

Translating molecular advances in Down syndrome and Fragile X syndrome into therapies.

Marie-Claude Potier<sup>a\*</sup>, Barbara Bardoni<sup>b</sup>, Victor Faundez<sup>c</sup>, Renata Bartesaghi<sup>d</sup>, Dean Nizetic<sup>e,f</sup>, Rafael de la Torre<sup>g</sup>, Roi Cohen Kadosh<sup>h</sup>, Yann Herault<sup>i</sup>, Mara Dierssen<sup>j,k,l\*</sup>, the Down Syndrome and Other Genetic Developmental Disorders ECNP Network<sup>m</sup>

<sup>a</sup>Institut du Cerveau et de la Moelle épinière, CNRS UMR7225, INSERM U1127, UPMC, Hôpital de la Pitié-Salpêtrière, 47 Bd de l'Hôpital, Paris, France.

<sup>b</sup>Institute of Molecular and Cellular Pharmacology, CNRS UMR 7275, Valbonne Sophia-Antipolis, France.

<sup>c</sup>Department of Cell Biology, Emory University, Atlanta, Georgia, USA.

<sup>d</sup>University of Bologna, Department of Biomedical and Neuromotor Sciences, Bologna, Italy.

<sup>e</sup>Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore.

<sup>f</sup>Barts and The London School of Medicine, Queen Mary University of London, United Kingdom

<sup>g</sup>Integrated Pharmacology and Neurosciences Systems Research Group, IMIM-Hospital del Mar Medical Research Institute, Barcelona, Spain. CIBEROBN, Madrid, Spain

<sup>h</sup>Department of Experimental Psychology, University of Oxford, Oxford, United Kingdom.

<sup>i</sup>Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS UMR7104, INSERM U964, Université de Strasbourg, 1 rue Laurent Fries, Illkirch, France

<sup>j</sup>Cellular and Systems Neurobiology, Center for Genomic Regulation, The Barcelona Institute of Science and Technology

<sup>k</sup>Universitat Pompeu Fabra (UPF), Barcelona, Spain

<sup>l</sup>Centro de Investigación Biomédica en Red CIBERER.

<sup>m</sup>The European Down syndrome and other genetic developmental disorders psychopharmacology network is a workgroup of the ECNP currently consisting of the following members: Stylianos

Antonarakis (Geneva, Switzerland), Renata Bartesaghi, Andrea Contestabile (Genova, Italy), Tonnie Coppus (Nijmegen, The Netherlands), Peter De Deyn (Antwerpen, Belgium), Alain Dekker (Groningen, The Netherlands), Jean-Maurice Delabar (Paris, France), Mara Dierssen, Elisabeth Fisher (London, United Kingdom), Yann Hérault, Annette Karmiloff- Smith (London, United Kingdom), Carmen Martinez-Cué (Santander, Spain), Marie-Claude Potier and Andre Strydom (London, United Kingdom). We dedicate this review to the memory of Annette Karmiloff- Smith who passed away at the beginning of year 2017.

\*Corresponding authors:

Marie-Claude Potier

ICM, Institut du Cerveau et de la Moelle

Hôpital de la Pitié Salpêtrière

47, Bd de l'Hôpital

75013 Paris, France

+33681998178

marie-claude.potier@upmc.fr

Mara Dierssen

Cellular and Systems Neuroscience

Center for Genomic Regulation

Barcelona, Spain.

C/ Dr. Aiguader, 88 PRBB Building

08003 Barcelona, Spain

+34933160140

mara.dierssen@crg.eu

## Abstract

Treatments for genetic developmental disorders of the central nervous system are mostly symptomatic and do not correct the genetic cause. Recent identification of common mechanisms between diseases has suggested that new therapeutic targets could be applied across intellectual disabilities. The European Down syndrome and other genetic developmental disorders (DSG2D) network has put together basic and clinical scientists to foster this research and carry out much needed clinical trials. Here we will discuss common mechanisms between Down syndrome and Fragile X syndrome, highlighting: i) how to model these complex diseases using induced iPS cells pluripotent stem cells, derived neuronal cells and brain organoids; ii) how to integrate genomic, proteomic and interactome data to help defining common mechanisms and boundaries between diseases; iii) how to target common pathways for designing clinical trials and assessing their efficacy; iv) how to bring new avenue such as noninvasive brain stimulations and cognitive training to clinical research. These new avenues have utterly transformed our understanding of the molecular pathology of these diseases and much is left to be done to bring them to newborn babies and children to improve their quality of life.

### **Common mechanisms causing intellectual disabilities.**

Down syndrome (DS) and Fragile X syndrome (FXS) are the most common genetic causes of intellectual disability for which no approved therapies are available yet. These disorders are associated with neurological complications including cognitive deficits that lead to mild to profound impairment in intellectual functioning. Current therapeutic approaches focus on behavioral therapy, educational mainstreaming and off-label medications that mitigate only a limited set of symptoms, such as hyperactivity, some cognitive deficits, seizure and anxiety. Individuals with DS and most males and approximately 30% of females affected by FXS have significant intellectual deficits and social dysfunctions in adulthood, with deleterious impact on affected individuals, families and society (Lott & Dierssen, 2010; Maurin *et al.*, 2014). DS and FXS show striking similarities and differences. Both intellectual disabilities are genetic developmental disorders characterized by defects in structural and synaptic plasticity due to alterations in specific molecular pathways leading to cognitive impairment.

It is interesting to underline that neuroanatomical abnormalities in the brain of patients and mouse model for DS and FXS are likely to be generated early during development and may be associated to defects in proliferation and/or differentiation of neural progenitors (Guidi *et al.*, 2014; Castren, 2016), pointing out a critical role of FMRP and human chromosome 21 genes during neurogenesis. Children with DS (aged between 5 and 23 years) have smaller overall brain volumes with smaller cerebellum and larger subcortical grey matter volumes (Pinter *et al.*, 2001) while children with FXS (aged between 18 and 42 months) have larger brain volumes and display enlargement in the temporal lobe white matter, cerebellar gray matter and caudate nucleus, but have a smaller amygdala (Hazlett *et al.*, 2012). In another study analyzing boys with FXS aged 1 to 3 years the authors found, as in the adult patients, increased caudate, fusiform gyrus, and thalamus grey matter volume (GMV) as well as reduced GMV in the superior temporal gyrus, hippocampus, insula, hypothalamus, and orbitofrontal cortex, and medial and lateral prefrontal cortices (Hoeft *et al.*, 2010). At the cellular level, dendritic atrophy is seen in children and adults with DS with a significant decrease in dendritic branching,

length and spine density (Takashima *et al.*, 1989). FXS patients exhibit immature dendritic spines that appear longer, denser and thinner (Irwin *et al.*, 2000a; Irwin *et al.*, 2000b). Similarly, pyramidal cells from mouse model of DS show significant differences in their dendritic arborization and branching pattern (Dierssen *et al.*, 2003). However, due to the alteration in dendritic spines, most of the studies on DS and FXS have been focusing on mature neurons so far. DS patients show reduced dendritic branching and complexity in pyramidal neurons along with fewer and abnormal spines with enlarged heads that could explain the cognitive deficits. This goes along, at the molecular level, with alterations in synaptic plasticity molecular pathways leading to long-term potentiation (LTP), deficits in DS mouse models, while long-term depression (LTD) is enhanced. Conversely, in FXS patients the cognitive impairment goes along with an increased density of thin and elongated spines in the same neurons and here while the role of LTP is controversial, LTD is strongly induced, due to the overactivation of glutamate receptors.

Those changes stem from very different genetic causes. DS is due to the presence of an extra copy of human chromosome 21 containing approximately 270 genes. While not all three copy genes are overexpressed in the DS conditions (Ait Yahya-Graison *et al.*, 2007) there are several candidate genes that are transcribed in the central nervous system and overexpressed in the DS condition whose expression and/or biological activity could be reduced. Among them, the kinase DYRK1A known to be involved in brain development is closely associated to DS phenotype and can be modulated using different classes of inhibitors (Duchon & Herault, 2016). Several mouse models of DS have been engineered that reproduce several phenotypes common with DS including cognitive impairments (Choong *et al.*, 2015). Using these mouse models more than 50 therapies targeting neurotransmission, neuroprotection or specific gene overexpression have been tested so far with a high rate of success (Stagni *et al.*, 2015).

FXS is due to the silencing of the Fragile X Mental Retardation gene encoding an RNA binding protein FMRP endowed with multiple RNA binding domains (two KH Domains and one RGG-bow),

a Nuclear Localization Signal (NLS) and a Nuclear Export Signal NES). This protein is mainly expressed in neurons and implicated in several steps of RNA metabolism including i) translational regulation, being part of ribonucleoproteic complexes associated to polyribosomes both in soma and at the synapse; ii) export of mRNA from nucleus to cytoplasm; iii) transport along dendrites and axons. The mRNA targets of FMRP have been extensively searched. Even if functional characterization has been performed only for some of them, it is clear that FMRP is mostly involved in regulating the levels of synaptic proteins implicated in autism (Maurin *et al.*, 2014). A mouse model for FXS exists, recapitulating the human phenotype of the disorder. By using this mouse, in recent years, the increased knowledge about the molecular pathways that are altered in FXS neurons has led to the identification of metabotropic glutamate receptor 5 (mGlu5) and GABA B receptors as potential pharmacological targets (Ellegood *et al.*, 2010; D'Antoni *et al.*, 2014; de Esch *et al.*, 2014; Lai *et al.*, 2016).

