13 th Summer school of Neuroscience
Cognition, the target Catania 11-17 Luglio 2015

Lessons from preclinical research to counteract neuro-behavioural and cognitive deficits in Rett syndrome, a complex neurodevelopmental disorder

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OVERVIEW

- Rett syndrome: brief history, temporal profile, clinical features
- MECP2 gene
- several mouse models available: the study of the neurobehavioural phenotype; what we know about cognitive performance in Rett mouse models?
- from neonatal motor to cognitive deficits: why mouse models can be useful in neurodevelopmental disorder contexts
- translational perspective: ongoing clinical trials and the attempts to raise the standards for preclinical research

Rett syndrome is 50-year-old

Andreas Rett (1924-1997)

1965

1966 Rett *Wien Med Wochenschr* (in German) N= 22;

1983 Habgerg et al. *Annals of Neurology* (in English) N=36;

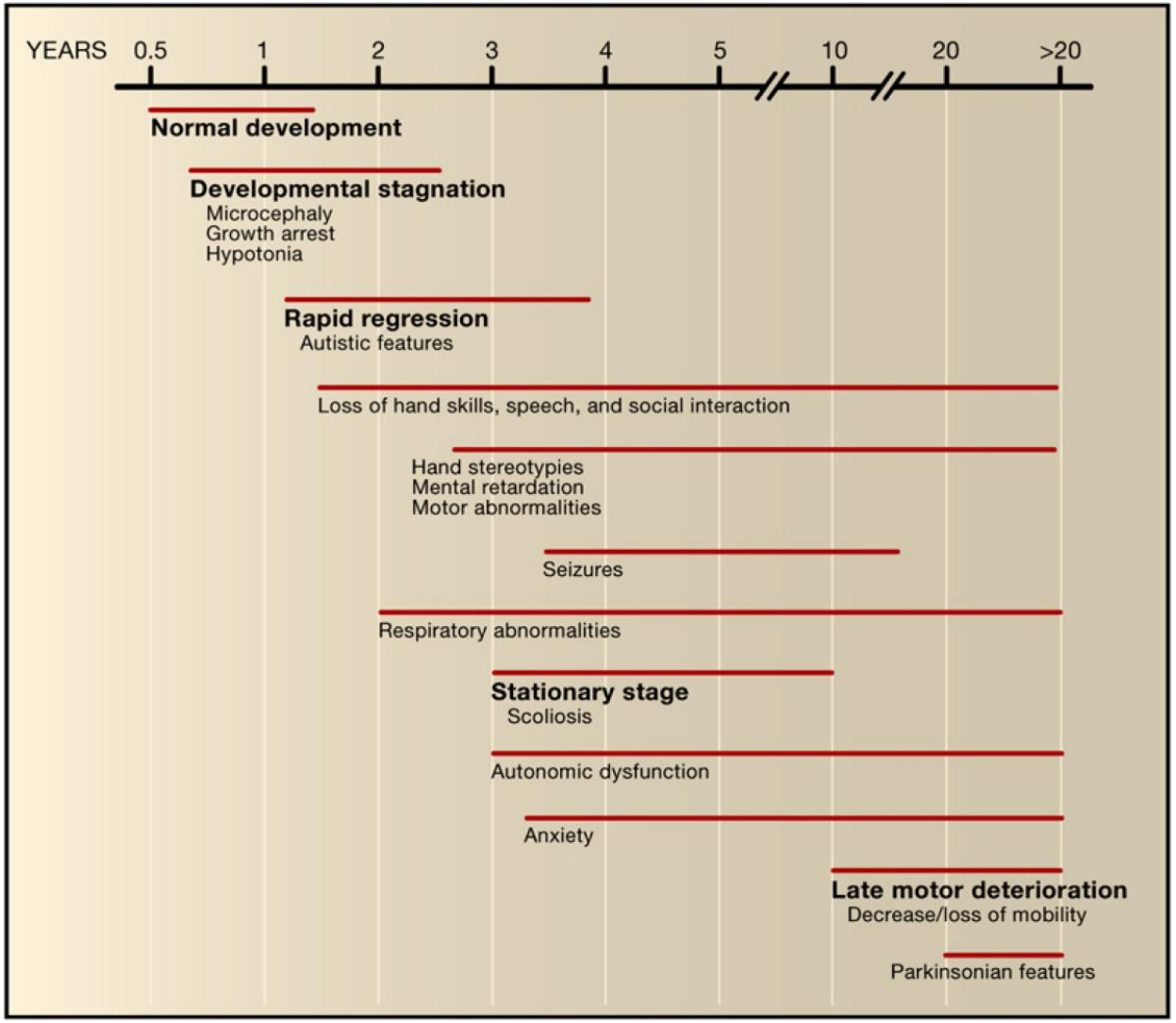
1999 Amir et al. *Nature Genetics,* N=9;

2015 about 2800 papers on Pubmed

Rett syndrome: temporal profile and clinical features

- Rare X-linked neurodevelopmental disorder, primarily affecting girls (1:10000/15000 female births).

- Peculiar temporal profile:
  - normal pregnancy, normal delivery, so-called normal psychomotor development for the first 6/18 months of life;
  - plateau or developmental stagnation
  - rapid regression (loss of purposeful hand movements;gait dyspraxia;loss of acquired cognitive and social skills;breathing irregularities;autistic-like features); additional features may also include: anxiety, seizures, growth failure, sleep disturbances.
  - stationary stage: established deficits persists through adulthood.



from Charour and Zoghbi *Neuron* 2007

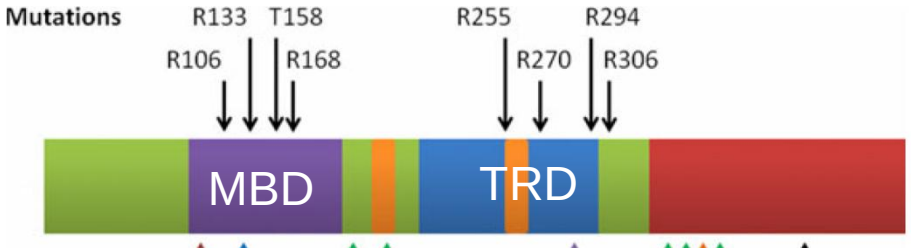
A great enthusiasm: Rett is a monogenic disorder (almost)

letter

Rett syndrome is caused by mutations in X-linked *MECP2*, encoding methyl-CpG-binding protein 2

Ruthie E. Amir[1], Ignatia B. Van den Veyver[2,3], Mimi Wan[5], Charles Q. Tran[2], Uta Francke[5,6] & Huda Y. Zoghbi[1,2,4]

Rett syndrome[1] (RTT, MIM 312750) is a progressive neurodevelopmental disorder and one of the most common causes of mental retardation in females, with an incidence of 1 in 10,000–15,000 (ref. 2). Patients with classic RTT appear to develop normally until 6–18 months of age, then gradually lose speech and purposeful hand use, and develop microcephaly, seizures, autism, ataxia, intermittent hyperventilation and stereotypic hand movements[3]. After initial regression, the condition stabilizes and patients usually survive into adulthood. As RTT occurs almost exclusively in females, it has been proposed that RTT is caused by an X-linked dominant mutation with lethality in hemizygous males[3–8]. Previous exclusion mapping studies using RTT families mapped the locus to Xq28 (refs 6,7,9–11). Using a systematic gene screening approach, we have identified mutations in the gene (*MECP2*) encoding X-linked methyl-CpG-binding protein 2 (MeCP2) as the cause of some cases of RTT. MeCP2 selectively binds CpG dinucleotides in the mammalian genome and mediates transcriptional patterns, but recently excluded these genes[14,15]. We then analysed the gene encoding methyl-CpG binding protein 2 (*MECP2*), which maps to Xq28 between *L1CAM* and the *RCP/GCP* loci and undergoes X inactivation[16]. MeCP2 is an abundant chromosome-binding protein that selectively binds 5-methyl cytosine residues in symmetrically positioned CpG dinucleotides in mammalian genomes[17]. These residues are preferentially located in the promoter regions of genes that are subject to transcriptional silencing after DNA methylation. MeCP2 contains two functional domains, an 85 amino acid methyl-CpG binding domain (MBD), essential for its binding to 5-methylcytosine[18], and a 104 amino acid transcriptional repression domain (TRD) that interacts with histone deacetylase and the transcriptional corepressor SIN3A. Interactions between this transcription repressor complex and chromatin-bound MeCP2 leads to deacetylation of core histones, which in turn leads to transcriptional repression[12,13]. This complex also can inhibit transcription from a promoter at a distance[13].



About 95% of individuals with Rett carry a mutation in the gene encoding the nuclear methyl-CpG-binding protein 2, located in the chromosome Xq28.

To date more than 200 mutations have been identified in MECP2, but 8 mutations account for approximately 60% of Rett cases.

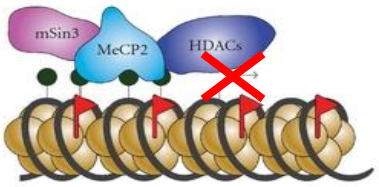
.

Most cases are de novo mutations in the paternal X chromosome, thus individuals with Rett syndrome are females who, due to X-chromosome inactivation, are somatic mosaic for normal and mutant MeCP2.

The clinical picture produced by a MECP2 mutation can be influenced considerably by X-inactivation patterns and degree of MeCP2 dysfunction conferred by the specific mutation.

MeCP2 functions are more complex than expected (1)

Initially, MeCP2 was considered as a transcriptional repressor of methylated promoters. Due to the association of highly methylated gene promoters with transcriptional silencing, it was assumed that *MECP2* mutation led to RTT because of a dysregulation of gene silencing.



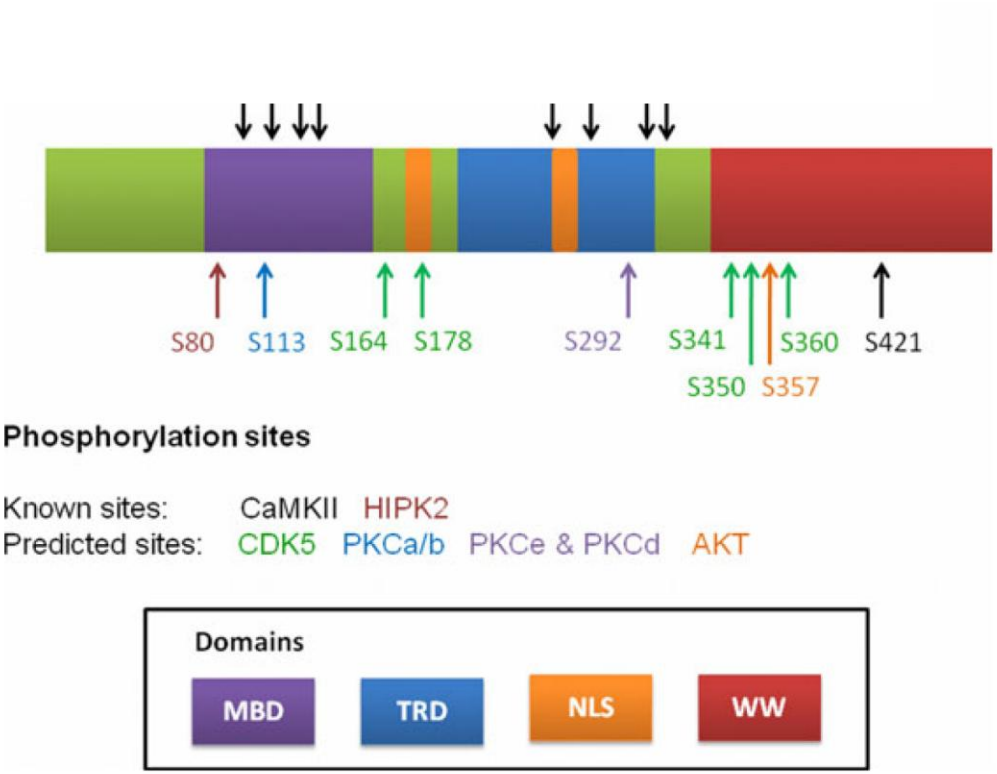
Transcriptional repression

It is now clear that MeCP2 plays an important role in activation of expression as well.

