SCUOLA NORMALE SUPERIORE

Pisa

CLASSE DI SCIENZE MATEMATICHE, FISICHE E NATURALI

CORSO DI PERFEZIONAMENTO IN NEUROBIOLOGIA

Triennio 2008-2010

Tesi di perfezionamento

ENVIRONMENTAL STIMULATION AND ENVIROMIMETICS: IMPACT ON A MURINE MODEL OF DOWN SYNDROME

Candidata: Tatjana Begenisic

Relatore: Dr. Alessandro Sale

INDEX

CHAPTER 1: DOWN SYNDROME – INSIGHTS FROM MURINE MODELS 6

1.1. The human trisomy 21 7

1.2. Modeling Down syndrome in mice 10

1.3. Central nervous system dysfunction in Down syndrome – comparison between affected humans and Ts65Dn mouse model 13

1.3.1. Cognitive and behavioral impairments 13

1.3.2. Motor and sensory abnormalities 19

1.3.3. Neuroanatomical correlates of neurophysiological impairments 22

1.3.4. Disruption of neurotransmitter systems and neurotrophic factors 30

1.4. Altered synaptic plasticity and disruption of excitatory-inhibitory balance as a central mechanism of neurophysiological impairments in Down syndrome 35

CHAPTER 2: EFFECTS OF ENVIRONMENTAL ENRICHMENT AND ENVIROMIMETICS ON BRAIN IN HEALTH AND DISEASE 42

2.1. Neural consequences of environmental enrichment 43

2.2. Influence of environmental enrichment on brain development 45

2.3. Maternal care, tactile stimulation and visual system development in environmental enrichment conditions 48

2.4. Rejuvenating the adult brain 50

2.5. Beneficial effects of environmental enrichment on functional outcome in models of brain disorders **53**

2.6. Enviromimetics – a novel class of therapeutics **57**

2.7. Antidepressant fluoxetine as a putative enviromimetic **58**

CHAPTER 3: AIM OF THE THESIS AND EXPERIMENTAL DESIGN **62**

CHAPTER 4: MATERIALS AND METHODS **66**

4.1. Animals **66**

4.2. DNA extraction **67**

4.3. Genotyping **67**

4.4. Screening for retinal degeneration **68**

4.5. Rearing environments **69**

4.6. Pharmacological treatment **70**

4.7. Maternal care observations **71**

4.8. Eye-opening observation **72**

4.9. Morris water maze **72**

4.10. Novel place recognition test **73**

4.11. In vitro electrophysiology: LTP recordings **74**

4.12. In vivo electrophysiology: assessment of visual acuity and binocularity 75

4.13. Analysis of GABA release in hippocampal and visual cortex synaptosomes 76

4.14. Statistics 78

CHAPTER 5: RESULTS 79

5.1. Effects of adult-onset environmental enrichment and fluoxetine treatment on Down syndrome related phenotypes displayed by Ts65Dn mice 79

5.1.1. Environmental enrichment promotes spatial learning and memory in Ts65Dn mice 79

5.1.2. Recovery of LTP at medial perforant path–granule cell synapses in Ts65Dn mice reared in environmental enrichment 81

5.1.3. Restoration of visual functions in Ts65Dn mice by environmental enrichment 83

5.1.4. Environmental enrichment normalizes excessive GABA release in the hippocampus and visual cortex of Ts65Dn mice 85

5.1.5. Evaluation of fluoxetine pro-convulsive side effects in Ts65Dn mice 86

5.1.7. Chronic treatment with fluoxetine improves short-term spatial memory impairment in Ts65Dn mice 91

5.1.8. Effects of chronic fluoxetine administration on hippocampal synaptic plasticity at the CA3-CA1 synapse 92

5.2. Impact of environmental enrichment on development in Ts65Dn mice 96

5.2.1. Maternal effects of environmental enrichment in Ts65Dn mice 96

5.2.3. Assessment of visual acuity development in Ts65Dn mice 101

CHAPTER 6: DISCUSSION 103

6.1. Environmental enrichment and enviromimetics: amelioration of neurological phenotypes associated with Down syndrome in adulthood 103

6. 1. 1. Effects of environmental enrichment on adult Ts65Dn mice 103

6. 1. 2. Effects of fluoxetine treatment on adult Ts65Dn mice 108

6.2. Developmental impact of environmental enrichment in Ts65Dn mice 113

6.2.1. Maternal effects of environmental enrichment in Ts65Dn mice 113

6.2.2. Influence of pre-weaning onset of environmental enrichment on visual system development in Ts65Dn mice 116

6.3. Conclusions 119

REFERENCES 121

CHAPTER 1

DOWN SYNDROME – INSIGHTS FROM MURINE MODELS

Down syndrome (DS) is a genetic pathology caused by triplication of chromosome 21 (Hsa21) and is characterized by a number of physical and mental abnormalities, with intellectual disability (ID) as the most serious health problem. Complexity of DS arises from the substantial genetic basis of syndrome, the dysfunction of multiple systems and the high phenotypic variability of its clinical manifestations among affected individuals (Antonarakis and Epstein, 2006). Virtually all people living with DS have a triplication of at least one segment of Hsa21 being responsible for inappropriate development and resulting in structural and functional anomalies that persist throughout the entire lifespan. Beside the disruption of development, trisomic gene expression affects also the properties of differentiated adult cells, compromising further their already altered functions.

Sequencing of Hsa21 and identification of its mouse ortholog genes have led to generation of transgenic mice that have enabled remarkable advances in better understanding of the DS nature. The mouse is the organism of choice in modern biomedical research as it allows a vast degree of genetic alteration, high reproducibility of experiments due to the 1breeding simplicity and use of intrusive studies. Respect to human genome, orthologs in mouse differ minimally in the structures of conserved genes (Gardiner and Davisson, 2000), although differences in regulation of gene expression may be expected (Gharib and Robinson-Rechavi, 2011). Importantly, developmental programs and basic mechanisms of mature functions are conserved across mammals. In line with this is the occurrence of DS like phenotype in mice trisomic for Hsa21 orthologs (Das and Reeves, 2011).

The advantage of DS mouse models for translational research is not only a successful

recapitulation of disease aspects, but also a predictive ability, i.e. certain phenotypic abnormalities for the first time observed in trisomic mice have been confirmed later in DS patients (Belichenko et al., 2004). Moreover, systematic investigation of these models enables a more precise definition of genotype-phenotype interaction, better understanding of altered molecular pathways and identification of potential therapeutic targets, and finally design of preclinical studies with treatments likely to be tested in clinical trials.

1.1. The human trisomy 21

DS is a complex clinical entity caused by trisomy of Hsa21 (T21) and characterized by numerous features affecting multiple systems, with cognitive impairment as the most prominent and deleterious symptom. With the prevalence of 1 in 850-1000 infants (Shin et al., 2009) DS represents the leading cause of ID of genetic origin and an important public health issue due to inability of affected individuals to live an independent life. DS was described for the first time in 1866 by John Langdon Down, but the link of its numerous features with T21 was revealed only later in 1959 by the cytological profiling study performed by Jerome Lejeune (Megarbane et al., 2009). In the majority of cases DS is caused by the full T21 (95%), but the occurrence of two other genetic variations including translocation T21 (4%) and mosaic T21 (1%) also gives rise to DS phenotype (Parker et al., 2010). The full T21 is due to a meiotic nondisjunction of Hsa21 that is related to an advanced age of mother in about 75% of DS cases, while in remaining 25% it has paternal origin (Turleau and Vekemans, 2010). This nondisjunction seems to be associated with errors in recombination and an age-dependent loss of meiotic chromosome cohesion (Hassold and Hunt, 2001, Hodges and Wallace, 2005), but why these recombinant mistakes take place and how their consequential burden of DS could be avoided it is still far away from being understood.

The presence of an additional copy of Hsa21 implicates a quantitative rather than a qualitative nature for DS. However, genes situated in Hsa21 account for a less than 2% of total genomic material (Gardiner and Davisson, 2000), leading to the intriguing question of how can such a relatively modest quantitative genomic alteration give rise to such serious pathological consequences. According to the ‘‘gene dosage effect hypothesis’’, particular phenotypic traits associated with T21 are direct consequences of the imbalance of individual genes located on the triplicate chromosome (Pritchard and Kola, 1999, Antonarakis et al., 2001, Antonarakis et al., 2004). In agreement with this hypothesis is the identification of several “dosage-sensitive” regions, including genes and noncoding conserved elements, across the length of Hsa21, that have been shown to be sufficient for induction of typical features of DS individuals (Korbel et al., 2009, Lyle et al., 2009). On the contrary, the “amplified developmental instability hypothesis” postulates that triplication of a relative small number of genes disrupts global gene expression and regulation of intracellular signaling pathways, leading to deleterious effects on development and function (Hall, 1965, Shapiro, 1983, Pritchard and Kola, 1999, Moldrich et al., 2007). The main strength of this hypothesis is in the interpretation of DS as a “genomic disorder”, which suggests overlapping underlying mechanisms between DS and other trisomies. A synthesis of these opposite approaches proposes that some dosage sensitive genes, whose triplication per se would have only modest effects, may account for the numerous DS phenotypes only in combination with other triplicate genes (Olson et al., 2004, Roper and Reeves, 2006).

Similar to other conditions caused by chromosome imbalance, DS afflicts structure and function of multiple systems, producing detrimental effects on physical and mental health. Additionally, the clinical manifestations of DS change across the lifespan, contributing further to the complexity of syndrome (Antonarakis and Epstein, 2006). The first line of health problems are congenital malformations that include craniofacial and skeletal

dysmorphic features with more cosmetic than functional effects (Frostad et al., 1971, Fischer-Brandies, 1988), and significant cardiovascular (Ferencz et al., 1989) and gastrointestinal (Levy, 1991) anomalies that, if not treated properly, could lead to serious morbidity. Presence of cancer phenotype is controversial, as at the birth there seems to exist an increased risk for myieloproliferative disorders, while in the adulthood prevalence of many malignant tumors is decreased (Wechsler et al., 2002). The second line of symptoms develops progressively during life and, beside growth retardation, obesity, thyroid dysfunction and male sterility (Antonarakis and Epstein, 2006), it refers mostly to central nervous system (CNS) dysfunctions. CNS symptomatology of DS starts with central hypotonia at birth and continues with delayed cognitive development in infancy and childhood, leading to mild to moderate mental retardation that aggravates in adulthood, with an additional loss of cognitive abilities due to precoocious development of Alzheimer disease (AD). Generally, the lifetime of DS individuals is shorter with respect to the normal population and its duration is strongly influenced by the constellation of clinical signs that vary enormously in prevalence and severity across DS population.

Despite the numerous health problems mentioned above, life quality of people living with DS has been improved significantly due to enhanced medical and social care, which also resulted in an increased life expectancy from 12 years in 1940s to the current 60 years and over (Glasson et al., 2002, Roizen and Patterson, 2003, Bittles and Glasson, 2004, Bittles et al., 2007). However, CNS abnormalities are still orphan of an effective therapy, with ID being the most significant obstacle to an independent life for DS individuals. The availability of animal models in the field of DS basic research has started to reveal underlying mechanisms of T21 that contribute to the etiology of CNS dysfunction and cognitive impairment, providing the basis for designing of clinical trials aiming at the amelioration of mental abilities (Reeves and Garner, 2007, Wetmore and Garner, 2010, Rissman and Mobley,

2011, Haydar and Reeves, 2012)

1.2. Modeling Down syndrome in mice

The basis for modeling DS in mice is a conserved synteny between the long arm of Hsa21 and mouse chromosomes 16 (Mmu16), 17 (Mmu17) and 10 (Mmu10) (Pletcher et al., 2001). The long arm of Hsa21 is approximately 33.7 Mb in length and contains ~ 430 protein-coding genes, of which ~175 have a homolog in the mouse genome. The distal end of Mmu16 carries the largest homolog region containing 37Mb. This region spans from *Rbm11* to *Znf295* and is composed of ~115 orthologous genes with a subset of them, such as *Ncam2, App, Grik, Sod, Synj1, Olig1, Olig2, Dyrk1a, Girk2, Bace2*, being involved in brain development and functioning (Haydar and Reeves, 2012). The two remaining homolog regions are identified on Mmu17 and Mmu10 and are much smaller, containing 1.1 Mb and 2.3 Mb and consisting of 19 and 41 orthologous genes, respectively (Das and Reeves, 2011).

The spontaneous Robertsonian translocations of Mmu16 naturally occurs in mice (Gropp et al., 1975) and this trisomy for Mmu16 (Ts16) has been considered the first model of DS. However, Ts16 model has a limited experimental use due to its very low postnatal viability, probably caused by trisomy of portions of Mmu16 that show sinteny with genes encoded by Hsa3, 8, 12, 6 and 22, which are not involved in etiology of DS (Moore and Roper, 2007). Genetic engineering of different segments of Hsa21-orthologues genes resulted in the availability of a comprehensive set of transgenic strains carrying those genes that show synteny with Hsa21 (Sérégaza et al., 2006). Importantly, these transgenic strains outlive Ts16 mice and may be studied at any developmental stage and across the entire lifespan.

The most important group of transgenic DS models encompasses mouse lines with triplication of various portions of the distal end of Mmu16. The most widely used and characterized model is Ts65Dn, which carries segmental trisomy in a form of small

chromosome produced by a Robertsonian translocation of Mmu16 to Mmu17. Trisomy in this model expands from *Mrpl39* to *Znf295* (Kahlem et al., 2004) and results in a dosage imbalance for ~104 genes conserved on Mmu16. Introduction of this model has been fundamental as it demonstrated for the first time that trisomy for syntenic genes of Hsa21 gives raise to DS-related outcomes in mice (Reeves et al., 1995). A detailed phenotypic characterization of Ts65Dn mice revealed a number of abnormalities in cognitive performance and brain morphology similar to those observed in people with DS. These findings have encouraged the use of Ts65Dn mouse as a standard to compare the incidence and severity of trisomic phenotypes reported in mouse models that came afterward (Holtzman et al., 1996, Baxter et al., 2000, Richtsmeier et al., 2000, Cooper et al., 2001, Rueda et al., 2005, Lorenzi and Reeves, 2006, Moore, 2006, Roper and Reeves, 2006).

Additionally developed partial trisomies carry smaller set of genes compared to Ts65Dn mice. In the case of the Ts1Cje model, the trisomic segment spans from *Sod1* to *Znf295* and counts for ~81 orthologous (Sago et al., 1998), while the Ts1Rhr model has a triplication for even a smaller region composed of ~33 genes expanding from *Cbr1* to *Orf9* (Olson et al., 2004). Ts1Cje and Ts1Rhr mice also show cognitive impairments, but the severity of their deficits is generally attenuated in comparison with Ts65Dn (Sago et al., 1998, Olson et al., 2007, Belichenko et al., 2009a). More complex models can be created when a third copy of a gene or chromosomal segment is subtracted from existing segmental trisomies by crossing them to a strain that carries a null allele/s for the gene/segment of interest thereby decreasing dosage of targeted gene/segment to euploid levels in the context of remaining trisomy (Salehi et al., 2006, Sussan et al., 2008). For instance, both Ms1Cje/Ts65Dn and Ms1Rhr/Ts65Dn were produced by breeding the corresponding monosomy of Ts1Cje and Ts1Rhr trisomies to the Ts65Dn mouse (Sago et al., 2000, Olson et al., 2004). These models have been developed in an attempt to understand the contribution of

specific orthologous genes to particular features of DS. However, the phenotypic analysis of these models revealed that the putative DS critical region (DSCR), believed to contain all essential genes responsible for the typical characteristics of DS, is not critically involved in the learning and memory deficit (Olson et al., 2007). These findings challenged the concept of specific dosage-sensitive genes as a primary cause of an abnormal phenotype of DS and rather supported the hypothesis that the over-expression of a large number of triplicated genes generally disrupts genomic homeostasis, leading to impaired regulation of developmental processes (Shapiro, 1997).

The models that are trisomic for the two remaining syntenic regions of Hsa21 mapped on Mmu17 and Mmu10 have been used to provide additional insights into the genetic basis of DS. The Ts1Yah mouse carries three copies of 12 genes, spanning from *U2af1* to *Abcg1*, of the Mmu17 syntenic to the sub-telomeric region of Hsa21 (Pereira et al., 2009). This model has been created in order to understand how the telomeric region of Hsa21 contributes to DS phenotype. The effects of trisomy on learning and memory in Ts1Yah model are contradictory as some aspects of cognitive performance are impaired, while other are even improved with respect to euploid controls, emphasizing the concept that a complex interaction between genes underlies behavioral alterations in trisomic mice (Pereira et al., 2009). Interestingly, the Ts2Yey model, that contains triplication for 41 genes of the Mmu10 region which is homologue to the Hsa21 telomeric end, does not show any cognitive impairment (Yu et al., 2010). Recently, a ‘triple trisomy’ model, the Ts1Yey;Ts2Yey;Ts3Yey mouse, has been developed. The Ts1Yey;Ts2Yey;Ts3Yey mouse is the first model with a dosage imbalance for all the Hsa21 orthologous found in the mouse genome and it carries in triplicate the syntenic segments from all three mouse chromosomes, Mmu16, Mmu17 and Mmu10 (Yu et al., 2010). Nevertheless, the revealed brain phenotype of this model is very close to the Ts65Dn justifying the use of the Ts65Dn mouse as the referent murine model for

DS (Yu et al., 2010). Beside DS models based on synteny between human and mouse genome, a transchromosomic model, Tc1, has been also introduced. Tc1 mice have a transferred copy of Hsa21, but the human chromosome is lost in a subset of cells from all tissues (O'Doherty et al., 2005). The mosaicism for Hsa21 reported in this model could account for the mild cognitive deficits display by Tc1 mice (O'Doherty et al., 2005).

Given that the cognitive performance is the most compromised function in DS, the behavioral phenotypes of DS models have been thoroughly assessed to establish their relevance for the human condition. Particularly, impairments of spatial and recognition learning and memory have been studied in depth by use of a battery of behavioral tests that have helped to identify the hippocampus as the brain region specifically affected by trisomy in mice (Das and Reeves, 2011). In addition to behavioral evaluations, hippocampal morphological and electrophysiological correlates of cognitive deficits have been studied in DS models revealing a clear relationship between the number of trisomic genes that are orthologous to Hsa21 and the level of expression of functional and structural abnormalities.

1.3. Central nervous system dysfunction in Down syndrome – comparison between affected humans and Ts65Dn mouse model

1.3.1. Cognitive and behavioral impairments

Humans

Difficulties in intellectual functioning are the most striking feature of DS. The major obstacle to an independent life in DS patients are limitations in cognitive performance that are additionally complicated by functional difficulties coming from various areas, such as linguistic, social, motor, and sensory. However, similarly to other genetic disorders associated with ID, different domains of intellectual processing in DS are not affected to the

same level by T21, leading to a complex cognitive profile characterized by specific weaknesses and strengths (Fidler et al., 2006).

Cognitive impairments in DS could be classified into those that are a product of disrupted neural development resulting in retardation of learning and memory from birth, and those that occur in adulthood as a consequence of accelerated neurodegenerative processes (Contestabile et al., 2010). DS individuals during the course of childhood and adolescence develop mild to severe ID that, when expressed with the Intelligence Quotient (IQ), falls into the range from 30 to 70 (Vicari et al., 2000, Vicari et al., 2004, Vicari et al., 2005). Cognitive development of DS infants begins relatively typically and the first delays are observed at the age of two years which may be associated with the myelination lag at this developmental stage (Koo et al., 1992). One of the key features of the overall slowdown in maturation of learning abilities for children with DS are difficulties in the maintenance of acquired skills and a persistent use of counterproductive strategies for novel problem solving tasks (Wishart, 1993).

Learning deficits in DS children refer to both short-term and long-term memory (Carlesimo et al., 1997, Vicari et al., 2000, Brown et al., 2003, Vicari et al., 2005). When assessed with tasks requiring low processing levels, visuospatial components of short-term and working memory tend to be speared in contrast to verbal processing, which instead demonstrates significant deficits respect to age-matched controls (Vicari et al., 1995, Jarrold et al., 1999, Vicari et al., 2006). As the task becomes more demanding, certain impairments emerge also in the visuospatial skills, and those reported for verbal processing worsen (Lanfranchi et al., 2004, Visu-Petra et al., 2007). Strengths and weaknesses are also present in domain of long-term memories. Explicit memory is significantly compromised in DS children due to poor information encoding, impaired retrieval abilities and attention deficits (Carlesimo et al., 1997, Brown et al., 2003, Krinsky-McHale et al., 2008), whereas learning

abilities for tasks requiring implicit memory processing seem to be preserved (Vicari et al., 2000). This is in agreement with a different mechanism underlying this two types of memory formation, as implicit memory is a more automatic process based on low levels of attention, while explicit memory requires a high degree of attention to carry on intentional learning and develop successful retrieval strategies.

