

Research Awardees: 2008

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Research Awards

Berube, Nathalie

Bissonnette, John

Chang, Qiang

Delaney, Kerry

Galanopoulou, Aristeia

Ravine, David

Yasui, Dag

Berube, Nathalie, University of Western Ontario, 2 years, \$100,000

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 expression 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 and is mutated in some forms of X-linked mental retardation syndromes, and is therefore another important regulator of brain development. In this study, we will start to examine the relationship between MeCP2 and ATRX using cultured cells and genetically engineered mice. We will examine whether MeCP2 and ATRX bind and regulate common genes in brain cells and will study how this could relate to abnormal DNA structure and 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, Oregon Health and Science University, 2 year, \$104,849

Title: Insufficient GABA inhibition underlies respiratory disorders in Mecp2 deficient mice

Lay Summary:

Disturbances in breathing characterized by periodic apneas and an irregular breath-to-breath interval are common and distressing features of Rett syndrome (RTT). Using the mouse model of RTT first described by Adrian Bird we have recently shown that increasing the amount of carbon dioxide in their environment eliminates periodic breathing. Thus the apneas are due to loss of carbon dioxide stimulation. The cause of the increased breathing that leads to low carbon dioxide has not been determined. Increasing the concentration of oxygen worsens periodic breathing, indicating that oxygen-sensing tissues outside the brain are not responsible. It is known that with each inspiration there is concurrent inhibition from γ -aminobutyric acid (GABA) along with excitation. Recent studies in RTT mice found that GABA inhibition in the cardiorespiratory areas of the brainstem is depressed. This allows RTT mice to “overshoot” in inspiration causing their respiratory disturbances. In preliminary studies we have shown that increasing GABA restores breathing in RTT mice to that of normal mice. These studies were done in awake freely moving animals. We now propose to use a reduced animal preparation that will allow us to study the phrenic nerve and individual inspiratory neurons to show the GABA defect and determine how best to correct it. We will also examine an investigational drug (now in phase 2 studies for schizophrenia) that enhances GABA receptors without causing sedation or leading to tolerance. These studies may lead to new treatments for the respiratory disorders in RTT.

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Chang, Qiang, University of Wisconsin, 2 years, \$100,000

Title: Administering novel small molecules that have been shown to specifically activate TrkB in MeCP2 mutant mice to evaluate the therapeutic potential of BDNF

Lay Summary:

Rett syndrome (RTT) is a devastating developmental disorder. The disease is caused by imperfect genetic makeup of the MECP2 gene. Researchers have created mouse models to study how the disruption of the Mecp2 gene lead to RTT like symptoms in mouse. Using such mouse models, we have previously found that losing the Mecp2 gene product in mouse results in insufficient supply of a neurotrophic factor called BDNF (brain-derived neurotrophic factor) in the brain. When we tricked the RTT mice to produce more BDNF, they lived longer and healthier lives. Because the physical properties of BDNF make it very difficult to administer, we plan to test whether we can replace BDNF with some custom designed small chemical compounds that have the same biological properties of BDNF (therefore behaving as functional replacement of BDNF) and still treat the RTT mice effectively. If successful, our research could potentially lead to

therapies for human RTT patients.

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Delaney, Kerry, University of Victoria, 2 years, \$100,000

Title: Systematic Evaluation of Cortical Neuronal Morphology in a Rett Syndrome Mouse Model

Lay Summary:

Molecular biological study of MeCP2 gene expression and function has proceeded rapidly with the cloning of the gene and the development of loss-of-function transgenic mouse lines. Detailed characterization of the physiological, behavioural and neuro-anatomical properties of these mouse models has recently begun but much work remains to maximize their utility to address problems related to finding treatments for RTT. One potentially useful biomarker is the reduced dendritic arborization and somata size that have been reported in a few studies pertaining to a few neuronal types in subregions of the neocortex. These alterations have been shown in human pathological studies in Rett patients (Armstrong et al., 1995; Belichenko et al., 1997). Both supporting and contradictory data exist for similar alterations in Mecp2 mutant mice (Chen et al., 2001; Moretti et al., 2006). Our premise is that evidence for effective reversal or amelioration of the RTT phenotype should be detectable in the form of improved neuronal morphology, quite possibly before clearly improved behavioral performance is seen.