Among intellectual disabilities from genetic origin, the chromosome 22q11.2 microdeletion is one of the strongest genetic risk factors for neurodevelopmental disorders, therefore an ideal genetic defect to study molecular pathways implicated in neurodevelopmental affections that result from imbalances in gene dosage. This microdeletion removes one copy of a chromosomal region spanning ~63 genes. This observation raises the question of whether there are single or diverse pathways downstream of this complex polygenic genetic defect and the identity of these pathways. To address these questions, we developed a pedigree-based quantitative mass spectrometry strategy, *genealogical proteomics*, where we compared the whole proteome of probands affected by 22q11.2 microdeletion syndrome and early childhood psychosis to the proteomes of their unaffected relatives. We analyzed homogenates of primary cultured skin fibroblasts from five affected individuals and four families using Tandem-Mass-Tagging (TMT), Triple SILAC, and label free quantitative (LFQ) MS/MS. We used bioinformatics to infer molecular mechanisms co-segregating with the microdeletion. We identified ~1,200 proteins whose expression was modified by the 22q11.2 microdeletion. 22q11.2-specific protein expression changes significantly enriched ontological categories of mitochondrion

and actin filament cytoskeleton. We confirmed these changes in a mouse model that genetically mimics the 22q11.2 microdeletion (*Df(16)A*) using similar proteomic and bioinformatics strategies. We focused on proteins expressed in mitochondria and confirmed that 22q11.2 mutant cells had distinctive respiration mitochondrial phenotypes. Moreover, we used *Drosophila* genetics and electrophysiology to demonstrate that gene dosage reductions in mitochondrial gene products identified in 22q11.2 cells affected synaptic transmission and behavior. These results demonstrate that several pathways are affected downstream the 22q11.2 mutation. One of these routes involves the mitochondria and its genetic manipulation affects synaptic function in *Drosophila*.

All the previous results have highlighted how common or divergent mechanisms can give rise to similar or opposite phenotypes. However, despite high hopes, preclinical studies have not always translated into a broadly effective treatment for intellectual disabilities of genetic origin. Even if some of these studies are still in progress, the search for new treatments able to modify the lifetime course of DS and FXS is urgently awaiting. It is essential that different molecules will be rendered available, to be administered to patients at different stages of life and to treat the multiple phenotypes alone or in combination. To this aim we need to advance the research on new disease models that warrant the best predictive validity for clinical trials, but also we need to be aware of the challenges that clinical trials pose to these disorders. In this review, we summarize some of the leading research efforts in this field.

### **Using induced pluripotent stem cells, derived neuronal cells and brain organoids to model DS and FXS**

As highlighted above, despite continuous efforts to improve the process of drug discovery achieving success at the clinical stage remains challenging. During the last years, the discovery of human induced pluripotent stem (iPS) cells has opened up the doors to a new era of disease modelling and brought new hope to the drug discovery field. For intellectual disabilities, availability of human iPS

cell-derived cells are enabling scientists to access a variety of human cellular models which can be used as tools to improve understanding of disease mechanisms and test therapeutic targets. However, their use in the field of translational medicine remains challenging and requires high levels of scrutiny and validation at each stage. These models have proven to be particularly useful in investigating single gene disorders, however, it is largely yet to be seen how useful iPSCs will be in modeling complex human diseases, such as DS. Here we will discuss some of the most recent advances in this field.

An isogenic DS induced pluripotent stem cells (iPSC) integration-free model was developed by using fibroblasts of an adult with constitutional mosaicism for trisomy 21 (T21). By comparing T21 and control isogenic neurons derived from iPSCs several DS phenotypes could be recapitulated such as increased  $\beta$ -amyloid, mitochondrial abnormalities, and increase in DNA double strand breaks indicating accelerated ageing (Murray *et al.*, 2015). 3D cerebral organoids differentiated from isogenic iPSCs were recently found to recapitulate aspects of human brain structure and layering (Sutcliffe & Lancaster, 2017). A collection of iPSCs has been generated from individuals with DS through the LonDownS consortium in London UK (<https://www.ucl.ac.uk/london-down-syndrome-consortium>). So far, >400 DS adults have been recruited with >120 lines isolated. These new human cellular models and organoids will be very useful to study pharmacotherapies aiming at correcting synaptopathies.

Mouse embryonic stem cell line displaying a reduced expression of *Fmr1* by stable transfection of a specific shRNA directed against *Fmr1* (*shFmr1* ES) were generated (Khalfallah *et al.*, 2017). These cells do not display any cell cycle variation or morphological abnormality but they exhibit altered expression of a subset of genes mainly involved in neuronal differentiation and maturation, suggesting a subjacent molecular pathology. For this reason, differentiation of *shFmr1* ES cells into the neuronal lineage was induced. After 4 days of *in vitro* differentiation an accelerated generation of neural progenitors and neurons with an increased expression of P27,  $\beta$ III-Tubulin and then a reduced



number of nuclei after 1 week of *in vitro* culture were observed. This phenotype is transient, as the final number of neurons is not affected at late phases of *in vitro* neurogenesis after 2-3 weeks of *in vitro* culture. Interestingly, neurogenesis is also accelerated in the embryonic brain of *Fmr1* KO mice, where an elevated level of p27 and  $\beta$ III-Tubulin both at E12.5 and E14.5 was present. These findings suggest that the sh*Fmr1* ES cell model recapitulates the molecular and cellular alterations present *in vivo* (Khalfallah *et al.*, 2017). The accelerated generation of neural progenitors and neurons during the first steps of neurogenesis of sh*Fmr1* ES cells is likely due to an elevated level of the Amyloid Precursor Protein (APP), whose mRNA is a known target of FMRP (Westmark & Malter, 2007). APP is processed by the BACE-1 enzyme, producing the  $\beta$ -amyloid (A $\beta$ ) peptide that is known to accelerate neurogenesis by activating the expression of *achaete-scute family bHLH transcription factor 1* (*Ascl1*) (Uchida *et al.*, 2007; Freude *et al.*, 2011), a factor that has also a pivotal role in neuronal differentiation. Thus in *Fmr1*-depleted ES cells the elevated level of A $\beta$  peptide is likely to induce the expression of *Ascl1* (Khalfallah *et al.*, 2017) that represents a surprising event and the key point to explain the subsequent accelerated neuronal differentiation (Khalfallah *et al.*, 2017). Consistently, the cell phenotype is rescued not only by re-expressing human FMRP, but also by reducing the processing of APP by the specific BACE-1 inhibitor LY2811376. The importance of the A $\beta$  peptide in the physiopathology of FXS as well in other forms of autism and intellectual disabilities (ID) has been extensively studied (Westmark *et al.*, 2016).

The phenotype of sh*Fmr1* neural progenitors appears surprising since cell models of neural precursors for genes involved in other forms of ID/autism and DS rather display a delay of neuronal differentiation or a disruption of neurogenesis (Jolly *et al.*, 2013; Jolly *et al.*, 2015; Fujitani *et al.*, 2016). An example of accelerated neuronal differentiation is provided by ATRX (Alpha thalassemia/mental retardation syndrome X-linked) intellectual disability. Indeed, premature neurogenesis has been associated to gross brain abnormalities consistently with the microcephaly observed in patients affected by this disorder (Ritchie *et al.*, 2014; Huh *et al.*, 2016). Conversely, the depletion of *Phosphatase and TENSin homolog* (*PTEN*) in postnatal/young neural stem cells produced

an altered neurogenesis characterized in a first step by an increased proliferation and differentiation rate of these cells (Amiri *et al.*, 2012) followed by an early loss of Neural Stem/progenitor Cells (NSCs). In this case, similarly to FXS, it is possible to observe an altered kinetics of neurogenesis. However, due to the severity of the cellular alterations, the morphological brain abnormality appears more evident than in FXS brains (Maurin *et al.*, 2014; Khalfallah *et al.*, 2017; Westmark, 2017). The sh*Fmr1* cell model will be a very useful tool to search for novel therapies for FXS. Indeed, it can be used for screening of bioactive molecules, including libraries of small bio-active molecules approved for clinical use. This screening is feasible considering that we have shown that the phenotype of the FXS cell model can be reverted by pharmacological tools and some outputs can be easily measured even in a high-throughput screening format leading to the identification of new drugs to treat FXS (Bardoni *et al.*, 2017). Overall, these results underline the importance to study embryonic neurogenesis in ID/autism animal models to decipher the physiopathology of these disorders and to identify helpful biomarkers for translational studies.

### **Computational modeling and new approaches to linking omic, data with disease mechanisms**

Probably, one of the most ambitious goals in biomedical research is to trace all the steps from gene expression to brain state. In the last years, computational approaches have been successfully used to unravel the pathophysiology in many different diseases. Models of neural diseases aim to establish a link between the biological and pathological abnormalities of the damaged brain, but to this aim computational neuroscience requires a battery of supporting data obtained in the same experimental context and covering different levels of detail (neural types, neuromodulators, stimulus specific responses, etc.). Regardless of the particularities of the model, all are network representations of computational units, i.e. excitable systems integrating several current sources. A neural network can be considered a function space that autonomously translates the domain of inputs into outputs. Due to the broad range of interacting variables (ions, neurotransmitters, proteins, etc.) and of brain

capabilities (learning, memory, consciousness, etc.) it is extremely challenging to capture the connections between the micro and macroscopic processes.

Computational models help to capture the essential features of the brain at multiple spatio-temporal scales, from molecular networks and protein interaction to membrane currents and chemical coupling to network oscillations. Therefore, they offer a powerful tool to relate these data to neurological dysfunction. Moreover models provide a theoretical framework where to control and manipulate neural networks under different “configurations” beyond the wet lab experiments, hence revealing unexpected results, otherwise counterintuitive or experimentally hard (or impossible) to obtain. In DS, due to the complexity of gene interactions and the outnumbered molecular pathways activated by the extra copy of HSA21, there has been an increasing interest on the over-expression of those genes that were particularly able to recapitulate the DS phenotype (Dierssen, 2012). On the other hand, research on DS patients is abundant and allow for model validation. However, despite the amount of experimental results available for data-driven models, DS has not driven the attention of computational neuroscience. According to the existing data on DS patients (and DS mouse models) covering network architecture (dendrite morphology) and macroscopic functional changes (brain activity alterations), we claim that both single-neuron-based and population networks can boost our understanding of DS. In addition, since the genetic causes of the disease are well known, computational models of DS can comprise another layer of biological description, i.e. the genetic regulatory network, that have remained so far detached from the modeling of neural circuits.