MeCP2 also binds throughout the genome, including intergenic regions thus regulating chromatin structure and long-range chromatin interactions

MeCP2 functions are more complex than expected (2)

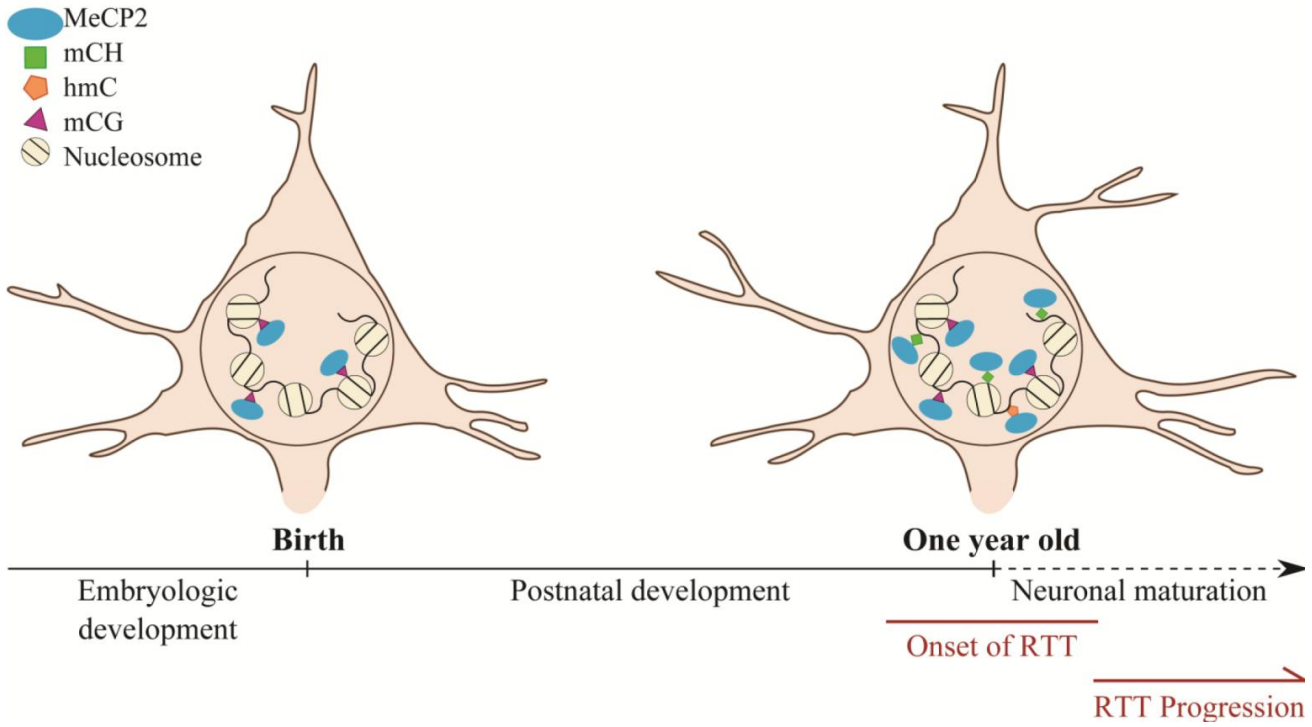
Post-translational modifications, such as phosphorylation, are a potential mechanism to provide localized functional specificity (activator or repressor) to the widely distributed MeCP2.



MeCP2 functions are more complex than expected (3)

Originally characterised as having high affinity for methylated **mCG**, MeCP2 binds also **mCH** (H=A, C or T) and hydroxymethylcytosine **hmC**:

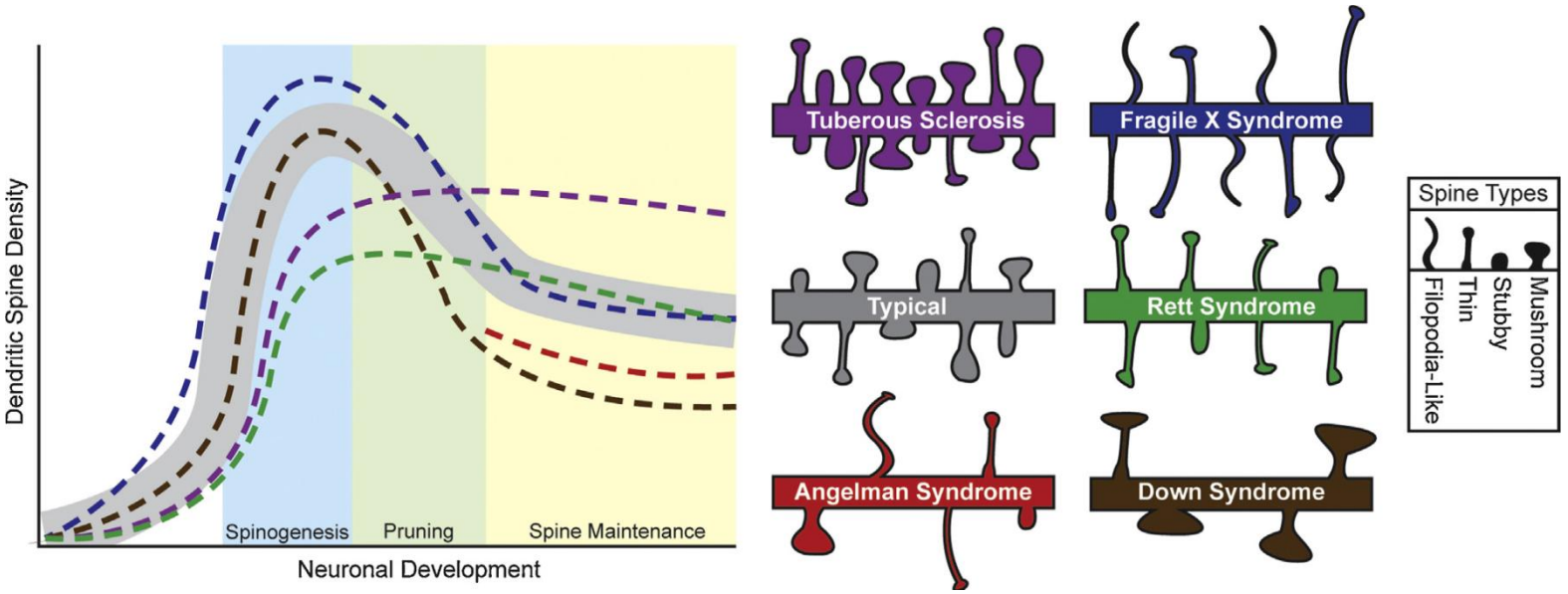
- **mCH** and **hmC** are **enriched** in mouse and human **brain** samples;
- **mCH** and **hmC** **accumulate postnatally** during neuronal maturation.



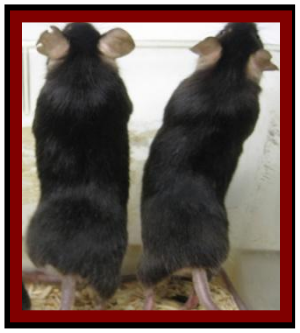
from Pohodich and Zoghbi *HMG* 2015

Dendritic spine dysgenesis in Rett syndrome

Neuropathology: neurones are smaller in size, density of dendritic spines is reduced in ctx and hipp



From Phillips & Pozzo-Miller, 2015



Rett mouse models recapitulate many of the phenotypes associated with Rett syndrome (*e.g.* motor and coordination impairments, altered emotional profile, respiration abnormalities)

Costitutive KO

MeCP2Bird
(Guy et al. 2001)

MeCP2Jae
(Chen et al. 2001)

MeCP2Tam
(Pelka et al. 2006)

Conditional KO

Forebrain knockout
(Chen et al. 2001)

CNS knockout
(Chen et al. 2001)

Hypothalamus knockout
(Fyffe et al., 2008)

GABAergic neurons knockout
(Chao et al., 2010)

Astrocytes
(Lioy et al., 2011)

Truncated

MeCP2-308 (*)
(Shahbazian et al. 2002)

- later onset of symptoms;
[10 weeks of age (Shahbazian *et al.* 2002)]

- longer lifespan than the null mutants

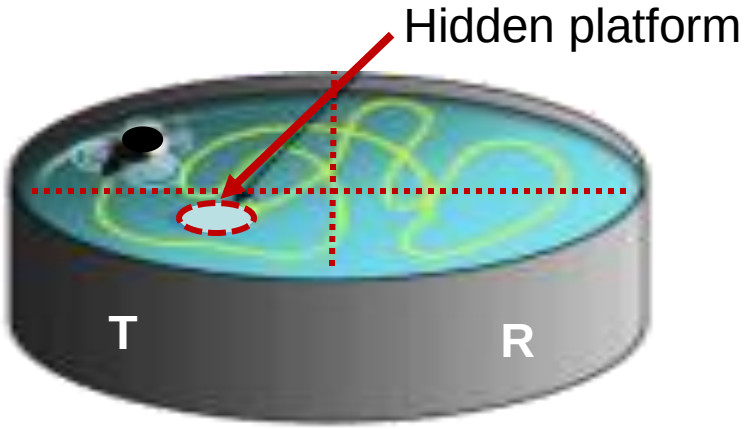
- motor and cognitive **impairments** (Moretti et al. 2006)

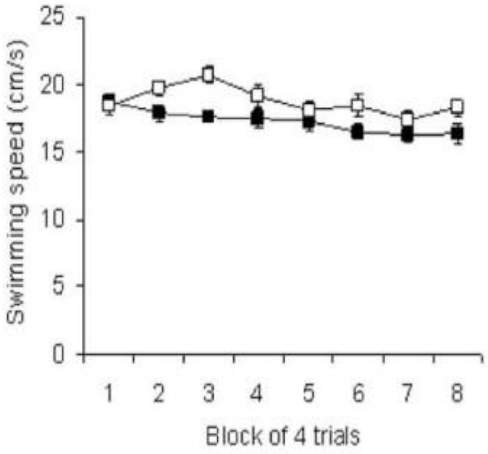
. *MeCP2-168*
(Lawson-Yuen et al. 2007)

