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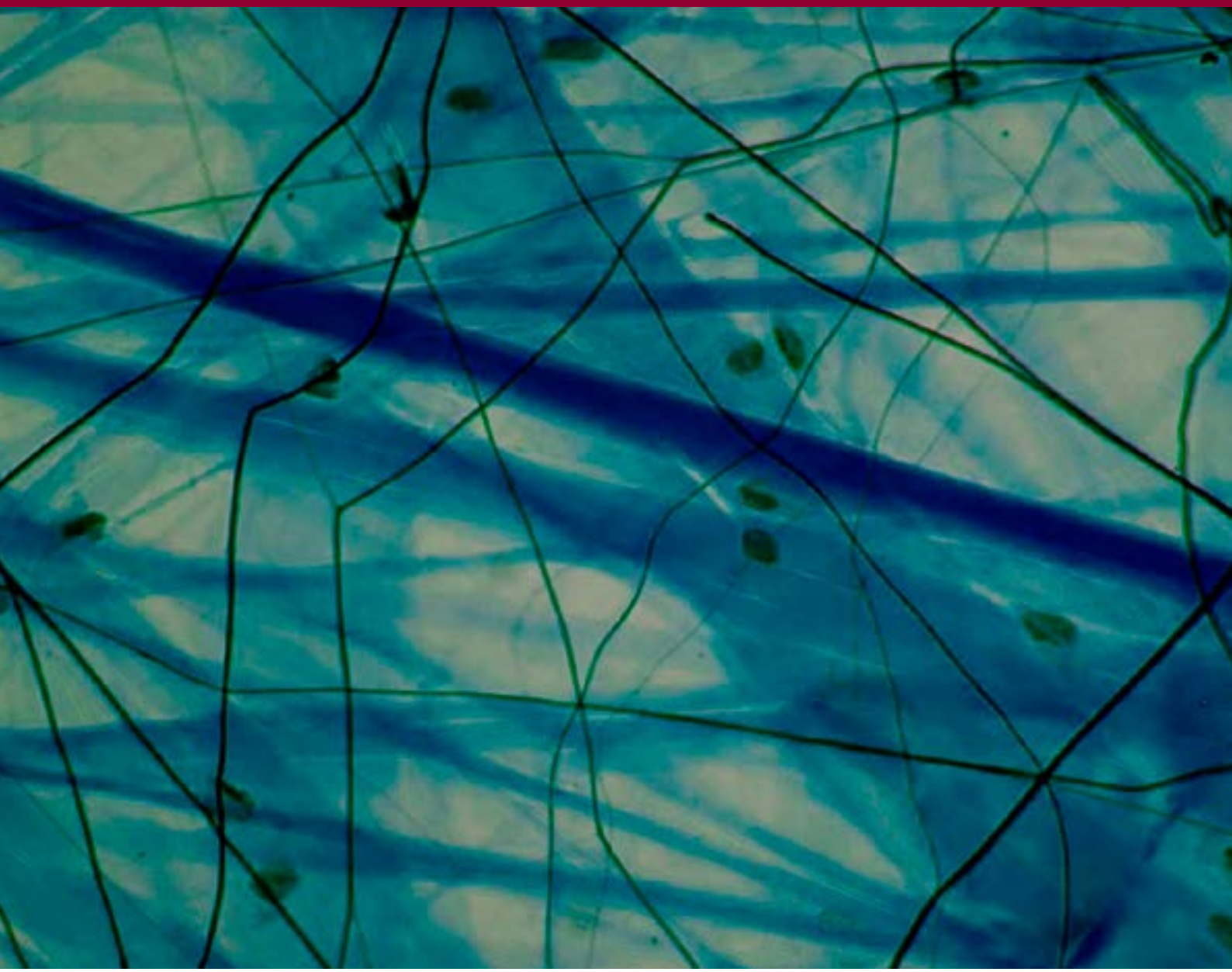

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RETT SYNDROME AND ENVIRONMENTAL ENRICHMENT AS A POTENTIAL THERAPY FOR ATTENUATING THE PATHOLOGY.