Impairment of long-term memory in DS has been associated with hippocampal and prefrontal lobe dysfunctions. Namely, deficit in spatial long-term memory reported in pre-school DS children assessed by delayed recall of place learning task (Pennington et al., 2003) pointed to altered hippocampal function, whereas impairment of non-verbal reasoning ability and attention has indicated a specific executive control defect due to abnormal prefrontal functioning, supporting the concept that ID in DS is differentially affected by the degree of required control (Rowe et al., 2006). A high prevalence of behavioral disinhibition, illustrated by hyperactivity, aggression, stubbornness, disobedience, impulsivity and already mentioned attention deficits (Cuskelly and Dadds, 1992, Dykens et al., 2002), is also indicative of prefrontal dysfunctions in DS. Recognition memory, another type of explicit memory partially dependent on hippocampus (Yonelinas et al., 2005) and frontal lobe (Neufang et al., 2006) integrity, is also affected in DS. DS children perform normally in the task of matching photographs of simultaneously presented faces, but perform significantly worse in the more demanding task of matching faces to non-present people (Wishart and Pitcairn, 2000). Thus, similarly to described working memory deficits, the level of task difficulty determines the quality of recognition memory performance, indicating that in DS cognitive deficits may be generally more pronounced in challenging situations.

In addition to developmental ID, DS patients may undergo additional cognitive decline with aging and develop precocious AD (Nieuwenhuis-Mark, 2009). Although at the neuropathological level virtually all subjects with DS develop typical hallmarks of AD during

adulthood (Ball and Nuttall, 1980, Hof et al., 1995, Folin et al., 2003, Nadel, 2003, Lott and Head, 2005), only a portion of patients demonstrate clinical signs of dementia (Devenny et al., 1996, Devenny et al., 2005). Clinical progression of AD in DS has some similarities with dementia onset in the general population, with confusion, forgetfulness, impairment of recent memories and a relative preservation of distant memories at early stages of disease (Deb et al., 2007). On the other hand, the signs of frontal lobe dysfunction, such as indifference, apathy, depression, socially-deficient communication and impaired adaptive functioning (Zigman et al., 1996, Lott and Head, 2001, Ball et al., 2006), may be expressed during initial phase of AD in some cases of DS (Deb et al., 2007, Ball et al., 2008), while among the general population these clinical manifestation typically occur only in a late phase of AD.

As discussed above, frontal lobe abnormalities and defective executive functions have been also reported in young DS subjects (Rowe et al., 2006), emphasizing the diagnostic difficulties in detecting cognitive decline caused by dementia in the context of ID (Nieuwenhuis-Mark, 2009). Moreover, in DS the risk of developing some forms of mental disease increases over time (Dykens et al., 2002), representing an additional confounding factor in the correct diagnosis of the cognitive deficit. Depression is the most common psychiatric problem among young adults affected by DS (Myers and Pueschel, 1991, Collacott et al., 1992) and its progression may lead to additional decline in adaptive behavior and cognitive performance during mid and late adulthood (Burt et al., 1992).

Ts65Dn

Similarly to human DS subjects, disruption of neural development processes leads to early cognitive impairments also in Ts65Dn mice (Bianchi et al., 2010b), with a further worsening occurring in adulthood due to neurodegenerative processes (Hyde and Crnic, 2001, Faizi et al., 2011).

Short-term working memory has been assessed by spontaneous alternation tasks, revealing a reduced performance in Ts65Dn mice (Fernandez et al., 2007, Belichenko et al., 2009a) and resembling short-term memory deficits observed in DS individuals (Lanfranchi et al., 2004, Visu-Petra et al., 2007). Furthermore, Ts65Dn mice, as well as persons with DS, are characterized by a functional dissociation between implicit and explicit long-term memory. Namely, Ts65Dn mice perform similarly to wild type (WT) mice in implicit memory tasks, as indicated by normal acquisition and maintenance of skills in the rotarod performance test (Hyde and Crnic, 2001, Fernandez et al., 2007).

On the other hand, Ts65Dn mice demonstrate selective impairments in explicit learning and memory tasks. Two prototypical domains of explicit memory that can be delineated in rodents through a variety of behavioral tasks are spatial and recognition learning and memory. These experimental paradigms depend on the functional integrity of the medial temporal lobe, composed of the hippocampus and the parahippocampal region, the same areas associated with deficits in declarative memory displayed by patients with DS (Nelson et al., 2005). A particular vulnerability of the trisomic hippocampus is well illustrated by the outcomes of two different forms of fear conditioning. In an acoustic fear conditioning, a behavioral test based on the amygdale (Anagnostaras et al., 1999), Ts65Dn mice do not show any significant defect (Salehi et al., 2009, Faizi et al., 2011), while there is a clear evidence of impaired performance in a context fear conditioning (Costa et al., 2008, Salehi et al., 2009, Bianchi et al., 2010b, Faizi et al., 2011), a paradigm mainly dependent on the hippocampus (Kim and Fanselow, 1992).

The most frequently used test to assess spatial long-term learning and memory in Ts65Dn mice is the Morris water maze (MWM). This paradigm evaluates visuospatial integration, as mice are trained to find a platform hidden in a tank filled with an opaque water, using a complex spatial mapping strategy based on extra- and intra-maze cues

(Morris, 1984, Redish and Touretzky, 1998). It has been repeatedly shown that Ts65Dn mice perform similarly to euploid mice in a cued task that does not involve visuospatial integration because the hidden platform is marked with visible cues such as a flag (Escorihuela et al., 1998, Sago et al., 2000, Bimonte-Nelson et al., 2003, Rueda et al., 2008). This indicates that motivation is not affected in Ts65Dn mice which have sufficient motor and visual skills to reach a visible platform. However, in the hidden-platform task Ts65Dn mice display significant navigational impairments and a general inability to create successful strategies to find and remember the position of the platform (Escorihuela et al., 1995, Reeves et al., 1995, Demas et al., 1996, Holtzman et al., 1996). It is important to note that deterioration in the performance of Ts65Dn mice in MWM may be partially due to a stressful nature of the test as a certain degree of aversiveness is an integral part of MWM (Stasko and Costa, 2004). This indicates that challenging situations are more prone to reveal deficits not evident in stress-free circumstances. This is also supported by the recent finding that Ts65Dn mice show identical outcomes as WT mice in place preference and place avoidance learning tasks when tested in the home cage environment, which can be considered a stress-free condition (Faizi et al., 2011). However, recognition memory evaluated by non aversive object recognition tests is also impaired in Ts65Dn mice, illustrating the persistence of some cognitive deficits even in low stress circumstances. Ts65Dn mice exhibit significant deficits in the simple version of the novel object recognition task (a simple protocol involving a pair of identical objects during the familiarization phase), but a complete inability to distinguish novel and familiar objects in the complex version of the test (a more challenging protocol involving a pair of different objects during the familiarization phase) (Fernandez et al., 2007). Importantly, the profile of this impairment is in accordance with previously described neuropsychological studies in human DS, reporting more pronounced difficulties in intellectual processing in DS patients with increasing task demands (Lanfranchi et al., 2004,

Visu-Petra et al., 2007).

The majority of the above mentioned behavioral tests refers to young adult Ts65Dn mice at the age of 3-4 months, but for some tasks longer follow up studies have been performed, allowing to uncover an additional amount of cognitive performance decline with age (Hyde and Crnic, 2001, Faizi et al., 2011). This is reminiscent of the cognitive deterioration due to an early onset of AD among DS individuals. It has been proposed that hyperactivity, frequently observed in Ts65Dn mice (Davisson et al., 1993, Reeves et al., 1995, Coussons-Read and Crnic, 1996, Stewart et al., 2007) may be an early sign of AD in this model. Namely, it has been shown that prefrontal cortex lesions leads to hyperactivity in rodents (Kolb, 1974, Takakusaki, 2008), and this type of disinhibited behavior in Ts65Dn mice could be driven by an altered prefrontal information processing, as it has been shown to be the case in the human DS (Rowe et al., 2006).

1.3.2. Motor and sensory abnormalities

Humans

Together with a poor cognitive performance, various motor and sensory deficits are associated with DS. Acquisition of motor milestones generally follows the same sequences found in typically developing children, but is characterized by significant delays that tend to be more pronounced for the latest milestones (Palisano et al., 2001, Vicari et al., 2006). Two typical characteristics of DS that significantly influence motor performance are low muscle tone and lack of control of muscle stiffness (Davis and Sinning, 1987, Vicari et al., 2006). Impairments are evident in all motor domains, as illustrated by a poor performance in tasks assessing fine and gross motor skills and motor planning (Jobling, 2001, Mon-Williams et al., 2001). However, performance in some specific skills, like running speed, agility and visual-motor control, reaches the level of age-matched controls (Jobling, 2001, Mon-Williams et al.,

2001) indicating that some qualities of motor function are preserved in DS.

The development and function of auditory and visual pathways are also affected by T21 (Folsom et al., 1983, Diaz and Zuron, 1995). Hearing loss occurs in approximately two thirds of DS children and is one of their most common sensory disabilities (Roizen et al., 1993). During infancy there is a high incidence of conductive hearing loss due to congenital malformation of the ear anatomy and repetitive ear infections, while progressive sensorineural loss is frequently found from middle childhood to adult life, as demonstrated by difficulties to evoke potentials by auditory stimulus in the brainstem (Jiang et al., 1990, Chen and Fang, 2005).

Although less frequent then auditory defects, visual system impairments are also an integral component of DS symptomatology. Visual acuity (VA), the ability to see fine details of objects, and contrast sensitivity, the ability to discriminate between different brightness levels, are reduced in infants and children with DS when compared with their normally developing peers (Courage et al., 1994, Woodhouse et al., 1996). Ophthalmic anomalies, such as refractive errors (Woodhouse et al., 1997), accommodative inaccuracy (Cregg et al., 2001), strabismus (Haugen and Hovding, 2001), and nystagmus (Wagner et al., 1990) are typical of DS patients, but cannot entirely account for their reduced vision, because children without such anomalies still show poor VA and contrast sensitivity (John et al., 2004). Abnormalities in cortical visual evoked potentials (VEPs) in DS patient rather point to a functional deficit of central visual pathways (Ellingson, 1986). The basis of this dysfunctional visual processing may be structural dendritic abnormalities characterized by progressive loss of dendritic branching and total length in the visual cortex, during the period from the first postnatal months to the two years of age (Becker et al., 1986). In adulthood, some DS patients show AD-like visual deficits (Rocco et al., 1997), as impaired color discrimination and stereoacuity, the ability to detect differences in distance, indicating that

both congenital abnormalities and age-related neuropathology within the visual pathways could contribute to sight defects.

Overall, the importance of motor and sensory deficits in DS is not only in the primary loss of their functions, but also in the capability to negatively influence the intellectual functions already compromised by trisomy.

Ts65Dn

Assessment of motor and sensory function in Ts65Dn mice has demonstrated that acquisition of developmental milestones in trisomic pups recapitulates the abnormal aqusition of motor and sensory skills of DS infants (Holtzman et al., 1996, Palisano et al., 2001, Vicari et al., 2006). Motor function has been evaluated in detail and it has been reported that young adult Ts65Dn mice display an altered motor performance in several paradigms including grip force, running and swimming speed and motor coordination in the rotarod test (Costa et al., 1999). The motor coordination deficit and its relevance for gait has been additionally investigated by motorized treadmills and the CatWalk automatic gait analysis system, revealing abnormalities in walking patterns and dynamics (Hampton et al., 2004, Faizi et al., 2011). The main deficits reported in Ts65Ds mice correspond to gait abnormalities observed also among DS children (Shumway-Cook and Woollacott, 1985, Parker et al., 1986, Galli et al., 2008).

While well studied in DS subjects, sensory deficits have been reported only recently in trisomic mice. Ts65Dn mice have elevated thresholds for auditory-evoked brainstem responses (ABR) compared to WT controls (Han et al., 2009). This finding faithfully recapitulates human deficits as an earlier clinical study found that ABR thresholds of DS infants are higher than those found in typically developing children (Werner et al., 1996). Similarly to the conductive nature of hearing loss among young DS population (Buchanan,

1990, Hassmann et al., 1998), auditory deficits in Ts65Dn mice have been correlated with a high prevalence of otitis media, thought to be provoked by altered craniofacial dimensions and middle ear anatomy (Hill et al., 2007) and depressed immune function (Han et al., 2009). However, while hearing loss aggravates with age in patients with DS due to additional sensorineural complications (Chen and Fang, 2005), ABR time-course experiments show a time-stable hearing loss in Ts65Dn mice (Han et al., 2009).

Loss of vision has been also recently reported for the first time in Ts65Dn mice. Similarly to persons with DS (John et al., 2004), recordings of VEPs revealed that Ts65Dn mice have lower spatial resolution, the electrophysiological correlate of VA, and higher contrast threshold, the electrophysiological inverse correlate of contrast sensitivity, than WT mice (Scott-McKean et al., 2010). Electroretinography assessments showed no significant deficits in retinal physiology in Ts65Dn mice in comparison with WT control mice (Scott-McKean et al., 2010), supporting the assumption based on clinical studies that a dysfunction of central components of visual processing may be involved in the visual deficits associated with DS (John et al., 2004).

1.3.3. Neuroanatomical correlates of neurophysiological impairments

Humans

Morphological alterations characteristic of the DS brain arise from a disruption of neural development leading to abnormal anatomy and connectivity of CNS, evident from the earliest evolutionary stages, and from a progressive atrophy believed to result from AD-like neurodegeneration, occurring during adulthood and superimposing on the existing neural changes.

Brain volume is smaller in DS subjects compared to age-matched healthy controls, even after normalizing for body size that is generally reduced in DS (Yoshimura et al., 1990,

Raz et al., 1995). Macroscopic analysis of postmortem brain tissue and ultrasonographical data have shown that the reduction of the overall volume of DS brain emerges during prenatal development (Winter et al., 2000, Guihard-Costa et al., 2006) and progresses further during the gestational period (Schmidt-Sidor et al., 1990, Golden and Hyman, 1994, Engidawork and Lubec, 2003). Singular brain areas are also modified by trisomy as illustrated by small cerebellum, frontal and temporal lobes, reduced number and depth of the cerebral sulci and a narrow superior temporal gyrus (Coyle et al., 1986, Wisniewski, 1990, Becker et al., 1991). Magnetic resonance imaging (MRI) studies have confirmed these regional brain abnormalities, emphasizing a disproportional reduction of cerebellar and hippocampal volumes in young DS subjects (Raz et al., 1995, Pearlson et al., 1998, Aylward et al., 1999, Pinter et al., 2001).

In agreement with the reduced volumes, lesser amount of neurons has been calculated in the hippocampus, the parahippocampal gyrus, cerebellum and neocortex of DS fetuses (Baxter et al., 2000, Guidi et al., 2008, Larsen et al., 2008) and in the cortex of DS children (Wisniewski, 1990), indicating that morphological alterations observed among young DS individuals may arise from decreased output of newborn neurons during development. Direct evidence that T21 disrupts neurogenesis at early developmental stages has come from the study showing that cell proliferation is impaired in the dentate gyrus (DG) and ventricular germinal matrix in the brains of DS fetuses at 17-21 weeks of gestation (Contestabile et al., 2007). Further analyses revealed that the G2 phase of neuronal cell cycle is prolonged in DS, probably accounting for slower proliferative rate during neurogenesis (Contestabile et al., 2007) and fewer numbers of differentiated neurons in the DS developing brain (Guidi et al., 2008). An increased apoptotic rate has been also reported in the hippocampus of DS fetuses contributing to the overall lack of neural cells in this brain structure (Guidi et al., 2008). Moreover, T21 significantly modifies hippocampal cell phenotypes producing more cells

with a glial phenotype and less cells expressing neuronal markers (Guidi et al., 2008). Significant neurogenesis impairments were also found in the cerebellum of fetuses with DS, while the rate of apoptotic cell death was similar to controls (Guidi et al., 2011), pointing to different mechanisms underlying reduced cell numbers in the hippocampus and cerebellum.

Numerous brain structures of DS individuals brain undergo further atrophic changes with age. Similarly to patients with sporadic AD, old DS subjects are characterized by extensive cerebral atrophy, extracellular deposition of the amyloid β peptide (Aβ) in the brain parenchyma and blood vessel walls (amyloid angiopathy) and accumulation of intraneuronal neurofibrillary tangles (NFT) composed of a hyperphosphorilated form of tau protein (Mann, 1988, Jellinger and Bancher, 1998). Neuropathological changes in sporadic AD typically occur over 65 years of age and follow a specific sequence, as in the preclinical stadium they affect transentorhinal and entorhinal cortex, while in more advanced stages of the disease they extend to the hippocampus and neocortical regions (Braak and Braak, 1997). On the contrary, almost all DS patients demonstrate AD pathology by the age of 30, with the earliest pathological markers occurring in the medial temporal lobe and spreading to the prefrontal cortex, basal ganglia, thalamus, hypothalamus and midbrain (Wisniewski et al., 1985). The pathological alterations of these regions are believed to underlie the age-dependent cognitive decline in DS patients, as indicated by a positive correlation between the number of plaques or tangles and the degree of dementia severity (Blessed et al., 1968, Ropper and Williams, 1980, Terry and Davies, 1980, Wilcock and Esiri, 1982, Ulrich, 1985, Wisniewski et al., 1985). Importantly, MRI studies have revealed that in some particularly vulnerable brain regions, such as the hippocampus and the amygdala, atrophic changes precede the clinical onset of dementia, emphasizing that evaluation of regional atrophy may help to identify people with DS in the prodromal stages of AD (Krasuski et al., 2002).

An important factor for the overall co-morbidity and early onset of AD in DS subjects

is an increased Aβ burden due to an additional copy of the amyloid precursor protein (APP) gene mapped on Hsa21 (Rumble et al., 1989). Aβ is the major constituent of extracellular insoluble aggregates that are considered to be key morphological markers for AD. Increasing evidence indicates that overproduction of Aβ, besides its role in the formation of fibrillar aggregates, interferes with physiological mechanisms underlying learning and memory, triggering an early onset of dementia in DS (Conti and Cattaneo, 2005, Gasparini and Dityatev, 2008). It has been demonstrated that Aβ oligomers, small soluble clusters of Aβ, alter synaptic plasticity of excitatory synapses in rodents (Lambert et al., 1998, Wang et al., 2002, Townsend et al., 2006, Shankar et al., 2008, Li et al., 2009), and clinical data illustrate that Aβ oligomers in AD patients are even better correlated with clinical symptoms then Aβ plaques (Mc Donald et al., 2010).

Deterioration of cognitive performance in old DS subjects has been also related to degeneration of basal forebrain cholinergic neurons (BFCNs). Deficits in the cholinergic system are very similar to those occurring in AD (Mufson et al., 2008, Schliebs and Arendt, 2011) and loss of BFCN and decreased activity of choline acetyltransferase (ChAT) have been observed in both DS and AD (Whitehouse et al., 1982, Yates et al., 1983, Casanova et al., 1985, Mann, 1988, Mufson et al., 1993). The function of basal forebrain cholinergic system is apparently normal in DS fetuses and infants (Kish et al., 1989, Lubec et al., 2001) and the number of neurons and ChAT activity start to decrease later during adolescence and adulthood (Yates et al., 1983, Casanova et al., 1985, Godridge et al., 1987, Mann, 1988, Mufson et al., 1993, Schneider et al., 1997), further supporting the view that degenerative processes occur in DS subjects during aging (Contestabile et al., 2010).