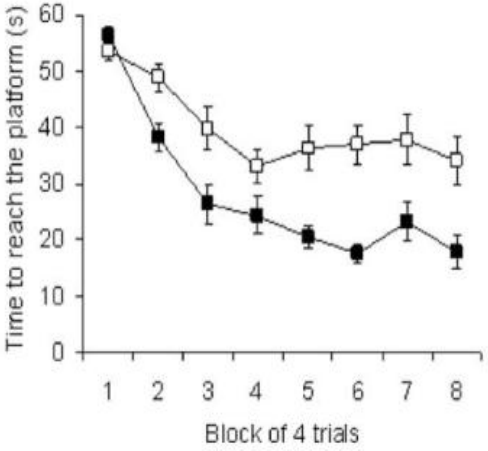
R255X mice
(Pitcher et al. 2015)

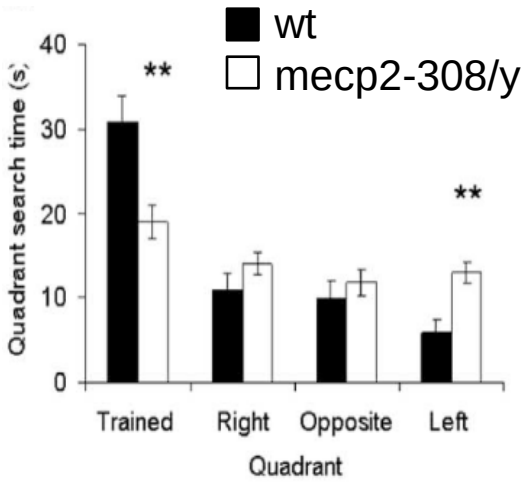
Neurobehavioural characterization of Mecp2-308 mice (1)

Evidence of **spatial learning and memory deficits** in Mecp2-308 male mice using the Morris water maze paradigm





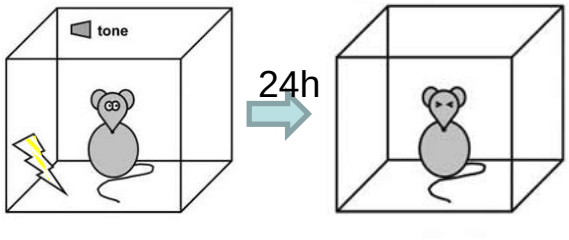




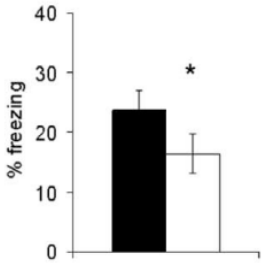
From *Moretti et al. 2006*

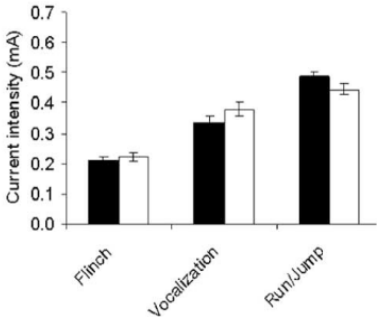
Neurobehavioural characterization of Mecp2-308 mice (2)

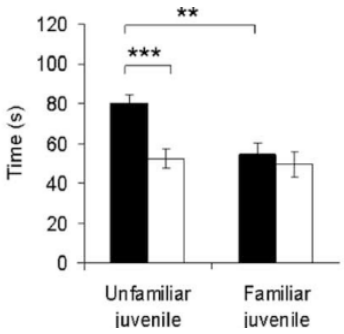
Evidence of impairments of **fear memory, social novelty discrimination and synaptic plasticity** in Mecp2-308 male mice (20-week-old)

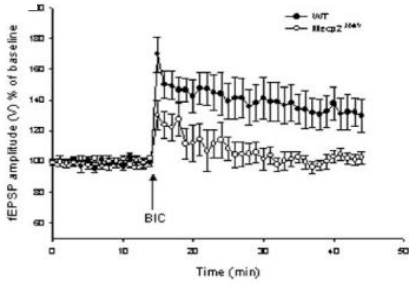


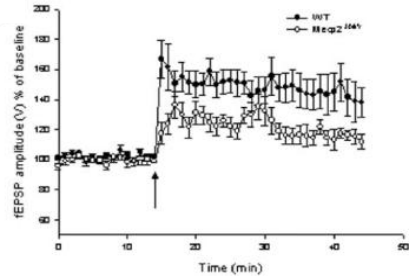
wt
mecp2-308/y









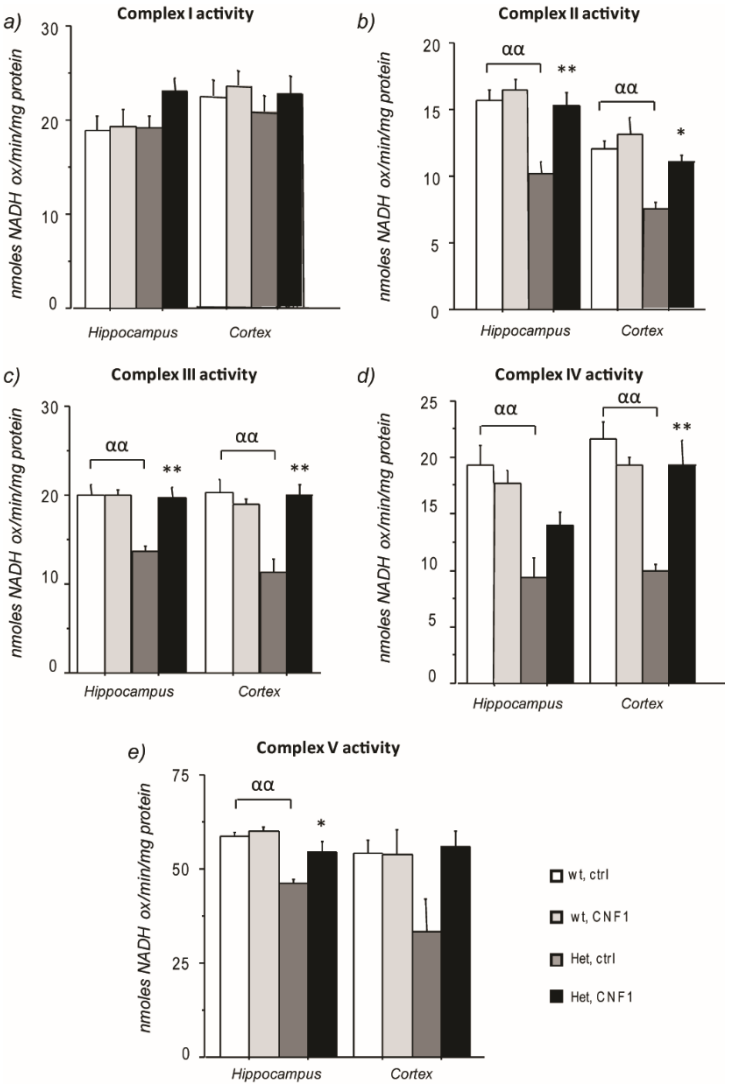


From Moretti et al. 2006

Preclinical testing in Mecp2-308 mice (CNF1)

Single intracerebroventricular (icv) injection of CNF1
(2 µl; 100 nM) in fully symptomatic MeCP2-308 mice

	Mitochondrial ATP synthesis rate (nmol/min/mg protein)	
	SUCC	GLU/MAL
wt, ctrl	86 ± 2	95 ± 2
wt, CNF1	84 ± 2	98 ± 2
Het, ctrl	53 ± 2 αα	93 ± 2
Het, CNF1	84 ± 1 **	97 ± 1

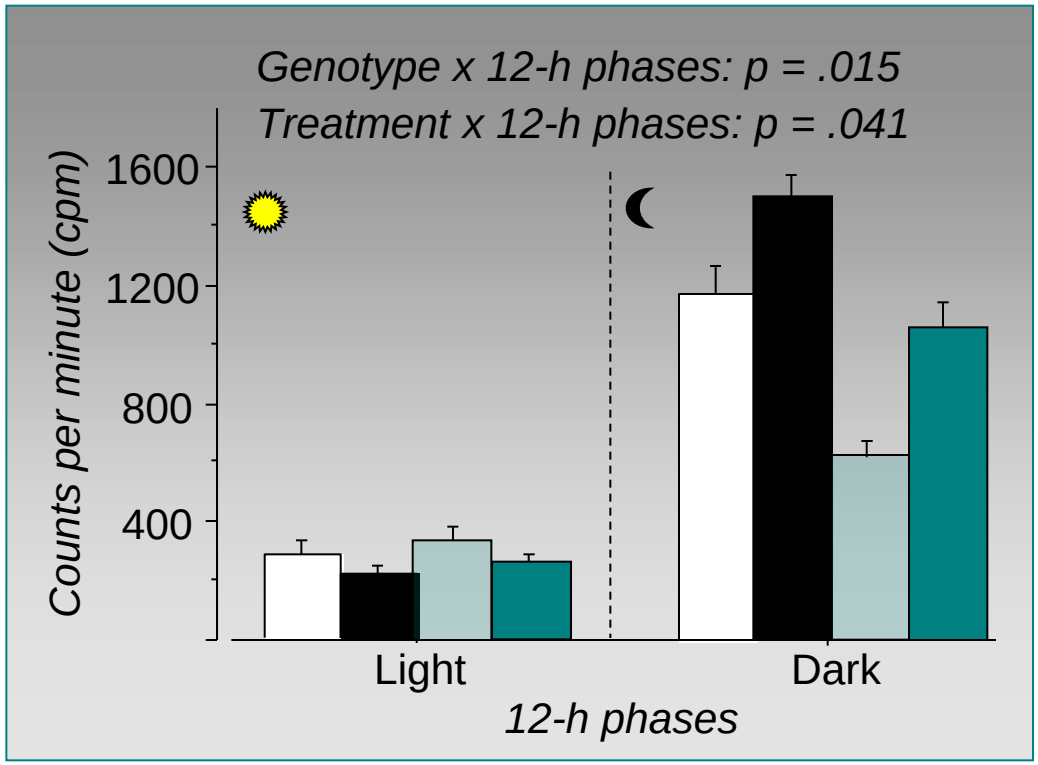


From De Filippis et al. 2015

Preclinical testing in Mecp2-308 mice (CNF1)

Home-cage locomotor activity

Spontaneous locomotor activity in the home-cages was automatically monitored continuously for 24 hours for 7 days. The infrared sensors (20 Hz) detected any movement of mice with a frequency of 20 events per second. Scores were obtained as counts per minute (cpm) expressed during 1-hour periods, and the 24-hour profile of daily activity was obtained by averaging seven days of continuous registration.