Another fundamental problem is that alterations of the nervous system leading to DS show a lifetime-specific development of neuropathological mechanisms that give raise to characteristic behavioral manifestations. Thus, we need to understand the progression of all the preclinical and clinical symptoms of such disorders, including patterns of behavior, what is commonly known as the natural history of the disorder, and the developmental and adult changes that produce specific cognitive disturbances. To this aim, using novel bioinformatics approaches to understand big behavioral data will be essential in the search of treatments.

## **Targeting common pathways for designing clinical trials for DS and other genetic developmental disorder of the central nervous system and assessing their efficacy**

During the last few years, many groups have been working intensively with the goal to clarify the molecular mechanisms underlying impaired brain functioning in DS and to devise targeted therapies. Neurogenesis and dendritogenesis alterations are the two major defects of the DS brain and are present at the very beginning of brain development. Thus, we believe that it is important to treat DS individuals during the perinatal period. It is relatively easy to test different interventions in the mouse models to identify drugs that eventually will be effective in individuals with DS.

Lithium was one of the first to be tested since it was known that in adult mice it increases neurogenesis in the subventricular zone of the lateral ventricle (Bianchi *et al.*, 2010). Lithium however, was not well tolerated by the pups that exhibited a very high death rate. In the following studies, fluoxetine, a selective serotonin reuptake inhibitor, was used based on the following considerations. Serotonin is crucial for neurogenesis and dendritogenesis, and the serotonergic system is impaired in DS. This impairment may thus contribute to the alteration of brain development. We found that in neonatally-treated Ts65Dn mice, one of the most widely used mouse model of Down syndrome, there was restoration of the development of the hippocampus (a structure that in rodents mainly develops postnatally) in terms of neurogenesis, dendritogenesis and connectivity. Importantly, these effects largely outlasted treatment cessation and led to restoration of hippocampus-dependent memory (Clark *et al.*, 2006). We then wondered whether embryonic treatment with fluoxetine also rescued other aspects of brain development. We treated pregnant Ts65Dn females from embryonic day ten to delivery and examined the progeny at P2 and P45. We found that embryonic treatment with fluoxetine restored neurogenesis and cellularity throughout the whole brain of P2 Ts65Dn mice and that this effect was retained after treatment cessation and led to restoration of memory in P45 mice (Guidi *et al.*, 2014). DS linked neurodevelopmental alterations were thought to be irreversible. This study demonstrates that it is possible, at least in the mouse model, to pharmacologically fully rescue brain

development. Fluoxetine is an antidepressant that is also prescribed in children. However, its use during pregnancy may not be free of side effects. Although the question is not completely settled, it appears that fluoxetine during pregnancy may have effects on development of the heart (Reefhuis *et al.*, 2015), a process that is impaired in many children with DS.

We had previously obtained evidence that in trisomic neural precursor cells excessive levels of APP cause excessive levels of the peptide AICD, one of its cleavage products. AICD increases the transcription of *Ptch1*, the repressor of the mitogenic Sonic Hedgehog pathway and that inhibition of this pathway is involved in neurogenesis impairment in DS (Giacomini *et al.*, 2015). Therefore, we thought that by reducing the levels of AICD we could reinstate the functionality of the Shh pathway and, consequently neurogenesis. Since AICD derives from the cleavage of the CTFs operated by gamma-secretase, in order to reduce AICD formation we used an inhibitor of gamma-secretase (ELND006). We found that neonatal treatment restored hippocampal neurogenesis and synaptic maturation (Giacomini *et al.*, 2015). This suggests that inhibitors of gamma-secretase may be used in order to restore brain development in DS. However, the inhibitor that we used was shown to cause adverse effects in a clinical trial in individuals with AD. Thus, our results provide proof-of principle demonstration of the usefulness of inhibitors of gamma-secretase in DS, but the transfer to humans requires the creation of safe inhibitors.

GSK3-beta is a kinase involved in many developmental processes, including neurogenesis and neuron differentiation (Jope & Johnson, 2004). Unlike other kinases, GSK3 becomes inactive when it is phosphorylated. We previously found that in trisomic neural precursor cells there are reduced levels of pGSK3beta and that treatment with lithium, an inhibitor of GSK3beta activity, restored proliferation (Trazzi *et al.*, 2014). Based on these premises we decided to use tideglusib, a new selective non-ATP competitive inhibitor of GSK3beta (Eldar-Finkelman & Martinez, 2011) in order to establish whether such a treatment may be useful to restore neurogenesis in DS. We found, however, that neonatal treatment with tideglusib had no positive impact on neurogenesis. In cultures of NPCs from Ts65Dn mice we tested a wide range of concentrations but none was effective. These

results are surprising because inhibition of GSK3beta by lithium has a positive impact on neurogenesis. It must be noted that lithium has additional effects in the cell (Can *et al.*, 2014), suggesting that its efficacy on proliferation may not be directly linked to GSK3beta inhibition. It is also possible that tideglusib has other cellular effects, not described so far, that counteract its effects on GSK3beta. In any case, although tideglusib appears to improve behavior in mouse models of AD, it is not a suitable drug for DS.

BDNF is a neurotrophin important for neurogenesis and neuron maturation. Since the DS brain exhibits reduced levels of BDNF, it is conceivable that this defect may contribute to brain development impairment and that a treatment targeted to the BDNF system may have a beneficial effect. BDNF crosses the blood-brain barrier poorly, but this problem can be circumvented by using small molecules that bind to its receptor TrkB. Based on these premises, we sought to establish whether treatment with 7,8-Dihydroxyflavone (7,8-DHF), a small flavonoid that binds with high specificity to TrkB (Liu *et al.*, 2010), positively impacts on neurogenesis and dendritic maturation. In Ts65Dn mice neonatally-treated with 7,8-DHF we found an increase in the proliferation rate of NPCs of the DG and an increase in spine density on the dendritic tree of granule neurons. These preliminary results show that pharmacotherapy targeted to the BDNF system has a positive impact on the two major defects of the trisomic brain. The duration of the effects of treatment remains to be established.

DYRK1A is one of the triplicated genes thought to be strongly involved in the brain phenotype of DS. This notion derives from studies in Dyrk1A transgenic models that show that these models have some features of DS. In these models, inhibition of Dyrk1A with epigallocatechin gallate (EGCG), that is a component of green tea extracts and is an inhibitor of DYRK1A (Tejedor & Hammerle, 2011), has been shown to restore many developmental defects and behavior (Guedj *et al.*, 2009; Pons-Espinal *et al.*, 2013; Thomazeau *et al.*, 2014). Importantly, the pioneer study by De La Torre *et al.* (De la Torre *et al.*, 2014) has shown that adult Ts65Dn mice treated with EGCG undergo memory restoration similarly to TgDyrk1A mice. Moreover, in a pilot study in young adults with DS, he

showed that EGCG induces a behavioral benefit in some memory domains. This benefit, however, tends to disappear with time. Based on this promising study, we wondered whether treatment with EGCG during a crucial phase of brain development, when most of the hippocampal neurons are generated, rescues hippocampal architecture and whether the hippocampus may remain in its restored state after treatment cessation. We found that neonatal treatment with EGCG fully restores neurogenesis and synaptic development. At one month after treatment cessation, these effects were no longer present and there was no behavioral improvement (Stagni *et al.*, 2016). These results show that EGCG is a very good therapy for restoration of neurogenesis in DS, although its effects are ephemeral. Yet, since EGCG appears to be a safe compound, it may be possible to envisage a protocol of EGCG administration in which EGCG is periodically administered at time intervals - to be established – or in conjunction with other intervention (e.g. cognitive training) in order to prevent the disappearance of its effects. It would also be important to establish a concomitant environmental stimulation since combined treatment was more effective in human studies than EGCG alone (de la Torre et al 2016).

Overexpression of *DYRK1A* is involved in several cognitive, electrophysiological and neuromorphological alterations found in mouse models of DS (Tejedor & Hammerle, 2011). New synthetic inhibitors of DYRK1A activity, with high purity and specificity, have been tested successfully showing recovery of cognition in DS mouse models (Gourdain *et al.*, 2013; Falke *et al.*, 2015; Kim *et al.*, 2016). Cognitive restoration in adult with DYRK1A inhibitors was similar to genetic rescue observed in DS mouse models when one functional copy of *Dyrk1a* was inactivated to bring back the DYRK1A dosage to 2 copies (Ortiz-Abalia *et al.*, 2008; Garcia-Cerro *et al.*, 2014). Nevertheless, the repetitive behavior observed in models with overexpression of DYRK1A was still observed after treatment suggesting that developmental defects due to DYRK1A triplication were not completely rescued by adult treatment. Earlier perinatal treatments should be explored to counteract DYRK1A overexpression in DS. In addition a quantitative phosphoproteomic approach identified several specific protein targets of DYRK1A activity involved in synaptic function, leading to a better

understanding of the pathophysiological alterations produced by DYRK1A overexpression (Y. Hérault *et al.*, in preparation). It will be important to find out whether similar molecular pathways are altered in various developmental disorders such as DS, FRX and 22q 11.2 deletion.

It is thus hoped that thanks to the efforts of preclinical studies it may be possible to offer to clinicians a series of compounds that may be worthwhile testing in children with DS. Since neurodevelopmental disorders share, sometimes, similar defects these compounds may also be exploited for other disorders. Given that they are effective, this achievement may offer a better life to affected children and their families.

### **Bringing new avenues to modulate neuroplasticity in intellectual disabilities: the promising case of neurotherapy**

As indicated in this review there is a strong motivation to improve ID, including DS and FXS by researchers. Intellectual disabilities are associated with abnormal brain activity, which might be linked to immature development of connectivity between distant brain regions (Anderson *et al.*, 2013). Such delay in brain development is likely to affect the development of coherent distributed networks (Anderson *et al.*, 2013). This abnormal neuroplasticity might be affected from cellular and molecular mechanisms, including excitation/inhibition, which plays a critical role in neuroplasticity during development and lead to delay in the acquisition of cognitive skills (Cohen Kadosh *et al.*, 2015; Werker & Hensch, 2015).