The aberrant developmental and degenerative mechanisms in DS act together at the cellular level to alter the morphological properties of dendrites. The receptive function of dendrites relies on the integrity of dendritic spines that are critically involved in brain

connectivity and plasticity (Sorra and Harris, 2000, Kasai et al., 2003, Newpher and Ehlers, 2009). Abnormalities in density and morphology of dendritic spines and reduced dendritic branching have been associated not only with DS but also with other forms of ID (Huttenlocher, 1990, Kaufmann and Moser, 2000, Dierssen and Ramakers, 2006). Spine density and length and branching of dendrites are reduced in hippocampus and cortex of DS brain (Suetsugu and Mehraein, 1980, Takashima et al., 1981, Becker et al., 1986, Takashima et al., 1989, Ferrer and Gullotta, 1990, Schulz and Scholz, 1992, Takashima et al., 1994). Interestingly, in the fetal period and during the earliest postnatal months, normal, or even increased, dendritic branching has been observed in the visual and prefrontal cortex of DS brain (Takashima et al., 1981, Becker et al., 1986, Vuksic et al., 2002) indicating that DS neurons reach the same structural complexity typical of age-matched controls. However, the period of early postnatal maturation is characterized by a greater dendritic retraction of DS neurons respect to typically developing infants, resulting in the gradual appearance of dendritic abnormalities during the first months of age (Takashima et al., 1981). Dendritic abnormalities emerging in childhood progress with age, as illustrated by additional reductions of spine density and dentridic length and branching in aged adults with DS (Takashima et al., 1989). This age-dependent deterioration of dendritic phenotype is consistent with the early onset of AD among DS patients as dendritic pathology accompanies also initial stages of AD and correlates significantly with the progressive decline of mental faculties (Baloyannis, 2009).

Ts65Dn

Differentially from human DS, the overall size of postnatal Ts65Dn brain is not altered (Reeves et al., 1995). On the contrary, anatomical changes have been detected in specific brain regions, such as cerebellum and hippocampus, indicating that cognitive deficits

displayed by this model are based on selective vulnerability of specific neural circuits rather than on gross anatomical changes.

A reduction of cerebellar volume has been found in trisomic pups (Roper and Reeves, 2006) indicating that, similarly to human DS, the neuroanatomical modifications of Ts65Dn mice emerge at early stage of neural development. Morphological alterations of cerebellum have been analyzed in depth, reveling that both the molecular layer and the internal granular layer of Ts65Dn mice are thinner respect to WT controls due to decreased densities of Purkinje neurons and granular cells (Baxter et al., 2000). These findings have a predictive value as similar deficits in cerebellum structure were found afterwards also among people with DS (Baxter et al., 2000). Interestingly, despite the structural deficit, Ts65Dn mice perform as well as control mice on tasks designed to evaluate typical cerebellar functions, such as balance and motor coordination (Baxter et al., 2000, Hyde and Crnic, 2001). Since, however, increasing findings suggest an involvement of the cerebellum in various learning and memory paradigms (Rondi-Reig and Burguiere, 2005, Burguiere et al., 2010), the contribution of described cerebellar developmental deficits to some aspects of cognitive dysfunction in DS should not be ruled out.

Structural abnormalities have been observed also in the hippocampus and they are in agreement with a poor performance in hippocampus-dependent cognitive tasks displayed by Ts65Dn mice. The overall volume of hippocampus does not seem altered in Ts65Dn mice up to 7 months of age (Insausti et al., 1998, Lorenzi and Reeves, 2006, Olson et al., 2007), but significant structural alterations are evident in the hippocampal subfields from early life (Lorenzi and Reeves, 2006, Costa et al., 2008, Salehi et al., 2009, Bianchi et al., 2010b, Faizi et al., 2011). In the DG of Ts65Dn mice the number of granule cells and the volume of hilus and the granule cell layer are slightly reduced already at postnatal day 6 (P6) indicating that this early developmental hypocellularity may contribute to the specific behavioral

impairments reported in young trisomic mice (Lorenzi and Reeves, 2006).

Similarly to human DS, there is a growing body of evidence suggesting that the major determinant of morphological brain alteration may be a defective neurogenesis process during early developmental stages. Substantial delays in prenatal growth of the Ts65Dn cerebral cortex and hippocampus have been linked to longer cell cycle duration and reduced neurogenesis from the ventricular zone (Chakrabarti et al., 2007), while the deficit in cerebellar formation has been correlated to defective response of granule cell precursors to the signaling pathway mediated by the sonic hedgehog (SHH) growth factor (Roper et al., 2006). Emerging evidence indicates that adult neurogenesis in both the DG and the subventricular zone is also altered in trisomic mice and it has been postulated that this deficit could be involved in ID, as therapies aimed at rescuing postnatal neurogenesis have been able to ameliorate some aspects of cognitive performance (Clark et al., 2006, Bianchi et al., 2010a, Bianchi et al., 2010b, Chakrabarti et al., 2011).

Additional morphological alterations associated with aging occur also in Ts65Dn mice. Elevated levels of APP (Seo and Isacson, 2005) and Aβ (Netzer et al., 2010) have been reported in the cortex and hippocampus of Ts65Dn mice, but this model does not develop typical pathological hallmarks of AD (Reeves et al., 1995, Kurt et al., 2004). The occurrence of age-related cognitive decline in the absence of plaques and tangles and the recent demonstration that lowering Aβ levels rescues learning and memory in Ts65Dn mice (Netzer et al., 2010) support the hypothesis that soluble Aβ forms may be crucially involved in the pathophysiology of AD. Similarly to human pathology, BFCN are also affected by trisomy in Ts65Dn mice. It has been reported that an elevated expression of APP in Ts65Dn mice provokes an enlargement of early endosomes employed in axonal trafficking and an impairment of retrograde transport of nerve growth factor (NGF) from the hippocampus to the basal forebrain, leading to degeneration of BFCNs (Cataldo et al., 2003, Salehi et al.,

2006, Chang and Gold, 2008). NGF is a neurotrophic factor that enhances the survival, differentiation and function of specific neurons of the peripheral and central nervous systems, including BFCNs (Sofroniew et al., 2001). A critical involvement of APP in the loss of BFCNs is illustrated by the reversion of the degenerative phenotype after infusion of NGF (Cooper et al., 2001). The degeneration of BFCN in Ts65Dn mice occurs from 6-10 up to 20 months of age (Holtzman et al., 1996, Granholm et al., 2000, Hunter et al., 2004, Lockrow et al., 2009) and may account for age-related impairments in learning and memory (Hyde and Crnic, 2001, Hunter et al., 2003). Given that BFCNs provide strong modulatory inputs to the hippocampus, the cholinergic deafferentation may in turn underlie age-related volume reductions of hippocampal subfields (Kurt et al., 2004). However, differentially from observations in humans, the activity of ChAT appears to be increased in the cortex and hippocampus of 10 months old Ts65Dn mice and remains stabile even in older mice (Cooper et al., 2001, Seo and Isacson, 2005, Contestabile et al., 2006, Contestabile et al., 2008, Chen et al., 2009) indicating a possible activation of some compensatory synaptic mechanisms that may attenuate the impact of cholinergic loss on cognitive decline in this model.

Similarly to human DS, significantly reduced spine density and dendritic complexity of the cortical pyramidal neurons and the hippocampal pyramidal and granular neurons have been observed in adult Ts65Dn mice compared to control littermates (Dierssen et al., 2003, Belichenko et al., 2004, Belichenko et al., 2007). Electron microscopy has revealed that these dendritic abnormalities are accompanied by a size increment of presynaptic boutons, postsynaptic spines and average length of synaptic cleft (Belichenko et al., 2004). Dendritic alterations are already present at P21 indicating that, similarly to human DS, trisomy alters spine dynamic in Ts65Dn mice from early stages of development. Importantly, it has been revealed that various types of synapses are differentially affected by the trisomic condition. In the dentate granule cells of the hippocampus, there is a shift of inhibitory synaptic

connections away from the dendritic shafts and onto the dendritic necks, which would be expected to facilitate inhibitory synaptic transmission given the significantly reduced volume of the spine neck compared to the shaft (Belichenko et al., 2004). Moreover, inhibitory synapses have greater apposition lengths in Ts65Dn mice while excitatory synapses are unaltered, indicating a shift towards excessive inhibition in these mice (Belichenko et al., 2009b). The altered spine morphology and shift towards excessive inhibition characterizing DS may directly affect information processing by suppressing not only excitatory synaptic transmission, but also plasticity related signaling cascades that frequently rely on depolarization-mediated calcium influx into the postsynaptic structural domains (Cramer and Galdzicki, 2012).

1.3.4. Disruption of neurotransmitter systems and neurotrophic factors

Humans

In addition to the above discussed alterations in the cholinergic system, the glutamatergic, serotonergic, noradrenergic and γ-aminobutyric acid (GABA)-ergic systems are also profoundly disrupted in DS individuals. Reduced levels of serotonin (5-HT), GABA and dopamine (DA) have been reported in the frontal cortex of trisomic fetuses in comparison to euploid fetuses (Whittle et al., 2007). The imbalance in these neurotransmitter systems that emerges from the prenatal period may be involved in the disruption of numerous aspects of DS brain development. It has been shown that 5-HT plays an important role in neurogenesis, neuronal differentiation, dendritic development, axon myelination and synaptogenesis (Whitaker-Azmitia, 2001). Additionally, there is a defect in a dynamic of 5-HT receptors illustrated by an earlier peak of serotonin 5-HT1_A receptors in developing DS brains (Bar-Peled et al., 1991). Reduction of GABA levels is also expected to impact the formation of DS brain as during development GABA acts as an epigenetic factor that controls cell

proliferation, neuroblast migration and dendritic maturation (Represa and Ben-Ari, 2005). The expression of three $GABA_A$ receptor subunits is also altered in the early prenatal life in DS, with up-regulation of subunit α2 and down-regulation of α5 and β3 subunits (Bhattacharyya et al., 2009). In the brain of adults with DS, 5-TH is below the levels of age-matched control group and is accompanied with a reduction in the levels of glutamate, aspartate and noradrenaline (NA) (Godridge et al., 1987, Risser et al., 1997). Reduced levels of 5-HT and NA in aged DS individuals are probable due to a degenerative loss of neurons from the dorsal raphe nucleus and locus coeruleus (Mann et al., 1985, Coyle et al., 1986), which send the serotonergic and noradrenergic inputs to the forebrain, respectively.

Disruption of neural development and accelerated neurodegenerative mechanisms in DS may be also affected by alterations of neurotrophin systems. Neurotrophins represent an important family of small proteins secreted in the vertebrates nervous system, that includes NGF, brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4) (Meakin and Shooter, 1992, Huang and Reichardt, 2001). During development, neurotrophins regulate cell survival and differentiation, axon growth, dendrite pruning, the innervation pattering and the expression of neurotransmitters and ion channels necessary for normal neural functions. In the mature nervous system, neurotrophins continue to modulate neural survival and also take part in the control of synaptic function and plasticity (Huang and Reichardt, 2001, Sofroniew et al., 2001). Neurotrophins exert their action throughout two different classes of receptors. While all trophic factors bind to the p75 neurotrophin receptor, each of them also binds to specific members of the surface tyrosine receptor kinases (Trks): NGF to TrkA, BDNF and NT-4 to TrkB, and NT-3 to TrkC and to TrkA (Meakin and Shooter, 1992, Meakin et al., 1992, Chao and Hempstead, 1995). So far, clinical studies concerning DS have been focused on NGF and BDNF expression. Reduced serum levels of NGF have been found in children with DS (Calamandrei et al., 2000). Similarly, in the DS

fetal brain samples it has been found a lower expression of BDNF and TrkB isoforms (Toiber et al., 2010). On the other hand, opposite results were reported from adult individuals with DS, as illustrated by a significant increase of their BDNF blood plasma level with respect to age-matched controls (Dogliotti et al., 2010). New studies are necessary to better understand this apparent discrepancy in BDNF levels (lower expression in fetuses and higher expression in adults) between developing and aging DS brain.

Ts65Dn

Neurotransmitter levels and receptor expressions have been also investigated in Ts65Dn mice, indicating an alteration of multiple systems. However, some data show discrepancies with respect to human findings. For example, there are no changes of 5-HT levels in the hippocampus of Ts65Dn mice (Bianchi et al., 2010b) as well as no alterations in the number of serotonergic neurons of the dorsal and medial nuclei (Megias et al., 1997). On the contrary, reduction has been found in the expression of 5-HT_{1A} receptors in hippocampal neurospheres and the hippocampus of newborn Ts65Dn mice (Bianchi et al., 2010b). As this receptors have been implicated in the regulation of neurogenesis (Malberg et al., 2000), their lower expression may contribute to the impairments of neural proliferation found in trisomic mice. This is supported by the rescue of early postnatal neurogenesis in Ts65Dn mice after treatment with the selective serotonin reuptake inhibitor (SSRI) fluoxetine that increases the expression of the 5-HT_{1A} receptors up to normal levels (Bianchi et al., 2010b). Analyses of inhibitory and excitatory neurotransmitter systems in Ts65Dn mice have been particularly focused on the alterations in receptor subunit composition. The hippocampal region-specific distributions of immunoreactivity for α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) receptor subunits GluR1 and GluR2, $GABA_A$ receptor subunits α1 and β2/3, and $GABA_B$ receptor subunits R1 or R2 were investigated at 3 and 8 months of age,

revealing significant alterations in the expression of AMPA GluR2, $GABA_A$ β2/3 and $GABA_B$ R1 subunits respect to euploid animals (Belichenko et al., 2009b). Namely, in young Ts65Dn mice an overall decrease for AMPA GluR2 and $GABA_A$ β2/3 subunits was reported, while in older Ts65Dn mice it was found a reduction of AMPA GluR2, $GABA_B$ R1 and $GABA_A$ β2/3. The most interesting result is probably the age-dependent diminution in the expression of GluR2 and $GABA_B$ receptor subunit R1, in contrast with an increase in the expression of $GABA_A$ receptor subunit β2/3. The composition of N-methyl-D-aspartate receptor (NMDA) receptors appears normal in neonatal and young adult brains of Ts65Dn mice (Pollonini et al., 2008, Fernandez et al., 2009), but there is a reduced brain expression of the subunits NR2A and NR2B at 10 months of age (Vink et al., 2009). Although the physiological consequences of these alterations in the expression of AMPA, GABA and NMDA receptors are still unclear, a contribution to the additional cognitive decline found in older Ts65Dn mice may be expected. The noradrenergic system is also affected in Ts65Dn mice. Although the number of β-adrenergic receptors in the cerebral cortex and hippocampus of Ts65Dn mice is similar to that of euploid mice, their function, as assessed through analysis of 3',5'-cyclic AMP (cAMP) formation, is impaired and there is a reduced basal and stimulated production of cAMP (Dierssen et al., 1997).

Expression of neurotrophic factors is also defective in Ts65Dn mice. Reduction of BDNF levels in Ts65Dn mice have been observed in the hippocampus at 15 days and 3 months of age (Bianchi et al., 2010b, Fukuda et al., 2010) and in the frontal cortex at 6 months of age (Bimonte-Nelson et al., 2003). Importantly, normalization of BDNF expression induced by treatment with fluoxetine was able to restore survival of newborn cells, differentiation, and granule cell number in Ts65Dn mice (Bianchi et al., 2010b). Recent evidence has established an important link between chromosome 21-derived microRNA mirna21 and above mentioned deficits in expression of 5-$HT1_A$ receptor and BDNF in

trisomic brains. It has been demonstrated that mirna21 are overexpressed in the DS brain and, consequentially, a downregulation in the expression of the transcriptor factor methyl-CpG-binding protein (MeCP2) does occur (Kuhn et al., 2010). Numerous mutations in the MeCP2 gene have been associated with the neurodevelopmental disorder Rett syndrome (RS) (Amir et al., 1999), indicating a possible involvement of this gene also in DS brain pathology. MeCP2 activates the expression of numerous genes, including 5-HT1_A receptor and BDNF (Chahrour et al., 2008), suggesting the possibility that reduced levels of these two proteins in Ts65Dn brain may be caused by silencing of MeCP2 gene. Given that fluoxetine administration increases MeCP2 expression in the DG (Cassel et al., 2006), it is possible to hypothesize that epigenetic modifications might also contribute to the restoration of a normal expression of 5-HT1_A receptor and BDNF in Ts65Dn mice after treatment with fluoxetine (Bianchi et al., 2010b).

The impact of abnormal retrograde transport of NGF on progressive age-dependent degeneration of BFCNs in adult Ts65Dn mice have been discussed in the previous sections. However, it seems that there is a deficit in the hippocampal NGF expression also in the early postnatal period (Bianchi et al., 2010b), indicating that abnormal NGF dynamics should be also investigated in the context of DS neurodevelopmental pathology. Finally, increased levels of NT-3 have been found in total brain extracts of neonate Ts65Dn mice compared to their littermates (Pollonini et al., 2008), suggesting a possible attempt of the nervous system to protect itself from the neuronal loss that occurs in DS throughout development. Enhanced secretion of NT-3 has been also found in the hippocampus of adult Ts65Dn mice (Pollonini et al., 2008). As NT-3 expression by cortical neurons serves to attract basal forebrain cholinergic projections (Robertson et al., 2006), an increase in NT-3 may be a compensatory response to the age-dependent BFCNs loss of DS (Cooper et al., 2001, Chang and Gold, 2008).

Overall, substantial morphological and molecular evidence demonstrates that trisomy disrupts CNS development and accelerates age-related neurodegeneration of individuals with DS and Ts65Dn mice in a very similar manner, resulting in specific cognitive, behavioral, motor and sensory deficits. This clearly illustrates that the Ts65Dn mouse is an excellent model for better understanding of pathological processes underlying DS and preclinical evaluation of new therapeutic strategies.

1.4. Altered synaptic plasticity and disruption of excitatory-inhibitory balance as a central mechanism of neurophysiological impairments in Down syndrome

Learning and memory, key cognitive processes affected by DS, have been strongly associated with synaptic plasticity mechanisms (Bliss and Collingridge, 1993, Hofer and Bonhoeffer, 2010). Synaptic plasticity refers to the physiological ability of synapses to alter their structure, composition or function in response to changes in neural activity. Depending on the timing and strength of pre- and postsynaptic activity, synapses can either be strengthened or weakened, providing a potential mechanism for memory formation and storage (Dan and Poo, 2006). The connection between synaptic plasticity and learning and memory is typically studied within the hippocampus, as this brain region is critically involved in acquisition, storage and recall of spatial information (Nadel and Bohbot, 2001, Kesner, 2007, Moser et al., 2008). Consequentially, any structural or functional abnormalities in hippocampus that limit the capability of synapses to undergo plastic changes would be expected to compromise spatial cognition. The described morphological and behavioral alterations associated with trisomic hippocampus, like dendritic pathology (Becker et al., 1986, Takashima et al., 1989) and impairments in spatial learning and memory (Pennington et al., 2003, Edgin et al., 2010),

suggest an ineffective synaptic plasticity in DS. There is evidence indicating that plasticity is reduced also in the motor cortex of DS individuals (Battaglia et al., 2008). Moreover, functional MRI (fMRI), performed during cognitive processing tasks involving multiple brain regions, reveals abnormal neural activation patterns in DS children and young adults (Losin et al., 2009, Jacola et al., 2011). These data suggest that an impairment of synaptic plasticity may not be exclusively an hippocampal phenomenon, but a general characteristic of DS brain. Consistent with this idea is also the general defect in synaptogenesis that starts to be evident from the fetal period (Becker et al., 1991) and persists until adult age (Petit et al., 1984, Takashima et al., 1994, Weitzdoerfer et al., 2001). Since it is very difficult to evaluate synaptic function in humans, the animal models have provided important insights into the neurobiology of aberrant plastic mechanisms in DS.

The best established experimental models for investigating the synaptic base of learning and memory in rodents are long-term potentiation (LTP) and long-term depression (LTD), that refer to long-lasting up- or downregulation of synaptic strength, respectively, after an appropriate electrical stimulus of hippocampal circuits in the brain slice preparation. It is conventionally believed that the ability of hippocampal circuits to undergo LTP and LTD is necessary for the processes of learning and memory; thus, alteration in the expression of these two model of synaptic plasticity is considered an electrophysiological correlate of cognitive deficits.

Multiple lines of evidence indicate that synaptic plasticity is altered in adult Ts65Dn mice. The phenomenon of LTP has been studied in detail in this mouse model and its reduction has been reported in the hippocampal CA1 and DG regions (Siarey et al., 1997, Kleschevnikov, 2004, Costa and Grybko, 2005, Das and Reeves, 2011, Kleschevnikov et al., 2012). Importantly, LTP abnormalities have been also observed in various rodent models of other forms of developmental ID (DID) (Costa et al., 2002, Asaka et al., 2006, Moretti et al.,

2006, Guy et al., 2007) and AD (Chapman et al., 1999, Larson et al., 1999, Moechars et al., 1999, Origlia et al., 2006, Houeland et al., 2010), indicating that the impairment of synaptic plasticity in the case of DS may underlie both ID emerging during childhood and additional cognitive decline due to precocious AD in adulthood. In addition to LTP failure, abnormal augmentation of LTD has been reported from hippocampal slices of Ts65Dn mice (Siarey et al., 1999, Scott-McKean and Costa, 2011) further strengthening a link between alteration of synaptic plasticity in hippocampus and poor cognitive performance displayed by this DS animal model.