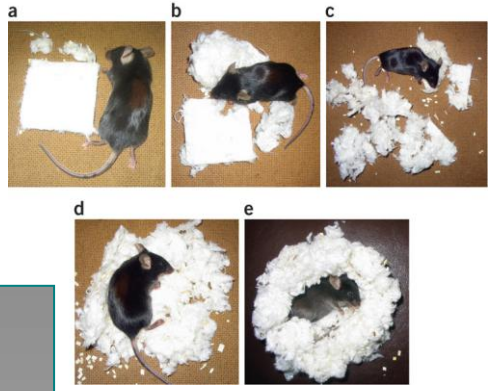
After CNF1, mutant mice reached a wt-like profile of circadian locomotor activity

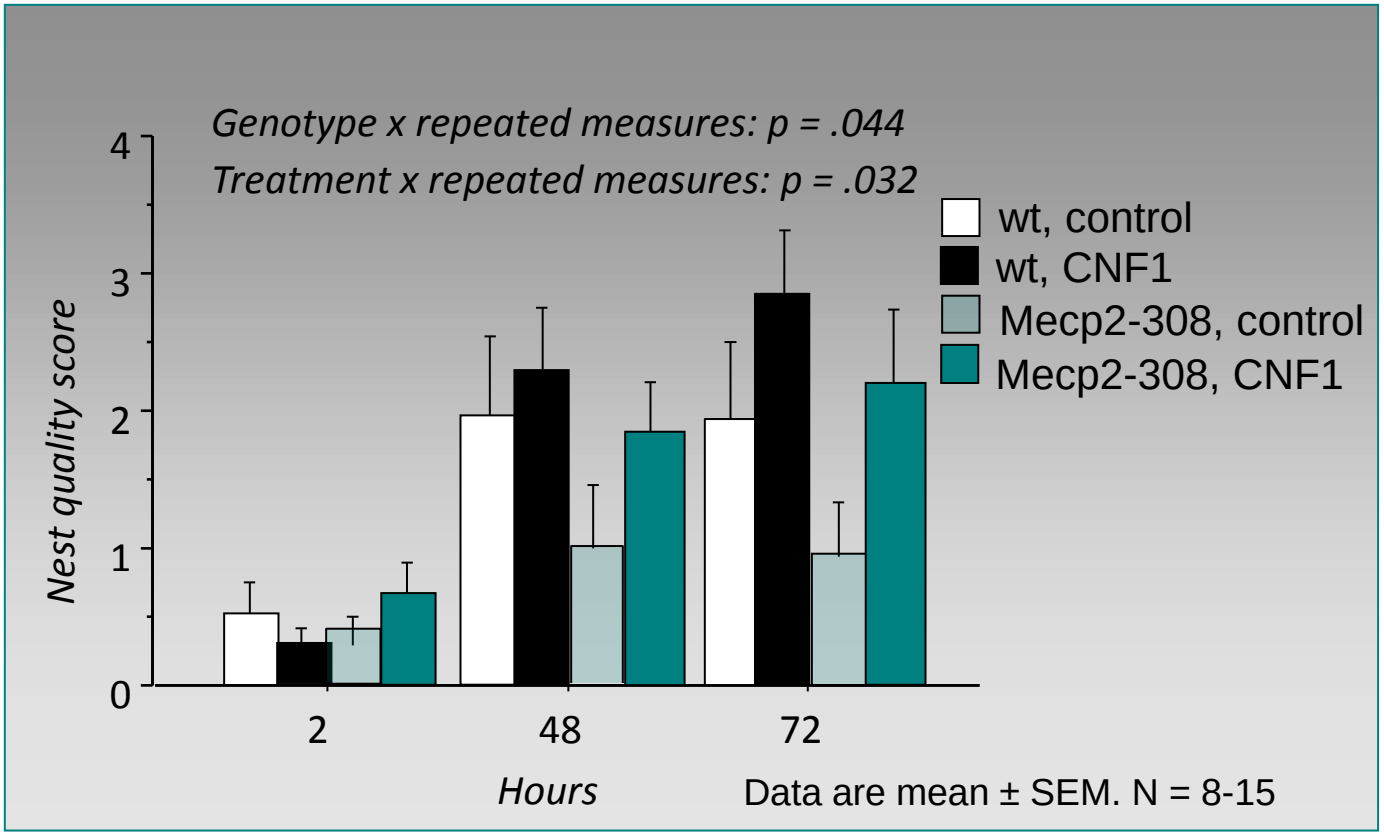
From De Filippis et al. 2012

Preclinical testing in Mecp2-308 mice (CNF1)

Nest building capacity

Position and quality of the nest were scored along 72 hours using a 4-point scale: 0: nest material untouched; **1:** nest material nearly untouched; **2:** nest material scattered; no clear shape evident; **3:** nest of intermediate quality; **4:** nest round and well built.

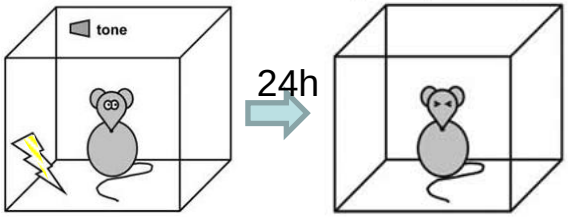




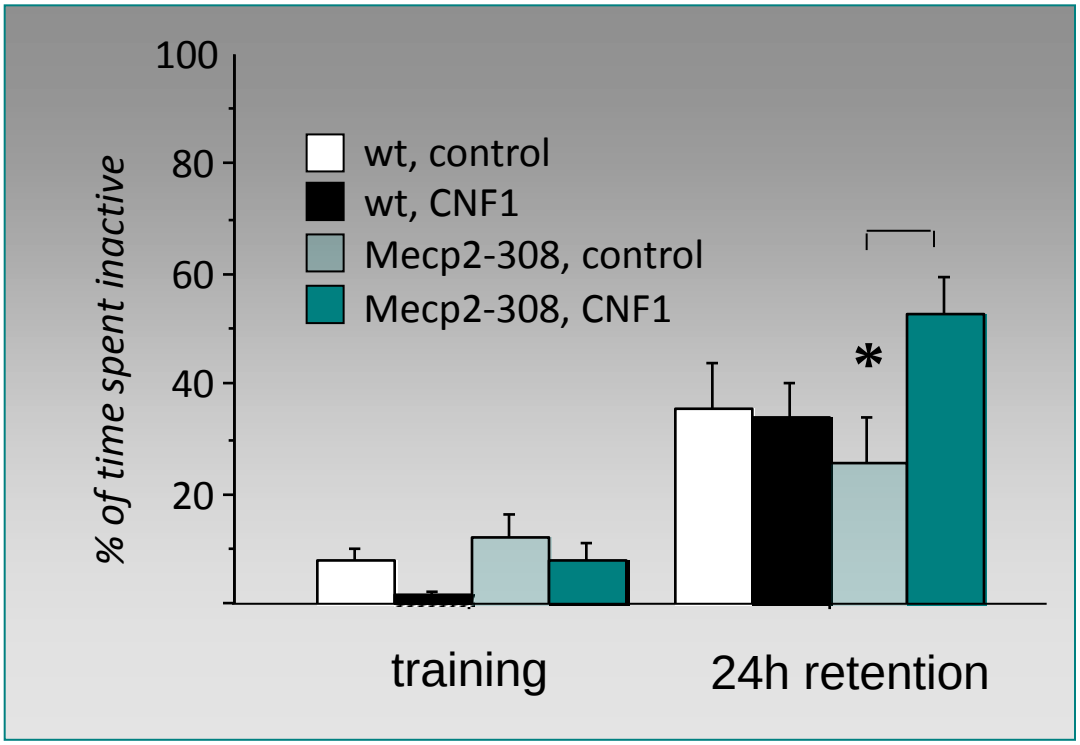
From De Filippis et al. 2012

Preclinical testing in Mecp2-308 mice (CNF1)

Contextual Fear-conditioning



Tone: 80 db,30 s
Electric shock: 0.5mA, 2 s



*CNF1 administration markedly improved the performance of mutant mice in the **contextual** fear-conditioning.*

From De Filippis et al. 2012

Preclinical testing in Mecp2-308 mice (CNF1)

Spatial reference memory (Barnes Maze)

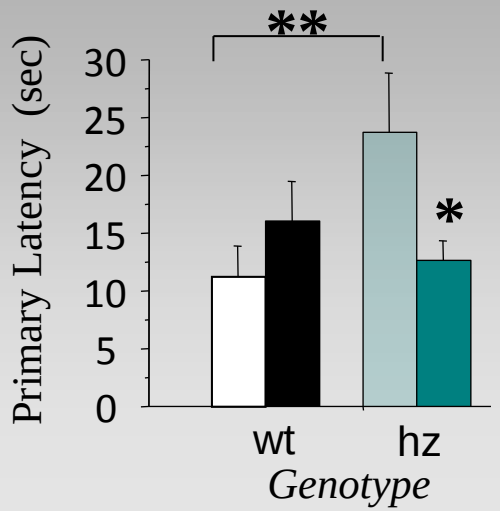
•**Acquisition phase (days 1-5,** two trials per day**):** mice were allowed to freely explore the platform. Latency to enter the target hole, total path length and the number of errors (i.e. nose pokes in holes other than the target) were counted.

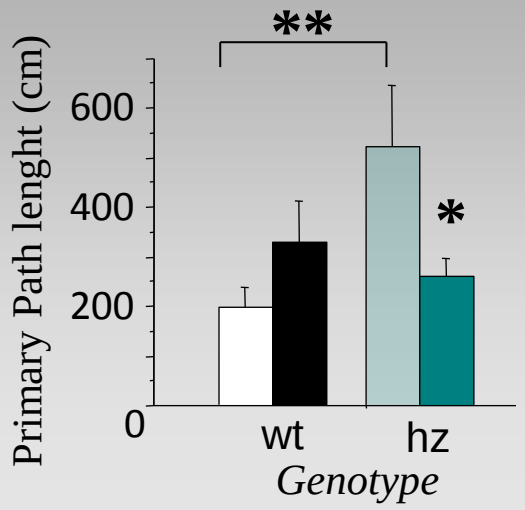
•**Probe test (day 6,** 24 h after the last training trial): to assess short-term reference memory retention, a 90-sec long session was used, during which the target hole was closed. Number of nose pokes (errors) in each hole and latency and path length to reach the virtual target hole were measured.