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ABSTRACT

Rett syndrome (RTT) is a neurological disorder affecting the development of the central nervous system and one of the leading causes of mental retardation among young women. RTT patients exhibit microcephaly, decreased neuronal size, shorter cortical dendrite, and a reduced dendritic spine density; evidence strongly suggesting that a synaptic disorder underlies the neurological RTT-associated phenotype. MECP2 is a transcription factor with multiple roles on gene expression, and mutations in its gene coding sequence have been identified as the major cause of RTT. The generation of transgenic mouse models lacking the expression of Mecp2 has allowed getting insight into the physiopathological events associated with the loss of a fully functional Mecp2 allele in RTT patients and it has been demonstrated that is possible to partially rescue, or reverse, the phenotype associated with RTT which opens a window to explore therapeutic approaches plausible to be utilized in RTT patients. Considering that RTT patients exhibit reduced neuronal plasticity and synaptic disorder, this mini-review is focused on studies demonstrating the positive effect of an enriched environment on the RTT-like phenotype exhibited by mouse models of the disease.

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Rett syndrome

Rett syndrome (RTT, OMIN #312750) is a neurological disorder affecting the development of the central nervous system [1]. This severe neuropathology was first described by the Austrian pediatrician Andreas Rett, who described a cohort of 20 female patients with similar neurological symptoms in 1966 [2]. Later in 1983, the Swedish pediatrician Bengt Hagberg described the neurological symptoms of another cohort of young females and recognized the manuscript published by Andreas Rett in a German medical journal naming the described pathology as RTT [3].

RTT has an incidence of 1 in 10,000-15,000 female birth and is one of the leading causes of mental retardation among young women [4]. Normal neurodevelopment characterizes the phenotype exhibited by RTT patients during the first 6-18 months of age, which is followed by a stagnation period accompanied by microcephaly, growth arrest, and hypotonia. The stagnation period is later followed by a development regression and the loss of most of the previously acquired skills and the onset of symptoms. In spite of the wide range of phenotypes characterizing this neurological disorder, there are common severe symptoms exhibited by RTT patients such as motor abnormalities that prevent walking, lack of adequate language, hand stereotypes movements, loss of hand skill, respiratory abnormalities, and scoliosis [1,4–6]. In patients exhibiting a mild phenotype, the onset of symptoms is usually later, and it is possible to observe the maintenance of walking, purposeful use of hand, autonomy to feed themselves, and some language [7].

RTT patients exhibit microcephaly, decreased neuronal size and shorter cortical dendrites [8], besides a reduced dendritic spine density in hippocampal neurons [9]; evidence strongly suggesting that a synaptic disorder underlies the neurological RTT-associated phenotype [10].

Mutation in Methyl CpG Binding Protein-2 coding gene

RTT patients are almost exclusively girls; the reason why this progressive neurological disorder was associated with the X chromosome; specifically, mapping studies demonstrated that this neuropathology was linked with the locus Xq28 [11–13]. Later, in the previously identified locus of both sporadic and familial RTT patients, Amir et al. identified mutations in the gene coding for the Methyl CpG Binding Protein-2 (*MECP2*) through a systematic mutational analysis [5]. MECP2 is a multifunctional protein described by Adrian Bird´s Lab and widely known for its initially described function, binds to methylated cytosine of CpG dinucleotide, and through the interaction with HDAC/SIN3a protein complex compacts the chromatin and silences the expression of its target genes [14]. It has been described that MECP2 regulates mRNA splicing [15], and it was later demonstrated that MECP2 is also able to activate the expression of some of its target genes through the interaction with CREB1, a transcription factor associated with the neuronal activity [16]. MECP2 is encoded by a four exon gene that, through alternative splicing, originates two spliced isoforms of 498 and 486 amino acid proteins identified as Mecp2-e1 and Mecp2-e2, respectively [17,18]. Mecp2-e1 is the most abundant of the isoforms in the central nervous system and it shares 477 amino acids with Mecp2-e2, but they differ in few amino acids in the amino-terminal

[19,20]. MECP2 has several functional domains, among them, a methyl-CpG-binding domain (MBD) and a transcriptional repressor domain (TRD) [21,22].

The identification of *MECP2* mutations as the main cause of RTT allowed the generation of transgenic mouse models to get insight into the physiopathological events associated with the loss of a fully functional Mecp2 allele. The first models generated lacked the MBD and TRD encoded by exons 3 and 4, respectively [23], and half of the MBD encoded by exon 3 [24]. Since the genetic similitudes between mouse and humans, both mouse models generated, and also others generated later, exhibit most of the phenotypes exhibited by RTT patients and have been widely used to study RTT. One of the most important advances achieved through the use of RTT mouse models was the finding that by conditionally re-expressing *Mecp2* in adult mice is possible to partially rescue or reverse the phenotype associated with RTT [25,26]. These findings opened a window to explore therapeutic approaches plausible to be utilized in RTT patients to ameliorate the symptoms and improve patients quality of life.