One promising methods to alter brain functions and modulate neuroplasticity is using transcranial stimulation. The two forms of transcranial stimulation that have been shown long-term effects at the behavioural and neural level spanning from days to months after the intervention are transcranial direct current stimulation (tDCS) and transcranial random noise stimulation (tRNS). Note that we do not review studies using transcranial magnetic stimulation (TMS), another form of transcranial stimulation. While the potential use of TMS in combination with cognitive training/intervention remains to be explored, tDCS and tRNS, have higher practical validity for intervention, as they are



portable, more comfortable for the participant, cheaper, easier to use in double-blind or sham-controlled studies, and more easily applied at the same time during training and for repeated use (Cohen Kadosh *et al.*, 2012; Krause & Cohen Kadosh, 2013). Moreover, when used within suggested guidelines, the acute safety risks (of seizures, for example) seem very low (in contrast to TMS, there are no reports of seizures) (Hummel & Cohen, 2006; Priori *et al.*, 2009). The issue of safety is important as individuals with Down syndrome might be at increased risk of epileptic seizures.

### *tDCS*

tDCS involves the application of weak electrical currents, typically between 1-2mA through saline-soaked sponge electrodes from a battery-driven stimulator. It is the most common form of transcranial electrical stimulation used in studies on cognitive enhancement (Kuo & Nitsche, 2012; Coffman *et al.*, 2014; Looi *et al.*, 2016) Harty *et al.* 2016; Sela *et al.* 2014) and neurointervention (Krause & Cohen Kadosh, 2013). In a typical setup, one electrode is placed over the scalp above cortical region of interest in accordance to the 10-20 electroencephalogram (EEG) system of electrode placement, while the other is placed to close the circuit. The latter electrode is referred in many studies as a “return” electrode and could be placed on the contralateral region of the region of interest, or usually over the supraorbital (region above the eye sockets) or extracephalic (e.g., shoulder) regions. The positioning of the return electrode could influence the overall current flow pattern through the brain and the consequent modulatory effects (Bikson *et al.*, 2004a). It is common for studies to assess the behavioural effects of tDCS by comparing at least two groups: a real vs. sham tDCS group. In the real group, participants would receive 1-2mA during a specific task (usually for around 20 minutes continuously), while the sham group would receive the same current intensity but only for a very short period of time (e.g., 15 seconds at the beginning and at the end of training). This short stimulation is sufficient to mimic the sensation of a real stimulation, but have negligible effects at the neuronal level (Gandiga *et al.*, 2006; Fritsch *et al.*, 2010).

Mechanistically, tDCS is known to operate on the basis of electrical polarity. Typically anodal tDCS typically facilitates neuronal firing, while cathodal tDCS inhibits neural firing of the cortical region

beneath the site of stimulation (Bindman *et al.*, 1964; Nitsche & Paulus, 2000b; Nitsche & Paulus, 2000a; Bikson *et al.*, 2004a; Bikson *et al.*, 2004b)(Bindman *et al.*, 1964; Nitsche & Paulus, 2000; Bikson *et al.*, 2004)[12-14](12-14)<sup>12-14</sup>(Bindman *et al.*, 1964; Nitsche & Paulus, 2000; Bikson *et al.*, 2004). Although, recent studies on humans have reported variability in these ‘expected’ facilitation and inhibition (Jacobson *et al.*, 2012). Pharmacological studies have indicated the potential involvement of ion channels (Dayan *et al.*, 2013) such as the voltage-dependent sodium channels (Liebetanz *et al.*, 2002; Nitsche *et al.*, 2003a), calcium channels and *N-methyl-d-aspartate* (NMDA) receptors (Nitsche *et al.*, 2003a) through the use of blockers or antagonists in examining the effects of tDCS. In addition, the effects of tDCS have also been linked with modulation of neurotransmitter systems such as dopamine (Nitsche *et al.*, 2006) and concentrations of  $\gamma$ -aminobutyric acid (GABA) (Stagg *et al.*, 2009; Clark *et al.*, 2011; Kim *et al.*, 2014) glutamate and glutamine (Clark *et al.*, 2011). It has been proposed that the excitability/inhibitory balance, as might be indicated by concentrations of glutamate/GABA in stimulated regions could mediate the effects of brain stimulation (Krause *et al.*, 2013). Others have also proposed that tDCS might improve cognition by directly targeting the intrinsic oscillatory activity, linked with a range of cognitive processes (Hoy *et al.*, 2013). Finally, at the network level, tDCS has been associated with significant changes in regional functional brain connectivity (Keeser *et al.*, 2011; Polania *et al.*, 2011; Meinzer *et al.*, 2012; Hunter *et al.*, 2013; Meinzer *et al.*, 2013; Stagg *et al.*, 2013) (for review on tDCS mechanisms, see also (Looi *et al.*, 2016)).

The effects of tDCS have shown to be long-lasting, spanning from weeks to months post-stimulation. This includes also improvement of high-level cognitive functions such as numerical abilities and executive functions, but also visuomotor abilities and language (e.g., (Floel *et al.*, 2008; Reis *et al.*, 2009; Cohen Kadosh *et al.*, 2010; Looi *et al.*, 2016). The long-term effect of tDCS were proposed to be supported by mechanisms with similar features to long-term synaptic plasticity (Stagg *et al.*, 2011) including processes that rely on protein (Nitsche *et al.*, 2009), protein synthesis (Gartside, 1968b; a), NMDA receptors known support long-term potentiation (Islam *et al.*, 1995; Nitsche *et al.*, 2003b)

and long-term depression, and mediation by polymorphisms in the brain-derived neurotrophic factor (BDNF) gene (Fritsch *et al.*, 2010).

### *tRNS*

tRNS is a relatively novel form of transcranial stimulation that was investigated experimentally in 2008 (Terney *et al.*, 2008). It involves the application of alternating currents (e.g., between  $-0.5$  to  $+0.5$ mA) at different frequencies to the scalp, typically between 0.1-640Hz or 100-640Hz, known to be safe for humans. Although it shares many similarities in the principles of its operation to tDCS, i.e., delivered to the scalp via electrodes that are attached to a stimulator and similar sham setup (by limiting the time of delivery enough to induce a ‘stimulation sensation’), this technique is preferred over tDCS for allowing better blinding (sham) conditions given its higher cutaneous perception threshold (Ambrus *et al.*, 2010; Fertonani *et al.*, 2015), and 2) for providing excitatory stimulation to the brain areas beneath the electrodes simultaneously (i.e., at the same time, on the same subject, rather than anodal and cathodal stimulation as in tDCS) as it is oscillatory current and hence, polarity-independent (Terney *et al.*, 2008). Furthermore, in a perceptual learning study, it has been shown to induce stronger behavioural effects than tDCS (Fertonani *et al.*, 2011).

As it was introduced fairly recently, the mechanisms of tRNS are fairly unexplored and hence, even less known compared to tDCS. It has been suggested that tRNS enhances neuronal excitability by increasing the activity of sodium ion channels (Terney *et al.*, 2008; Fertonani *et al.*, 2011) and stochastic resonance, whereby signal detection is enhanced when noise is introduced into the neural system (Ward *et al.*, 2006; Miniussi *et al.*, 2010; van der Groen & Wenderoth, 2016). In line with both theories, the effects of tRNS have been shown to produce transient modulation in blood oxygenation-level dependent (BOLD) response (Chaieb *et al.*, 2009), possibly reflecting less noise in the system (Matsuoka *et al.*, 2000) and/or a change in synaptic activity. A recent study has also reported more efficient neurovascular coupling in stimulated regions post-tRNS with training, consistent with observed behavioural improvements (Snowball *et al.*, 2013). Through mechanisms of stochastic resonance, the authors suggested that the amount of endogenous electrical noise might have

been reduced, resulting in a decreased level of responses in regional cerebral blood flow to maintain neural activity. In the same study, the effects of tRNS were maintained up to 6 months. It was proposed that such longevity of effects in the absence of further stimulation might have been sustained by indirect mechanisms such as structural changes to the cerebrovasculature. This idea is in line with previous animal studies that showed significant angiogenesis and upregulation of angiogenic vascular endothelial growth factor post-electrical stimulation (Baba *et al.*, 2009).

Studies that used tRNS to affect cognitive functions have shown improvement in skill acquisition, and mathematical and numerical abilities (Fertonani *et al.*, 2011; Popescu *et al.*, 2016), in some cases with effects lasting months after the end of the intervention (Cappelletti *et al.*, 2013; Snowball *et al.*, 2013; Cappelletti *et al.*, 2015).

While the mechanisms of both techniques warrant further investigation, tDCS and tRNS have been shown to enhance various human motor and cognitive abilities (see (Paulus, 2011) and (Cohen Kadosh *et al.*, 2015) for a collection of reviews) with minimal discomfort or adverse side effects (Poreisz *et al.*, 2007; Fertonani *et al.*, 2015).

Given the current results, and the strong potential in long-term effect, the potential for applying such methods to improve intellectual abilities, such as Down syndrome is appealing. However, the application of tDCS and tRNS to the developing brain is quite sparse. While there is a higher risk-benefit ratio in the case of intervention in atypical development than cognitive enhancement in typical development (Maslen *et al.*, 2014a; Maslen *et al.*, 2014b), there is a need for more caution when applying such stimulation and monitor the benefit as well as potential side effects (Krause & Cohen Kadosh, 2013; Davis, 2014). In addition, studies on developing animals are important in order to assess whether there might be a potential side effect that might be overseen when stimulation is applied to the child's brain, as currently the work on safety on animal models are based mainly on adults. Future studies would allow assessing the efficacy, safety, and also provide more causal evidence for the neural factors that are involved in intellectual disabilities, and therefore have both basic and translational impact.

