the most frequent ones. In addition, T158 is located in the domain of MeCP2 that is responsible for methyl-DNA binding. Thus, to verify the genetic cause of T158 mutation in RTT and to understand the role of methyl-DNA binding of MeCP2 in the pathogenesis of RTT, we have developed a knockin

mouse that carries a point mutation of MeCP2 at T158. With this newly developed mouse model, we plan to address the role of methyl-- DNA binding in MeCP2-- mediated gene regulation and synapse development, and ultimately obtain a genome-- wide view of MeCP2 function. We hope to uncover novel therapeutic to cure RTT.

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#### Post Doctoral Fellowships

Amendola, Elena

Han, Jing

Krishnan, Keerthi

Kron, Miriam

Amendola, Elena, European Molecular Biology Laboratory, 2 years, \$100,000

Title: A Mouse Model of CDKL5 Rett syndrome

Lay Summary: Rett syndrome is a neurodevelopmental disorder most commonly caused by mutations in the MECP2 gene. However, some persons with Rett do not carry mutations in MECP2 and recently mutations in the cyclin dependent kinase-like 5 (CDKL5) gene have been found in persons having a Rett-like disorder that includes seizures during the first six months of life. There are currently over 50 reported persons with such Rett-like disorders caused by mutations in CDKL5 and all children with CDKL5 mutations show similar features: seizures in the first months of life and subsequent development of Rett-like features. These observations indicate that CDKL5 might play a role in brain development similar to that of MECP2. They also suggest that a better understanding of the function of CDKL5 might help to better understand all forms of Rett. Interestingly, experiments in the laboratory have shown that CDKL5 can bind to and alter the function of MECP2 supporting the idea that they might act on similar brain functions to cause Rett. Nevertheless, little is known otherwise about the function of CDKL5 and a number of basic research tools are urgently needed to learn more about what this gene does in the brain and what goes wrong when it is mutated. Here we propose the development of several critical research tools and their use to address important questions about CDKL5 Rett. First, we plan to construct a mouse model of CDKL5 Rett; no mouse model of the disorder exists to date. Second, we will develop monoclonal antibodies, a renewable resource for distribution to Rett researchers that can be used to find out where CDKL5 functions in the brain. Third, we will perform experiments to find new molecules that help CDKL5 carry out its function and therefore might be defective in Rett. Together, these experiments aim to establish essential knowledge about the role of CDKL5 in brain development and will offer a platform for the testing of drugs to treat Rett.

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Han, Jing, Baylor College of Medicine, 1 year, \$50,000

Title: Investigating the Role of the Neuroendocrine-Hypothalamic System in Rett Syndrome

**Lay Summary:** Rett syndrome (RTT) is a postnatal neurodevelopmental disorder occurring predominantly in females. It is caused by mutations in MECP2, a modulator of gene expression. Our previous gene expression study and behavior analysis of MeCP2 mutant mice indicated that one brain region, the hypothalamus, plays an important role in RTT pathogenesis. The mammalian hypothalamus has a dominant influence on behavior, as it serves as the control center of the body, regulating sleep, mood, social function, stress response, and gut motility. Given the critical function of the hypothalamus, it is important to thoroughly examine the role of the hypothalamus in RTT. In my current work funded by IRSF, we removed MeCP2 from nearly all the critical areas of the hypothalamus and analyzed the behavioral and physiological changes in these mice. We found these mice exhibit many RTT features including low bone density, abnormal breathing pattern, and low body temperature, etc. In the coming year, we will focus on one feature, the low bone mineral density phenotype because it also manifests in RTT patients resulting in early childhood bone fractures. The critical question is why does loss of MeCP2 in the hypothalamus lead to abnormal bone formation? To answer this question, we will examine the features of bone formation, resorption, and central control of BMD in these mice. These experiments will help us gain a deeper understanding of the origin of RTT phenotypes and provide insight into potential new therapeutic avenues.