The small number of previous studies that have examined RTT neuronal structure have used Golgi impregnation techniques to reveal single neuron morphology, with analysis limited to a small number of neurons in a few brain areas. While providing detailed cell morphology this technique is not compatible with fluorescence-based immunohistochemistry. We present a proposed series of experiments using a variety of cell staining techniques to examine the dendritic morphology of neurons in several layers of primary motor, somatosensory and visual cortex. We will use mouse lines created by crossing MecP2-null mice with mice expressing YFP in subsets of layer 5 pyramidal neurons that provide Golgi-like staining of individual neuronal branching patterns. We will use single cell injection and retrograde filling via axonal projections to label neurons in ways that allow full reconstruction of their dendritic arbors to compare these structures in mutant male mice to wild-type littermates. Combining these single cell labeling techniques with immunohistochemistry for MeCP2 we will compare the structure of neurons in female brain for which the active X chromosome has a wild-type Mecp2 gene to neighbouring neurons that have the mutant Mecp2 as the active allele. We will test the hypothesis that the phenotype of the individual neuron is the dominant factor affecting the maturation of its dendritic branching. In collaboration with Dr. Samulski's laboratory at UNC we will examine whether adeno-associated viral vector can be effective in replacing Mecp2 genes into mutant neurons in mouse brain in vivo using neuronal branching pattern as a measure of MeCP2 phenotype.

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Galanopoulou, Aristeia, Albert Einstein School of Medicine, 2 years, \$95,504

Title: Identify the chloride cotransporters involved in the premature switch of GABAA signaling to hyperpolarizing in SNC neurons

Lay Summary:

Rett syndrome is associated with early life regression of brain development, motoric dysfunction, seizures, and neurodevelopmental deficits. Neuropathological studies demonstrate profound abnormalities in cortical and subcortical dendritogenesis and neurodegeneration of the dopaminergic neurons of the substantia nigra pars compacta (SNc). In preliminary studies, I have found that in the SNc of neonatal mice with MeCP2 mutations, there is absence of the normal depolarizing GABAA signaling, which can deprive their brain from an important neurotrophic signaling pathway and can potentially predispose and contribute to the above pathologies. In this proposal, I plan to study whether interventions that aim to correct the abnormal GABAA signaling in Rett null mice can be used as new therapeutic interventions to restore normal function and development.

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Ravine, David, University of Western Australia, 2 years, \$80,000

Title: Role of Methyl CpG binding protein 2 (MeCP2) in microtubule dynamics

Lay Summary:

We have preliminary data indicating that mutations known to cause Rett syndrome impair normal microtubule function. We have found that the functional defect is aggravated by a microtubule destabilising drug and improved by a microtubule stabilizing drug. We aim to characterise the mechanism underlying these observations, which offer the possibility of rational therapy for this group of neurodevelopmental disorders.

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Yasui, Dag, University of California - Davis, 2 years, \$78,699

Title: The role of MeCP2e1 in brain development and function

Lay Summary:

MeCP2e1 is the primary MeCP2 protein isoform found in the brain and is also likely the ancestral form in vertebrate animals. Mutations in MECP2 exon 1 are associated with Rett syndrome and mental retardation. Therefore we want to determine the normal function of MeCP2e1 in the brain. To do this we propose creating a mouse where the expression of MeCP2e1 protein is blocked. These mice will allow us to look for gene expression and behavioral defects caused by loss of MeCP2e1 by comparing them with their normal siblings. This MeCPe1 genetic model is expected to help understand the defects underlying Rett syndrome and other human neurologic disorders.

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Post Doctoral Fellowship Awards

Blanchi, Bruno

Calfa, Gaston

Durand, Severine

Gonzalez, Michael

Han, Jing

Li, Yong

Lin, Ying

Blanchi, Bruno, University of California - Los Angeles, 2 years, \$100,000

Title: Unraveling Rett Syndrome Molecular Mechanisms using Gene and MicroRNA Expression Analysis and Promoter Tilling Array on Phenotypic MeCP2 Knockdown Human Neurons Derived from Human Embryonic Stem Cells

Lay Summary:

Rett syndrome is a devastating neuro-developmental disorder, but recent studies that have shown that neurological defects can be reversed in animal models by re-expressing MeCP2 are very promising since they suggest that normal

neuronal function could be restored in RTT patients. However, direct use of MeCP2 as a therapeutic agent might be difficult, since excessive expression of MeCP2 is also detrimental for neuronal function. Our approach aims to characterize the direct effectors responsible for the neurological defects of RTT, downstream of MeCP2, which could become valuable therapeutic targets.