The breakthrough discovery in this field was the demonstration that the reduction of LTP in adult Ts65Dn mice is due to an imbalance between excitatory and inhibitory inputs to the hippocampus. Acute additions of inhibitors of $GABA_A$ receptors in the brain slice preparation rescued LTP expression in the DG (Kleschevnikov, 2004), indirectly pointing at excessive hippocampal inhibition in trisomic mice. More direct evidence for increased GABAergic transmission came from the recent report of significantly increased evoked inhibitory postsynaptic currents (IPSCs) mediated by $GABA_A$ and $GABA_B$ receptors in the DG, suggesting an enhancement in presynaptic GABA release probability (Kleschevnikov et al., 2012). While this study indicates the involvement of both types of GABA receptors in the modulation of DG synaptic efficiency, another recently published paper reported that in the CA1 region the balance between $GABA_A$ and $GABA_B$ receptor-mediated inhibition is shifted in favor of the latter in a pathway-specific manner (Best et al., 2012). Increased $GABA_B$ receptor-mediated signaling has been linked to DS specific triplication of G-protein-activated inwardly rectifying potassium channel 2 *(Girk2)* gene. The Girk channels are the postsynaptic effectors of $GABA_B$ receptors and it has been hypothesized that overexpression of Girk2 gene may be directly responsible for LTP failure in trisomic mice (Harashima et al., 2006, Best et al., 2007). In line with this functional evidence for an increased inhibitory tone in the

hippocampus of Ts65Dn mice are the above discussed changes in synaptic structure that favor GABAergic transmission (Belichenko et al., 2004, Belichenko et al., 2009b), supporting the view that hippocampal LTP failure and increased inhibition may be a key mediator of cognitive dysfunction in DS.

Given that DS patients demonstrate susceptibility to epilepsy (Stafstrom, 1993), it has been speculated that the increment in the level of inhibition could reflect an attempt by the system to cope with over-excitation (Pérez-Cremades et al., 2010). However, it has been demonstrated that the origin of altered excitatory/inhibitory ratio dates back to the prenatal period, suggesting that excessive inhibition is not a compensatory mechanism but rather the direct pathological output of abnormal cortical development in Ts65Dn mice. During the prenatal period, longer cell cycle durations in the ventricular and subventricular zone of Ts65Dn might lead to reduced overall production of excitatory neurons at the cortical plate, with a delay in their arrival and differentiation (Haydar et al., 1996, Cheng et al., 2004, Chakrabarti et al., 2007). It has been shown that this perturbation of neural developmental pathways may be due to overexpression of the dual-specificity tyrosine phosphorylation-regulated kinase 1A *(Dyrk1a)* gene (Altafaj et al., 2001, Martinez de Lagran et al., 2004). The *Dyrk1a* gene encodes a kinase that is strongly expressed in neural structures, especially within neural precursor populations during embryonic neurogenesis (Okui et al., 1999, Hammerle et al., 2002, Hammerle et al., 2008) and interacts with a wide variety of growth factors, transcription factors and cell-cycle regulatory proteins known for their roles in neural cell proliferation and specification (Yang et al., 2001, Maenz et al., 2008, Fernandez-Martinez et al., 2009, Lepagnol-Bestel et al., 2009, Tejedor and Hammerle, 2011). Additionally, prenatal overexpression of the *Olig1* and *Olig2* transcription factor genes in the germinal zones of the ventral telencephalon results in an elevated rate of cell production from the neural precursors and leads to increased number of parvalbumin- and somatostatin-

expressing interneurons in the cerebral cortex and hippocampus of Ts65Dn mice (Chakrabarti et al., 2010). *Olig1* and *Olig2* genes are typically expressed in the progenitors of the medial ganglionic eminence, the specific germinal zone for parvalbumin- and somatostanin-expressing interneurons, and not in the progenitors of the lateral and caudal ganglionic eminence specific for calretinin-expressing interneurons, resulting in an increased neurogenesis for only the first two specific groups of interneurons (Chakrabarti et al., 2010). Taken together, these impairments in neurogenesis alter the ratio between excitatory and inhibitory neurons leading to the onset of excessive inhibition during early phases of prenatal forebrain development (Haydar and Reeves, 2012).

A fine tuning of the ratio between excitatory and inhibitory drive within neural networks is necessary for an appropriate function of both the developing and mature brain (Bradford, 1995, Thompson et al., 1996, Fagiolini and Hensch, 2000, Jamain et al., 2003, Haydar, 2005, Sale et al., 2007b). Anatomical and physiological evidence from all so far tested species indicates that in the neocortex inhibitory circuits mature later and slower than excitatory connections (Blue and Parnavelas, 1983, Luhmann and Prince, 1991, Benevento et al., 1992, Guo et al., 1997, Micheva and Beaulieu, 1997, Gao et al., 2000, Mower and Guo, 2001, Murphy et al., 2005). This gradual maturation of GABAergic inhibition appears to be a key determinant in the initiation and closure of critical periods (CPs) of experience-dependent developmental plasticity in cortical circuits (Huang et al., 1999). CPs are time windows in early postnatal development characterized by heightened brain plasticity, during which experience can produce permanent, large-scale modifications in neuronal circuits (Berardi et al., 2000, Hensch, 2004). By their ability to control the onset and offset of CPs, GABAergic interneurons may directly affect how experience shapes brain connections during early life and adolescence. The capacity for adaptive change of neural networks wanes after the closure of CPs and the limited ability of adult circuits to undergo plastic responses is also controlled

by proper inhibitory drive (Harauzov et al., 2010, Sale et al., 2010). Moreover, beside the engagement in the control of neuronal excitability, a proper GABAergic tone is also required for the generation of temporal synchrony and oscillatory behavior among networks of pyramidal neurons. Such oscillations within and across neural systems are believed to serve various complex functions, such as memory, perception and movement initiation (Chattopadhyaya and Cristo, 2012).

Based on this important role of finely tuned GABA tone in modulating network dynamics of neocortical circuits, the mismatch in the maturation of excitation and inhibition and the long-term changes in neural excitability in the DS brain may affect multiple neural functions (Baroncelli et al., 2011). The results from Ts65Dn mice clearly illustrate that an enhanced GABAergic inhibition in hippocampal circuits leads to prominent reductions of LTP levels. It has been hypothesized that the impairment of this form of activity-dependent plasticity displayed by Ts65Dn mice may be actually linked to dysfunctional homeostatic plasticity (Fernandez and Garner, 2007). Homeostatic plasticity refers to remodeling of neural connections in response to chronic excitation or inhibition of neuronal networks and it occurs over broad time scales to protect the system from excessive activity of excitatory or inhibitory neurons (Turrigiano, 1999, Turrigiano and Nelson, 2004, Davis, 2006). Absence of such resetting of neural activity in DS brain as a consequence of excessive inhibition from early stages of development may result in the establishment of neural circuits incapable to experience synaptic plasticity and properly adapt to novel demands of the system.

It has been suggested that therapeutic strategies targeting over-inhibition in DS should be based on the concept of adaptive change in order to safely and permanently counteract excitatory-inhibitory imbalance and promote homeostatic plasticity. The concept of adaptive change alludes to long-term alterations at molecular, physiological, anatomical and behavioral levels that occur not through acute modifications of synaptic function, but through

lasting alterations within neural circuits (Fernandez and Garner, 2007). This possibility for DS treatment has been successfully explored on Ts65Dn mice by administrating a non-epileptic dose of noncompetitive $GABA_A$ antagonists. It has been found that chronic, but not acute, treatment resulted in amelioration of learning and memory deficits in Ts65Dn mice, accompanied by rescue of impaired LTP in the DG (Fernandez et al., 2007). This pharmacological approach led to a persistent recovery of Ts65Dn cognition and LTP lasting for several months after the cessation of treatment, indicating that this therapeutic protocol was able to elicit neuroadaptive processes promoting both homeostatic and synaptic plasticity (Fernandez et al., 2007).

Altogether these data strongly suggest that dysfunctional synaptic plasticity may be the major determinant of DS. A growing body of evidence demonstrates the tight link between synaptic failure and disrupted excitatory-inhibitory balance driven by molecular and cellular perturbations associated with overexpression of genes involved in forebrain development. Theoretically, the early onset of excessive inhibition in DS brain may be prevented by prenatal interventions aiming at the correction of such genetic perturbations, but the application of this gene-based therapy on human fetuses is not feasible at the present due to technical limitations and ethical concerns (Haydar and Reeves, 2012). On the other hand, over-inhibition as the final outcome of mixed gene dosage effects during early development has started to emerge as a novel therapeutic target.

CHAPTER 2

EFFECTS OF ENVIRONMENTAL ENRICHMENT AND ENVIROMIMETICS ON BRAIN IN HEALTH AND DISEASE

The development and maturation of the mammalian brain are governed by complex genetic and epigenetic programs that enable temporal and spatial integration of molecular and cellular signals necessary for the construction of appropriate neural networks. Once formed, neural circuitries undergo continuous refinements through motor, sensory, social and cognitive interactions with the environment during the whole lifespan. In this way, genes and environment act together to establish and maintain optimal brain function. Thus, any modification of this complex gene-environment interaction would be expected to have profound impact on CNS. This chapter will address the spectrum of beneficial effects elicited by environmental enrichment (EE) paradigm. EE refers to the housing conditions that enable enhanced cognitive, motor, social and sensory stimulation of animals in comparison with standard laboratory environment (Rosenzweig and Bennett, 1996, van Praag et al., 2000, Sale et al., 2009).

It has been clearly demonstrated that EE is able to dramatically affect the functional, anatomical and molecular properties of the CNS, both during the CPs and in adulthood (Baroncelli et al., 2010a). Important insights into the mechanisms underlying these remarkable effects have been provided by studies focused on the visual system (Sale et al., 2009). At early stages of brain development, EE triggers a marked acceleration in the maturation of the visual system with enhanced maternal care acting as a fundamental mediator of the enriched experience in the newborn. In the adult brain, EE stimulates plasticity in the cerebral cortex, promoting the recovery of visual functions in amblyopic

animals. The broad spectrum of neural effects exerted by EE is considerable given that it activates multiple molecular responses including reduced intracerebral inhibition, enhanced neurotrophin expression and epigenetic changes at the level of chromatin structure.

These results shed new light on the potential of EE as a non invasive strategy to ameliorate deficits in the development of the CNS and to treat neurodegenerative disorders associated with aging (Nithianantharajah and Hannan, 2006, Baroncelli et al., 2010a). Moreover, it has been proposed that the molecular and cellular systems which mediate the therapeutic effects of EE may provide novel targets for development of new pharmacologic therapies. Based on this assumption, a novel class of therapeutics, called enviromimetics, has been introduced as a promising therapeutic approach for a wide range of nervous system disorders (McOmish and Hannan, 2007).

2.1. Neural consequences of environmental enrichment

EE is classically defined as "a combination of complex inanimate and social stimulation" (Rosenzweig et al., 1978). Enriched animals are reared in large groups and housed in spacious cages where running wheals and a variety of differently shaped objects are introduced and changed frequently. In this way the animals experience a multi-sensory/cognitive stimulation, increased physical activity and enhanced social interactions that improve the quality of animals' life and promote their natural explorative behaviors. Thus, EE has been created as a gain-of-function paradigm that allows to understand how increased levels of environmental stimulation may influence brain development and plasticity.

EE exerts profound effects on the adult healthy brain. The most prominent outcome of this experimental paradigm is an enhancement of neural plasticity, the ability of the nervous system to change in response to experience. While neural plasticity is a fundamental property

of the brain, it has been shown that EE is able to elicit structural and functional changes in the brain that promote brain plasticity (van Praag et al., 2000).

Multiple studies reported that EE is able to modify animals' behavior leading to an improvement in complex cognitive functions, particularly learning and memory (Rampon and Tsien, 2000), and positively affecting the animals' emotional and stress reactivity (Chapillon et al., 2002). In line with these ameliorations of cognitive performance is the increased potential for synaptic refinement displayed by enriched animals, as illustrated by facilitation of LTP induction in the hippocampus (van Praag et al., 2000).

This functional improvement is accompanied by prominent changes at the anatomical level. Robust increments in cortical thickness and weight and modifications of neuronal morphology, including increased dendritic arborisation, number of dendritic spines, synaptic density and postsynaptic thickening, have been observed in several regions of the brain, particularly in the hippocampus and the occipital cortex (Mohammed et al., 2002). Moreover, exposure to EE enhances hippocampal neurogenesis and the integration of newly born cells into functional circuits, which in turn may increase the existing potential of mature brain for learning and memory (van Praag et al., 2000).

At the molecular level, EE causes a significant change in the expression of a large set of genes involved in neuronal structure, excitability, synaptic transmission and plasticity (Rampon et al., 2000). Further, EE modulates the synthesis and secretion of neurotrophic factors throughout the brain and affects the cholinergic, serotoninergic and noradrenergic signaling (Rosenzweig and Bennett, 1969, Rasmuson et al., 1998, Ickes et al., 2000, Naka et al., 2002). Although the major part of EE studies have been mainly focused on rodents, similar effects have been reported in several species of mammals, such as gerbils, ground squirrels, rabbits, cats and primates (Rosenzweig and Bennett, 1969, Cornwell and Overman, 1981, Hansen and Berthelsen, 2000, Kozorovitskiy et al., 2005, Jansen et al., 2009).

2.2. Influence of environmental enrichment on brain development

Despite the large body of evidence concerning the effects of EE on the adult brain, until recently the influence of an enhanced stimulation on the developmental physiology and plasticity of the CNS has remained only poorly investigated. This topic has been addressed in the past few years with a series of studies focusing on the visual system, the paradigmatic model for studying experience-dependent plasticity, as it allows easy manipulations of visual experience and follow-up of the elicited changes at the molecular, cellular and anatomical level. Although the maturation of visual system starts before eye opening and the initial targeting of neural connections is subjected to either genetic programs or spontaneous activity, a proper development of the visual system requires sensory experience (Crowley and Katz, 2002, Leamey et al., 2009). A total absence of sensory input leads to a delay in the functional and anatomical maturation of the visual cortex as illustrated by a spectrum of serious physiological deficits, like immature ocular dominance (OD) distribution in the primary visual cortex (V1) and lower VA, displayed by adult animals reared in darkness (dark rearing, DR) from birth (Fregnac and Imbert, 1978, Timney et al., 1978, Benevento et al., 1992, Fagiolini et al., 1994). Experience-dependent plasticity in V1 is typically investigated throughout analysis of binocularity expressed by the OD index. More precisely, OD plasticity refers to the rapid change in visual cortex physiology resulting from unbalanced inputs from the two eyes and it is easily induced only in developing brain. The first demonstration of this phenomenon has been provided by Hubel and Wiesel, who reported that reduced input from one eye by lid suture (monocular deprivation, MD) during development results in the loss of cortical responses to closed eye and gain of activity of neurons preferentially stimulated by the open eye. Consequentially, this visual manipulation dramatically affects the binocularity of V1 and makes the deprived eye amblyopic, with a significantly reduced VA and blunted contrast sensitivity (Wiesel and Hubel, 1963, Hubel

and Wiesel, 1970). Based on this experiment, the effects of MD and the existence of a CP for OD plasticity have been subsequently described in different species of mammals (Van Sluyters and Stewart, 1974, Hubel et al., 1977, Emerson et al., 1982, Fagiolini et al., 1994, Gordon and Stryker, 1996, Issa et al., 1999).

The most relevant result regarding the effects of EE from birth on visual system development is a marked acceleration in the maturation of VA respect to animals reared in standard conditions (Prusky et al., 2000, Cancedda et al., 2004, Sale et al., 2004, Landi et al., 2007a, Landi et al., 2007b). Moreover, rearing animals from birth in EE results in an accelerated decline of white matter-induced LTP (WM-LTP), a well-established in vitro model of developmental plasticity (Kirkwood et al., 1995), in visual cortical slices after theta-burst stimulation (Cancedda et al., 2004). Importantly, EE is also able to compensate the deficits caused by a complete lack of visual experience from birth. Namely, DR rats maintained in EE conditions show a normal VA development and closure of the CP for OD plasticity (Bartoletti et al., 2004). Similar findings have been reported in the auditory system. It has been found that pre-weaning EE improves spatial localization abilities and enhances directional sensitivity of A1 neurons (Cai et al., 2009), although it remains still unexplored whether exposure to EE conditions counteract the delay in A1 maturation provoked by white noise rearing.

Retina development is also affected by high levels of environmental stimulation. EE accelerates the segregation of retinal ganglionic cell (RGC) dendrites into ON and OFF sublaminae and the maturation of retinal acuity during development (Landi et al., 2007a, Landi et al., 2007b). Strikingly, it has been shown that retinal development is profoundly affected by EE also during embryonic life. Exposing pregnant females to EE is able to accelerate structural processes critical for retinal maturation, such as the migration of neural progenitors and the time-course of naturally occurring cell death in the RGC layer (Sale et al.,

2007a), indicating that even indirect forms of environmental stimulation may influence development of the nervous system during prenatal life. The influence of increased female stimulation during pregnancy is not restricted only to the visual system of their offspring. Voluntary wheel running of pregnant mice leads to a two-fold increase in hippocampal precursor-cell proliferation in their pups (Bick-Sander et al., 2006), while maternal swimming improves short-term memory abilities of the newborns (Lee et al., 2006).

BDNF and the insulin-like growth factor (IGF-I) have emerged as crucial molecular mediators of the EE effects on the development of visual system. Mice raised in EE showed increased levels of the BDNF protein in their visual cortex at P7 (Cancedda et al., 2004, Sale et al., 2004). Higher BDNF levels in EE pups were shown to trigger the maturation of the inhibitory GABAergic system, which in turn, by affecting the formation of receptive field and synaptic plasticity, may determine both the accelerated development of VA and the decline of cortical plasticity (Huang et al., 1999, Cancedda et al., 2004, Sale et al., 2004). Similarly to BDNF increase in mice, IGF-I expression is higher at P18 in the visual cortex of EE rats. Importantly, exogenous IGF-I supply mimics, whereas blocking of its action prevents, the EE effects on VA maturation. As in the case of BDNF, these effects are mediated by activation of the inhibitory system, as GABAergic interneurons respond to IGF-I with a glutamic acid decarboxylase 65 (GAD65) increase in their synaptic terminals (Ciucci et al., 2007). IGF-I emerged also as a key player in the mediation of the effects of maternal enrichment on prenatal retinal development. The previously described anatomical modifications of retinal structure are accompanied by a marked increase of IGF-I levels in the retinas of EE pups and in the maternal milk. Furthermore, if IGF-I is infused during late pregnancy to non-EE females, their foetuses undergo all the reported changes elicited by EE, while neutralization of IGF-I action in EE mothers prevents the effects of maternal EE on retinal development (Sale et al., 2007a).

The aforementioned studies of the effects of EE on retinal development in the newborn have suggested that BDNF and IGF-I may activate the same intracellular pathways. It has been revealed that retinal levels of both proteins are precociously increased in the RGC layer of developing EE rats and blocking either IGF-I or BDNF action in EE animals counteracts the acceleration of retinal maturation (Landi et al., 2007a, Landi et al., 2007b, Landi et al., 2009). Importantly, BDNF turned out to be a downstream target of IGF-I (Landi et al., 2009). BDNF and IGF-I signalling may eventually converge on the activation of intracellular pathways, leading to the phosphorylation of the transcription factor CREB. The wave of CREB/CRE-mediated gene expression in the visual cortex is accelerated in EE mice and chronic administration of rolipram, a drug that increases the phosphorylation of CREB, partially mimic the EE outcome on VA maturation in non-EE animals (Cancedda et al., 2004). Thus, activation of CREB/CRE transcription pathway was identified as a key molecular target of EE in the visual system during development.

2.3. Maternal care, tactile stimulation and visual system development in environmental enrichment conditions

The aforementioned studies demonstrate that the CNS responds readily to environmental manipulations already at very early stages of development. However, given that during the first days of life rodents spend their whole time in the nest, it was crucial to understand how EE is able to elicit its marked effects on visual system development, in a period when a direct interaction between the pup and the external environment is missing. During the early postnatal period, the mother is the most important source of sensory experience; thus, it has been soon realized that differences in maternal behaviour between EE and non-EE conditions could be a fundamental mediator of the earliest effects of EE on visual system development.