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Krishnan, Keerthi, Cold Spring Harbor Laboratory, 2 years, \$100,000

Title: Role of MeCP2 in the maturation of neocortical GABA interneurons and critical period of plasticity

**Lay Summary:** Rett syndrome, caused by mutations in the X-linked gene encoding methyl-CpG-binding protein 2, is the best characterized of the autism spectrum disorders. Our general hypothesis is that MeCP2 mutations perturb the connectivity, function, and plasticity in subsets of inhibitory interneurons in distributed brain areas, leading to altered development and maladaptive plasticity of neural circuits and behavioral deficits. Using mouse genetics, our lab has established powerful experimental systems to study the function and dysfunction of GABAergic circuits in neocortex with cell type and synapse type resolution. GABA-mediated inhibition is crucial in nearly all aspects of neural circuit operations. In this proposal, we focus on a particular developmental time window, the critical period. This is a highly sensitized period during early development, when experience-dependent wiring of the brain takes place. Experience-dependent matching of different streams of information both within a sensory modality and between modalities (visual and auditory, visual and social) are crucial in forming proper perception and in guiding adaptive behaviors. Elucidating the cellular mechanisms underlying how MeCP2 regulates experience dependent circuit development in a well-established paradigm in visual cortex will have implications in other cortical areas and brain systems; this will also provide fresh insight into the pathogenic mechanisms of RTT, both in sensory perception (e.g. strabismus and autistic patients) and social interactions that require proper sensing and interpreting of different streams of information. If successful, the project would result in a major breakthrough in RTT research that might have therapeutic implications targeting defined neural circuits.

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Kron, Miriam, Case Western Reserve University, School of Medicine, 2 years, \$99,990

Title: Synaptic mechanisms of apnea in Rett syndrome

**Lay Summary:** Many patients with Rett syndrome (RTT) suffer from severe respiratory problems characterized by

alternating periods of hyperventilation and apneas (arrest of breathing) which can severely affect quality of life and be life-threatening. However, the cause of breathing dysfunction in RTT is largely unknown, thereby hampering our ability to design effective treatments. The proposed research is designed to test a specific hypothesis regarding the mechanism responsible for apneas in RTT and to test molecules for their ability to prevent apnea, using mouse models of the disease. I hypothesize that apneas are generated, or exacerbated, by abnormalities in a specific region of the brainstem called the nucleus tractus solitarius (nTS) that plays a critical role in regulating the timing of inspiration and expiration. Previous work in Dr. Katz's laboratory demonstrated that neuronal signaling in some pathways in nTS is hyperexcitable in mouse models of RTT. If this is also true in pathways that control the timing of inspiration and expiration, this could account for the generation or exacerbation of apneas in RTT. Therefore, I plan to use electrophysiological techniques to determine whether or not these respiratory control pathways are hyperexcitable in a mouse model of RTT and, if so, to determine the mechanisms responsible for this hyperexcitability. In addition, I will determine whether or not any observed abnormalities can be reversed with molecules that are designed to restore normal chemical signaling in neural pathways in nTS. Thus, the ultimate goal of the proposed studies is to develop potential new treatments for apnea in RTT.

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#### HeART Awards

Adachi, Megumi

Bissonnette, John M.

Delaney, Kerry

Djukic, Aleksandra

Lomvardas, Stavros

Ratan, Rajiv

Segal, David

Sun, Yi Eve

Zhang, Liang

Zhao, Xinyu

Adachi, Megumi, University of Texas Southwestern Medical Center, 1 year \$50,000

Title: Evaluation of Antidepressants as Potential Therapeutic Intervention for Rett Syndrome