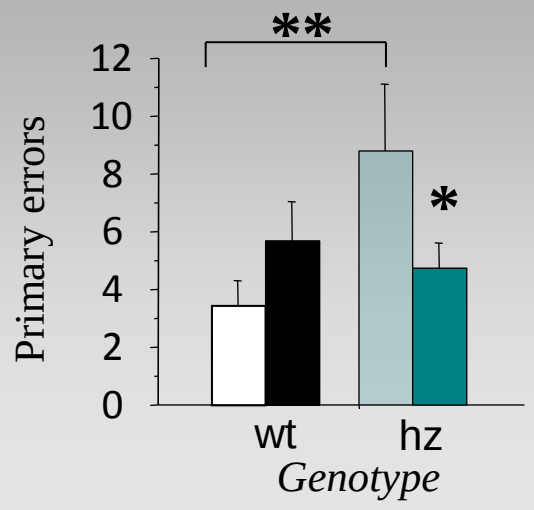
The deficit exhibited by mutant mice in spatial reference memory was efficaciously reversed by CNF1 administration.

wt, control
wt, CNF1
Mecp2-308 control
Mecp2-308, CNF1

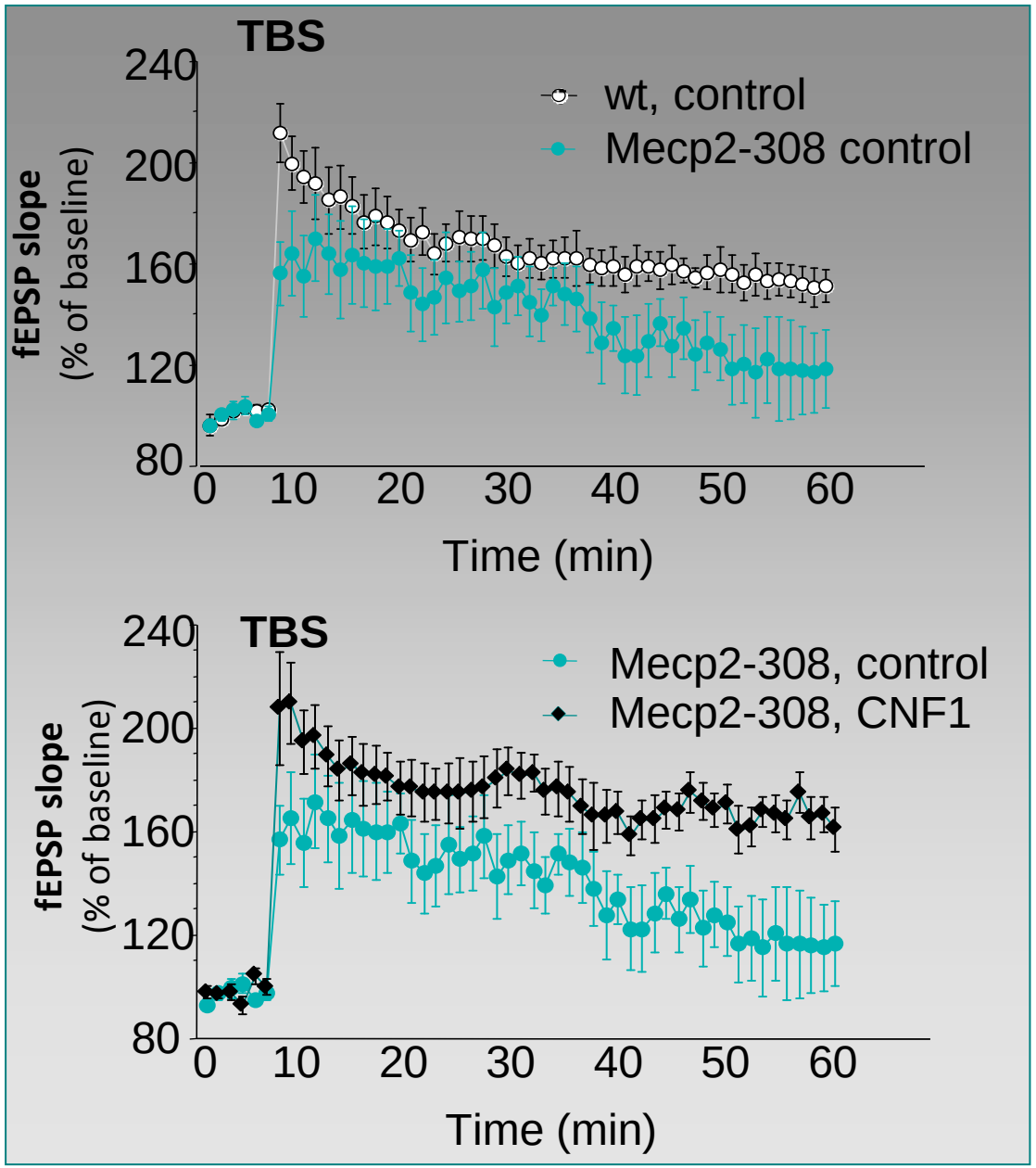
Probe test (day 6):







Preclinical testing in Mecp2-308 mice (CNF1)



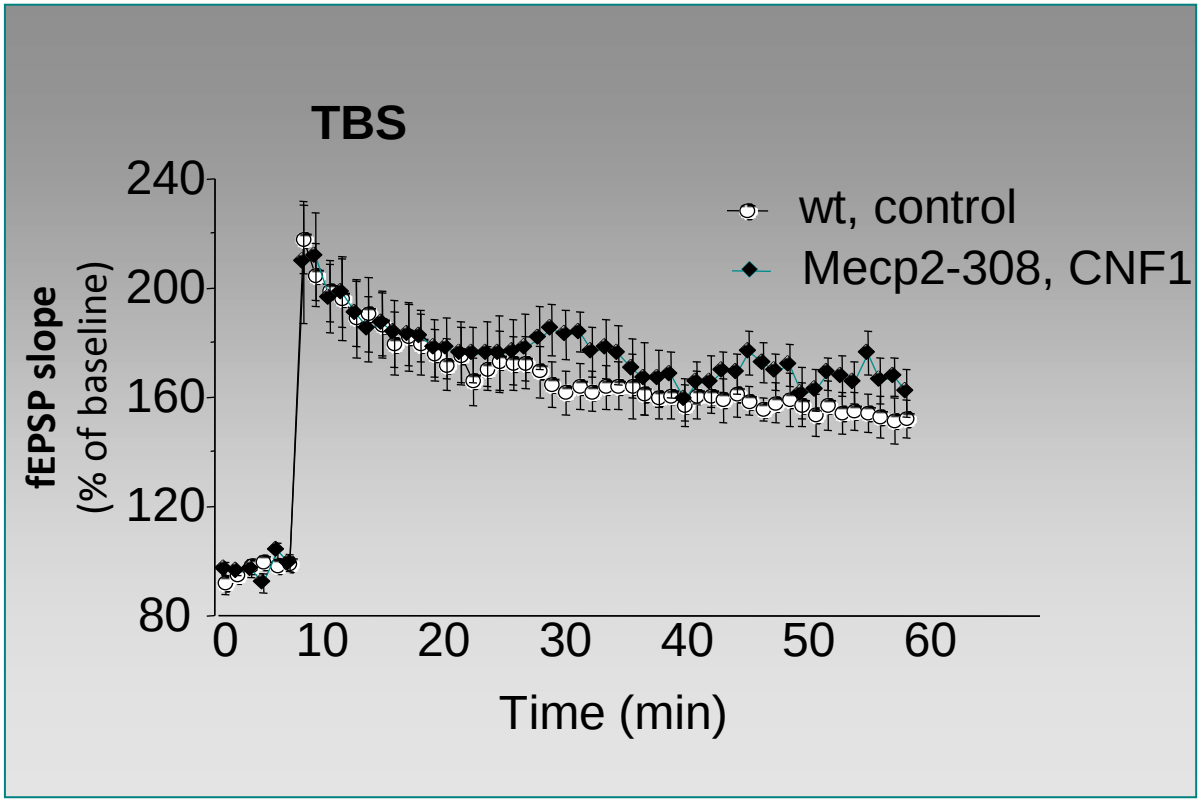
Long-term potentiation in the hippocampus

Long-term potentiation (LTP) of the field excitatory post-synaptic potential (fEPSP) was induced by administering a theta-burst stimulation (TBS) consisting in 2 trains of 5 sets of bursts (four stimuli, 100 Hz) with an interburst interval of 200 ms and a 20 s interval between each stimulus train.

Each point represents the mean of three responses evoked by stimulation of the Schaffer collaterals at 0.05 Hz.

Preclinical testing in Mecp2-308 mice (CNF1)

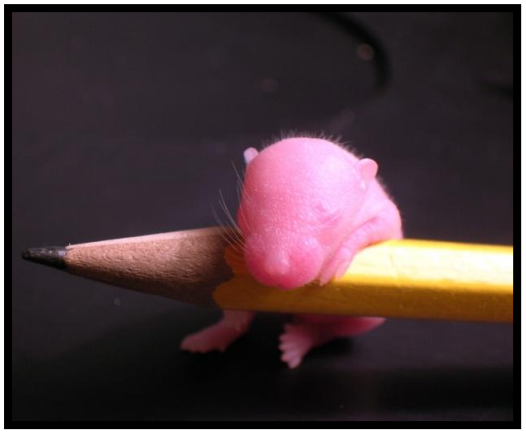
Long-term potentiation in the hippocampus



Treatment with CNF1 rescued the impairment of long-term potentiation in CA1 area of the hippocampus of mutant mice

From De Filippis et al. 2015

RTT mouse models have been used to assess **early phases of neuro-behavioural development**, an approach possible only retrospectively in RTT patients



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MONOAMINE DEFICITS IN THE BRAIN OF METHYL-CpG BINDING

Neuroscience 165 (2010) 1254–1260

J Neurophysiol 99: 112–121, 2008.
First published November 21, 2007; doi:10.1152/jn.00826.2007.

Early Defects of GABAergic Synapses in the Brain Stem of a MeCP2 Mouse Model of Rett Syndrome

L. Medrihan,[1] E. Tantalaki,[1] G. Aramuni,[1] V. Sargsyan,[1] I. Dudanova,[1,2] M. Missler,[1,2] and W. Zhang[1]
[1]*Center for Physiology and Pathophysiology, Georg-August University of Göttingen and Deutsche Forschungsgemeinschaft Research Center of Molecular Physiology of Brain, Göttingen; and* [2]*Department of Anatomy and Molecular Neurobiology, Westfälische Wilhelms-University, Münster, Germany*

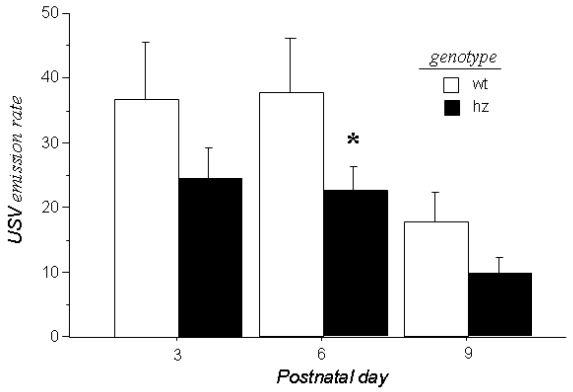
Submitted 25 July 2007; accepted in final form 16 November 2007

Medrihan L, Tantalaki E, Aramuni G, Sargsyan V, Dudanova I, Missler M, Zhang W. Early defects of GABAergic synapses in the brain stem of a MeCP2 mouse model of Rett syndrome. *J Neurophysiol* 99: 112–121, 2008. First published November 21, 2007; doi:10.1152/jn.00826.2007. Rett syndrome is a neurodevelopmental disorder caused by mutations in the transcriptional repressor methyl-CpG-binding protein 2 (MeCP2) and represents the leading genetic cause for mental retardation in girls. MeCP2-mutant mice have been generated to study the molecular mechanisms of the disease. It was suggested that an imbalance between excitatory and inhibitory neurotransmission is responsible for the behavioral abnormalities, although it remained largely unclear which synaptic components are

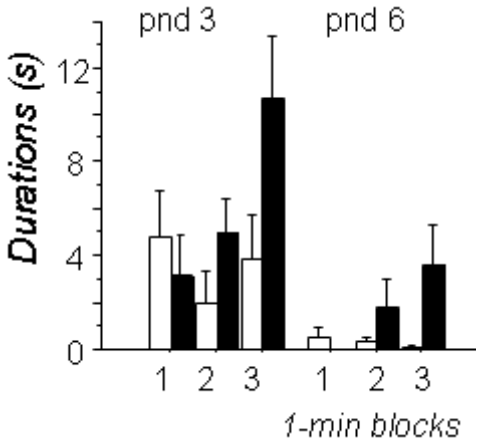
are apparently normal until about 5 wk of age, when they begin to exhibit abnormal motor and social behavior, including respiratory irregularities (Stettner et al. 2007; Viemari et al. 2005). Since morphological alterations in brain architecture are subtle in MeCP2 mutants (Chen et al. 2001), most studies have focused on functional impairments to explain their profound behavioral abnormalities. Previous electrophysiological studies of adult mutant MeCP2 mice demonstrated an enhanced excitatory neurotransmission (Moretti et al. 2006) and reduced synaptic plasticity in the hippocampus (Asaka et al. 2006; Moretti et al. 2006). In addition, it was shown that deletion of