### **Why translational research fails in intellectual disability?**

Advances in understanding molecular and synaptic mechanisms of ID in FXS and DS syndromes through animal models have led to targeted controlled trials with pharmacological agents designed to normalize these underlying mechanisms and find molecular targets to improve clinical outcomes. However, several clinical trials have failed to demonstrate efficacy of these targeted treatments to improve surrogate behavioral/cognitive endpoints. These failures relate to the difficulties in establishing neuropsychological measures for cognitive rehabilitation in DS. In the absence of a gold standard method to examine the therapeutic effects of an intervention on cognition within a short examination, researchers are pushed to test for efficacy in a variety of domains using an assortment of assessments. This approach inevitably leads to problems in translating significant findings from mouse models. Because the ultimate index of disease modification in these disorders is amelioration of ID, the validation of cognitive measures for tracking treatment response is essential. One major obstacle to the demonstration of efficacy in human trials has been the lack of generally accepted endpoints to assess improvement in function in individuals with intellectual disability. The recently developed National Institutes of Health Toolbox Cognitive Battery (NIH-TCB) for ID has potential for assessing important dimensions of cognition in persons with ID, and several tests may be useful for tracking response to intervention. However, more extensive psychometric studies, and evaluation of its sensitivity to developmental and treatment-related change, will determine the true utility of the battery as a set of outcome measures (Hessl *et al.*, 2016).

#### *Individual variability in intellectual disability: the case of DS.*

The second aspect to be taken into account is the broad inter individual variability in brain disorders leading to ID. We cannot consider patients with ID or even more specifically with a defined syndrome, as a homogeneous group, since individual differences influence the relationships between genotype and the emerging phenotype. If we aspire at precision Medicine, one extremely important issue is to understand that differences in the cognitive and functional evolution of each individual are

conditioned by individual differences at different levels: genetic (genetic polymorphisms, such as DA, 5-HT, APOE), biochemical (A $\beta$ -peptides), physiological (sleep disorders), medical comorbidities (hypothyroidism, depression), sociodemographic characteristics (gender), or life style (diet). Those differences may also modulate the response to treatment and may lead to unresponsive, responsive patients or even to individual-specific adverse events.

In the case of DS we have several examples of these factors. The first is driven by the fact that nearly all adults with DS show neuropathology of Alzheimer's disease (AD), including amyloid deposition by their fourth-fifth decade of life. In DS, higher plasma A $\beta$ 42 concentration is associated to a higher score in the Dementia Questionnaire for People with Learning Disabilities (DLD; formerly known as DMR) and impaired communication skills in the Adaptive Behavior Assessment System (ABAS-II; (Hoyo *et al.*, 2015). Semantic verbal fluency in young DS adults is specifically influenced, so that the larger the A $\beta$ 42 concentrations the worse the performance of such tasks (Hoyo *et al.*, 2015).

Modifiable risk factors for cognitive impairment have also received attention, and there is a growing literature of metabolic risk factors for cognitive impairment and therapeutic management. One example is thyroid dysfunction. Overt hypothyroidism is a well-known reversible factor causing cognitive impairment including dementia (Moon, 2016). Hypothyroidism is the most common endocrine problem in DS, and approximately 10% of children and between 13% and 50% adults with DS have congenital or acquired thyroid disease being the incidence hypothyroidism high. Serum TSH levels during hypothyroidism are inversely proportional to performance on word fluency and working memory tasks, and years of treatment with L-thyroxine are predictive of better performance in object recognition tests in the DS population (Xicota *et al.*, in preparation).

One of the challenges in cognitive neuroscience is to delineate the genetic determinants of inter individual variations. In the euploid population, polymorphisms involved in dopaminergic activity have consequences on cognition. Specifically, genetic variants of COMT Val158Met and VNTR-DAT1 polymorphisms have been shown to contribute to prefrontal cortex-dependent cognition in healthy population. However, there are few data about how such genetic variants may influence the

DS phenotype that is mostly explained by the overexpression of genes encoded by chromosome 21. In fact, it is known that the genetic background modulates the phenotypic consequences of genetic variants, and thus, polymorphisms may have differential consequences in DS. In DS, genotypes conferring higher dopamine availability as Met carriers and 10-repeat homozygotes result in improved executive function tasks that require mental flexibility (Del Hoyo *et al.*, 2016). Met carriers also present worse social skills and self-direction, along with higher social deterioration as measured by the Dementia Questionnaire for People with Intellectual Disabilities (DMR). This suggests that COMTVal158Met and VNTR-DAT1 polymorphisms may interact with the trisomic genetic background to influence DS phenotypes.

Other factors may also confer individual variability to the DS population. For example, sleep quality. Individuals with DS are particularly vulnerable to sleep-related disturbances including, snoring, cough, choke and exhibiting signs of restlessness, unusual sleeping positions, excessive sweating and periods of sleep apnea. Poor sleep quality was associated to worse visual memory skills in DS and daily living functioning, and early conversion to AD using the Pittsburgh Sleep Quality Index (PSQI). Apnea and snoring were associated to a worse adaptive behavior, while only snoring was associated to higher rates in the DMR and higher A $\beta$ 42/ A $\beta$ 40 ratio in plasma (de la Torre *et al.*, 2016).

Variability at different levels may overlap in each individual with DS in different ways, and as a result may end in different cognitive phenotypes. These myriads of differential interactions may translate into convergent or divergent cognitive and functional outcomes. For example, in some subjects with congenital hypothyroidism, the consequent cognitive impairment could be compensated if bearing genetic or environmental modifiers moderating APP metabolism. Instead, a trisomic subgroup with increased A $\beta$  concentrations, which at the same time would be suffering from obstructive sleep apnea or respiratory distress, could have more deleterious consequences on cognitive decline. Besides, despite the similar neuropsychological and functional pattern in DS at a group level, it is likely that a particular level of performance is achieved by different developmental trajectories in each individual (D'Souza & Karmiloff-Smith, 2011).

In conclusion, DS individuals might not show similar response to the same pharmacological treatment, and therefore cannot be directly compared to one another, which is an important consideration for establishing drug efficacy in DS clinical trials. We need to redefine primary endpoints to assess improvement in function in individuals with ID and consider the factors, which explain part of the phenotype variability. Those would possibly configure a set of biomarkers that need to be assessed in the screening visit in the context of a drug-efficacy clinical trial for cognitive, behavioral and/or functional enhancement.



## **Conclusions**

Current therapeutic approaches for DS and FXS focus on behavioral therapy, educational mainstreaming and off-label medications that mitigate only a limited set of symptoms but no approved pharmacological therapies are yet available. The incomplete understanding of individual phenotypic variability, natural history, and causes of differential response to inform trial design have limited our capacity to succeed, even when very promising drugs are tested, and are possibly dismissing good therapeutic opportunities. This underlines the requirement for more basic research, new tools and models that will allow a better understanding of the pathophysiology of those syndromes. In the recent years the use of iPS cells reprogrammed from patient fibroblasts and further derived into neuronal cells and organoids, has boosted research on mechanisms involved in genetic developmental disorders leading to intellectual disabilities. Some examples come from the field of DS and FXS. Using these cellular models as well as validated mouse models, genomic, proteomic and interactome studies has led to the discovery of common pathways across diseases that can now be targeted for clinical studies. On the other extreme is clinical research that has somehow been disappointing likely because of the heterogeneity inside specific intellectual disabilities of genetic origins taking place during development. Commonalities and grounds for stratification in this heterogeneous landscape still need to be defined. Nevertheless, it is also necessary to create more sensitive and realistic outcome measures to quantify disease and therapeutic efficacy, for improving patient recruitment strategies and access to resources required to mount a clinical trial (including funding). Solutions will require multicenter collaboration, partnership with patient organizations, training new generations of researchers and increasing the public resources dedicated to this field. More fundamental, applied and clinical research will need to be performed and various domains scrutinized for possible applications in various IDs.

## Bibliography

- Ait Yahya-Graison, E., Aubert, J., Dauphinot, L., Rivals, I., Prieur, M., Golfier, G., Rossier, J., Personnaz, L., Creau, N., Blehaut, H., Robin, S., Delabar, J.M. & Potier, M.C. (2007) Classification of human chromosome 21 gene-expression variations in Down syndrome: impact on disease phenotypes. *Am J Hum Genet*, **81**, 475-491.
- Ambrus, G.G., Paulus, W. & Antal, A. (2010) Cutaneous perception thresholds of electrical stimulation methods: comparison of tDCS and tRNS. *Clin Neurophysiol*, **121**, 1908-1914.
- Amiri, A., Cho, W., Zhou, J., Birnbaum, S.G., Sinton, C.M., McKay, R.M. & Parada, L.F. (2012) Pten deletion in adult hippocampal neural stem/progenitor cells causes cellular abnormalities and alters neurogenesis. *J Neurosci*, **32**, 5880-5890.
- Anderson, J.S., Nielsen, J.A., Ferguson, M.A., Burbach, M.C., Cox, E.T., Dai, L., Gerig, G., Edgin, J.O. & Korenberg, J.R. (2013) Abnormal brain synchrony in Down Syndrome. *Neuroimage Clin*, **2**, 703-715.
- Baba, T., Kameda, M., Yasuhara, T., Morimoto, T., Kondo, A., Shingo, T., Tajiri, N., Wang, F., Miyoshi, Y., Borlongan, C.V., Matsumae, M. & Date, I. (2009) Electrical stimulation of the cerebral cortex exerts antiapoptotic, angiogenic, and anti-inflammatory effects in ischemic stroke rats through phosphoinositide 3-kinase/Akt signaling pathway. *Stroke*, **40**, e598-605.
- Bardoni, B., Capovilla, M. & Lalli, E. (2017) Modeling Fragile X syndrome in neurogenesis: An unexpected phenotype and a novel tool for future therapies. *Neurogenesis (Austin)*, **4**, e1270384.
- Bianchi, P., Ciani, E., Contestabile, A., Guidi, S. & Bartesaghi, R. (2010) Lithium restores neurogenesis in the subventricular zone of the Ts65Dn mouse, a model for Down syndrome. *Brain Pathol*, **20**, 106-118.
- Bikson, M., Inoue, M., Akiyama, H., Deans, J.K., Fox, J.E., Miyakawa, H. & Jefferys, J.G. (2004a) Effects of uniform extracellular DC electric fields on excitability in rat hippocampal slices in vitro. *J Physiol*, **557**, 175-190.
- Bikson, M., Inoue, M., Akiyama, H., Deans, J.K., Fox, J.E., Miyakawa, H. & Jefferys, J.G. (2004b) Effects of uniform extracellular DC electric fields on excitability in rat hippocampal slices in vitro. . *J. Physiol.* , **557**, 175.
- Bindman, J.L., Lippold, O.C.J. & Redfearn, J.W.T. (1964) The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after-effects. *J. Physiol.*, **172**, 369-382.
- Can, A., Schulze, T.G. & Gould, T.D. (2014) Molecular actions and clinical pharmacogenetics of lithium therapy. *Pharmacol Biochem Behav*, **123**, 3-16.