Using human embryonic stem cells and specific inhibition of MeCP2 expression, we have generated cultures of human neurons largely deficient in MeCP2. Interestingly, these cultured neurons can form synapses, but their spontaneous synaptic activity is altered with an imbalance of the ratio between excitatory and inhibitory activity as compared to control human neurons. We propose to analyze gene expression in order to identify effectors of the synaptic function which are abnormally expressed in MeCP2 deficient neurons. We will also determine whether their expression is directly regulated by MeCP2, or whether their mis-regulation is an indirect consequence of MeCP2 deficiency. Finally, we will manipulate the expression of the most relevant candidates, in cultured human neurons, in order to determine whether they can reverse the synaptic defects caused by MeCP2 deficiency. Ultimately, we aim to characterize the cascade of molecular events responsible for the synaptic defects of our hESC-derived MeCP2 deficient neurons, and therefore potentially involved in the neurological defects of RTT, and identify therapeutic targets for the treatment of the disease.

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Calfa, Gaston, University of Alabama - Birmingham, 2 years, \$100,000

Title: Hippocampal network excitability in MeCP2 deficient mice; a voltage sensitive dye study

Lay Summary:

Major features of epilepsy disorders are spontaneous and periodic seizures, a recurrent clinical manifestation highly prevalent (80%) in RTT patients that significantly affect their quality of life and that of their caretakers. The proposed studies will investigate the cellular basis of these recurrent seizures using a well-established mouse model of RTT. Using Mecp2 deficient mice and state-of-the-art imaging techniques to measure neuronal and synaptic activity, we have observed hyperexcitable neuronal networks in one of the most seizure prone regions of the brain, the hippocampus. We will test whether this hyperexcitability originates from too much synaptic excitation, too little synaptic inhibition, or a combination of both. We will also test whether the well-known antiepileptic actions of adenosine reduce hippocampal hyperexcitability in Mecp2 null slices, thus providing support for a potential therapeutic strategy to reduce seizures in RTT patients.

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Durand, Severine, Children's Hospital, Boston, 2 years, \$99,450

Title: Role of MeCP2 in experience-dependent development and plasticity of cortical circuits

Lay Summary:

The complexity of Autism-like disorders may lie in the formation of excitatory/inhibitory circuit balance during critical periods of development (Rubenstein and Merzenich, 2003). In the present grant, we propose to test the hypothesis that neuronal activity driven by sensory experience creates an optimal excitatory/inhibitory circuit balance during critical periods of heightened cortical plasticity in infancy. Circuit disruption leads to the complex behavioral phenotype of neurodevelopmental disorders such as Rett Syndrome (RTT). By probing systems physiology, we aim to inquire about the functional development of cortical circuits in animal models of RTT. Direct manipulation of excitatory/inhibitory circuit balance will be used to rescue the cortical impairments observed in these mice. The results will provide potential new therapeutic strategies for reactivating brain plasticity in animal models of Rett Syndrome.

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Gonzalez, Michael, University of California - Davis, 2 years, \$100,000

Title: Identification and Characterization of MeCP2 Post Translational Modifications

Lay Summary:

It is clear that mutations in the gene encoding the MeCP2 protein are the major cause of the neurodevelopmental disorder Rett Syndrome and that these mutations lead to impaired neuronal development. However, it is not clear exactly how these mutations hinder neuronal development and lead to Rett syndrome. Likewise it is unclear exactly how normal MeCP2 function(s) in the brain. Determining the mechanisms by which MeCP2 is functioning in normal brains will allow for a deeper understanding of how MeCP2 mutations are contributing to the pathogenesis of Rett Syndrome. This fellowship proposal attempts to determine the mechanism(s) of MeCP2 function by studying the effects of MeCP2 post translational modifications. Post translational modifications are molecular switches for proteins. They can be quickly added and removed from proteins and allow for very quick responses to the environment. Among other functions, cells use them to turn the activities of proteins on and off, to direct proteins to specific sites within cells, and to regulate interactions between two proteins. MeCP2 appears to contain a large number of these modifications. Identifying these modifications and determining their effects on MeCP2 will allow for a more detailed understanding of the mechanisms by which MeCP2 functions. Understanding MeCP2 post-translational modifications may also be important for developing effective Rett Syndrome therapies. While deficiencies in functional MeCP2 cause Rett Syndrome, the presence of excessive amounts of MeCP2 in the brain causes a different series of neurological defects. Therefore it is clear that any attempts to alleviate the symptoms using gene therapy approaches will have to exactly balance the levels of MeCP2. However, in addition to overall levels of MeCP2 it may also be important to consider the amount and types of

modifications of the MeCP2 used in these therapies. It may be that modified forms of MeCP2 will be more effective at rescuing the Rett phenotype and/or less likely to cause adverse effects when expressed at levels beyond normal MeCP2. Overall the study of MeCP2 post-translational modifications will be important both for the understanding of how MeCP2 is contributing to the pathogenesis of Rett Syndrome as well as for the implementation of potential gene therapy techniques.