Neonatal behavioural testing in Mecp2-308 mice

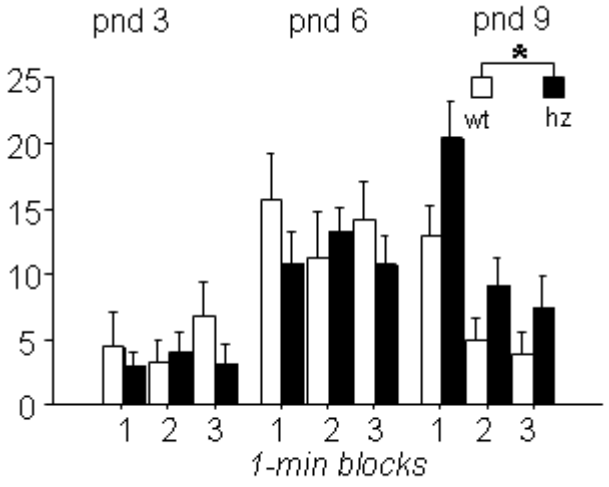
Ultrasonic vocalization rate



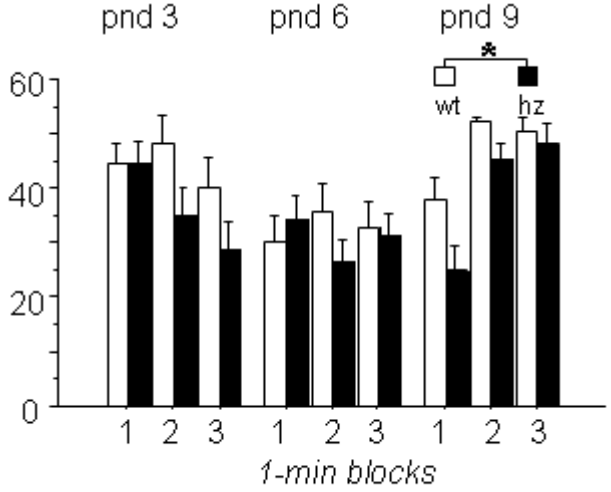
Curling



Head rising

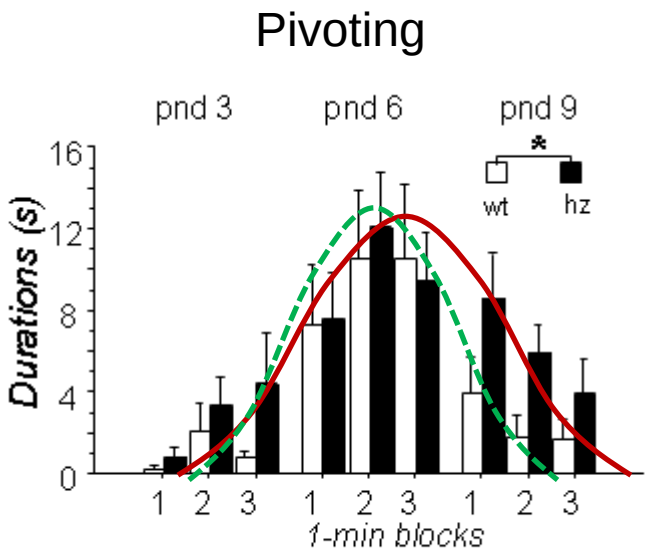


Immobility



From De Filippis et al. 2010

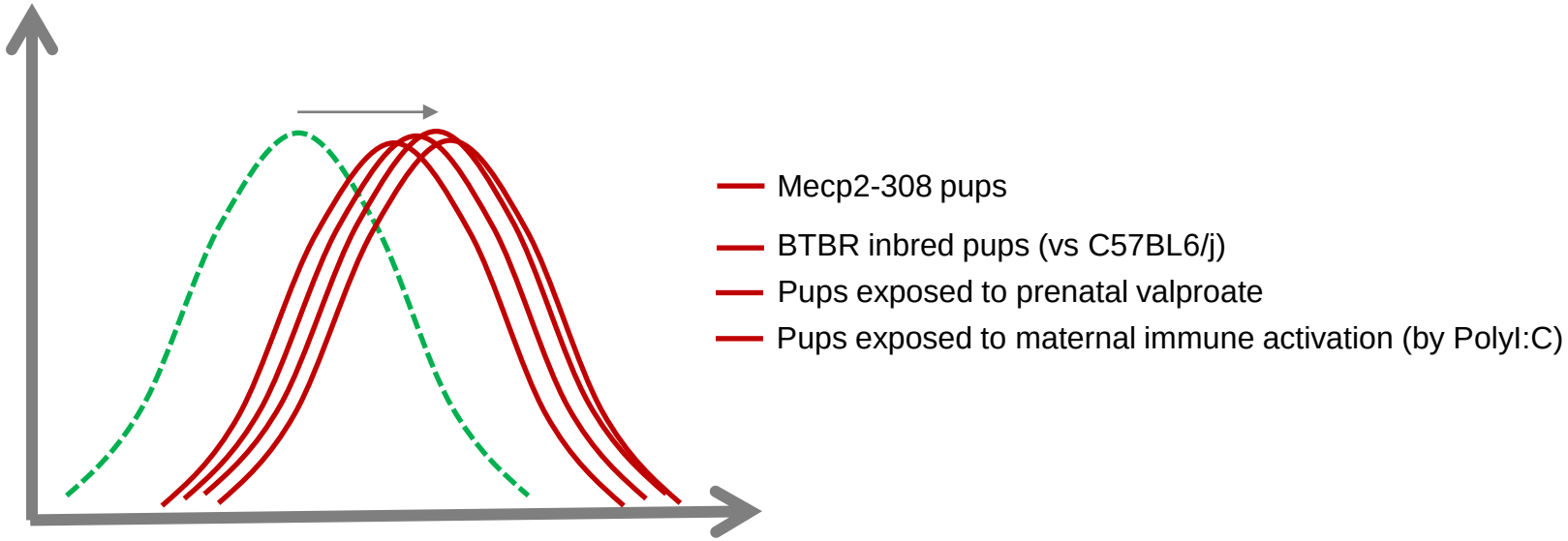
Neonatal behavioural testing in Mecp2-308 mice



Pivoting is an immature locomotor response involving the front limbs alone (resulting in circular movements) that is replaced by full locomotion at the end of the first postnatal week in wt mice.

Modified from De Filippis et al. 2010

Delays in development of adult-like motor patterns are common to different mouse models of neurodevelopmental disorders: an alternative trajectory?



Why could these neonatal motor abormalities be relevant from a translational viewpoint?

1) they could represent early markers

autism

Article

Motor development in children at risk of autism: A follow-up study of infant siblings

Autism
0(0) 1–11

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SAGE

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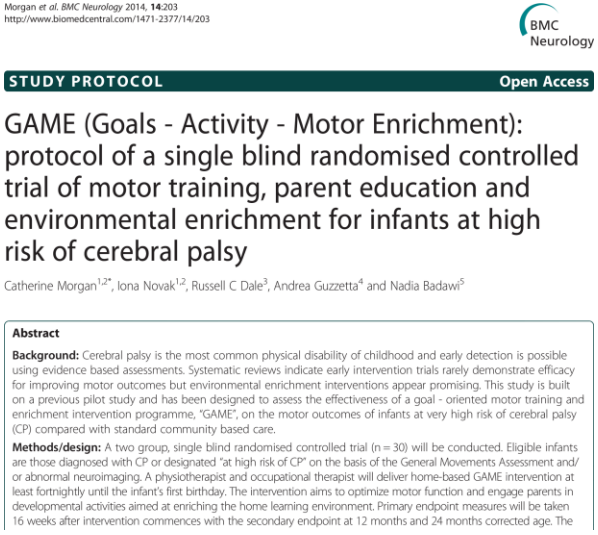
The BASIS team

Abstract

Recently, evidence of poor or atypical motor skills in autism spectrum disorder has led some to argue that motor impairment is a core feature of the condition. The current study uses a longitudinal prospective design to assess the development of motor skills of 20 children at increased risk of developing autism spectrum disorder, who were recruited and tested at 9 and 40 months of age, on the basis of having an older sibling diagnosed with the condition. All children completed a range of motor, face processing, IQ and diagnostic assessments at a follow-up visit (aged 5–7 years), providing a detailed profile of development in this group from a number of standardised, parental report and experimental measures. A higher proportion of children than expected demonstrated motor difficulties at the follow-up visit and those highlighted by parental report as having poor motor skills as infants and toddlers were also more likely to have lower face processing scores and elevated autism-related social symptoms at 5–7 years, despite having similar IQ levels. These data lend support to the argument that early motor difficulties may be a risk factor for later motor impairment as well as differences in social communication and cognition, traits that are related to autism spectrum disorder.

2) they could represent targets for early intervention



Morgan *et al. BMC Neurology* 2014, **14**:203
http://www.biomedcentral.com/1471-2377/14/203

BMC Neurology

STUDY PROTOCOL Open Access

GAME (Goals - Activity - Motor Enrichment): protocol of a single blind randomised controlled trial of motor training, parent education and environmental enrichment for infants at high risk of cerebral palsy

Catherine Morgan[1,2*], Iona Novak[1,2], Russell C Dale[3], Andrea Guzzetta[4] and Nadia Badawi[5]

Abstract

Background: Cerebral palsy is the most common physical disability of childhood and early detection is possible using evidence based assessments. Systematic reviews indicate early intervention trials rarely demonstrate efficacy for improving motor outcomes but environmental enrichment interventions appear promising. This study is built on a previous pilot study and has been designed to assess the effectiveness of a goal - oriented motor training and enrichment intervention programme, "GAME", on the motor outcomes of infants at very high risk of cerebral palsy (CP) compared with standard community based care.