Lay Summary: Rett Syndrome (RTT) is a neurological disorder involving cognitive and motor dysfunctions in young females and mostly results from mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2). MeCP2 is a protein that binds to DNA and regulates levels of genes expression. The mutations in the MECP2 gene, which are predicted to result in loss of MeCP2 function, have been suggested to cause a global imbalance of gene expression that may contribute to the neurological symptoms seen in RTT patients. Much effort has been devoted to identify MeCP2 target genes that are relevant to RTT. One of the potential targets is brain derived neurotrophic factor (BDNF), a growth

factor found in the brain that supports survival, growth, and differentiation of neurons and its impaired activity is often associated with several psychiatric illnesses. In mouse models of RTT, abnormal BDNF signaling as well as its reduced expression has been observed in independent studies. More importantly, normalization of BDNF expression reversed the abnormal deficits observed in a mouse model of RTT further supporting BDNF as a crucial mediator underlying pathophysiology of RTT. The present proposal aims to evaluate whether antidepressants alleviate abnormal behavioral deficits observed in mouse models of Rett syndrome (RTT). Chronic, but not acute, antidepressant administration increases BDNF expression in the brain, which is believed to be an essential step to achieve clinical efficacy of antidepressant responses. Therefore, we hypothesize that the chronic

antidepressant treatment will increase BDNF expression sufficiently to rescue some of the behavioral abnormalities seen in mouse models of RTT. We will also evaluate the therapeutic benefit of a low dose ketamine for the treatment of RTT as it produces a fast-acting and long-lasting antidepressant response in patients suffering from major depressive disorder. Recent data from our lab demonstrates that a single administration of ketamine in mice produces a fast acting antidepressant response, which is accompanied by a rapid increase in BDNF protein in the brain. We will examine whether antidepressants, by increasing BDNF levels in the brain, can rescue the behavioral abnormalities seen in mouse models of RTT.

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Bissonnette, John M., Oregon Health and Science University, 1 year \$50,000

Title: Serotonin and small molecule treatment of respiratory disorders in a mouse model of Rett syndrome

Lay Summary: Disturbances in breathing are common and disturbing occurrences in Rett syndrome (RTT). In previous studies in a mouse model of RTT we have found that a compound that activates serotonin receptors of the 1a type effectively treats their respiratory disorders. This compound is not available for human use. There is, however, a serotonin medication with similar mode of action that has been used in adults to treat complications of Parkinson disease. In this project we will evaluate the effectiveness of this serotonin medication in correcting respiratory problems in RTT mice. Serotonin drugs probably correct breathing in RTT mice by stimulation a certain type of potassium channel in brain respiratory neurons. In collaboration with Jerod Denton, PhD at Vanderbilt we will examine small molecules that he has determined stimulates these potassium channels. This stimulation acts directly on these channels, not through nerve cell receptors and potentially could have fewer side effects. Either or both of these two compounds may be effective in treating the respiratory problems in RTT.

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Delaney, Kerry, University of Victoria, 1 year \$50,000

Title: MeCP2 Replacement Using "Trojan Horse" Pegylated Immunoliposomes

Lay Summary: Rett Syndrome (RTT) is neurodevelopmental disorder primarily caused by mutations in the X-linked gene MECP2. A distinctive characteristic of this disorder is the complexity and variability of symptoms seen in RTT patients. This is due in part to the variety of different mutations that occur in the MeCP2 gene, but also due to the many different functions MeCP2 plays within brain cells. This remarkable protein regulates the expression of thousands of other genes,



and does so in different ways, depending on the brain region involved. It is this carefully balanced and regulated functional complexity that makes the design of new therapeutics for RTT patients so challenging. If the full suite of MeCP2 functionality cannot be replicated, then the considerable task of identifying and testing the essential pharmacological targets “downstream” of MeCP2 still remains. The most direct approach to this challenge, however, is to try to reintroduce functional MeCP2 itself back into the deficient cells. Studies with mutant mice have shown that expressing normal MeCP2 can reverse many of the RTT-like symptoms, even in highly symptomatic animals that have grown to maturity completely deficient in MeCP2. The development of therapeutic applications for these exciting findings has been complicated by other studies, which show that long-term overexpression of MeCP2 also has deleterious effects. If MeCP2 gene replacement therapy is going to be viable, it has to be done in a way that allows some control over the “dosage” of gene expression.