- Cappelletti, M., Gessaroli, E., Hithersay, R., Mitolo, M., Didino, D., Kanai, R., Cohen Kadosh, R. & Walsh, V. (2013) Transfer of cognitive training across magnitude dimensions achieved with concurrent brain stimulation of the parietal lobe. *J Neurosci*, **33**, 14899-14907.
- Cappelletti, M., Pikkat, H., Upstill, E., Speekenbrink, M. & Walsh, V. (2015) Learning to integrate versus inhibiting information is modulated by age. *J Neurosci*, **35**, 2213-2225.
- Castren, M.L. (2016) Cortical neurogenesis in fragile X syndrome. *Front Biosci (Schol Ed)*, **8**, 160-168.
- Chaieb, L., Kovacs, G., Cziraki, C., Greenlee, M., Paulus, W. & Antal, A. (2009) Short-duration transcranial random noise stimulation induces blood oxygenation level dependent response attenuation in the human motor cortex. *Exp Brain Res*, **198**, 439-444.
- Choong, X.Y., Tosh, J.L., Pulford, L.J. & Fisher, E.M. (2015) Dissecting Alzheimer disease in Down syndrome using mouse models. *Front Behav Neurosci*, **9**, 268.
- Clark, S., Schwalbe, J., Stasko, M.R., Yarowsky, P.J. & Costa, A.C. (2006) Fluoxetine rescues deficient neurogenesis in hippocampus of the Ts65Dn mouse model for Down syndrome. *Exp Neurol*, **200**, 256-261.
- Clark, V.P., Coffman, B.A., Trumbo, M.C. & Gasparovic, C. (2011) Transcranial direct current stimulation (tDCS) produces localized and specific alterations in neurochemistry: a (1)H magnetic resonance spectroscopy study. *Neurosci Lett*, **500**, 67-71.
- Coffman, B.A., Clark, V.P. & Parasuraman, R. (2014) Battery powered thought: enhancement of attention, learning, and memory in healthy adults using transcranial direct current stimulation. *Neuroimage*, **85 Pt 3**, 895-908.
- Cohen Kadosh, K., Krause, B., King, A.J., Near, J. & Cohen Kadosh, R. (2015) Linking GABA and glutamate levels to cognitive skill acquisition during development. *Hum Brain Mapp*, **36**, 4334-4345.
- Cohen Kadosh, R., Levy, N., O'Shea, J., Shea, N. & Savulescu, J. (2012) The neuroethics of non-invasive brain stimulation. *Curr Biol*, **22**, R108-111.
- Cohen Kadosh, R., Soskic, S., Iuculano, T., Kanai, R. & Walsh, V. (2010) Modulating neuronal activity produces specific and long-lasting changes in numerical competence. *Curr Biol*, **20**, 2016-2020.
- D'Antoni, S., Spatuzza, M., Bonaccorso, C.M., Musumeci, S.A., Ciranna, L., Nicoletti, F., Huber, K.M. & Catania, M.V. (2014) Dysregulation of group-I metabotropic glutamate (mGlu) receptor mediated signalling in disorders associated with Intellectual Disability and Autism. *Neurosci Biobehav Rev*, **46 Pt 2**, 228-241.

- D'Souza, D. & Karmiloff-Smith, A. (2011) When modularization fails to occur: a developmental perspective. *Cogn Neuropsychol*, **28**, 276-287.
- Davis, N.J. (2014) Transcranial stimulation of the developing brain: a plea for extreme caution. *Front Hum Neurosci*, **8**, 600.
- Dayan, E., Censor, N., Buch, E.R., Sandrini, M. & Cohen, L.G. (2013) Noninvasive brain stimulation: from physiology to network dynamics and back. *Nat Neurosci*, **16**, 838-844.
- de Esch, C.E., Zeidler, S. & Willemsen, R. (2014) Translational endpoints in fragile X syndrome. *Neurosci Biobehav Rev*, **46 Pt 2**, 256-269.
- de la Torre, R., de Sola, S., Hernandez, G., Farre, M., Pujol, J., Rodriguez, J., Espadaler, J.M., Langohr, K., Cuenca-Royo, A., Principe, A., Xicota, L., Janel, N., Catuara-Solarz, S., Sanchez-Benavides, G., Blehaut, H., Duenas-Espin, I., Del Hoyo, L., Benejam, B., Blanco-Hinojo, L., Videla, S., Fito, M., Delabar, J.M., Dierssen, M. & group, T.s. (2016) Safety and efficacy of cognitive training plus epigallocatechin-3-gallate in young adults with Down's syndrome (TESDAD): a double-blind, randomised, placebo-controlled, phase 2 trial. *Lancet Neurol*, **15**, 801-810.
- De la Torre, R., De Sola, S., Pons, M., Duchon, A., de Lagran, M.M., Farre, M., Fito, M., Benejam, B., Langohr, K., Rodriguez, J., Pujadas, M., Bizot, J.C., Cuenca, A., Janel, N., Catuara, S., Covas, M.I., Blehaut, H., Herault, Y., Delabar, J.M. & Dierssen, M. (2014) Epigallocatechin-3-gallate, a DYRK1A inhibitor, rescues cognitive deficits in Down syndrome mouse models and in humans. *Mol Nutr Food Res*, **58**, 278-288.
- Del Hoyo, L., Xicota, L., Langohr, K., Sanchez-Benavides, G., de Sola, S., Cuenca-Royo, A., Rodriguez, J., Rodriguez-Morato, J., Farre, M., Dierssen, M., de la Torre, R. & Group, T.S. (2016) VNTR-DAT1 and COMTVal158Met Genotypes Modulate Mental Flexibility and Adaptive Behavior Skills in Down Syndrome. *Front Behav Neurosci*, **10**, 193.
- Dierssen, M., Benavides-Piccione, R., Martinez-Cue, C., Estivill, X., Florez, J., Elston, G.N. & DeFelipe, J. (2003) Alterations of neocortical pyramidal cell phenotype in the Ts65Dn mouse model of Down syndrome: effects of environmental enrichment. *Cereb Cortex*, **13**, 758-764.
- Duchon, A. & Herault, Y. (2016) DYRK1A, a Dosage-Sensitive Gene Involved in Neurodevelopmental Disorders, Is a Target for Drug Development in Down Syndrome. *Front Behav Neurosci*, **10**, 104.
- Eldar-Finkelman, H. & Martinez, A. (2011) GSK-3 Inhibitors: Preclinical and Clinical Focus on CNS. *Front Mol Neurosci*, **4**, 32.
- Ellegood, J., Pacey, L.K., Hampson, D.R., Lerch, J.P. & Henkelman, R.M. (2010) Anatomical phenotyping in a mouse model of fragile X syndrome with magnetic resonance imaging. *Neuroimage*, **53**, 1023-1029.

- Falke, H., Chaikuad, A., Becker, A., Loaec, N., Lozach, O., Abu Jhaisha, S., Becker, W., Jones, P.G., Preu, L., Baumann, K., Knapp, S., Meijer, L. & Kunick, C. (2015) 10-iodo-11H-indolo[3,2-c]quinoline-6-carboxylic acids are selective inhibitors of DYRK1A. *J Med Chem*, **58**, 3131-3143.
- Fertonani, A., Ferrari, C. & Miniussi, C. (2015) What do you feel if I apply transcranial electric stimulation? Safety, sensations and secondary induced effects. *Clin Neurophysiol*, **126**, 2181-2188.
- Fertonani, A., Pirulli, C. & Miniussi, C. (2011) Random noise stimulation improves neuroplasticity in perceptual learning. *J Neurosci*, **31**, 15416-15423.
- Floel, A., Rosser, N., Michka, O., Knecht, S. & Breitenstein, C. (2008) Noninvasive brain stimulation improves language learning. *J Cogn Neurosci*, **20**, 1415-1422.
- Freude, K.K., Penjwini, M., Davis, J.L., LaFerla, F.M. & Blurton-Jones, M. (2011) Soluble amyloid precursor protein induces rapid neural differentiation of human embryonic stem cells. *J Biol Chem*, **286**, 24264-24274.
- Fritsch, B., Reis, J., Martinowich, K., Schambra, H.M., Ji, Y., Cohen, L.G. & Lu, B. (2010) Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. *Neuron*, **66**, 198-204.
- Fujitani, M., Zhang, S., Fujiki, R., Fujihara, Y. & Yamashita, T. (2016) A chromosome 16p13.11 microduplication causes hyperactivity through dysregulation of miR-484/protocadherin-19 signaling. *Mol Psychiatry*.
- Gandiga, P.C., Hummel, F.C. & Cohen, L.G. (2006) Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. *Clin Neurophysiol*, **117**, 845-850.
- Garcia-Cerro, S., Martinez, P., Vidal, V., Corrales, A., Florez, J., Vidal, R., Rueda, N., Arbones, M.L. & Martinez-Cue, C. (2014) Overexpression of Dyrk1A is implicated in several cognitive, electrophysiological and neuromorphological alterations found in a mouse model of Down syndrome. *PLoS One*, **9**, e106572.
- Gartside, I.B. (1968a) Mechanisms of sustained increases of firing rate of neurones in the rat cerebral cortex after polarization: role of protein synthesis. *Nature*, **220**, 383-384.
- Gartside, I.B. (1968b) Mechanisms of sustained increases of firing rate of neurons in the rat cerebral cortex after polarization: reverberating circuits or modification of synaptic conductance? *Nature*, **220**, 382-383.
- Giacomini, A., Stagni, F., Trazzi, S., Guidi, S., Emili, M., Brigham, E., Ciani, E. & Bartesaghi, R. (2015) Inhibition of APP gamma-secretase restores Sonic Hedgehog signaling and neurogenesis in the Ts65Dn mouse model of Down syndrome. *Neurobiol Dis*, **82**, 385-396.