Methods/design: A two group, single blind randomised controlled trial (n = 30) will be conducted. Eligible infants are those diagnosed with CP or designated "at high risk of CP" on the basis of the General Movements Assessment and/or abnormal neuroimaging. A physiotherapist and occupational therapist will deliver home-based GAME intervention at least fortnightly until the infant's first birthday. The intervention aims to optimize motor function and engage parents in developmental activities aimed at enriching the home learning environment. Primary endpoint measures will be taken 16 weeks after intervention commences with the secondary endpoint at 12 months and 24 months corrected age. The

RTT mouse models have been used to test different therapeutic strategies

Gene replacement (primarily in MeCP2 knock out models):

-global MeCP2 replacement in in MeCP2 knockout males (Guy et al. 2007);

-delayed MeCP2 gene re-activation in MeCP2 knockout females (Lang et al. 2013);

-replacement of MeCP2 in R255X mice (Pitcher et al. 2015);

-viral delivery of MeCP2 (scAAV9) in males and females (Garb et al. 2013);

Downstream MeCP2:

-exogenous growth factor administration IGF (1-3) BDNF and BDNF analougs;

-NMDA receptor antagonists (ketamine Kron et al. 2012);

-other factors targeting mitochondrial functions;

RTT therapy downstream of the Mecp2 gene

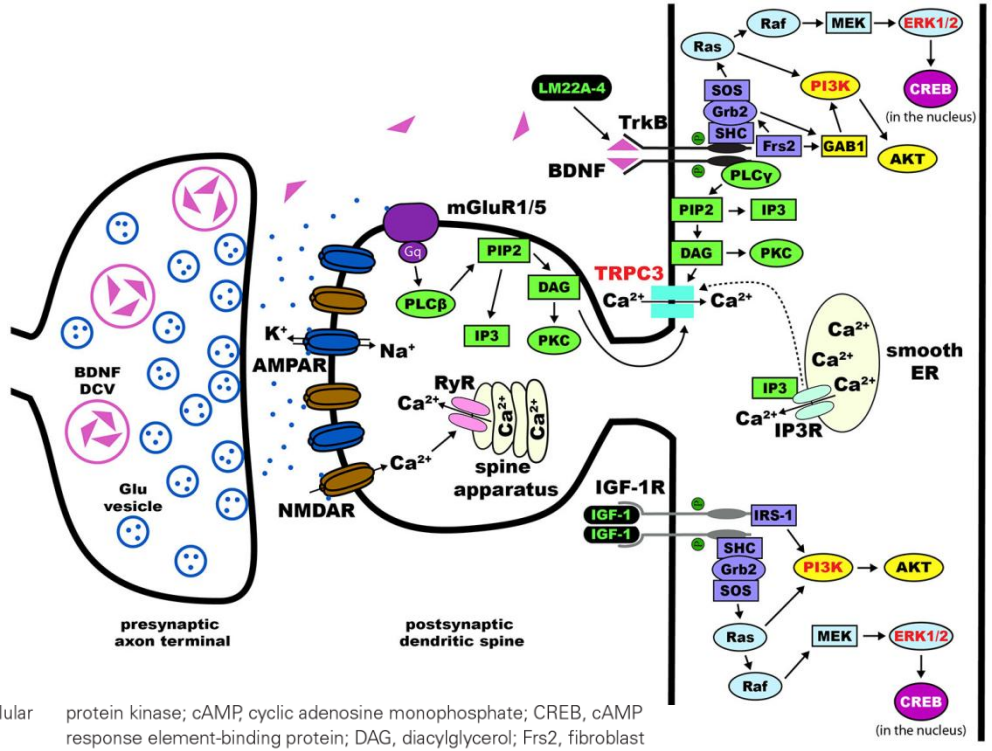
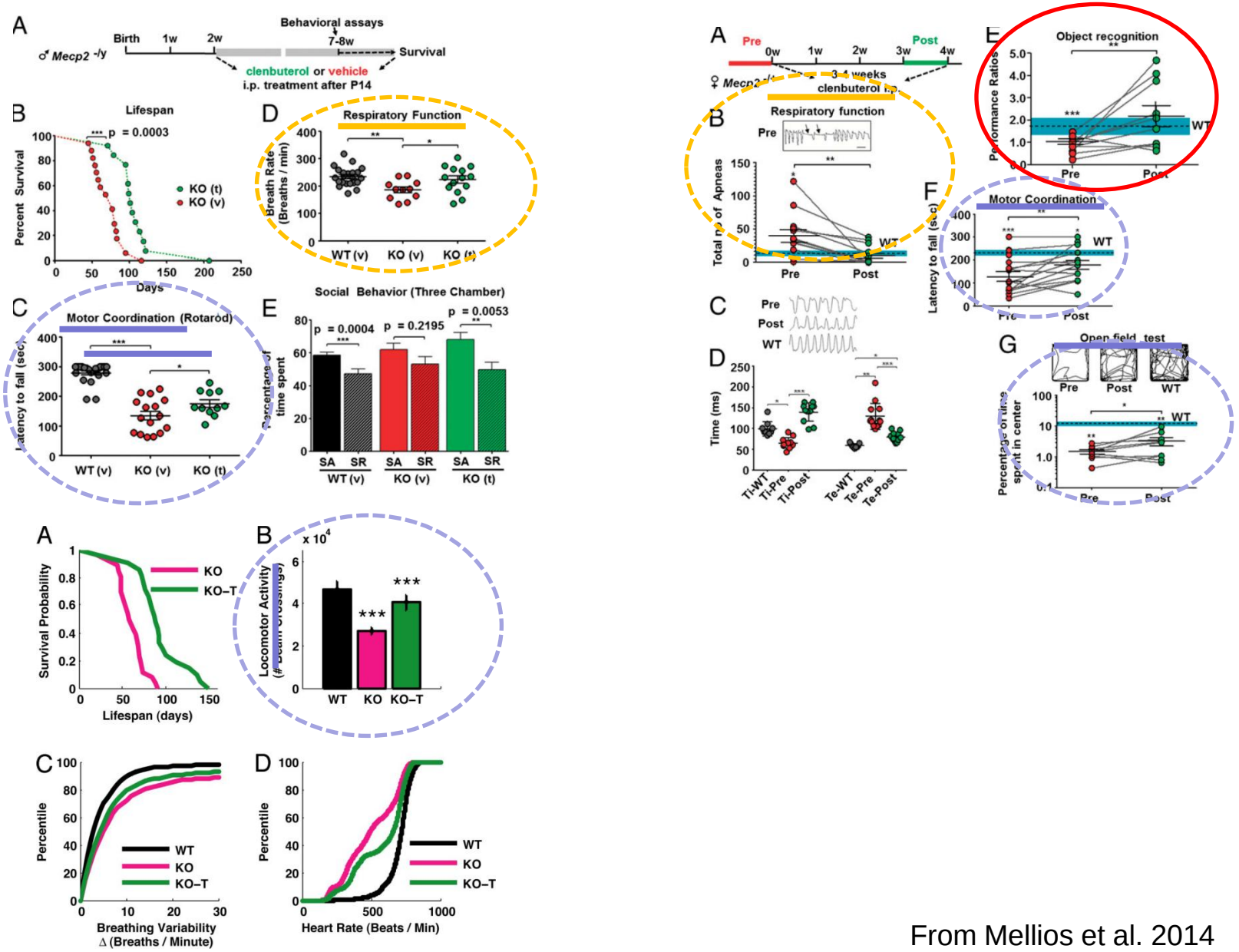


FIGURE 2 | Dendritic spines in Rett syndrome (RTT). Proposed intracellular mechanisms that mediate the effects of BDNF/TrkB on dendritic spine density and morphology. Trk receptors are activated upon binding of neurotophic factors, leading to dimerization and auto-phosphorylation. This process allows for the intracellular binding of adaptor proteins to Trk and activation of major pathways including Ras/ERK, PI3K, and PLCγ. Components of each of these three pathways have been implicated in the effects of BDNF on dendritic spines (highlighted in red text). Potential therapies for the treatment of RTT act on these pathways (highlighted in green text)—LM22A-4 directly phosphorylates TrkB and [1,3]IGF-1 activates the PI3K and Ras/ERK pathways. Abbreviations: AKT(PKB), protein kinase B; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; BDNF, brain-derived neurotrophic factor; CaMK, Ca2+/calmodulin-dependent protein kinase; cAMP, cyclic adenosine monophosphate; CREB, cAMP response element-binding protein; DAG, diacylglycerol; Frs2, fibroblast growth factor receptor substrate 2; GAB1, GRB2-associated-binding protein 1; Grb2, growth factor receptor-binding protein 2; IGF-1, insulin-like growth factor 1; IGF-1R, IGF-1 receptor; IP3, inositol triphosphate; MAPK, mitogen-activated protein kinase; MEK, MAPK//ERK kinase; NMDAR, *N*-methyl-D-aspartate receptor; PI3K, phosphoinositide 3-kinase; PIP2, phospohatidylinositol 4, 5 bisphosphate; PKC, protein kinase C; PLCγ, phospholipase C-γ; Raf, proto-oncogenic serine/threonine protein kinase; Ras, rat sarcoma proto-oncogenic G-protein; SHC, SH-2-containing protein; SH-2, src homology domain 2; SOS, nucleotide exchange factor son of sevenless; TrkB, tropomyosin related kinase B receptor; TRPC, transient receptor potential channel.

From Wang H et al. 2015

Agent or Approach	Model	Sponsor	Action	Effect
BDNF mimetic or booster				
LM22A-4	Mouse	Sigma-Aldrich; RSRT	Partial agonist of TrkB receptors	Improves breathing
RP103 (Cysteamine)	Mouse	Raptor Pharmaceuticals	Increase BDNF expression and release	Improves breathing
5-HT1a receptor agonists				
Sarizotan	Mouse	Newron Pharmaceuticals	Agonist of 5HT1 receptors	Improves breathing
NLX-101	Mouse	Neurolixis	Agonist of 5HT1 receptors	Improves breathing
NMDA receptor modulators				
GluN2A negative allosteric modulator	Mouse	Mnemosyne Pharmaceuticals	negative allosteric modulator of GluN2A-containing NMDA receptors	Improved visual cortical function
Ketamine	Mouse	Approved drug	Non-competitive antagonist of NMDA receptors	Improved breathing and locomotor function
Antidepressant				
REV-003 (Tianeptine)	Mouse	Revive Therapeutics	Atypical tricyclic antidepressant	Improves breathing
Gene therapy, and "read-through" drugs				
Gene replacement	Mouse	Research laboratories	Improvement of symptoms	Improvement of life span and behavioral symptoms
Read-through agents	*In vitro* (lymphocyte cell line from RTT; fibroblasts from knockin mice)	Research laboratories	Skip premature STOP codon in nonsense mutations	*In vitro* expression of full length MeCP2 protein

Modified from Pozzo-Miller et al. 2015



From Mellios et al. 2014

Agent	Results	Trial Outcome	Sponsor	Type
Growth factors				
IGF-1 (Mecasermin)	Phase 2	Safety confirmed On-going; scheduled through Fall 2015	Approved drug in children with growth failure	Double-blind; placebo controlled; injection; 3-10 years of age
NNZ-2566	Phase 2	Safety confirmed Short-term trial positive	Neuren Pharmaceuticals	Double-blind; placebo controlled; oral; 16-45 years of age
BDNF boosters				
Copaxone (Glatiramer acetate)	Phase 2	Improved ambulation	Approved drug	Open-label; injection
Fingolimod	Phase 1	On-going; scheduled through August 2017	Novartis Pharmaceuticals; Approved drug-adults	Open-label; oral
NMDA antagonist				
Dextromethorphan	Phase 2	On-going; scheduled through June 2015	Approved drug in cough suppressant	Double-blind; placebo controlled
Norepinephrine reuptake inhibitor				
Desipramine	Phase 2	On-going; scheduled through December 2014	Approved drug in Europe	Double-blind; placebo controlled
Mitochondrial effector				
EPI-743	Phase 2	Safety confirmed Improved head circumference growth	Edison Pharmaceuticals	Double-blind; placebo controlled; oral