We are proposing to test a new “Trojan Horse” gene delivery technology that uses particles called pegylated immunoliposomes (PILs). In a PIL, a drug (e.g. a therapeutic DNA) is encapsulated in a small lipid sphere. Attached to the outside of the sphere are specific antibodies, which target receptors on the blood-brain barrier (BBB) and on brain cells. PILs are called molecular Trojan Horses because when the antibody binds its target receptor, it triggers a transport mechanism that normally functions to shuttle a particular protein across the BBB. This allows intravenously injected PILs to deliver the therapeutic gene to virtually every neuron in the brain, because the central nervous system is so highly vascularized. Our general hypothesis is that because PIL-mediated gene expression is transient, administering low doses at an appropriate frequency may be able to mitigate the effects of MeCP2 overexpression. PIL-delivered DNA has a built-in safety mechanism in this regard, because it does not integrate into the chromosomal DNA of the host cell and is instead broken down after several days. Our current aim is to assess this new technology in mice by studying the efficiency and cellular distribution patterns of PIL-delivered MeCP2. If we are successful we would to expand these studies to include assessing symptom amelioration in MeCP2 mutant mice.

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Djukic, Aleksandra, Montefiore Medical Center, Albert Einstein College of Medicine, 1 year \$50,000

Title: Evaluation of Nonverbal Cognitive Abilities in Patients with Rett syndrome (Visual Attention and Recognition)

Lay Summary: Attempts to assess cognitive functioning in girls with Rett Syndrome (RTT) have been extremely difficult. We are proposing a pilot study to examine the cognitive development of patients with RTT in a structured way using eye gaze and eye tracking technology. The area of development that would be included is visual memory as part of the cognitive assessment. Due to the role that vision seems to play for people with RTT, the assessment of the ability to remember visually presented material seems a reasonable choice to examine visual recognition memory as part of their cognitive assessment. We will examine visual recognition memory and visual attention using a test already developed by a group of investigators, the paired-comparison paradigm. We are planning to include 50 patients to participate in this pilot study using eye tracker technology (Tobii software) at the Rett Syndrome Center at Montefiore, Albert Einstein College of Medicine. We will record information from the tests of visual perception and visual memory, including duration of looks, number of shifts in gaze from one target to another, the percentage of total looking time on the test that was spent looking at the novel stimulus and how the stimuli is inspected. Besides we will interview parents and record adaptive functioning.

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Lomvardas, Stavros, University of California, San Francisco, 1 year \$50,000

Title: Developing a cell culture system for high-throughput screening of Rett syndrome therapeutics

Lay Summary: Rett syndrome is a devastating form of Autism that affects 1/50,000 girls born in the US. Mutations on the gene that encodes for the methyl-DNA binding protein MeCP2 account for more than 90% of the diagnosed cases of Rett syndrome. However, the downstream targets of MeCP2 and the molecular consequences of the identified mutations remain elusive. My laboratory studies the mechanisms that regulate the monogenic and monoallelic expression of olfactory receptor genes in the mouse. Using the MeCP2 knockout mouse model we discovered a very subtle, but extremely significant consequence of MeCP2 deletion; the inappropriate expression of more than one olfactory receptor genes in each olfactory sensory neuron. Using this olfactory receptor gene miss-expression phenotype as a molecular assay, we designed a high throughput screen for small molecules that can reverse the consequences of MeCP2 deletion and therefore can potentially function as Rett syndrome therapeutics. Therefore we propose to use cultured olfactory neurons as “biosensors” for highthroughput pharmacological screens for Rett syndrome. With the generous support of the Heart grant we will be able to establish the proposed assay.