- Gourdain, S., Dairou, J., Denhez, C., Bui, L.C., Rodrigues-Lima, F., Janel, N., Delabar, J.M., Cariou, K. & Dodd, R.H. (2013) Development of DANDYs, new 3,5-diaryl-7-azaindoles demonstrating potent DYRK1A kinase inhibitory activity. *J Med Chem*, **56**, 9569-9585.
- Guedj, F., Sebrerie, C., Rivals, I., Ledru, A., Paly, E., Bizot, J.C., Smith, D., Rubin, E., Gillet, B., Arbones, M. & Delabar, J.M. (2009) Green tea polyphenols rescue of brain defects induced by overexpression of DYRK1A. *PLoS One*, **4**, e4606.
- Guidi, S., Stagni, F., Bianchi, P., Ciani, E., Giacomini, A., De Franceschi, M., Moldrich, R., Kurniawan, N., Mardon, K., Giuliani, A., Calza, L. & Bartesaghi, R. (2014) Prenatal pharmacotherapy rescues brain development in a Down's syndrome mouse model. *Brain*, **137**, 380-401.
- Hazlett, H.C., Poe, M.D., Lightbody, A.A., Styner, M., MacFall, J.R., Reiss, A.L. & Piven, J. (2012) Trajectories of early brain volume development in fragile X syndrome and autism. *J Am Acad Child Adolesc Psychiatry*, **51**, 921-933.
- Hessl, D., Sansone, S.M., Berry-Kravis, E., Riley, K., Widaman, K.F., Abbeduto, L., Schneider, A., Coleman, J., Oaklander, D., Rhodes, K.C. & Gershon, R.C. (2016) The NIH Toolbox Cognitive Battery for intellectual disabilities: three preliminary studies and future directions. *J Neurodev Disord*, **8**, 35.
- Hoeft, F., Carter, J.C., Lightbody, A.A., Cody Hazlett, H., Piven, J. & Reiss, A.L. (2010) Region-specific alterations in brain development in one- to three-year-old boys with fragile X syndrome. *Proc Natl Acad Sci U S A*, **107**, 9335-9339.
- Hoy, K.E., Emonson, M.R., Arnold, S.L., Thomson, R.H., Daskalakis, Z.J. & Fitzgerald, P.B. (2013) Testing the limits: Investigating the effect of tDCS dose on working memory enhancement in healthy controls. *Neuropsychologia*, **51**, 1777-1784.
- Hoyo, L.D., Xicota, L., Sanchez-Benavides, G., Cuenca-Royo, A., de Sola, S., Langohr, K., Fagundo, A.B., Farre, M., Dierssen, M. & de la Torre, R. (2015) Semantic Verbal Fluency Pattern, Dementia Rating Scores and Adaptive Behavior Correlate With Plasma Abeta42 Concentrations in Down Syndrome Young Adults. *Front Behav Neurosci*, **9**, 301.
- Huh, M.S., Ivanochko, D., Hashem, L.E., Curtin, M., Delorme, M., Goodall, E., Yan, K. & Picketts, D.J. (2016) Stalled replication forks within heterochromatin require ATRX for protection. *Cell Death Dis*, **7**, e2220.
- Hummel, F.C. & Cohen, L.G. (2006) Non-invasive brain stimulation: a new strategy to improve neurorehabilitation after stroke? *Lancet Neurol*, **5**, 708-712.
- Hunter, M.A., Coffman, B.A., Trumbo, M.C. & Clark, V.P. (2013) Tracking the neuroplastic changes associated with transcranial direct current stimulation: a push for multimodal imaging. *Front Hum Neurosci*, **7**, 495.

- Irwin, S.A., Galvez, R. & Greenough, W.T. (2000a) Dendritic spine structural anomalies in fragile-X mental retardation syndrome. *Cereb Cortex*, **10**, 1038-1044.
- Irwin, S.A., Swain, R.A., Christmon, C.A., Chakravarti, A., Weiler, I.J. & Greenough, W.T. (2000b) Evidence for altered Fragile-X mental retardation protein expression in response to behavioral stimulation. *Neurobiol Learn Mem*, **74**, 87-93.
- Islam, N., Aftabuddin, M., Moriwaki, A., Hattori, Y. & Hori, Y. (1995) Increase in the calcium level following anodal polarization in the rat brain. *Brain Res*, **684**, 206-208.
- Jacobson, L., Koslowsky, M. & Lavidor, M. (2012) tDCS polarity effects in motor and cognitive domains: a meta-analytical review. *Exp Brain Res*, **216**, 1-10.
- Jolly, L.A., Homan, C.C., Jacob, R., Barry, S. & Gecz, J. (2013) The UPF3B gene, implicated in intellectual disability, autism, ADHD and childhood onset schizophrenia regulates neural progenitor cell behaviour and neuronal outgrowth. *Hum Mol Genet*, **22**, 4673-4687.
- Jolly, L.A., Nguyen, L.S., Domingo, D., Sun, Y., Barry, S., Hancarova, M., Plevova, P., Vlckova, M., Havlovicova, M., Kalscheuer, V.M., Graziano, C., Pippucci, T., Bonora, E., Sedlacek, Z. & Gecz, J. (2015) HCFC1 loss-of-function mutations disrupt neuronal and neural progenitor cells of the developing brain. *Hum Mol Genet*, **24**, 3335-3347.
- Joep, R.S. & Johnson, G.V. (2004) The glamour and gloom of glycogen synthase kinase-3. *Trends Biochem Sci*, **29**, 95-102.
- Keeser, D., Meindl, T., Bor, J., Palm, U., Pogarell, O., Mulert, C., Brunelin, J., Moller, H.J., Reiser, M. & Padberg, F. (2011) Prefrontal transcranial direct current stimulation changes connectivity of resting-state networks during fMRI. *J Neurosci*, **31**, 15284-15293.
- Khalfallah, O., Jarjat, M., Davidovic, L., Nottet, N., Cestele, S., Mantegazza, M. & Bardoni, B. (2017) Depletion of the Fragile X Mental Retardation Protein in Embryonic Stem Cells Alters the Kinetics of Neurogenesis. *Stem Cells*, **35**, 374-385.
- Kim, H., Lee, K.S., Kim, A.K., Choi, M., Choi, K., Kang, M., Chi, S.W., Lee, M.S., Lee, J.S., Lee, S.Y., Song, W.J., Yu, K. & Cho, S. (2016) A chemical with proven clinical safety rescues Down-syndrome-related phenotypes in through DYRK1A inhibition. *Dis Model Mech*, **9**, 839-848.
- Kim, S., Stephenson, M.C., Morris, P.G. & Jackson, S.R. (2014) tDCS-induced alterations in GABA concentration within primary motor cortex predict motor learning and motor memory: a 7 T magnetic resonance spectroscopy study. *Neuroimage*, **99**, 237-243.

- Krause, B. & Cohen Kadosh, R. (2013) Can transcranial electrical stimulation improve learning difficulties in atypical brain development? A future possibility for cognitive training. *Dev Cogn Neurosci*, **6**, 176-194.
- Krause, B., Marquez-Ruiz, J. & Cohen Kadosh, R. (2013) The effect of transcranial direct current stimulation: a role for cortical excitation/inhibition balance? *Front Hum Neurosci*, **7**, 602.
- Kuo, M.F. & Nitsche, M.A. (2012) Effects of transcranial electrical stimulation on cognition. *Clin EEG Neurosci*, **43**, 192-199.
- Lai, J.K., Lerch, J.P., Doering, L.C., Foster, J.A. & Ellegood, J. (2016) Regional brain volumes changes in adult male FMR1-KO mouse on the FVB strain. *Neuroscience*, **318**, 12-21.
- Liebetanz, D., Nitsche, M.A., Tergau, F. & Paulus, W. (2002) Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain*, **125**, 2238-2247.
- Liu, X., Chan, C.-B., Jang, S.-W., Pradoldej, S., Huang, J., He, K., Phun, L.H., France, S., Xiao, G., Jia, Y., Luo, H.R. & Ye, K. (2010) A synthetic 7,8-dihydroxyflavone derivative promotes neurogenesis and exhibits potent antidepressant effect. *J Med Chem*, **53**, 8274-8286.
- Looi, C.Y., Duta, M., Brem, A.K., Huber, S., Nuerk, H.C. & Cohen Kadosh, R. (2016) Combining brain stimulation and video game to promote long-term transfer of learning and cognitive enhancement. *Sci Rep*, **6**, 22003.
- Lott, I.T. & Dierssen, M. (2010) Cognitive deficits and associated neurological complications in individuals with Down's syndrome. *Lancet Neurol*, **9**, 623-633.
- Maslen, H., Douglas, T., Cohen Kadosh, R., Levy, N. & Savulescu, J. (2014a) The regulation of cognitive enhancement devices: extending the medical model. *J Law Biosci*, **1**, 68-93.
- Maslen, H., Earp, B.D., Cohen Kadosh, R. & Savulescu, J. (2014b) Brain stimulation for treatment and enhancement in children: an ethical analysis. *Front Hum Neurosci*, **8**, 953.
- Matsuoka, A.J., Abbas, P.J., Rubinstein, J.T. & Miller, C.A. (2000) The neuronal response to electrical constant-amplitude pulse train stimulation: additive Gaussian noise. *Hear Res*, **149**, 129-137.
- Maurin, T., Zongaro, S. & Bardoni, B. (2014) Fragile X Syndrome: from molecular pathology to therapy. *Neurosci Biobehav Rev*, **46 Pt 2**, 242-255.
- Meinzer, M., Antonenko, D., Lindenberg, R., Hetzer, S., Ulm, L., Avirame, K., Flaisch, T. & Floel, A. (2012) Electrical brain stimulation improves cognitive performance by modulating functional connectivity and task-specific activation. *J Neurosci*, **32**, 1859-1866.