Modified from Pozzo-Miller et al. 2015

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SPECIAL ARTICLE

Preclinical research in Rett syndrome: setting the foundation for translational success

David M. Katz[1,*,‡], Joanne E. Berger-Sweeney[2,‡], James H. Eubanks[3,‡], Monica J. Justice[4,‡], Jeffrey L. Neul[4,5,‡], Lucas Pozzo-Miller[6,‡], Mary E. Blue[7], Diana Christian[1], Jacqueline N. Crawley[8], Maurizio Giustetto[9], Jacky Guy[10], C. James Howell[1], Miriam Kron[1], Sacha B. Nelson[11], Rodney C. Samaco[5], Laura R. Schaevitz[2], Coryse St. Hillaire-Clarke[12], Juan L. Young[13], Huda Y. Zoghbi[5,14,*,‡] and Laura A. Mamounas[12,*,‡]

In September of 2011, the National Institute of Neurological Disorders and Stroke (NINDS), the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), the International Rett Syndrome Foundation (IRSF) and the Rett Syndrome Research Trust (RSRT) convened a workshop involving a broad cross-section of basic scientists, clinicians and representatives from the National Institutes of Health (NIH), the US Food and Drug Administration (FDA), the pharmaceutical industry and private foundations to assess the state of the art in animal studies of Rett syndrome (RTT). The aim of the workshop was to identify crucial knowledge gaps and to suggest scientific priorities and best practices for the use of animal models in preclinical evaluation of potential new RTT therapeutics. This review summarizes outcomes from the workshop and extensive follow-up discussions among participants, and includes: (1) a comprehensive summary of the physiological and behavioral phenotypes of RTT mouse models to date, and areas in which further phenotypic analyses are required to enhance the utility of these models for translational studies; (2) discussion of the impact of genetic differences among mouse models, and methodological differences among laboratories, on the expression and analysis, respectively, of phenotypic traits; and (3) definitions of the standards that the community of RTT researchers can implement for rigorous preclinical study design and transparent reporting to ensure that decisions to initiate costly clinical trials are grounded in reliable preclinical data.

Introduction

Rett syndrome (RTT) is a prototype childhood neurological disease characterized by features that are observed in many other disorders ranging from autism to Parkinson's disease and dystonia. The disorder affects ~1 in 10,000 females and is most often caused by mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2), a transcriptional regulatory protein. It has been shown that many of the features of RTT are reversible in mice (Gadalla et al., 2011; Guy et al., 2007), and that these features are probably due to dysfunction of neurons and supporting cells, rather than neural degeneration (Armstrong, 2002). These findings provide hope that some and perhaps most symptoms can be reversed in affected individuals if we discover effective therapies that can overcome the consequences of loss of function or dysfunction of MeCP2.

Table 2. Comparison of phenotypes in RTT individuals and *Mecp2*-mutant mice

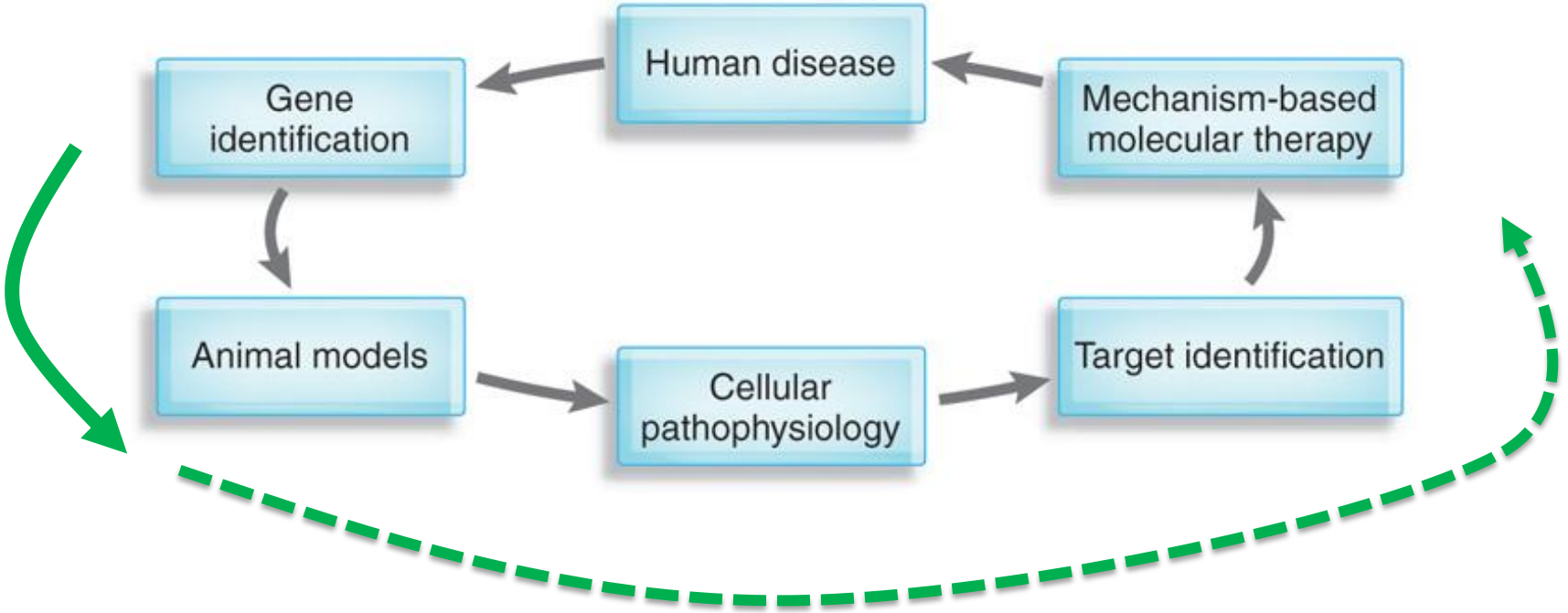
Core phenotypes in RTT individuals	Similar phenotypes in *Mecp2*-mutant mice
Morphological	
Microcephaly	++
Neuronal hypotrophy (reduced neuronal soma size, smaller dendritic arbors and lower spine density)	++
Respiratory and autonomic control	
Abnormal breathing (irregular pattern, respiratory pauses, increased mean frequency)	++
Prolonged QTc	+
Vasomotor disturbances	nd
Gastrointestinal dysmotility	nd
Motor function	
Gait abnormalities	++
Stereotypies	+
Tremors	++
Early hypotonia	nd
Lack of purposeful hand movements	? (poor nest building might reflect decreased forepaw function)
Parkinsonian features	? (mice exhibit hypokinesis; mechanism unknown)
Dystonia	? (hindlimb clasping might be a sign of dystonia)
Cognition and behavior	
Cognitive deficits	++
Social withdrawal	+/–
Increased anxiety	+/–
Loss of speech	?
Repetitive behaviors	? (some indication of repetitive and/or excessive grooming)
Other	
Reduced lifespan	++
EEG abnormalities and seizures	++
Neurological regression	++
Sleep cycle disturbances	nd
Scoliosis	nd

++, phenotypes similar to human and reported in more than one mouse model; +, phenotypes similar to human and reported in only one mouse model to date; +/–, conflicting data in different mouse models; ?, suggestive or inconclusive data; nd, not determined. Detailed mouse phenotyping data are available in online supplementary material Tables S3–S6.

Table 3. Best-practice guidelines for preclinical animal trials testing therapeutic compounds in RTT

Parameter	Recommendations
Study design	
Sample size	Because many studies are frequently underpowered, sample size calculations, based on expected variance and effect size, should be performed before the animal trial starts (i.e. mice should not be added during the trial, once data analysis has begun, in an attempt to increase statistical significance). For most RTT trials, sample sizes should include at least 12–16 mice per group
Dose-response data	In addition to the optimal dose, the minimally effective and maximally tolerated dose should be determined. Early evidence that the drug reaches the tissue target (including drug concentration at the target) and preliminary safety and tolerability data can help to support continued development of the candidate therapeutic (e.g. more comprehensive preclinical pharmacokinetics/pharmacodynamics studies)
Timing and duration of treatment	Treatment onset (e.g. in presymptomatic vs symptomatic mice) and duration (acute vs chronic) should correspond as closely as possible to the expected clinical trial indication. For example, in early Phase 2 RTT clinical trials, the intervention will probably start in individuals who are already symptomatic and will involve chronic intervention. In addition, data on the therapeutic window (e.g. whether the treatment is more effective when initiated in presymptomatic mice) and acute vs chronic treatment effects can be informative in interpreting clinical efficacy data and/or planning later-phase clinical trials
Outcome measures	Assessing multiple outcome measures can be informative, especially in exploratory studies. However, for the preclinical animal trial, primary and secondary outcome measures and/or end points should be established in advance, and incorporated into the statistical design of the study. The selection of outcome measure(s) should be as relevant to the expected clinical measure(s) as possible
Biomarkers	If available, biomarkers that measure target engagement or activity, and disease progression or amelioration, can be informative in animal studies, especially if the biomarker is translatable to human studies
Statistics and minimizing bias	
Statistical design	Statistical methods should be chosen before the study has begun. Once the outcome variability has been characterized, it is a good idea to consult with a statistician on the design of the trial
Randomization and balancing	The study design should include how mice will be randomly allocated to treatment groups (e.g. computer-generated randomization schedules). Picking animals 'at random' from a cage is likely to introduce bias based on subtle (or not so subtle) characteristics of the animal. Treatment groups should be balanced by age and sex of mice, if relevant, and to prevent over-representation of sibs in any experimental cohort
Blinding	Wherever possible, individuals conducting the experiments and those analyzing the results should be blinded to the experimental group (i.e. allocation concealment and blinded assessment of outcome, respectively)
Inclusion/exclusion criteria	Determine in advance the criteria by which animals will be included or excluded from the study, or discarded from analysis (e.g. based on phenotype, disease severity, sickness or other factors that could skew the outcomes). Post-hoc exclusion of animals or data after the analysis (before unblinding) should be justified and reported
Reporting	
ARRIVE guidelines	We suggest consulting the ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines to report RTT preclinical animal trial results, and to include details on mouse model and genetic background, animal housing and handling, sample size calculations and statistical methods, methods to minimize bias (randomization, blinding), inclusion/exclusion criteria, animals and data excluded from analysis, and other relevant items on the checklist provided in the ARRIVE guidelines
Negative results	Publish all results (positive and negative outcomes) to avoid publication bias
Conflict of interest	Report any relationship that could be perceived as a conflict of interest, or that could potentially bias the study outcomes
Replication	
Multiple RTT models	To evaluate the robustness and generality of the original findings, we suggest validating promising treatment outcomes in more than one RTT mouse model/strain (e.g. comparing the *Mecp2* null and a disease-specific mutation, mice with different genetic backgrounds, etc.). Moreover, validating findings in female heterozygous *Mecp2* mice is imperative
Independent replication	Ideally, an independent laboratory (or a contract research organization) with no financial, scientific or other vested interest in the original study should conduct the replication study. The replication study can attempt to duplicate exactly the original experimental design or modify the procedures (e.g. using a different RTT mouse model/strain) to evaluate the robustness and generality of the findings. All results (positive and negative) should be reported

The translational *state of the art* for Rett syndrome



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CNF1 studies
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