A detailed quantitative study of maternal behaviour in different environmental conditions has demonstrated that enriched pups receive higher levels of maternal care when compared with standard-reared pups (Sale et al., 2004). More specifically, EE newborns experience a continuous physical contact due to the presence of adult females in the nest and also receive increased levels of active tactile stimulation in the form of licking and stepping (Sale et al., 2009). Beside the visual cortex, increased amounts of maternal care can also influence the development of hippocampus, affecting molecular factors crucial for plasticity, such as BDNF and NMDA receptors (Liu et al., 2000).

Tactile stimulation has been identified as a crucial component of maternal care during early postnatal period. It has been demonstrated that daily artificial tactile stimulation of newborn rats is effective as well as EE in accelerating the maturation of visual functions, in particular VA. Moreover, this protocol of tactile stimulation increases IGF-I levels in the visual cortex exactly at P18, as reported in EE animals, and blocking IGF-I action prevents the effects of massage on VA development (Guzzetta et al., 2009). Tactile stimulation may also successfully compensate the deleterious effects of inadequate maternal care on developing pups. It has been shown that artificial massage is able to counteract the negative consequences of different types of perinatal stress on neonates growth, as illustrated by rescue of hormonal balance, normalization of hypothalamus-pituitary-adrenal axis activity and BDNF expression in massaged newborns (Schanberg and Field, 1987, Burton et al., 2007, Chatterjee et al., 2007). Altogether, these results provide a remarkable example of cross-modal plasticity by which an increased input in a single modality reverberates as a driving force for the whole brain (Baroncelli et al., 2010a).

Strikingly, brain development of healthy preterm infants is also highly responsive to tactile stimulation. Daily massage therapy of preterm neonates induces an earlier shortening of the interbust intervals in the EEG, a robust index of the developmental stage of the brain, a

significantly reduction in the latency of flash VEPs and an increase in VA that outlasts the end of the treatment, all together indicating an accelerated CNS maturation (Guzzetta et al., 2009). Moreover, massaged infants displayed increased levels of plasma IGF-I, suggesting that this molecule has a critical role in mediating the effects of an enhanced sensory stimulation also in humans. These findings underline the importance of environmental stimulation for early postnatal CNS development and promote the implementation of massage therapy in the protocols of intensive care for preterm babies as a potential strategy to counteract the onset of neurological pathologies associated with a precocious delivery (Guzzetta et al., 2009).

2.4. Rejuvenating the adult brain

The developing brain is characterized by high levels of plasticity that dramatically decline after the end of CPs. This limits the capability of the adult brain to effectively rearrange its circuits in response to external stimuli. The visual system has emerged again as a valid experimental model for a better understanding of neural plasticity limitations in adulthood. It is well known that early abnormal visual experience caused by anisometropia (unequal refractive power in the two eyes), strabismus (abnormal alignment of one or both eyes), congenital cataract or, in animal models MD, results in a functional imbalance between the two eyes leading to amblyopia, a widely diffused pathology in the human population (Holmes and Clarke, 2006). Amblyopia compromises numerous aspects of visual processing resulting in a dramatic loss of VA, deficits in stereopsis and poor contrast sensitivity in an apparently healthy eye (Lewis and Maurer, 2005, Levi, 2006). The classic hallmarks of amblyopia are recapitulated in rodent models and include permanent loss of VA in the affected eye and a pronounced OD shift of visual cortical neurons in favour of the normal eye (Timney, 1983, Maurer et al., 1999, Prusky et al., 2000, Prusky and Douglas, 2003, Kiorpes, 2006). Based on

clinical experience it has been widely accepted that reinstatement of visual functions is possible only if amblyopia is diagnosed and corrective therapy is started early in development (Wu and Hunter, 2006).

Recent studies in rodents have revealed a previously unsuspected potential for recovery of visual processing well after the end of the CP, suggesting a possibility to treat amblyopia even in adulthood (Spolidoro et al., 2009). It has been demonstrated that a brief exposure, two-three weeks, of adult amblyopic rats to EE is sufficient to completely rescue both VA and OD (Sale et al., 2007b). In vivo brain microdyalisis revealed that the improvement of visual functions is accompanied with a threefold reduction in the basal levels of GABA in the visual cortex. Moreover, the decreased cortical inhibition associated with EE treatment enabled induction of WM-LTP in visual cortical slices (Sale et al., 2007b), a form of synaptic plasticity that is normally occluded in adulthood as a result of the maturation of inhibitory circuits (Kirkwood et al., 1995, Huang et al., 1999). Restoration of plasticity is totally prevented by benzodiazepine cortical infusion during the period of enhanced stimulation, indentifying the reduction of inhibitory drive as a key molecular mechanism at the basis of heightened plasticity levels induced by EE (Sale et al., 2007b). This data represent an additional evidence for the crucial role of the excitatory-inhibitory balance of cortical activity in regulating plasticity in the developing and adult brain (Huang et al., 1999, Fagiolini and Hensch, 2000, He et al., 2006, He et al., 2007). The reduction of cortical inhibition in the visual cortex of enriched rats is paralleled by an increased expression of BDNF (Sale et al., 2007b), a neurotrophic factor critical for experience-dependent plasticity (Huang et al., 1999). The potential of EE to reinstate juvenile-like plasticity in the adult visual cortex is not limited to its effects on amblyopia. Remarkable reactivation of OD plasticity in response to MD, accompanied by a marked reduction of the inhibitory tone and an increase in BDNF signalling in the visual cortex, has been also reported in healthy

enriched rats (Baroncelli et al., 2010b). Moreover, it has been demonstrated that EE elicits a twofold enhancement of serotoninergic transmission in the visual cortex. Importantly, infusion of a serotonin synthesis inhibitor not only blocks OD plasticity in response to MD, but also completely counteracts the effects produced by EE on GABAergic inhibition and BDNF synthesis, indicating a critical role for this transmitter in triggering the plastic changes provoked by enhanced stimulation (Baroncelli et al., 2010b).

EE in adulthood also influences the regulation of gene expression. One mechanism of action is the activation of specific transcription factors. Enhancement of BDNF intracellular signalling following EE stimulates phosphorylation and activation of CREB (Finkbeiner et al., 1997, Pizzorusso et al., 2000, Ratto and Pizzorusso, 2006), which plays a pivotal role in various forms of plasticity in the visual cortex (Pham et al., 1999a, Mower et al., 2002, Pham et al., 2004) and other brain structures (Lonze and Ginty, 2002). EE regulates gene expression also at the epigenetic level, resulting in the enhancement of histone acetylation in the hippocampus and neocortex, a process that generally promotes gene transcription (Fischer et al., 2007). A similar relationship between histone acetylation and EE may be present in the adult visual system, as pharmacological treatment with inhibitors of histone deacetylases restores OD plasticity (Putignano et al., 2007). On the basis of these results, a possible involvement of epigenetic mechanisms in the control of CPs for OD plasticity has been suggested (Medini and Pizzorusso, 2008, Fagiolini et al., 2009).

Beside modulation of the excitatory/inhibitory balance and gene expression, EE affects plasticity in the adult brain also through alteration of synaptic structures. It has been reported that amblyopic rats exposed to EE have reduced density of chondroitin-sulfate proteoglycan (CSPG) perineuronal nets (PNNs) in the visual cortex (Sale et al., 2007b). This is consistent with pharmacological studies that have reported reactivation of OD plasticity in monocularly deprived adult rats after removal of crucial components of PNNs from the

mature extracellular matrix by means of the enzyme chondroitinase ABC, suggesting that CSPGs exert a powerful repressive control on adult plasticity (Pizzorusso et al., 2002, Pizzorusso et al., 2006). Moreover, this treatment counteracted the consequences of early visual deprivation on both VA and binocularity. These functional effects are accompanied by a normalization of dendritic-spine density indicating that removal of CSPGs may favour remodelling of synaptic contacts onto visual-cortex pyramidal neurons (Pizzorusso et al., 2006). A very recent study reported that EE increases density and turnover of dendritic spines in the somatosensory cortex (Jung and Herms, 2012). In vivo microscopy shown that EE induces both transient and long-lasting gain of novel spines. Based on these results, authors have proposed that the cognitive benefits seen in environmental-enriched animals might be a consequence of both, a higher connectivity of the neuronal network due to more established synapses and an enhanced flexibility due to more transient spines (Jung and Herms, 2012).

2.5. Beneficial effects of environmental enrichment on functional outcome in models of brain disorders

The aforementioned studies focused on the visual system have provided important insights into the nature of the neural benefits of EE and offered a new hope for amblyopia treatment in adulthood. However, the positive effects of this paradigm exceed sensory processing and occur also in the areas of motor performance, social-emotional behaviour and learning and memory. Deficits in these functional domains may be diagnosed in neurodevelopmental and neurodegenerative disorders and are often associated with an altered potential of neural circuits to undergo synaptic modifications (Battaglia et al., 2007, Bagetta et al., 2010, Orth et al., 2010, West and Greenberg, 2011, Zoghbi and Bear, 2012). Given that EE normalizes brain plasticity through multiple mechanisms, such as correction of excitatory/inhibitory

disbalance, enhancement of neurotrophin signalling and epigenetic modifications, it is not surprising that this non-pharmacological strategy may be successfully employed to prevent, delay and/or ameliorate the symptoms of many neurodevelopmental and neurodegenerative disorders, despite their quite various aetiologies (Nithianantharajah and Hannan, 2006, Baroncelli et al., 2010a).

Significant deterioration of neural functions may occur in apparently healthy individuals during the aging process. It has been proposed that cognitive and physical stimulation through EE may be neuroprotective in the elderly by preventing the age–related cognitive decline and reducing the risk of neurodegenerative disease (La Rue, 2010). It has been shown that long-term continuous EE improves performance of healthy aged mice in MWM and radial arm maze tasks (Bennett et al., 2006). Similarly, old rats housed in enriched conditions for their whole life perform better on a delayed sample to match task (Soffie et al., 1999). However, recent evidence suggests that it is necessary to expose animals to EE before the median of their lifespan in order to gain the protective effects against the age-related cognitive decay (Freret et al., 2012).

Beside this important preventive potential of EE, there are numerous studies demonstrating the remarkable capacity of this paradigm not only to delay onset and slow progression of degenerative brain disorders, but also to rescue their cognitive phenotype. The first demonstration that this is the case has come from studies on Huntington's disease (HD) (van Dellen et al., 2000), a genetic brain syndrome manifested by cognitive deficits leading to dementia, psychiatric disorders and motor symptoms due to a progressive process of neurodegeneration in the cerebral cortex and striatum (Laviola et al., 2008). Mouse models of HD display cognitive impairment together with abnormal synaptic plasticity and decreased hippocampal neurogenesis (Murphy et al., 2000, Lazic et al., 2004, Mazarakis et al., 2005). EE has been shown to delay the onset and progression of motor symptoms of transgenic

models of HD (van Dellen et al., 2000, Hockly et al., 2002, Spires et al., 2004) as well as their cognitive deficits, accompanied by rescue of synaptic plasticity and neurogenesis (Grote et al., 2005, Nithianantharajah et al., 2008).

Another important example of the therapeutic effectiveness of EE comes from preclinical research focused on transgenic models of AD. It has been demonstrated that EE enhances cognitive performance in a variety of AD transgenic mice (Berardi et al., 2000, Arendash et al., 2004, Jankowsky et al., 2005, Wolf et al., 2006, Costa et al., 2007, Cracchiolo et al., 2007). In addition to cognitive improvement, some studies have reported positive effects of EE on adult hippocampal neurogenesis (Wolf et al., 2006, Herring et al., 2009, Mirochnic et al., 2009), angiogenesis and Aβ clearence (Herring et al., 2009). Finally, it has been shown that EE also upregulates the expression of trophic factors, such as NT-3, BDNF, IGF-I and VEGF, in the hippocampus (Wolf et al., 2006) and attenuates pro-oxidative processes (Herring et al., 2009) in AD mouse models. Thus, EE profoundly alters cognitive, cellular and molecular aspects of pathogenetic processes associated with AD.

An important line of research deals with the potential therapeutic effects of EE on experimental models of DID. There is evidence demonstrating that EE may improve functional outcome not only in animal models of diseases resulting from alteration of environmental factors during early phases of development, such as Fetal Alcohol Spectrum Disorders (FASDs), but also in the case of genetically programmed syndromes, like RS and DS (Reynolds et al., 2010).

FASDs can occur in children whose mothers consume alcohol during pregnancy and behavioural manifestations of disease generally include deficits in cognitive performance, executive functioning, and sensory-motor functions (Jirikowic et al., 2008, Kodituwakku, 2009). Rodent offspring whose mothers are exposed to ethanol display similar deficits accompanied by hippocampal damage and LTP failure (Puglia and Valenzuela, 2009, 2010).

It has been shown that post-weaning exposure to EE improves spatial learning and normalizes atypical performance on a structured learning task in affected mice (Hannigan et al., 1993, Wainwright et al., 1993).

RS is a genetic disorder characterized by a pattern of apparently typical development for the first few years of life, followed by a progressive decline in motor and cognitive functions (Hagberg et al., 1983). Several mouse models of RS have been developed, recapitulating motor, cognitive, and behavioural features typical for the human syndrome (Stearns et al., 2007) and demonstrating a LTP impairment in the hippocampus and the motor and sensory cortex (Asaka et al., 2006, Moretti et al., 2006). Also in this case, EE has proved beneficial, resulting in a reduction of anxiety-related behaviours, improvement of motor coordination and learning, locomotor activity and memory deficits and normalization of LTP expression in different models of RS (Kondo et al., 2008, Kerr et al., 2010, Lonetti et al., 2010).

Finally, the Ts65Dn mice, the animal model of DS addressed in detail in the first chapter, have been exposed to EE. EE resulted in an improvement of spatial cognitive abilities in MWM in a sex-specific manner, with EE-female Ts65Dn mice performing better than untreated experimental group and EE-male Ts65Dn mice being incapable to learn the task (Martinez-Cue et al., 2002). On the contrary, there was no sex difference in rescue of hippocampal neurogenesis deficit after enrichment in adult Ts65Dn mice (Chakrabarti et al., 2011). However, little is known about the effects of EE on synaptic plasticity and GABAergic signaling in the brain, indicating that further studies are necessary to better understand how increased environmental stimulation may impact disrupted neural development in DS.

2.6. Enviromimetics – a novel class of therapeutics

The impressive results coming from animal studies that illustrate the activation of an array of molecular pathways in response to EE encourage the application of these findings to the human population (Greenwood, 2007, Greenwood and Parasuraman, 2010). Important questions are how the environmental stimulation of animals in a laboratory setting relates to the complexity and diversity of human lives and how this therapeutic strategy should be translated to clinical trials. Most humans experience high levels of novelty throughout life, and there is a notable difference in the quantity and quality of mental stimulation, physical activity and social engagement between persons. This implies that it may be not always feasible to identify the level of richness experienced by individuals and to optimize their living conditions and life style in order to obtain benefits for brain function. Moreover, some pathological conditions described previously are characterized by low motivation, already compromised neural functions and/or other health problems that may impede active involvement of these patients in stimulating programs (McOmish and Hannan, 2007) . Thus, it has been proposed that pharmacological targeting of the molecules that mediate the benefits of EE, aiming at reproduction or strengthening of its positive effects, may be a good alternative strategy to promote brain health and plasticity (McOmish and Hannan, 2007, Pang and Hannan, 2013).

The animal models described above are ideally suited for screening and characterization of enviromimetics, a novel class of therapeutics capable of mimicking the neural outcomes of EE. Given that EE results in an enhancement of neural plasticity, a candidate drug would be expected to induce cellular changes, such as facilitation of synaptic plasticity, synaptogenesis or adult neurogenesis, in a manner analogous to environmental stimulation (McOmish and Hannan, 2007). One possibility, in line with a classical aetiology-based therapeutic approach, is to search for the disease specific molecular disruptions and to

target them directly in order to correct the deficit and obtain a clinical improvement. An example of this approach may be a drug that mimics or activates neprilysin, an enzyme thought to be a limiting step in Aβ degradation (Iwata et al., 2001); neprilysin has been shown to be up-regulated in enriched AD mice, in association with reduction of amyloid load and plaques (Lazarov et al., 2005). Another appealing possibility is to focus on a pharmacological normalization of pathophysiological mechanisms that seem to overlap in a wide range of nervous system disorders. An example in this case could be targeting of over-inhibition in various forms of DIDs, as there is mounting evidence that these disorders are associated with excessive GABAergic transmission (Fernandez and Garner, 2007).

Plausible pharmaceuticals that would meet the criteria for enviromimetics do not refer exclusively to novel compounds, they may also include drugs already in the clinical use. Indeed, it has been demonstrated that the molecular pathways involved in the regulation of neuronal plasticity in models of learning and memory are also activated by drugs used for the treatment of depression and bipolar disorder (Duman et al., 2000).

2.7. Antidepressant fluoxetine as a putative enviromimetic

The selective serotonine reuptake inhibitor fluoxetine is a commonly prescribed drug for the treatment of mood and anxiety disorders. Fluoxetine enhances serotonergic transmission by blocking the serotonin transporter (5-HTT) located on plasma membrane of serotonergic neurons, leading to increased 5-HT extracellular levels and sustained activation of pre- and postsynaptic 5-HT receptors (Tatsumi et al., 1997). 5-HT is produced by serotonergic neurons located in the raphe nuclei in the brain stem, which send their diffuse projections throughout the whole brain affecting multiple processes such as learning and memory (Buhot, 1997, Meneses, 1999, Riedel et al., 1999, Buhot et al., 2000, Schmitt et al., 2000),

emotion (Asberg et al., 1976, Meneses and Liy-Salmeron, 2012), feeding (Tecott, 2007), sleep (Trivedi et al., 1999, Ivarsson et al., 2005, Silber and Schmitt, 2010), sexual and other social behaviours (Ferrari et al., 2005, Chan et al., 2008, Carrillo et al., 2009) and sensory perception (Wei et al., 2010).

Animal studies have shown that fluoxetine treatment enhances neuronal plasticity processes in adult brain, such as BDNF signalling and hippocampal neurogenesis (Duman and Monteggia, 2006, Martinowich et al., 2007). It has been demonstrated that chronic, but not acute, antidepressant administration enhances CREB expression and phosphorylation in limbic brain structures, including hippocampus and cerebral cortex (Nibuya et al., 1996, Thome et al., 2000) and leads to up-regulation of BDNF (Nibuya et al., 1995, Russo-Neustadt et al., 1999). It is believed that an increased CREB and BDNF expression contributes to the effects of fluoxetine on neural plasticity, including synaptic remodeling and increased neurogenesis in WT animals (Duman, 2002). Moreover, it has been shown that treatment with fluoxetine is able to rescue the neurogenesis impairment in the mouse models of HD, leading to improvement of cognitive deficit displayed by these animals (Grote et al., 2005).

The potential of fluoxetine to induce plastic responses in the adult brain has been also evaluated in the visual system. Studies performed on adult amblyopic rats have shown that chronic fluoxetine administration induces a plastic response in the mature visual cortex that closely resemble that observed at the peak of the critical period (Maya Vetencourt et al., 2008, Castren et al., 2012). Furthermore, fluoxetine treatment during adulthood, in combination with the encouragement of the use of the weak eye, fully restored VA of the amblyopic eye (Maya Vetencourt et al., 2008, Castren et al., 2012). The enhancement of adult plasticity induced by fluoxetine is accompanied by a reduction of GABA-mediated inhibition, together with increased BDNF signalling and activation of $5HT1_A$ serotonin receptors (Maya Vetencourt et al., 2008, Maya Vetencourt et al., 2011). An independent study based on two-

photon imaging in vivo reported that pharmacological reduction of intracortical inhibition by fluoxetine was able to promote parameters of structural plasticity in the adult brain. In this study fluoxetine treatment, in a combination with a brief MD, led to increased dendritic branch dynamics of superficial L2/3 interneurons in the adult visual cortex, facilitating in this manner circuit specific modification driven by experience (Chen et al., 2011).