Environmental enrichment attenuates RTT-like phenotype.

In spite of all the efforts made by the scientific and clinical community, up to date, there is no cure for RTT. During the last years, several clinical studies aimed to attenuates RTT phenotype have been started ; although some of them seem to be promising, there is still no approved pharmacological therapy for RTT patients. However, as an alternative, there are some non-pharmacological approaches proved to be able to attenuate RTT phenotype progression. Considering that RTT patients exhibit reduced neuronal plasticity and synaptic disorder as mentioned above, several studies have been focusing on demonstrating the positive effect of an enriched environment (EE) on the RTT-like phenotype exhibited by a mouse model of the disease. It is worthy to note that EE is a paradigm widely used to increased synaptic plasticity in models of neurological disorders and it consists of a combination of social and inanimate stimuli to increase neuronal plasticity. It usually includes a free-running wheel and different materials for bedding, which as a whole, promote locomotor activity and increase cognitive processes. Besides, inanimate stimuli are periodically changed to increase novelty and maintain motivation for exploring the new environment and thus locomotion [27,28].

Kondo et al. demonstrated that the permanent exposure of 4 week-old Mecp2tm1Tam RTT mouse model [29] in a mostly C57BL6 genetic background for 6 weeks to EE improved motor coordination in both hemizygotes males and heterozygotes female, and in male, it was associated with an increased expression of brain-derived neurotrophic factor (BDNF) in the cerebellum [30]. BDNF is an important neurotrophin whose expression is decreased in RTT patients and mouse models of the disease and it has been described as one of the main targets of MECP2 [31,32]. Later, Nag et al. described that exposure of 3 week-old $Mecp2^{1lox}$ RTT mouse model for a shorter period also attenuates the onset of locomotor phenotype, a result that was also associated with a reduction of brain ventricles volume [33]. To get insight into the mechanism through which EE improves RTT-like phenotype, Kerr et al. housed $Mecp2^{bird}$ RTT mouse model in a homogeneous genetic background C57BL/6J.129Svj to EE

cages for 2 weeks since weaning, and they described that this period was enough to prevent the RTT locomotor phenotype at 7 weeks of age. Besides, they also described that this beneficial effect of EE exposure was not related to an increase in neuronal plasticity-associated genes, as it was expected [34], but it was related to an increased synaptic plasticity and synapsis formation in B6.129SF1-Mecp2^{tm1Jae} RTT mouse model housed in EE conditions since 10 days of age for 50 days [35].

More recently, Torres et al. described that one of the mechanisms through which the EE exposure attenuates the RTT-like phenotype is by driving the expression of *Ryr3* [36], an intracellular calcium released channel expressed in the forebrain and its increased expression in response to environmental stimulation is essential for memory formation [37]. Interestingly, in RTT patients an EE intervention improved gross motor skills and increased BDNF levels in blood, supporting the use of this paradigm of environmental stimulation for clinical purposes [38].

The complexity of spine dysgenesis exhibited by neurons of both RTT patients and mouse models, strongly suggest that Mecp2 is required for the proper dendritic spine formation during the early postnatal neurodevelopment, but later a compensatoty mechanism start driving spinogenesis as suggested by Xu et al. [39]. The studies presented in this mini-review allow us to propose that this compensatory mechanism is activated by environmental stimuli. Besides, they demonstrate the beneficial effect of EE exposure on the RTT-like phenotype, giving some light about the mechanism underlying this effect. However, some questions remain open, such as the Mecp2-independent transcriptional mechanisms through which the RTT-like phynotepe is attenuated in mouse models of the disease after being exposed to EE. Besides, these results allow us to gain a deeper insight into the physiopathology of RTT and increase our understanding about the transcriptional mechanism underlying neuronal response to environmental stimuli, not only in the context of a neurological disorder but also in physiological conditions.