- Meinzer, M., Lindenbergh, R., Antonenko, D., Flaisch, T. & Floel, A. (2013) Anodal transcranial direct current stimulation temporarily reverses age-associated cognitive decline and functional brain activity changes. *J Neurosci*, **33**, 12470-12478.
- Miniussi, C., Ruzzoli, M. & Walsh, V. (2010) The mechanism of transcranial magnetic stimulation in cognition. *Cortex*, **46**, 128-130.
- Moon, J.H. (2016) Endocrine Risk Factors for Cognitive Impairment. *Endocrinol Metab (Seoul)*, **31**, 185-192.
- Murray, A., Letourneau, A., Canzonetta, C., Stathaki, E., Gimelli, S., Sloan-Bena, F., Abreghart, R., Goh, P., Lim, S., Baldo, C., Dagna-Bicarelli, F., Hannan, S., Mortensen, M., Ballard, D., Syndercombe Court, D., Fusaki, N., Hasegawa, M., Smart, T.G., Bishop, C., Antonarakis, S.E., Groet, J. & Nizetic, D. (2015) Brief report: isogenic induced pluripotent stem cell lines from an adult with mosaic down syndrome model accelerated neuronal ageing and neurodegeneration. *Stem Cells*, **33**, 2077-2084.
- Nitsche, M.A., Boggio, P.S., Fregni, F. & Pascual-Leone, A. (2009) Treatment of depression with transcranial direct current stimulation (tDCS): a review. *Exp Neurol*, **219**, 14-19.
- Nitsche, M.A., Fricke, K., Henschke, U., Schlitterlau, A., Liebetanz, D., Lang, N., Henning, S., Tergau, F. & Paulus, W. (2003a) Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. *J Physiol*, **553**, 293-301.
- Nitsche, M.A., Lampe, C., Antal, A., Liebetanz, D., Lang, N., Tergau, F. & Paulus, W. (2006) Dopaminergic modulation of long-lasting direct current-induced cortical excitability changes in the human motor cortex. *Eur J Neurosci*, **23**, 1651-1657.
- Nitsche, M.A., Nitsche, M.S., Klein, C.C., Tergau, F., Rothwell, J.C. & Paulus, W. (2003b) Level of action of cathodal DC polarisation induced inhibition of the human motor cortex. *Clin Neurophysiol*, **114**, 600-604.
- Nitsche, M.A. & Paulus, W. (2000a) Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *Journal of Physiology-London*, **527**, 633-639.
- Nitsche, M.A. & Paulus, W. (2000b) Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J Physiol*, **527 Pt 3**, 633-639.
- Ortiz-Abalia, J., Sahun, I., Altafaj, X., Andreu, N., Estivill, X., Dierssen, M. & Fillat, C. (2008) Targeting Dyrk1A with AAVshRNA attenuates motor alterations in TgDyrk1A, a mouse model of Down syndrome. *Am J Hum Genet*, **83**, 479-488.
- Paulus, W. (2011) Transcranial electrical stimulation (tES - tDCS; tRNS, tACS) methods. *Neuropsychol Rehabil*, **21**, 602-617.

- Pinter, J.D., Eliez, S., Schmitt, J.E., Capone, G.T. & Reiss, A.L. (2001) Neuroanatomy of Down's syndrome: a high-resolution MRI study. *Am J Psychiatry*, **158**, 1659-1665.
- Polania, R., Paulus, W., Antal, A. & Nitsche, M.A. (2011) Introducing graph theory to track for neuroplastic alterations in the resting human brain: a transcranial direct current stimulation study. *Neuroimage*, **54**, 2287-2296.
- Pons-Espinal, M., Martinez de Lagran, M. & Dierssen, M. (2013) Environmental enrichment rescues DYRK1A activity and hippocampal adult neurogenesis in TgDyrk1A. *Neurobiol Dis*, **60**, 18-31.
- Popescu, T., Krause, B., Terhune, D.B., Twose, O., Page, T., Humphreys, G. & Cohen Kadosh, R. (2016) Transcranial random noise stimulation mitigates increased difficulty in an arithmetic learning task. *Neuropsychologia*, **81**, 255-264.
- Poreisz, C., Boros, K., Antal, A. & Paulus, W. (2007) Safety aspects of transcranial direct current stimulation concerning healthy subjects and patients. *Brain Res Bull*, **72**, 208-214.
- Priori, A., Hallett, M. & Rothwell, J.C. (2009) Repetitive transcranial magnetic stimulation or transcranial direct current stimulation? *Brain Stimul*, **2**, 241-245.
- Reefhuis, J., Devine, O., Friedman, J.M., Louik, C., Honein, M.A. & National Birth Defects Prevention, S. (2015) Specific SSRIs and birth defects: Bayesian analysis to interpret new data in the context of previous reports. *BMJ*, **351**, h3190.
- Reis, J., Schambra, H.M., Cohen, L.G., Buch, E.R., Fritsch, B., Zarahn, E., Celnik, P.A. & Krakauer, J.W. (2009) Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. *Proc Natl Acad Sci U S A*, **106**, 1590-1595.
- Ritchie, K., Watson, L.A., Davidson, B., Jiang, Y. & Berube, N.G. (2014) ATRX is required for maintenance of the neuroprogenitor cell pool in the embryonic mouse brain. *Biol Open*, **3**, 1158-1163.
- Snowball, A., Tachtsidis, I., Popescu, T., Thompson, J., Delazer, M., Zamarian, L., Zhu, T. & Cohen Kadosh, R. (2013) Long-term enhancement of brain function and cognition using cognitive training and brain stimulation. *Curr Biol*, **23**, 987-992.
- Stagg, C.J., Bachtir, V. & Johansen-Berg, H. (2011) The role of GABA in human motor learning. *Curr Biol*, **21**, 480-484.
- Stagg, C.J., Best, J.G., Stephenson, M.C., O'Shea, J., Wylezinska, M., Kincses, Z.T., Morris, P.G., Matthews, P.M. & Johansen-Berg, H. (2009) Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. *J Neurosci*, **29**, 5202-5206.

- Stagg, C.J., Lin, R.L., Mezue, M., Segerdahl, A., Kong, Y., Xie, J. & Tracey, I. (2013) Widespread modulation of cerebral perfusion induced during and after transcranial direct current stimulation applied to the left dorsolateral prefrontal cortex. *J Neurosci*, **33**, 11425-11431.
- Stagni, F., Giacomini, A., Emili, M., Trazzi, S., Guidi, S., Sassi, M., Ciani, E., Rimondini, R. & Bartesaghi, R. (2016) Short- and long-term effects of neonatal pharmacotherapy with epigallocatechin-3-gallate on hippocampal development in the Ts65Dn mouse model of Down syndrome. *Neuroscience*, **333**, 277-301.
- Stagni, F., Giacomini, A., Guidi, S., Ciani, E. & Bartesaghi, R. (2015) Timing of therapies for Down syndrome: the sooner, the better. *Front Behav Neurosci*, **9**, 265.
- Sutcliffe, M. & Lancaster, M.A. (2017) A Simple Method of Generating 3D Brain Organoids Using Standard Laboratory Equipment. *Methods Mol Biol*.
- Takashima, S., Ieshima, A., Nakamura, H. & Becker, L.E. (1989) Dendrites, dementia and the Down syndrome. *Brain Dev*, **11**, 131-133.
- Tejedor, F.J. & Hammerle, B. (2011) MNB/DYRK1A as a multiple regulator of neuronal development. *FEBS J*, **278**, 223-235.
- Terney, D., Chaieb, L., Moliadze, V., Antal, A. & Paulus, W. (2008) Increasing human brain excitability by transcranial high-frequency random noise stimulation. *J Neurosci*, **28**, 14147-14155.
- Thomazeau, A., Lassalle, O., Iafrati, J., Souchet, B., Guedj, F., Janel, N., Chavis, P., Delabar, J. & Manzoni, O.J. (2014) Prefrontal deficits in a murine model overexpressing the down syndrome candidate gene *dyrk1a*. *J Neurosci*, **34**, 1138-1147.
- Trazzi, S., Fuchs, C., De Franceschi, M., Mitrugno, V.M., Bartesaghi, R. & Ciani, E. (2014) APP-dependent alteration of GSK3 $\beta$  activity impairs neurogenesis in the Ts65Dn mouse model of Down syndrome. *Neurobiol Dis*, **67**, 24-36.
- Uchida, Y., Nakano, S., Gomi, F. & Takahashi, H. (2007) Differential regulation of basic helix-loop-helix factors Mash1 and Olig2 by beta-amyloid accelerates both differentiation and death of cultured neural stem/progenitor cells. *J Biol Chem*, **282**, 19700-19709.
- van der Groen, O. & Wenderoth, N. (2016) Transcranial Random Noise Stimulation of Visual Cortex: Stochastic Resonance Enhances Central Mechanisms of Perception. *J Neurosci*, **36**, 5289-5298.
- Ward, L.M., Doesburg, S.M., Kitajo, K., MacLean, S.E. & Roggeveen, A.B. (2006) Neural synchrony in stochastic resonance, attention, and consciousness. *Can J Exp Psychol*, **60**, 319-326.

- Werker, J.F. & Hensch, T.K. (2015) Critical periods in speech perception: new directions. *Annu Rev Psychol*, **66**, 173-196.
- Westmark, C.J. (2017) Commentary: Depletion of the Fragile X Mental Retardation Protein in Embryonic Stem Cells Alters the Kinetics of Neurogenesis. *Front Mol Neurosci*, **10**, 29.
- Westmark, C.J. & Malter, J.S. (2007) FMRP mediates mGluR5-dependent translation of amyloid precursor protein. *PLoS Biol*, **5**, e52.
- Westmark, C.J., Sokol, D.K., Maloney, B. & Lahiri, D.K. (2016) Novel roles of amyloid-beta precursor protein metabolites in fragile X syndrome and autism. *Mol Psychiatry*, **21**, 1333-1341.