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Ratan, Rajiv, Winifred Masterson Burke Medical Research Institute, Weill Medical College of Cornell University, 1 year \$50,000

Title: Novel screening methods for quantitative, homeostatic regulation of MeCP2

Lay Summary: Rett Syndrome afflicts young girls with motor, cognitive, and autonomic abnormalities. Remarkably restoration in MeCP2 levels in afflicted female rodents can completely reverse symptoms in all of these arenas. A natural strategy to treat Rett Syndrome is to develop drugs that can restore MeCP2 levels in the nucleus of patients. We have developed a screening strategy that will allow us to identify drugs that modulate MeCP2 stability or synthesis in human cells. Drugs that normalize MeCP2 levels and increase BDNF release from cortical neurons will also be identified. It is very important that our drugs do not increase MeCP2 levels beyond a homeostatic range. Our screening strategy permits high fidelity methods to modulate MeCP2 levels, thus avoiding this potentially tragic outcome. Drugs identified in this screen will be ready for testing in complex models of Rett Syndrome. Positive hits will also undergo optimization by medicinal chemists, including Dr. Alan Kozikowski, a long standing collaborator of the group at Burke. A pluralistic effort among multiple labs is the surest way to cure this devastating condition.

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Segal, David Jay, University of California, Davis, 1 year \$50,000

Title: Epigenetic Therapy for Rett Syndrome

Lay Summary: Rett syndrome is caused by mutations in the MECP2 gene on the X chromosome. Females have two X chromosomes. Typically, only one X chromosome carries the mutation, the other has a normal MECP2 gene. However, only one of the X chromosomes is active in any cell. The other is compacted by an epigenetic mechanism, and its genes are turned off and silenced. In some cells, the normal MECP2 gene will be on the active X chromosome, and the mutant gene will be silenced on the inactive X chromosome. Cells with this arrangement will be normal. Unfortunately, in other cells, the mutant MECP2 gene will be on the active X chromosome, and the normal gene will be silenced on the inactive

X chromosome. Cells with this arrangement give rise to Rett syndrome, because they can not produce the MECP2 protein. However, these cells still have a perfectly normal MECP2 gene, but it is silenced with the other genes on the inactive X chromosome. We are developing a method to reactivate this silenced MECP2 gene on the inactive X chromosome. We proposed to do that by engineering an artificial transcription factor (ATF) that will bind to the silenced MECP2 gene and turn it on. Similar ATFs been previously shown to turn on other epigenetically silenced genes. Our preliminary studies show that at least one of our ATFs seems to work. In the current proposal, we aim to verify that it is activating the silenced MECP2 gene, and to create similar ATFs to the mouse *Mecp2* gene so that we can begin pre-clinical testing in a mouse model of Rett syndrome.

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Sun, Yi Eve, University of California, Los Angeles, 1 year \$50,000

Title: A high throughput small molecule screening platform for potential Rett Syndrome MBD mutation therapeutics

Lay Summary: Rett Syndrome (RTT) is mostly caused by mutations of a gene called MECP2. The MeCP2 protein recognizes and binds to methylated DNA region in the genome. More than half of all known RTT patients mutations occur in the methyl-CpG binding domain (MBD). T158M and R106W, among the most common mutations, are suggested to have decreased binding affinity to methylated DNA. Here we propose to search for small molecules that may enhance the binding affinity of the mutant MeCP2 protein. The screenings will be done in both test tube and cell culture. This approach may lead to drug-discovery for RTT patients with MBD mutations.