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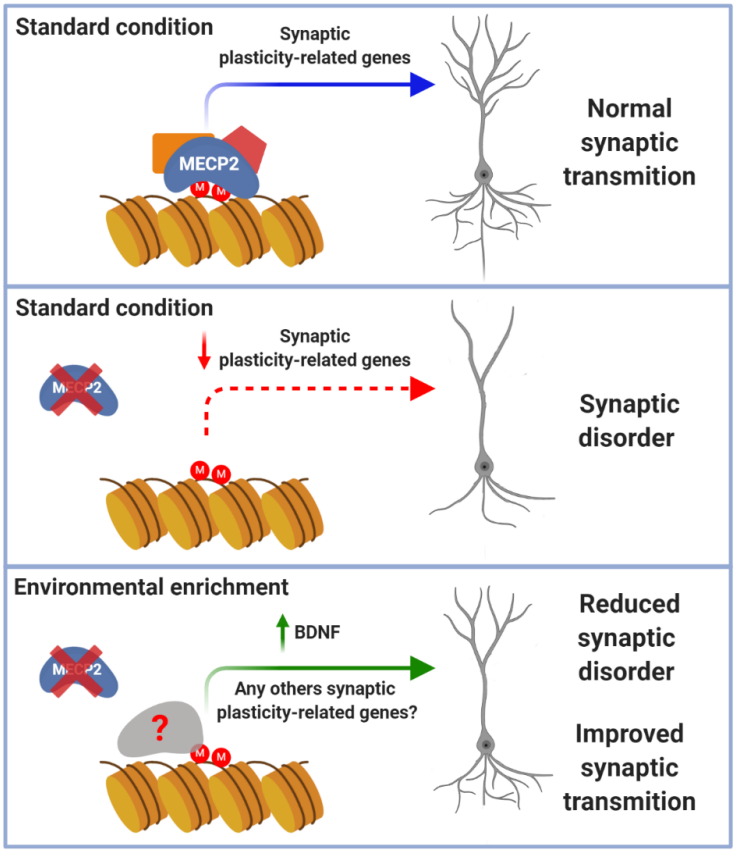

Figure 1. Environmental enrichment attenuates Rett syndrome-like phenotype. Under standard conditions, the presence of MECP2 allows the normal expression of synaptic plasticity-related genes and thus synaptic transmission. The lack of MECP2 causes the deregulation of gene expression underlying the synaptic disorder observed in RTT patients and RTT mouse models. In spite of lacking the expression of Mecp2, the exposure to environmental enrichment allows an increase in BDNF, reducing the synaptic disorder and improving synaptic transmission, probably through the expression of other still unknown synaptic plasticity-related genes. MECP2: Methyl CpG Binding Protein-2. BDNF: Brain-derived neurotrophic factor. Created by Dr. Pablo Tapia with BioRender.com.

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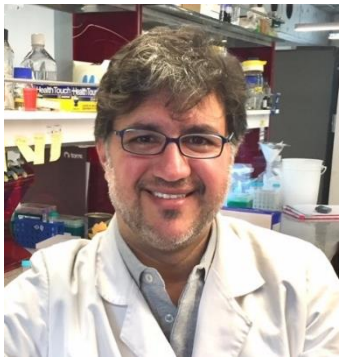
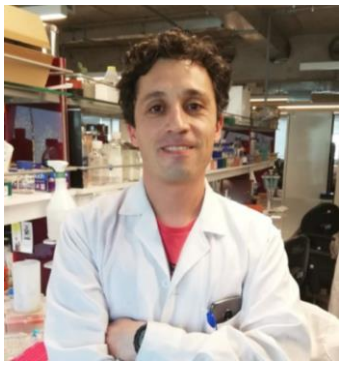


Dr. Bredford Kerr received his Ph.D. in Physiological Science from the Pontifical Catholic University of Chile. He was Fogarty/NIH fellow for postdoctoral training at the Oregon National Primate Research Center in Portland, USA, under the mentoring of Dr. Sergio Ojeda. After three years of training, he went back to Chile to join Dr. Juan Young´s lab for a second postdoctoral training at the Centro de Estudios Científicos-CECs in Valdivia; institution in which he subsequently formed his working group under a tenure track position. Dr. Kerr has currently an Associated Professor at Centro de Biología Celular y Biomedicina-Universidad San Sebastián in Santiago, Chile, where head the lab of Neuroendocrinology and Epigenetics. Besides, Dr. Kerr is former President of the Chilean Society of Physiological Science and a member of the Executive Council of Pan American Neuroendocrine Society.

The research of his lab is aimed to elucidate the role of gene-environment interaction in commanding neurological processes in areas of the central nervous system that maintain high levels of neural plasticity and for which cellular communication is essential such as body weight control and endocrine regulation of energy homeostasis. His lab is also involved in unveiling the mechanism through which DNA methylation and miRNA underlie the neuronal function of highly plastic brain areas, whose importance is evident in Rett syndrome, an epigenetic based neurological disorder. The work done at Dr. Kerr´s Lab is based on integrative results obtained by using techniques of cell / molecular biology, physiology, behavioral tests, murine models of epigenetic disruption and the generation of genetically modified mice to conditionally change gene expression.



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