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Han, Jing, Baylor College of Medicine, 2 years, \$100,000

Title: Determine
the pathogenic mechanisms of MeCP2 mutations on development and
function of the inhibitory circuits in RTT mouse models

Lay Summary:

Rett syndrome (RTT) is postnatal neurodevelopmental disorder affecting mostly females. It is caused by mutations in MECP2, a modulator of gene expression. RTT girls appear normal during the first 6 to 18 months of life then fall into developmental stagnation. The progressive clinical phenotypes of RTT include small brain size, growth and mental retardation, loss of hand use skills, speech and social interaction deficits, hand stereotypies, motor abnormalities, seizures, respiratory abnormalities, anxiety, parkinsonian features and autonomic abnormalities. How do MECP2 mutations cause so many behavioral impairments in RTT? We believe that MeCP2 plays a role in particular brain regions or cell types. Our gene expression study and behavior analysis of MeCP2 mutant mice indicated that one brain region, the hypothalamus, plays an important role in RTT pathogenesis. What is the role of hypothalamus in RTT? In my proposed work, I will apply two approaches to answer this question: 1) The mammalian hypothalamus has a dominant influence on behavior, as it serves as the control center of the body, regulating its stable state of equilibrium with regard to sleep, mood, social function, stress response, and gut motility. We will remove MeCP2 from almost all the important parts of the hypothalamus and analyze the behavioral and physiological changes of the mice. 2) The hypothalamus generates many signal molecules, called neuropeptides, to regulate and coordinate the activity of the central nervous and peripheral nervous systems. Dysfunction of MeCP2 in mice causes gene expression changes of these neuropeptides, e.g. RFRP, a hypothalamic neuropeptide, is up-regulated in the MeCP2 mutant mice. To study the function of RFRP in RTT, I will genetically normalize the altered function caused by increased RFRP levels to seek a potential rescue of some RTT phenotypes. These experiments will help us gain a deeper understanding of the origin of RTT phenotypes and provide an inroad to new therapeutic avenues.

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Li, Yong, University of Alabama - Birmingham, 2 years, \$100,000

Title: The consequences of genetic deletion on MeCP2 in BDNF-induced membrane currents in hippocampal neurons

Lay Summary:

Rett syndrome (RTT) is a X-linked-dominant disorder caused by loss-of-function mutations in the gene encoding methyl-CpG-binding protein-2 (MECP2), a transcriptional repressor that binds to methylated DNA. It is unclear how MECP2 mutations cause the nervous system dysfunction in RTT, and no effective treatments for RTT are available. The gene encoding for brain-derived neurotrophic factor (Bdnf) was identified as a target of MECP2. The major goal of this proposal is to understand the role of endogenous BDNF modulation of activity-dependent neuronal and synaptic functions. By recording a membrane current in hippocampal CA3 pyramidal neurons that reflects the release of endogenous (native) BDNF from presynaptic mossy fiber terminals, these experiments will address the apparent discrepancy between the total BDNF levels versus the releasable BDNF content in a mouse model lacking *Mecp2*, a transcriptional repressor of the *Bdnf* gene. Thus, these experiments will reveal the consequences of *Mecp2* deletion for BDNF function, uncovering fundamental brain mechanisms and evaluating a potential therapeutic strategy relevant to Rett syndrome.

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Lin, Ying, Cold Spring Harbor Laboratory, 2 years, \$100,000

Title: Determine the pathogenic mechanisms of MeCP2 mutations on development and function of the inhibitory circuits in RTT mouse models

Lay Summary:

Rett Syndrome (RTT) patients usually achieve developmental milestones until 6-18 months old and then start to regress and display cognitive and motor deficits, autistic features and seizures. It is hypothesized that RTT results from inappropriate neuronal connectivity and communication, possibly through abnormal experience-dependent maturation, refinement, and maintenance of neural circuits. RTT is primarily caused by mutations in the X chromosome-linked gene encoding a transcriptional repressor methyl-CpG-binding protein 2 (MeCP2). One of the target genes of MeCP2 is *Gad67*, which encodes the rate-limiting enzyme for GABA synthesis and has been shown to play an important role in maturation and function of inhibitory neural circuits. Therefore, we hypothesize that aberrant expression of *Gad67* by

MeCP2 mutation affects inhibitory transmission and the maturation and plasticity of inhibitory circuits and underlie the pathologies observed in RTT. First of all, we will visualize and characterize where, when and how MeCP2 mutations influence Gad67 expression with cellular resolution in “Gad67-d2GFP” reporter mice. Then we will examine the precise morphological and physiological consequences of MeCP2 mutations on inhibitory neurons and circuits. The findings from the proposed studies will not only have implications in the pathogenic mechanisms of RTT, but will also aid in the advancement of diagnosis and treatment of this disease.

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