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Zhang, Liang, University Health Network - Toronto Western Hospital, 1 year \$50,000

Title: Evaluating carbonic anhydrase inhibitors as potential treatments for Rett syndrome

Lay Summary: Rett syndrome is a devastating genetic condition caused primarily by mutations of the MECP2 gene. There are no cures presently, and the best treatments for Rett syndrome generally attempt to manage specific symptoms associated with the condition. There is growing evidence, though, that Rett syndrome is a treatable condition, and that therapeutic strategies can be developed to significantly help patients. To aid in the identification of promising treatment strategies, several research groups have generated mouse models of Rett syndrome. These mice develop many Rett-like deficits, and have proven highly useful for unraveling how the loss of MeCP2 function affects the brain and body. Importantly, work on these mice has shown that their Rett-like conditions can be improved or even corrected by both genetic and pharmacological procedures. While the genetic procedures used to cure these mice hold little if any clinical applicability, the pharmacological studies certainly do – it is now clear that the Rett-like condition of these mice can be improved by drug treatments. Now we need to identify quickly additional drugs that will provide benefit in these mice so we can expedite their path to the clinic. The most attractive drugs or combinations of drugs would be those that have already been approved for use. Based on converging lines of data, we hypothesize that an anti-convulsive drug, namely acetazolamide, functions in a way that would improve the neural and behavioral deficits we and others have identified in MeCP2-deficient mice. We further hypothesize that including a second drug, namely valproate, will complement the actions of acetazolamide, and increase the overall level of improvement seen in the mice. If our results show significant improvements in these mice as anticipated, our study would provide the foundation necessary for testing these drugs immediately in Rett patients, as each of these drugs is already approved for clinical use in children.

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Zhao, Xinyu, University of New Mexico Health Sciences Center, 1 year \$50,000

Title: Novel method for enhancing BDNF protein expression

Lay Summary: Although the mutation of X-linked MECP2 gene is known to cause Rett syndrome (RTT), effective therapeutic treatment for this devastating disorder is lacking. Extensive evidence has identified BDNF as a downstream effector of MeCP2. Reduced expression of BDNF protein in the brain is characteristic of RTT and enhancing BDNF can alleviate neurological symptoms associated with MeCP2 deficiency. However, an effective method for elevating BDNF protein levels in the brain is still lacking. We have found that MeCP2-deficiency leads to both up and down regulation of noncoding small RNAs (nsRNAs) in neural stem cells and neurons and some of the microRNAs are predicted to target BDNF mRNA and potentially regulate BDNF protein translation. Here, we propose a one-year pilot project to explore a novel therapeutic idea. Our hypothesis is that some MeCP2-regulated nsRNAs can modulate BDNF protein translation and manipulation of these nsRNAs may be used as therapeutic methods for treating RTT. To achieve the goal of this project, we propose to establish a screening systems that can be used to identify nsRNAs with the ability in promoting BDNF levels in neural cells. Then I will use this system to identify nsRNAs and nsRNA inhibitors that may promote BDNF protein translation. Finally, we would like to determine whether manipulation of nsRNAs can promote BDNF protein expression in the brain with MeCP2 deficiency. If this pilot project indicates positive outcome, further translational investigations will sprout from this effort, leading to potential new treatments for RTT.

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ANGEL Awards

Justice, Monica J.

Katz, David M.

Justice, Monica J., Baylor College of Medicine, 1 year \$100,474

Title: Developing new therapeutic targets for amelioration of Rett Syndrome from the identification of genetic suppressors in mice

Lay Summary: The ability to rescue symptoms in MeCP2 mutant mice by reintroduction of the gene has provided tremendous hope to families of RTT patients. However, current approaches to rescue symptoms using re-introduction of

MeCP2 in humans are problematic. We have used genetic strategies in mice to identify genes that when altered, ameliorate the symptoms caused by mutation of MeCP2 in mice. The first of these “suppressors” shows promise for developing therapeutics to alleviate symptoms. This proposal will focus on developing therapeutics based on our knowledge of the first modifier locus, while we learn more about the second modifier, and determine if it could be another candidate for therapeutic approaches.