The pharmacological enhancement of neural plasticity elicited by fluoxetine is not limited to the hippocampus and the visual cortex. A recent study performed on mice rendered anxious by fear conditioning and afterwards treated with a protocol of fear extinction and fluoxetine explains why an optimal therapeutic outcome is achieved only when the pharmacological and cognitive behavioural approach are combined. Specifically, it has been reported that chronic fluoxetine treatment reactivates a juvenile-like plastic state in the basolateral amygdala of anxious mice, enabling a permissive environment for re-adaptation of affected neural circuits driven by instructive stimuli deriving from psychotherapy (Karpova et al., 2011). In a similar manner, pharmacological reactivation of juvenile-like plasticity induced by SSRIs may be responsible for facilitation of the rehabilitation and functional recovery after an ischemic brain injury in rodents (Acler et al., 2009, Jorge et al., 2010, Chollet et al., 2011). Finally, very recent data suggest that chronic fluoxetine administration also promotes the restoration of motor function after a spinal cord lesion (Scali et al., 2013).

Based on the capability to enhance neural plasticity, fluoxetine has started to emerge as a potential pharmacological treatment also for DS. It has been shown that four weeks of adult-onset fluoxetine treatment are sufficient to normalize deficient adult hippocampal neurogenesis in Ts65Dn mice, although it was not tested whether this increase of neurogenesis was accompanied by a recovery of hippocampus-dependent cognitive deficits (Clark et al., 2006). A more recent study has reported that fluoxetine administration for 13

days to neonate Ts65Dn mice fully rescues postnatal neurogenesis in DG and subventricular zone (SVZ), restores expression of BDNF and 5-HT1_A receptors and improves contextual fear conditioning impairment in juvenile trisomic mice (Bianchi et al., 2010b). Beside the positive effects on neurogenesis, early postnatal fluoxetine treatment also favours dendritic arborisation in young Ts65Dn mice (Guidi et al., 2013). Given that DS is characterized by developmental deficits in dendritic maturation, it is expected that restoration of this process by early-onset fluoxetine treatment may lead to optimization of synaptic connectivity and neural function (Bartesaghi et al., 2011).

All together these data indicate that an enhancement of serotonergic transmission elicited by fluoxetine may favour plasticity of multiple neural networks involved in cognition, sensory perception, emotions and motor performance. Thus, fluoxetine treatment emerges as a promising therapeutic opportunity for a wide spectrum of CNS pathologies. The ongoing clinical trials will reveal whether encouraging findings from animal studies can be successfully translated to the human condition.

CHAPTER 3

AIM OF THE THESIS AND EXPERIMENTAL DESIGN

DS is a genetic neurodevelopmental disorder characterized by a set of clinical features with CNS abnormalities and ID as the most limiting aspects of the pathology. While improved medical and social care in the last two decades resulted in a significant improvement of physical health and life quality in DS patients, there is still need for interventions aiming at the prevention and/or amelioration of neurocognitive dysfunction associated with DS.

In the first part of my Thesis, I addressed the possibility to ameliorate DS-related neurocognitive phenotypes in adulthood. In Ts65Dn mice abnormally high levels of inhibitory synaptic transmission have been associated with deficient hippocampal synaptic plasticity (Kleschevnikov, 2004), an important cellular mechanism for learning and memory formation. A ground-breaking study demonstrated that chronic treatments with $GABA_A$ receptor antagonists reversed synaptic plasticity impairments and cognitive deficits displayed by adult Ts65Dn mice (Fernandez et al., 2007), indicating that lowering of inhibitory tone may be a promising therapeutic strategy. However, since persons with DS are more susceptible to convulsions (Menendez, 2005), concerns can be raised on the clinical application of $GABA_A$ receptor antagonists, which are characterized by a narrow therapeutic window and pro-convulsive action. Thus, I investigated the possibility to lower GABAergic transmission and promote plasticity processes in adult Ts65Dn mice with treatments more eligible for human application, such as EE, a behavioral paradigm of increased sensory-motor stimulation, and pharmacotherapy with fluoxetine, a commonly prescribed antidepressant for the treatment of mood and anxiety disorders. Using the visual system as a model for the study of experience-dependent plasticity, it has been demonstrated that these two therapeutic

strategies can be efficiently employed to reduce cortical inhibition (Sale et al., 2007b, Maya Vetencourt et al., 2008, Baroncelli et al., 2010b), enhancing neuronal plasticity in the adult brain.

First, I studied the effects of EE on adult Ts65Dn mice of both sexes. It has been already reported that exposing Ts65Dn mice to EE in large groups (8-10 mice per cage) improves their spatial learning abilities in a sex-specific manner, with rescue of performance in females and deterioration in males (Martinez-Cue et al., 2002). As there is a tendency toward social subordination in Ts65Dn male mice (Martinez-Cue et al., 2006), disruption of spatial learning and memory in trisomic males has been attributed to elevated level of perceived stress due to excessive social stimulation as a component of EE (Martinez-Cue et al., 2005). Thus, to optimize the social configuration of housing environment for male mice, I used a protocol of EE in which only two-three Ts65Dn males from the same litter were reared together; trisomic females were housed in group of 6-8 mice per cage. After 6 weeks of enrichment, I evaluated spatial learning and memory, synaptic plasticity, visual functions and GABAergic transmission in adult Ts65Dn mice.

For assessment of the behavioral response to EE, I used the MWM test, a hippocampus-related task that measures spatial learning and memory, in which Ts65Dn mice have impaired performance (Escorihuela et al., 1995, Reeves et al., 1995, Demas et al., 1996, Holtzman et al., 1996). Since poor performance in MWM displayed by Ts65Dn mice has been associated with deficient synaptic plasticity (Siarey et al., 1997, Siarey et al., 1999, Kleschevnikov, 2004, Fernandez et al., 2007), I evaluated, for the first time in this model, the effects of EE on hippocampal LTP. Specifically, I studied LTP in response to high frequency stimulation in DG, a hippocampal region severely affected by excessive levels of inhibition in Ts65Dn mice (Belichenko et al., 2004, Fernandez et al., 2007, Kleschevnikov et al., 2012).

Since it has been recently reported that Ts65Dn mice have visual deficits similar to

those seen in DS patients (Scott-McKean et al., 2010), I also investigated the therapeutic potential of EE to rescue these visual impairments. I used in vivo electrophysiological recordings of VEPs from V1 to assess the effects of EE on the following visual functions: VA, binocularity (by calculating the contralateral-to-ipsilateral VEP ratio) and VEP latencies.

Finally, I used a biochemical model of neurotransmitter release from synaptosomes, preparations of synaptic terminals that remain intact after homogenization of nerve tissue, to analyze the properties of inhibitory transmission in Ts65Dn mice after exposure to EE. I measured the depolarization-evoked release of GABA from synaptosomes in the hippocampus and in V1. It is important to note that enhanced levels of inhibition in Ts65Dn mice have been mainly documented in the hippocampus; thus I provided the first evidence regarding GABAergic transmission in the visual cortex of trisomic mice.

Since a recently published paper reported severe side effects of a high-dose fluoxetine administration (80 mg/kg/day) in Ts65Dn mice (Heinen et al., 2012), I performed a pilot experiment aimed at assessing the occurrence of fluoxetine side effects in animals treated with 3 different dosage schemes, that is 40, 20 and 10 mg/kg/day. This study demonstrated that 10 mg/kg/day is a safe dose in Ts65Dn mice, in line with the dosage used in previous studies that reported beneficial effects of fluoxetine treatment on neurogenesis deficits (Clark et al., 2006, Bianchi et al., 2010b**.** Then, I used this dosage to assess the effects of chronic fluoxetine administration (8 weeks) on behavior, synaptic plasticity and inhibitory transmission in Ts65Dn and WT mice.

Initially, I investigated the impact of adult-onset fluoxetine treatment on spatial learning and memory in MWM. Then, I also used the novelty place recognition (NPR) test, a hippocampal-based recognition memory test, in which the performance of Ts65Dn mice is also impaired (Kleschevnikov et al., 2012).

Afterwards, I examined whether fluoxetine treatment was able to restore deficient

hippocampal LTP. Specifically, I studied LTP at the CA3-CA1 hippocampal synapses, another hippocampal region with aberrant synaptic plasticity in Ts65Dn mice (Siarey et al., 1997, Kleschevnikov, 2004, Costa and Grybko, 2005), in response to theta burst stimulation.

Finally, I measured depolarization-evoked GABA release from hippocampal synaptosomes, in order to evaluate the effects of a chronic fluoxetine administration on inhibitory transmission strength in Ts65Dn mice.

In the second part of my Thesis, I studied brain development in Ts65Dn mice. I focused on the visual system development, as it is currently not known if the deficits of visual functions displayed by adult Ts65Dn mice originate from disrupted developmental mechanisms or rather represent a sign of early-onset neurodegeneration. Since it has been shown that rearing WT animals from birth in EE accelerates visual system development (Cancedda et al., 2004, Sale et al., 2004), I investigated whether a complex sensory-motor stimulation provided by preweaning exposure to EE affects the maturation of visual system in Ts65Dn offspring.

As an integral part of the developmental study, I analyzed postpartum maternal care in Ts65Dn mice. Since it has been demonstrated in WT animals that EE promotes maternal care (Sale et al., 2004), I performed a set of behavioral observations in Ts65Dn females during the first eleven postpartum days, in both enriched and standard conditions. The EE procedure was started since pregnancy, thus avoiding possible stress that could have been experienced by Ts65Dn mothers if housing conditions were changed immediately before delivery. I weaned and sexed pups at P28 and reared them in EE until the end of experimentation. Then I monitored the timing of eye-opening and the maturation of VA at three different time points, that is P28, P35 and P60.

CHAPTER 4

MATERIALS AND METHODS

4.1. Animals

The transgenic line Ts65Dn was used in this study. Ts65Dn mice carry segmental trisomy in a form of Robertsonian translocation of Mmu16 to Mmu17 (17^{16}). Segmental trisomy provokes male sterility, therefore female carriers of the 17^{16} chromosome (B6EiC3H – a/ATs65Dn) are used to generate trisomic offspring by intercross with (C57BL/6JEi x C3H/HeJ) F1 hybrid male mice. Ts65Dn mice are thus maintained on the B6/C3H background (Davisson et al., 1993). The marker chromosome 17^{16} is inherited at expected Mendelian frequency, but, due to the perinatal loss, only 20-40% of the offspring of Ts65Dn mothers are trisomic at weaning. Moreover, the proportion of trisomic mice per litter decreases with the age of the Ts65Dn mother (Roper, 2005). Diploid (WT) mice of the same litter were used as a control experimental group.

The low reproductive outcome of Ts65Dn mice is further compromised by the recessive retinal degeneration (*rd*) mutation that segregates in C3H/HeJ background and leads to blindness by ~1 month of age in about 25% of newborns from Ts65Dn mothers. This limits the use of *rd* positive homozygotes in behavioral experiments (Reeves et al., 1995, Das and Reeves, 2011). This problem has been recently overwhelmed through the breeding of Ts65Dn extra chromosome across many generations into a closely related genetic background free of *rd* mutation. Analysis of the effects of this new genetic background on mice performance revealed no significant differences in behavioral phenotype respect to the original Ts65Dn model (Costa et al., 2010).

In the present study the effects of EE in adulthood were investigated on Ts65Dn mice

maintained on background with segregating *rd* mutation, while the impact of EE on CNS development and the response of adult mice to pharmacotherapy with fluoxetine were evaluated in Ts65Dn strain free of *rd* mutation. Ts65Dn mating couples were originally obtained from The Jackson Laboratory (Bar Harbor, Maine, USA) and colonies of both type of Ts65Dn strain, with- and free of *rd* mutation, were established. The progeny of colonies was genotyped by a quantitative PCR (qPCR) protocol developed by The Jackson Laboratory (http://www.jax.org/cyto/quanpcr.html). For the pharmacological part of the study adult Ts65Dn and WT mice were acquired directly from The Jackson Laboratory. All mice arriving from The Jackson Laboratory were acclimated to the controlled housing conditions for at least one week before inclusion in the study.

4.2. DNA extraction

Genomic DNA was extracted according to the protocol developed by The Jackson Laboratory. Tail tips (3 mm) were collected and subsequently digested with 300 ul of 50 mM NaOH for 1 h at 98°C, with a brief resuspension after 30min. After incubation, DNA was dissolved in 30 ul of 1 M Tris (pH8), resuspended and centrifuged for 6 min at 14000 rpm. The supernatant was used for qPCR genotyping and PCR screening for retinal degeneration.

4.3. Genotyping

All mice obtained from internal colonies were genotyped using real-time qPCR with TaqMan probes (Applied Biosystems) specific for the following genes: *App*, apolipoprotein B (*ApoB*) and myxovirus resistance 1 (*Mx1*). Genes for *App* and *Mx1* were used as the target (marker) genes, whereas the *ApoB* gene was used as internal control. The rationale is that *App* and *Mx1* are located in the chromosome segment triplicated in Ts65Dn, whereas *ApoB* is mapped on Mmu12. Thus, Ts65Dn samples have three copies of *App* and *Mx1*, whereas WT samples

have only two copies. Both Ts65Dn and WT animals have two copies of *ApoB*. The extra copy of *App* or *Mx1* in trisomic samples is detected by qPCR. The reaction was performed on StepOne™ instrument at 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15s and 60°C for 1 min. After amplification, the average change in cycle threshold (ΔΔCT) of the target gene *App/Mx1* from that of *ApoB* in sample animals with respect to controls was calculated:

$$\Delta\Delta CT = (CT\mathit{App/Mx1} - CT\mathit{ApoB})_{trisomic} - (CT\mathit{App/Mx1} - CT\mathit{ApoB})_{euploid})$$

The ΔΔCT value for *App* or *Mx1* in trisomic samples is < -0.3. The sequences of primers and probes were as follows:

ApoB	forward primer	5'-CACGTGGGCTCCAGCATT-3'
	reverse primer	5'-TCACCAGTCATTTCTGCCTTTG-3'
	probe	5'-CCAATGGTCGGGCACT-3'
App	forward primer	5'-TGCTGAAGATGTGGGTTCGA-3';
	reverse primer	5'-GACAATCACGGTTGCTATGACAA-3';
	probe	5'-CCATCATCGGACTCAT-3';
Mx1	forward primer	5'-TCTCCGATTAACCAGGCTAGCTAT-3'
	reverse primer	5'-GACATAAGGTTAGCAGCTAAAGGATCA-3'
	probe	5'-TGGCTTTCCTGGTCGC-3'

4.4. Screening for retinal degeneration

All genotyped mice of Ts65Dn strain with *rd* mutation were screened for the presence of retinal degeneration using a qualitative PCR protocol. Retinal degeneration in the Ts65Dn mouse is inherited as an autosomal recessive trait and is caused by a mutation in the *rd* gene mapped on Mmu5 encoding the β subunit of cGMP phosphodiesterase (β-PDE) expressed in

rod photoreceptors (Bowes et al., 1993). PCR screening is based on a difference between length of normal and aberrant *rd* genes, as the wt gene is 260 pb long, while the mutated gene is 560 pb long. The gene of interest was amplified from genomic DNA by Taq polymerase and gene specific forward and reverse primers (Sigma). After amplification performed in a thermal cycler at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 65°C for 30 s and 72°C for 1min 30 s, PCR products were identified by their size using agarose gel electrophoresis. The $rd^{-/-}$ samples displayed only low weight band relating to wt *rd* gene, the $rd^{+/+}$ samples displayed only high weight band relating to mutant *rd* gene, while $rd^{+/-}$ samples displayed both low weight and high weight band. Homozygotes $rd^{+/+}$ were excluded from the experiments.

4.5. Rearing environments

EE, a complex rearing environment composed of motor, sensory, cognitive and social stimulation, consisted of a large Plexiglas cage (44x62x28 cm) with a wire mesh lid containing standard laboratory bedding, several food hoppers, running wheels, and various objects of different shape (e.g. tunnels, shelters, stairs) and material (e.g. wood, plastic, glass). Once per week the position of food hoppers were changed and the objects were completely replaced with others. Standard conditions (SC) consisted of a standard Plexiglas laboratory cage (26x42x18 cm) with a wire mesh lid containing only standard laboratory bedding. In both experimental conditions mice had ad libitum access to a standard laboratory chow diet and tap water throughout the study. All animals were maintained at 12h/12h light-dark cycle with lights on at 6 a.m..

Rearing conditions for the study of the effects of EE on Ts65Dn mice in adulthood

WT mice (> P60) were maintained in SC cages housing a maximum of 3 females or brother males. Age-matched Ts65Dn mice were reared for 6 weeks in either EE or in SC cages.

Every EE cage housed at least six females; since it is known that Ts65Dn males have a tendency toward social subordination and that an excess of social stimulation can disturb their behavioral and learning skills (Martinez-Cue et al., 2005), I used a protocol of EE in which only 2-3 Ts65Dn males from the same litter were reared together.

Rearing conditions for the study of the effects of EE on the development of Ts65Dn mice

Female Ts65Dn mice were assigned to either enriched (3 adult females or 2 adult females and 1 helper adult female or 1 adult female and 2 adult females) or to standard cages (1 female). One male was added to every mating cage. For all breeding, males were removed from the mating cage at least 7 days before delivery and mothers and offspring left undisturbed until P11, at which time an evaluation of eye opening was started. Pups were weaned and sexed at P28. Rearing conditions (SC and EE) after weaning corresponded to those at the birth.

Rearing conditions for the pharmacological study

Adult WT and Ts65Dn mice were maintained in SC cages. Males were singly housed, while females were reared in small groups composed of a maximum of 4 animals per cage.

4.6. Pharmacological treatment

Age-matched Ts65Dn and WT mice of both genders were either systemically treated with fluoxetine (Fluoxetine-hydrochloride, Galeno, Prato-Italy) dissolved in tap water for 8 weeks, or subjected to a normal water drinking regimen. Solutions were prepared fresh twice a week. As a preliminary study aimed at optimizing fluoxetine dose, I evaluated three decreasing drug concentrations within the range of fluoxetine dosing schemes generally used in mice and rats (Rantamaki et al., 2007, Maya Vetencourt et al., 2008, Karpova et al., 2011), that is 40, 20 and 10 mg/kg/day (fluoxetine was dissolved in tap water at the following, respective, concentrations: of 0.10, 0.05 and 0.025 mg/ml), and I monitored, for the entire length of the treatment, animal survival and seizure susceptibility through behavioral observations (two

weekly observation sessions of 60 min each for the 8 weeks of treatment). I analyzed the following 8 different experimental groups (numbers after fluox indicate the corresponding drug doses for each group): Ts65Dn-fluox40; Ts65Dn-fluox20; Ts65Dn-fluox10; Ts65Dn-water; WT-fluox40; WT-fluox20; WT-fluox10 and WT-water.

To control drug assumption, I performed a pilot experiment in which I quantified the consumption of drinking water and fluoxetine solution (0.025 mg/ml) in a cohort of adult Ts65Dn animals, for five consecutive days. I found that the amounts of consumed water and fluoxetine solution were not statistically different among different individuals (Ts65Dn-water, $n = 4$, I day: 10.8 ± 0.9 ml, II day: 10.3 ± 0.9 ml, III day 11.8 ± 0.3 ml, IV: 9.5 ± 1.2 ml, V: 11.3 ± 0.6 ml; Ts65Dn-fluox, $n = 4$, I day: 10.8 ± 1.4 ml, II day: 9.0 ± 2.5 ml, III day 10.0 ± 1.6 ml, IV: 9.3 ± 1.1 ml, V: 10.3 ± 0.6 ml) (Two-way RM ANOVA, $p = 0.537$). Moreover, also the individual weights of mice did not significantly differ among each others (Ts65Dn-water: 22.50 ± 1.02; Ts65Dn-fluoxetine: 23.01 ± 0.39; t-test, $p = 0.653$). Thus, delivering fluoxetine in the drinking water did not result in substantial differences in the reliable average doses of the compound taken in by different individuals.

4.7. Maternal care observations

Postpartum maternal and helper female behaviors were observed inside mating EE and SC cages, applying an observational protocol modified from Cancedda et al. (2004) and Sale et al. (2004). Behavioral observations started at P1 and were conducted every second day until P11. Maternal/helper female behaviors were scored during 4 daily observation sessions of 45 min. The observation sessions occurred at 10 a.m., 1 p.m., 4 p.m. and 7 p.m.; the last sessions was during dark phase of the daily cycle and was performed under dim red light. During each session the behavior of each female was scored every 3 min, recording whether the target behavior was present or not.

Scored behaviors (not mutually exclusive):

Mother/helper female in the nest (passive stimulation) – the mother and/or the helper is touching of at least one pup with a part of her body other then the tail.

Stepping – the adult female steps gently over the pups' body and stimulates them.

Licking – general licking of any part of a pup body.

Nest building – activities aimed at nest construction and reconsolidation.

Arched back nursing – the mother is immobile and in a characteristic high upright dorsal arched posture with all or most pups attached to the nipples; this position provides the most effective nourishment for the pups.

Data are reported as the percentage of observations in which pups received the target behavior (Liu et al., 2000).

4.8. Eye-opening observation

Starting from P11, pups were daily inspected for eye-opening at 4 p.m. Eye-opening was defined as the initial break in the membrane sealing the lids of both eyes.

4.9. Morris water maze

Mice were trained for 2 trials per day and for a total of 10 days in a circular water tank, made from grey polypropylene (diameter, 100 cm; height, 40 cm), filled to a depth of 25 cm with water (23°C) rendered opaque by the addition of a small amount of atoxic white paint. Four positions around the edge of the tank were arbitrarily designated North (N), South (S), East (E), and West (W), which provided four alternative start positions and also defined the division of the tank into 4 quadrants: NE, SE, SW, and NW. To avoid possible confounding effects due to reduced visual acuity in Ts65Dn mice, the tank was surrounded by a set of

extra-maze cues in the visual discrimination range detectable by all experimental groups. A circular clear Perspex escape platform (diameter, 10 cm; height, 2 cm) was submerged 0.5 cm below the water surface and placed at the midpoint of one of the four quadrants. The hidden platform remained in the same quadrant during training, while the start positions (N, S, E, or W) were randomized across trials. Mice were allowed to locate the escape platform up to 60 s, and their escape latency was automatically recorded by the Noldus Ethovision system. In the last trial of the last training day, mice received a single probe trial, during which the escape platform was removed from the tank and the swimming paths were recorded over 60 s while mice searched for the missing platform. The swimming paths were recorded and analyzed with the Noldus Ethovision system.

4.10. Novel place recognition test

Performance in the NPR test was tested using a modified protocol from Kleschevnikov et al. (2012). Mice were habituated to a Plexiglas rectangular chamber (30 x 22 x 20 cm) for 10 min on 2 consecutive days under dim ambient light conditions. The activity of mice was recorded with a video camera. Test had 2 phases: acquisition and testing. For acquisition, 2 identical objects were placed in corners of the chamber, 2 – 3 cm from the walls. A mouse was placed at the midpoint between the objects. After allowing 10 min to explore the objects, the mouse was returned to the colony. For the test phase, one of the objects was moved in a new location at the center of the chamber, while the other object remained at its former spatial location. Testing was performed 10 min after the acquisition, the mouse was again placed in the chamber and allowed to explore the objects for 3 min. The amount of time spent exploring each object (nose sniffing and head orientation within < 1 cm) was recorded.

Data are reported as the discrimination index. Discrimination index was computed as:

$$\text{Discrimination index (\%)} = T_{new} * 100/(T_{new} + T_{old})$$

T new is the time spent exploring the object with a new position, and T old is the time spent exploring the object with the unchanged position.

4.11. In vitro electrophysiology: LTP recordings

The brain was rapidly removed and immersed in ice-cold cutting artificial cerebrospinal fluid (ACSF) solution containing (in mM): 130 NaCl, 3.1 KCl, 1.0 K_2HPO_4, 4.0 $NaHCO_3$, 5.0 dextrose, 2.0 $MgCl_2$, 1.0 $CaCl_2$, 10 HEPES, 1.0 ascorbic acid, 0.5 myo-Inositol, 2.0 pyruvic acid, 0.01 glycine and 1.0 kynurenate, pH 7.35. Both hippocampi were extracted and cut with a tissue chopper into 400µm thick slices. Hippocampal slices were allowed to recover in oxygenated cutting ACSF free of kynurenate at room temperature for at least 2 h before recordings.

A slice was transferred into the recording chamber and perfused at a constant rate of 4 ml/min with 30°C oxygenated recording ACSF solution. The recording ACSF solution was composed as the cutting ACSF solution with the following differences (in mM): 1.0 $MgCl_2$, 2.0 $CaCl_2$, and no kynurenate. Electrical stimulation (100 µsec duration) was delivered with a bipolar concentric stimulating electrode (FHC, St. Bowdoinham, ME). The stimulating electrode was inserted into the middle molecular layer of the DG (stimulation of the perforant path projections) or into CA3 (stimulation of Schaffer collateral fibers); dendritic field excitatory post-synaptic potentials (fEPSPs) were recorded by a micropipette (1–3MΩ) filled with the recording ACSF solution and positioned into the granule cell layer of the DG upper blade or into CA1 stratum radiatum, respectively. An input/output curve was generated by delivering stimuli of increasingly higher intensities and recording the corresponding fEPSP amplitudes until a plateau was reached. Baseline responses were obtained every 30 s with a stimulation intensity that yielded responses of 50-60% of saturation.

After achievement of a 15 min stable baseline (field potential amplitude within 15% of change and with no evident increasing or decreasing trends), LTP was induced as follows:

DG: high frequency stimulation (HFS) (4 trains at 100 Hz of 0.5 s duration; inter-train interval – 30 s);

CA3: theta burst stimulation (TBS) (4 trains of 10 bursts delivered at 5 Hz; burst composition – 4 stimuli at 100 Hz of 1 ms duration; inter-train interval – 15 s).

After delivery of high frequency/theta burs stimulus, the fEPSP amplitudes were recorded for following 60 min. Field recordings were filtered and digitized with an A/D board (National Instruments) driven by a custom acquisition software. LTP graphs were generated by averaging the amplitude of the fEPSPs in 1 min bins, and expressing data as percentage of the averaged baseline collected before LTP induction. Field potential potentiation was evaluated by comparing the last 20 minutes post-theta to baseline.

4.12. In vivo electrophysiology: assessment of visual acuity and binocularity

Mice were anesthetized by i.p. injection with Zoletil-100 (40 mg/kg, Virbac), and Xilor (10 mg/kg, Sigma) and placed in a stereotaxic frame. Additional doses of anesthetic were used to keep the anesthesia level stable throughout the experiment. Body temperature was continuously monitored and maintained at ~37°C by a thermostated electric blanket. A hole was drilled in the skull, corresponding to the binocular portion of the primary visual cortex (binocular area Oc1B). After exposure of the brain surface, a micropipette filled with NaCl (3M) was inserted into the cortex 2.7-3.5 mm from λ (intersection between sagittal and lambdoid sutures). Both eyes were fixed and kept open by means of adjustable metal rings surrounding the external portion of the eye bulb. The eyes were frequently inspected and rinsed with physiological solution to prevent the formation of cataracts.

VA was measured using VEPs. To record VEPs, the electrode was advanced at a

depth of 100 or 400 µm within the cortex. At these depths, VEPs had their maximal amplitude. Signals were band-pass-filtered (0.1-100 Hz), amplified and fed to a computer for analysis. At least 50 events were averaged in synchrony with the stimulus contrast reversal. Transient VEPs in response to abrupt contrast reversal (1 Hz) were evaluated in the time domain by measuring the peak-to-baseline amplitude and peak latency of the major positive (at 100 µm depth) or negative (at 400 µm depth) component. Visual stimuli were horizontal sinusoidal gratings of different spatial frequencies, generated by a VSG2/2 card running custom software and presented on a monitor (20 x 22 cm; luminance 15 cd/m^2) positioned 20 cm from the mouse eyes. VA was obtained by extrapolation to zero amplitude of the linear regression through the data points in a curve where VEP amplitude is plotted against log spatial frequency.

Binocularity was assessed calculating the contralateral to ipsilateral (C/I) VEP ratio at 0.05 c/deg, i.e. the ratio of VEP amplitudes recorded by stimulating the contralateral and ipsilateral eye with respect to the brain side where recording is performed. For each animal, at least 12 independent C/I VEP ratio values were calculated and averaged together from 3 well-spaced traces along the medio-lateral and antero-posterior axes of the primary visual cortex. Care was taken to equally sample VEPs across the two cortical depths so that all layers contributed to the analysis.

4.13. Analysis of GABA release in hippocampal and visual cortex synaptosomes

Animals were sacrificed and the brain regions corresponding to the primary visual cortex and to the hippocampus were removed. Synaptosomes were prepared essentially as previously described (Stigliani et al., 2006). The tissue was homogenized at 4°C, utilizing a

homogenizer Teflon/glass (clearance 0.25 mm), in 10 volumes of sucrose 0.32M, buffered with Tris-HCl at pH 7.4. The homogenized tissue was centrifuged (5 min, 1000 x g a 4°C) in order to remove all nuclei and cellular fragments. Then, the supernatant was gently stratified on a discontinuous Percoll gradient (2, 6, 10, 20% v/v in tris HCl/sucrose) and again centrifuged (33500 x g per 5 min a 4°C). After centrifugation, the stratified fraction of synaptosomes, leaning between 10% and 20% Percoll, was collected, washed by centrifugation (20200 x g per 15 min a 4°C) and then resuspended in a physiologic medium, containing: NaCl 140mM; KCl 3mM; $MgCl_2$ 1.2mM; $CaCl_2$ 1.2mM; NaH_2PO4 1.2mM; HEPES 10mM; glucose 10mM; pH 7.4.

Synaptosomes were incubated at 37°C for 15 min with the radioactive tracers [^{3}H]D-GABA, at a final concentration of 0.05μM (plus 50 μM of the GABA transaminase inhibitor amino-oxyacetic acid). Aliquots of the synaptosomal suspensions were layered on microporous filters at the bottom of a set of parallel superfusion chambers (Superfusion System, Ugo Basile, Comerio, Varese, Italy) maintained at 37°C. Superfusion was started at a rate of 0.5 ml/min with standard medium supplemented with 50 μM amino-oxyacetic acid. After 36 min of superfusion, to equilibrate the system, samples were collected according to the following scheme: one sample collected for 3-min (t = 36-39 min; basal outflow); one sample collected for 6-min (t = 39-45 min; stimulus-evoked release); one sample collected for 3-min (t = 45-48 min; basal outflow after stimulus-evoked release). A 90-s period of stimulation was applied at t = 39 min, after the first sample has been collected. Stimulation of synaptosomes was performed with 15 mM KCl, substituting for equimolar concentration of NaCl. Radioactivity was determined in each sample collected and superfused filters by liquid scintillation counting. Tritium released in each sample was calculated as percentage of the total synaptosomal tritium content at the beginning of the respective sample collection (fractional rate x 100). The stimulus-evoked overflow was estimated by subtracting

transmitter content of the two 3-min samples (basal outflow) from release evoked in the 6-min sample collected during and after the depolarization pulse (stimulus evoked release).

4.14. Statistics

All statistical analysis was done using SigmaStat Software. Differences between two groups were assessed with a two-tailed t test. One-way ANOVA, Two way ANOVA and Two way RM ANOVA, followed by multiple comparison procedures Holm–Sidak or Tukey test, were used to compare data belonging to more than two experimental groups.

CHAPTER 5

RESULTS

5.1. Effects of adult-onset environmental enrichment and fluoxetine treatment on Down syndrome related phenotypes displayed by Ts65Dn mice

In the first part of the presented work I investigated the impact of adult-onset EE and fluoxetine administration, two therapeutic strategies shown to be efficacious in treatment of neurodevelopmental disorders in adulthood (Castren et al., 2012), on DS-related phenotypes displayed by Ts65Dn mice. My results suggest that these two treatments can be successfully employed to favour recovery from cognitive impairments and synaptic plasticity failure in adult Ts65Dn mice, with EE being effective also in counteracting the visual deficits observed in adult trisomic mice. I also report that levels of GABA release are markedly increased in the hippocampus and in the visual cortex of Ts65Dn mice compared to WT animals, and that exposure to both EE and fluoxetine reduces the inhibitory transmission, normalizing GABA release in the synaptosomes of trisomic mice.

5.1.1. Environmental enrichment promotes spatial learning and memory in Ts65Dn mice

I initially assessed spatial memory abilities in MWM task, a cognitive paradigm in which Ts65Dn mice are known to be severely impaired (Escorihuela et al., 1995, Stasko and Costa, 2004, Fernandez et al., 2007). The latency to locate the submerged platform on the ninth day of training was longer in Ts65Dn mice reared in standard environmental conditions (Ts65Dn-SC, 33.02 ± 8.24 s, n = 8) compared to both WT (WT, 14.52 ± 3.34 s, n = 9) and Ts65Dn

enriched mice (Ts65Dn-EE, 15.61 ± 2.53, n = 5), while these two latter groups did not differ between each other (Figure 1).

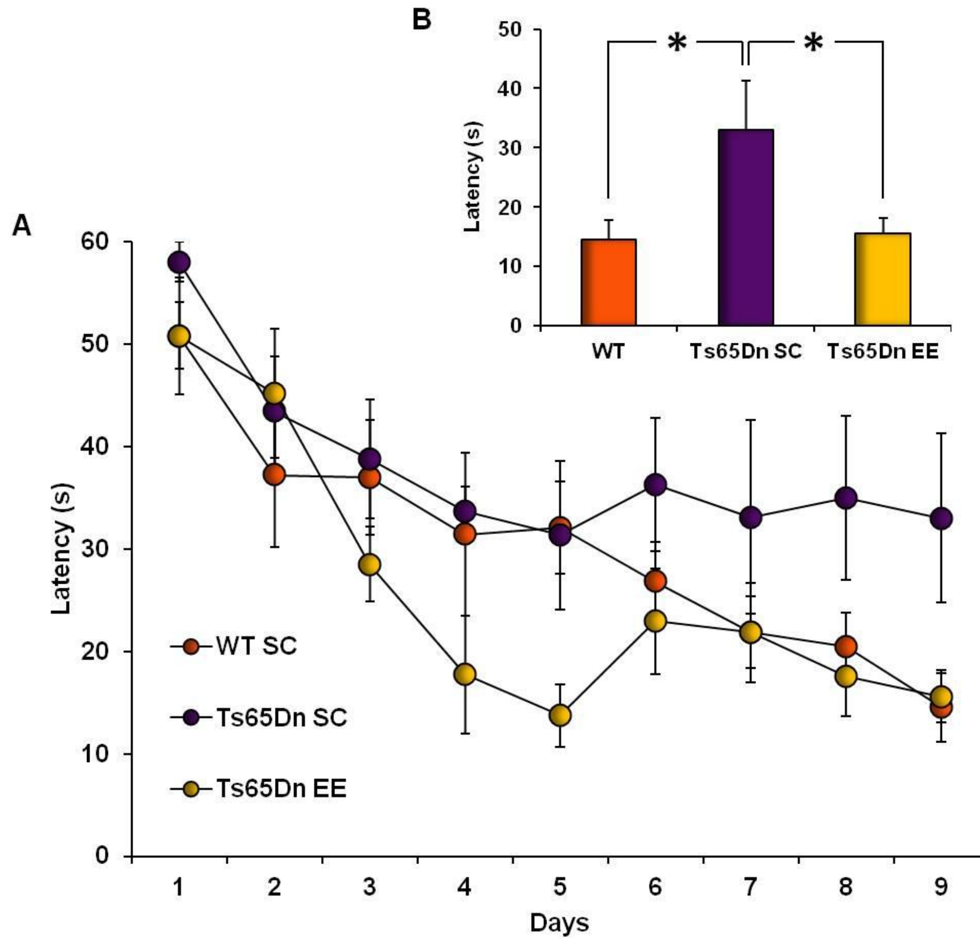


Figure 1. Environmental enrichment promotes spatial learning in Ts65Dn mice. (**A**) Learning curves for WT (orange), Ts65Dn-SC (violet) and Ts65Dn-EE (yellow). (**B**) The histogram shows latency to locate the submerged platform on the last day of training for the three groups. One Way ANOVA followed by a multiple comparison procedure (Holm-Sidak method) showed a statistical difference between WT and Ts65Dn-SC mice and Ts65Dn-SC and Ts65Dn-EE ($p < 0.05$), but not between WT and Ts65Dn-EE mice ($p = 0.885$). *, statistical significance; error bars, s.e.m.

To assess the strength of spatial learning, I performed on the last trial of the last (tenth) training day a probe trial in which the hidden platform was removed and the amount of time spent in the former region of platform was measured. The probe test confirmed the spatial memory impairment of Ts65Dn mice: WT mice spent significantly longer time in the quadrant where the platform was located during the days of training; in contrast, Ts65Dn-SC

mice showed no preference for the target quadrant, indicating that they did not remember the location of the hidden platform. Also in this case, EE was able to completely counteract the cognitive deficit displayed by Ts65Dn mice (Figure 2).

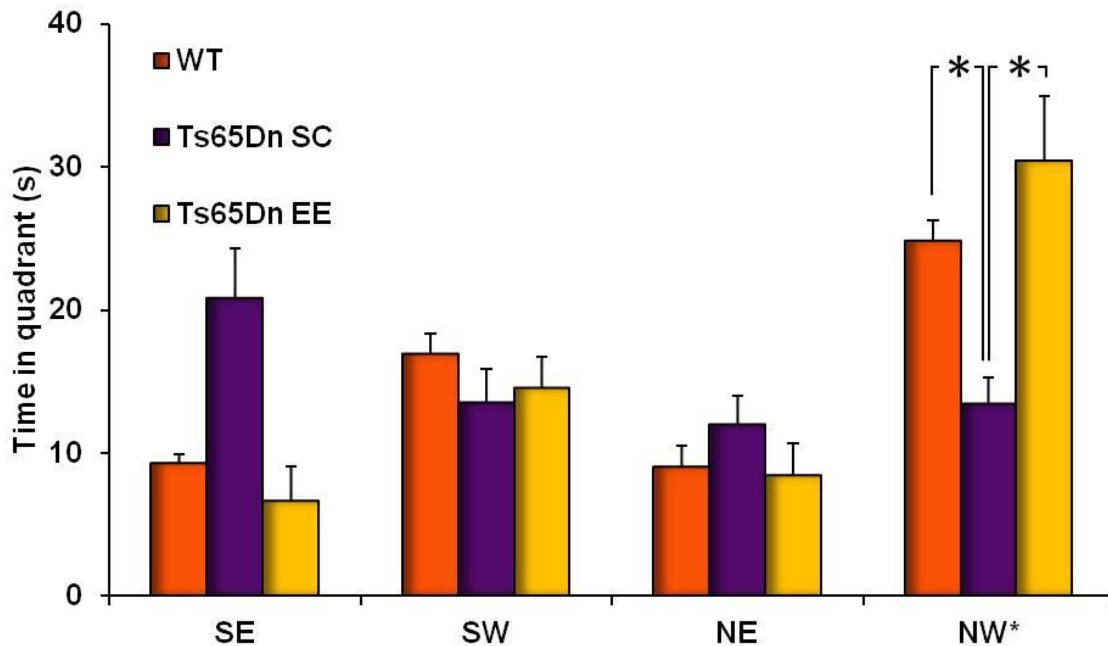


Figure 2. Environmental enrichment promotes spatial memory in Ts65Dn mice. Probe trial for WT (orange), Ts65Dn-SC (violet) and Ts65Dn-EE (yellow) mice. Two Way RM ANOVA revealed a statistically significant interaction between the genotype group and the pool quadrant ($p < 0.001$). A Holm-Sidak multiple comparison procedure revealed that while Ts65Dn-SC did not show any preference for the target (NW) quadrant, both WT and Ts65Dn-EE mice spent significantly more time in the NW quadrant than in the other quadrants. Moreover, the time spent in the target quadrant was shorter in Ts65Dn-SC mice than in the other two groups, which instead did not differ between each other ($p = 0.1$). *, statistical significance; error bars, s.e.m.

5.1.2. Recovery of LTP at medial perforant path–granule cell synapses in Ts65Dn mice reared in environmental enrichment

Since the spatial memory impairment displayed by Ts65Dn mice has been repeatedly related to synaptic plasticity deficits in the hippocampus (Siarey et al., 1997, Siarey et al., 1999, Kleschevnikov, 2004, Fernandez et al., 2007), I studied LTP in the DG in response to HFS of

the perforant path, the circuitry most severely affected by excessive levels of inhibition in the Ts65Dn mouse brain (Belichenko et al., 2004, Fernandez et al., 2007, Kleschevnikov et al., 2012). While I found a strong potentiation of the response after HFS in the DG of WT mice (132.04 ± 7.13%, n = 12 slices and 8 animals), no potentiation of field EPSPs with respect to baseline was recorded in Ts65Dn-SC mice (105.38 ± 5.85%, n = 10 slices and 5 animals). In contrast, a robust rescue of LTP was evident in Ts65Dn-EE mice (124.5 ± 7.67 %, n = 13 slices and 7 animals) (Figure 3). These results demonstrate for the first time that EE in adult Ts65Dn mice is able to restore long-term synaptic plasticity in a neural circuit critically involved in spatial learning and memory.