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Katz, David M., Case Western Reserve University, School of Medicine, 2 years \$446,176

Title: Preclinical Evaluation of BDNF-Targeted Therapies for Rett Syndrome

Lay Summary: The aim of this proposal is to evaluate Brain-Derived Neurotrophic Factor (BDNF)-based therapies for Rett syndrome (RTT) in a well characterized mouse model of the disease. BDNF is a molecule that is absolutely critical for the development and/or function of many neurons in the brain and peripheral nervous system. BDNF has emerged as a candidate target molecule for treatment of RTT based on observations in this and other laboratories that 1) BDNF levels are markedly reduced in mouse models of RTT and RTT patients and 2) BDNF is required for maturation and function of neural systems that are affected in RTT, such as the network of neurons that controls breathing. Furthermore, we and others have shown that increasing BDNF levels in RTT mice by either genetic or pharmacological means is associated with improvements in physiologic function and/or survival.

Our lab has recently entered into collaborations that give us access to novel reagents that can compensate for deficits in BDNF. The proposed research is designed to build on this unique opportunity by evaluating the ability of these compounds to improve respiratory and other functions in a mouse model of RTT. The goal of these studies is to determine the ability of BDNF-targeted therapies to provide long-term improvement in physiologic outcomes in a mouse model of RTT, with the hope of identifying candidate molecules for subsequent clinical studies.

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Ad Hoc Awards

Christodoulou, John C.

Gaisina, Irina

Glaze, Daniel G.

Kozikowski, Alan P.

Neul, Jeffrey L.

Christodoulou, John C., Children's Hospital at Westmead, Contract, 1 year \$35,165

Title: Rettbase Database

Lay Summary: Rett syndrome is a severe genetic form of autism in girls. Girls with RTT have abnormal growth, movement problems and abnormal breathing and heart rate patterns. There is no treatment for RTT. Mice with the equivalent genetic change have similar symptoms to human patients. Giving these mice a drug called IGF1 relieves a large number of these symptoms. IGF1 is already available for use in children. We wish to evaluate the safety and effectiveness of IGF1 when given to girls with RTT. We will use an non-invasive instrument to measure improvements in breathing and heart rate during treatment with IGF1.

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Gaisina, Irina, University of Chicago, Illinois, Contract, 1 year \$125,000

Title: Selected Molecular Agents for Rett Therapy – The IRSF SMART Initiative

Lay Summary: We propose to create a SMART [Selected Molecular Agents for Rett Therapy] library of FDA approved drugs and non-FDA approved chemical entities that are of mechanistic interest to RTT investigators. Although there exist many commercial libraries of compounds that have been optimized for their structural diversity and drug-like properties, these libraries do not directly address the real needs in RTT drug discovery. Our aim is to facilitate the elucidation and repair of the biological events that go awry during the development of Rett syndrome and to ameliorate the symptoms of the disease. A library of 300-400 FDA approved drugs and experimental drugs will be assembled and renewed as necessary over a two year period. Additional library expected to be about 300 compounds with growth to 1000 likely. Compounds that show interesting activities will be prepared or acquired for in vivo work. Compounds will be centrally housed and distributed, upon request, to RTT investigators. The IRSF Science Advisory Board (SAB) has already made recommendations concerning a number of known and experimental drugs that operate by diverse mechanism [e.g., ampakines, growth hormone secretagogues, monoamine reuptake inhibitors, carbonic anhydrase inhibitors, histone deacetylase inhibitors, inducers of BDNF synthesis and release, and others], and, as such, we believe that we will be able to populate a rich library of compounds that work through diverse mechanisms.

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Glaze, Daniel G., Baylor College of Medicine, Contract, 1 year \$50,000

Title: Sleep Characterization in Rare Disease Pilot Study

Lay Summary: Coming Soon!

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Kozikowski, Alan P., University of Chicago, Illinois, Contract, 1 year \$25,000

Title: Rett Syndrome Proposal for Limited Medicinal Chemistry and In Vitro Cell Studies - Prelude to In Vivo Rett Animal Studies

Lay Summary: Coming Soon!

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Neul, Jeffrey L., Baylor College of Medicine, Contract, 1 year \$25,000

Title: Rare Diseases Training

Lay Summary: Coming Soon!

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