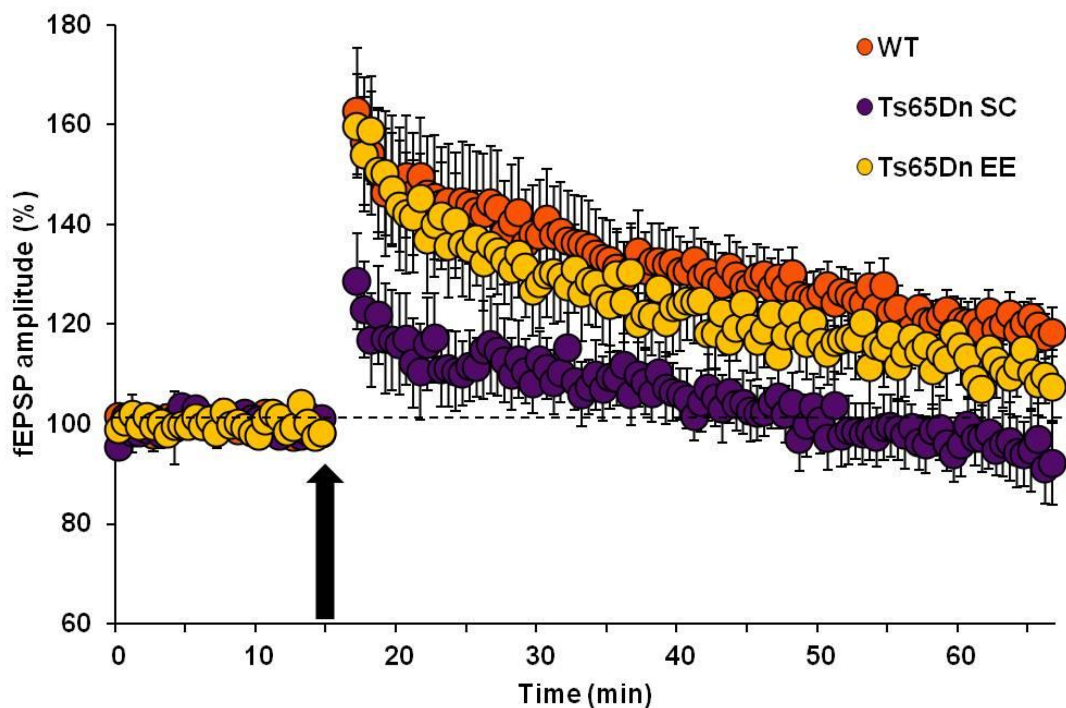


Figure 3. Recovery of LTP at medial perforant path–granule cell synapses in Ts65Dn mice reared in environmental enrichment. Averaged data for LTP induced in WT (orange circles), Ts65Dn-SC (violet circles) and Ts65Dn-EE (yellow circles) mice. Slices from WT and Ts65Dn-EE mice showed potentiation of the response after high frequency stimulation HFS (Two Way RM ANOVA, baseline vs. the last 20 min post-HFS, $p < 0.001$), while slices from Ts65Dn-SC did not show potentiation (Two Way RM ANOVA, baseline vs. the last 20 min post-HFS, $p = 1$). A multiple comparison procedure (Holm-Sidak method) showed a statistical difference in LTP levels between WT and Ts65Dn-SC mice, between Ts65Dn-SC mice and Ts65Dn-EE mice and between WT and Ts65Dn-EE mice ($p < 0.001$). The arrow indicates HFS; error bars, s.e.m.

5.1.3. Restoration of visual functions in Ts65Dn mice by environmental enrichment

It has been recently reported that Ts65Dn mice exhibit a number of visual deficits similar to those reported in individuals with DS (Scott-McKean et al., 2010). Therefore, I moved to the visual system with the aim to investigate whether the beneficial effects exerted by EE are specific to the hippocampus or they are also detectable in the sensory cortices.

Using electrophysiological recordings of VEPs from V1, VA of WT (n = 6), Ts65Dn-SC (n = 13) and Ts65Dn-EE (n = 5) mice was measured and, for the first time in this model, the OD properties of visual cortical neurons was analyzed by calculating the C/I VEP ratio as an index of V1 binocularity (Sale et al., 2007). VA of Ts65Dn-SC mice was lower (0.45 ± 0.04c/deg) than that of WT controls (0.57 ± 0.02 c/deg) (Figure 4A). While it is well known that the C/I VEP ratio is in the 2.0–3.0 range in adult normal mice (Porciatti et al., 1999), reflecting the predominance of crossed fibres in retinal projections, I found that the visual cortex of Ts65Dn-SC mice was not dominated by the contralateral eye, with a marked reduction in C/I VEP ratio compared to WT animals (C/I VEP ratio of Ts65Dn-SC: 1.26 ± 0.18; C/I VEP ratio of WT mice: 2.36 ± 0.16) (Figure 4B). Moreover, I also report, in agreement with data from patients with DS, that Ts65Dn-SC mice did also display a robust increase in VEP latencies compared to WT controls in response to visual gratings of 0.05, 0.1, 0.2, 0.3 and 0.4 spatial frequencies (WT 0.05: 125.99 ± 3.28 ms; 0.1: 130.56 ± 5.92 ms; 0.2: 146.24 ± 9.26 ms; 0.3: 144.18 ± 8.85 ms; 0.4: 147.24 ± 8.72 ms; Ts65Dn-SC 0.05: 132.79 ± 9.51 ms; 0.1: 145.33 ± 16.38 ms; 0.2: 152.42 ± 8.04 ms; 0.3: 161.03 ± 4.12 ms; 0.4: 168.27 ± 12.89 ms) (Figure 4C).

EE completely reversed all visual function deficits displayed by Ts65Dn mice (Figure 4 A-C): the VA (0.59 ± 0.06) and the C/I VEP ratio (2.81 ± 0.27) of Ts65Dn-EE

mice, indeed, were not statistically different from those of WT mice; in addition, I found that VEP latencies in Ts65Dn-EE mice were significantly shorter than of Ts65Dn-SC mice, but did not differ from those recorded in WT controls (Ts65Dn-EE 0.05: 115.35 ± 16.50 ms; 0.1: 126.33 ± 12.83 ms; 0.2: 132.25 ± 9.56 ms; 0.3: 132.93 ± 10.04 ms; 0.4: 137.30 ± 10.83 ms).

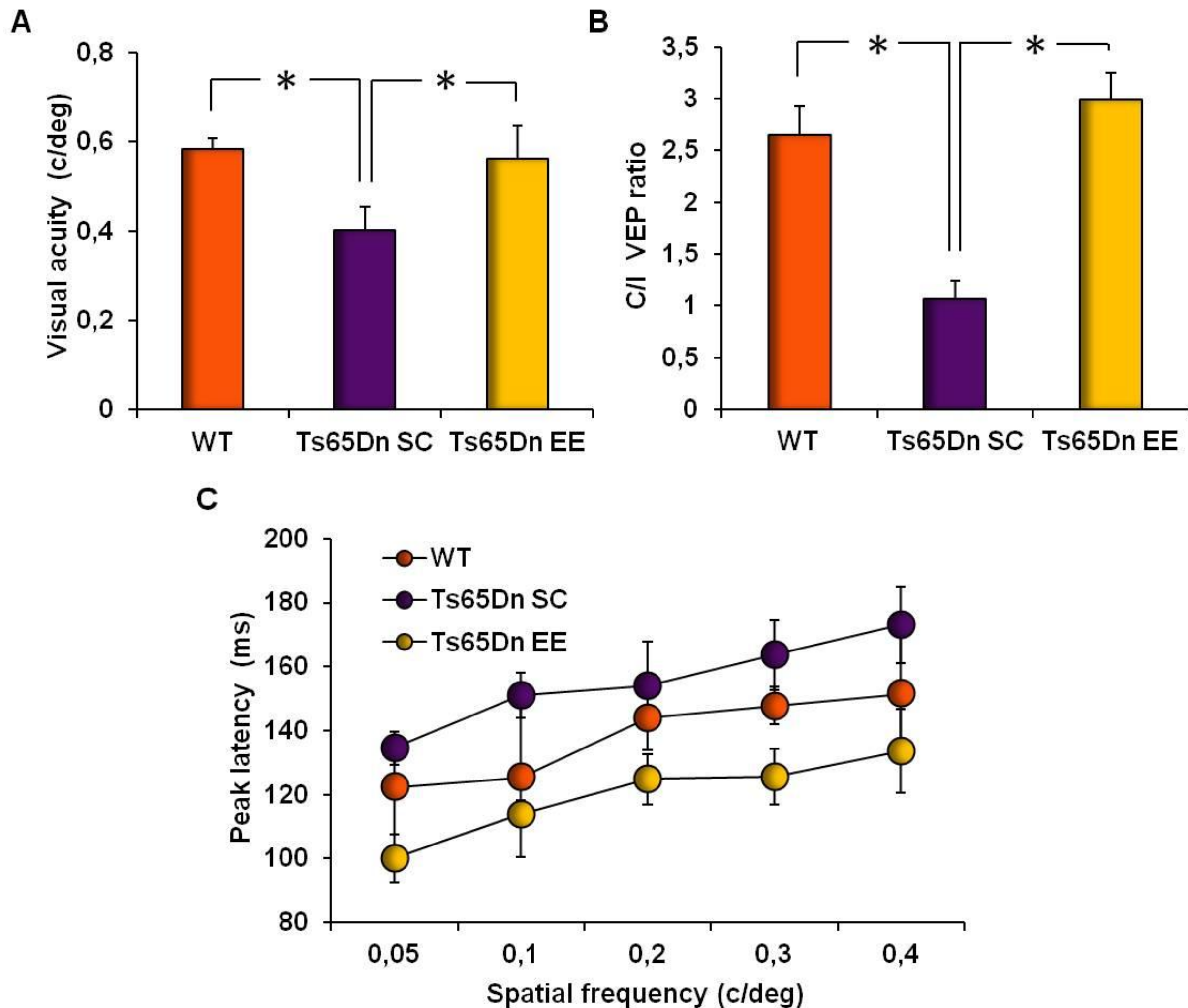


Figure 4. Restoration of visual functions in Ts65Dn mice by environmental enrichment. Visual acuity **(A)**,ocular dominance **(B)** and peak latency **(C)** assessed by electrophysiological recordings of VEPs from the primary visual cortex in WT (orange), Ts65Dn-SC (violet) and Ts65Dn-EE (yellow) mice. One Way ANOVA showed that a statistical difference in the mean values was present among the three groups for both visual acuity and C/I VEP ratio ($p < 0.05$ and $p < 0.001$, respectively); a multiple comparison procedure (Holm-Sidak method) showed a statistical difference between WT and Ts65Dn-SC mice and between Ts65Dn-nonEE mice and Ts65Dn-EE mice, but not between WT and Ts65Dn-EE mice ($p = 0.82$ for visual acuity; $p = 0.14$ for C/I VEP ratio). VEP latencies of Ts65Dn-EE mice were statistically different from Ts65Dn-SC animals, but not from WT mice (Two Way RM ANOVA). *, statistical significance; error bars, s.e.m

5.1.4. Environmental enrichment normalizes excessive GABA release in the hippocampus and visual cortex of Ts65Dn mice

Because substantial evidence suggests that excessive inhibition is critically involved in the cognitive deficits displayed by Ts65Dn mice (Siarey et al., 1997, Siarey et al., 1999, Kleschevnikov, 2004, Fernandez et al., 2007, Belichenko et al., 2009b), I investigated whether restoration of spatial learning abilities and DG long-term plasticity elicited by EE was accompanied by a reduced GABA release in the hippocampus.

Specifically, KCl-evoked release of [^{3}H]GABA from synaptosomes in superfusion was measured in the hippocampus of WT (n = 16), Ts65Dn-SC (n = 7) and Ts65Dn-EE mice (n = 7) (Figure 5A). Moreover, since it was previously showed that EE is is able to reinstate plasticity in the visual cortex of adult animals through a reduction of GABAergic inhibition (Sale et al., 2007b, Baroncelli et al., 2010b), I also performed synaptosome analysis for GABA release in the primary visual cortex of the same animals (Figure 5B). I found that the stimulus-evoked release of GABA was markedly increased in both the hippocampus and the visual cortex of Ts65Dn-SC mice (hippocampus: 7.30 ± 0.85%; visual cortex: 10.63 ± 0.850%) compared to WT animals (hippocampus: 4.71 ± 0.67%; visual cortex: 7.26 ± 0.90%). In contrast, levels of depolarization-evoked overflow of GABA were markedly decreased in Ts65Dn-EE animals (hippocampus: 5.04 ± 1.02%; visual cortex: 7.22 ± 120%) and did not statistically differ from those of WT mice.

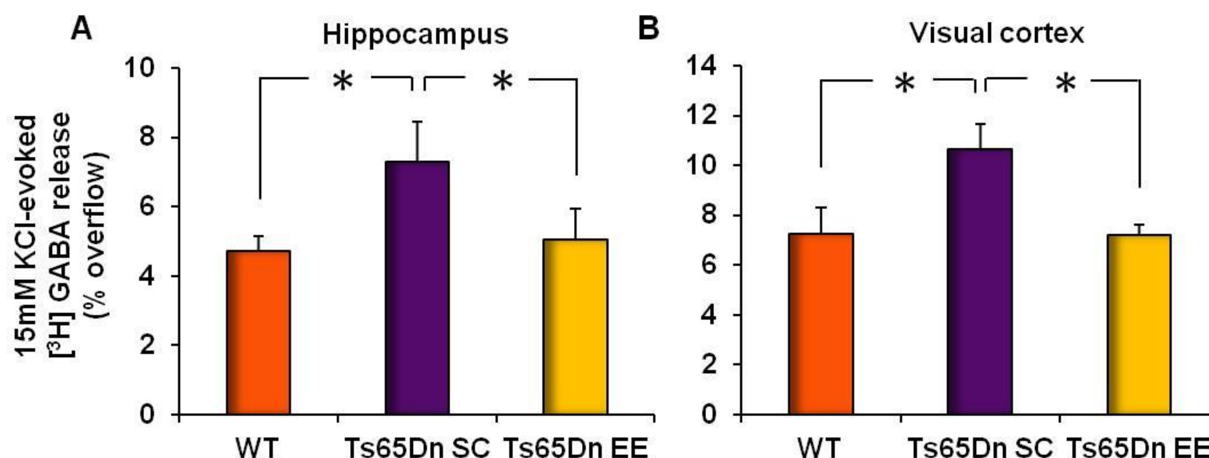


Figure 5. Normalization of depolarization-evoked release of GABA from synaptosomes after exposure to environmental enrichment. 15 mM KCl evoked GABA release from hippocampal (**A**) and visual cortex (**B**) synaptosomes of WT (orange), Ts65Dn-SC (violet) and Ts65Dn-EE (yellow) mice. Two Way ANOVA showed a significant difference among the different levels of group ($p < 0.001$); a multiple comparison procedure (Holm-Sidak method) showed that levels of GABA were significantly higher, for both the hippocampus and the visual cortex, in Ts65Dn-SC mice compared to WT animals, while no statistical difference was present between Ts65Dn-EE animals and WT mice ($p = 0.79$ for the hippocampus, $p = 0.98$ for the visual cortex). *, statistical significance;errorbars,s.e.m.

5.1.5. Evaluation of fluoxetine pro-convulsive side effects in Ts65Dn mice

Based on the potential to induce plastic responses in adult brain in a manner analogous to EE, fluoxetine has emerged as a putative pharmacological treatment for DS. Since previous studies in humans and animal models have reported that high doses of fluoxetine administration could have a pro-convulsive action (Rosenstein et al., 1993, Oke et al., 2001, Zienowicz et al., 2005, Suchard, 2008, Heinen et al., 2012), I performed a preliminary analysis aimed at establishing a safe fluoxetine dose in my experimental model. I evaluated three decreasing drug concentrations within the range of fluoxetine dosing schemes generally used in laboratory rodents (see Methods for references), that is 40, 20 and 10 mg/kg/day, administered to both trisomic and WT mice. For the entire length of the treatment (8 weeks) I monitored, via behavioral observations, mouse survival and seizure susceptibility (Figure 6).

In WT mice, only one case of death (1 out of 7) was registered, when the maximum dose (WT-fluox40) was used, but no one case of seizures was ever observed. In trisomic mice, 4 out 10 of Ts65Dn-fluox40 mice and 1 out 7 of Ts65Dn-fluox20 died during the 8-week observation period. There were no deaths among Ts65Dn-fluox10 mice (0 out of 8 mice), water-treated Ts65Dn mice (0 out of 7 mice) and water-treated WT animals (0 out of 9 mice) (Figure 6A).

Tonic-clonic seizures were observed in a few animals (a total of 5 mice). All seizure episodes occurred in Ts65Dn-fluox40 and Ts65Dn-fluox20 mice (4 out of 10 and 1 out of 7, respectively) (Figure 6B).

Taken together, these results indicate that a 10 mg/kg/day dose of fluoxetine did not result in any sudden death and was not associated with pro-convulsive side effects in the Ts65Dn background. Thus, I used a 10 mg/kg/day dose to test the effects of fluoxetine on the phenotype of euploid and trisomic mice.

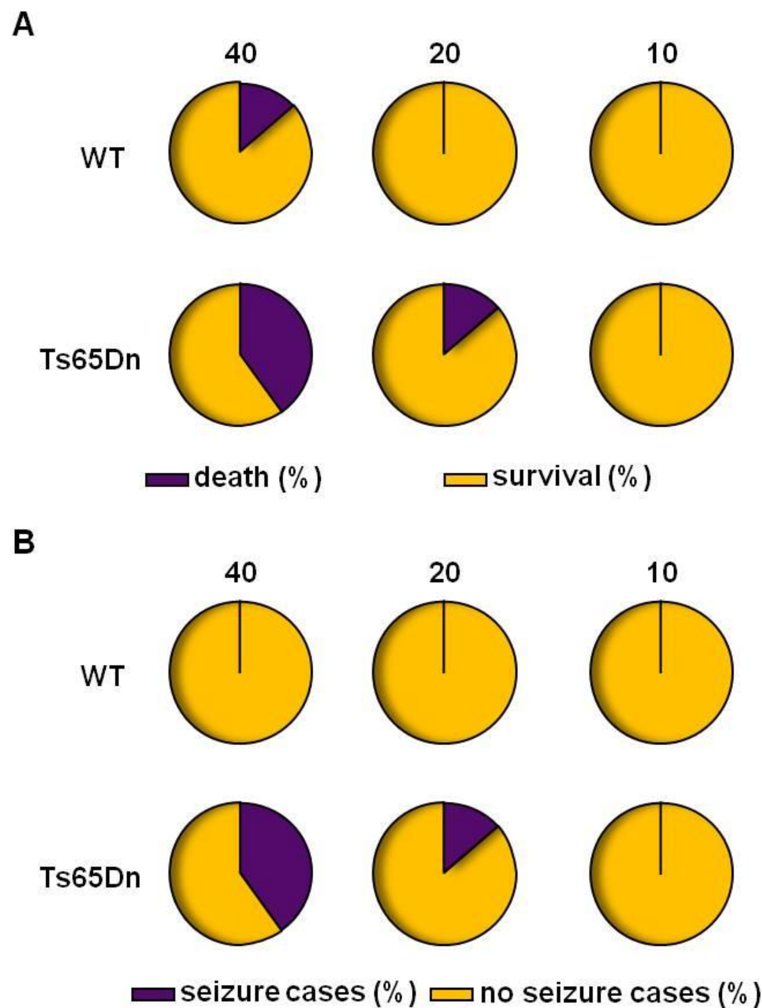


Figure 6. Assessment of severe side effects in fluoxetine treated Ts65Dn and WT mice. The percentage of unexpected and sudden death (**A**) and seizure (**B**) cases found in the four experimental groups of mice with three different dosing protocols of fluoxetine administered in the drinking water. In both analyses, no adverse cases were recorded for Ts65Dn-fluox animals treated for 8 weeks with 10 mg/kg/day fluoxetine.

5.1.6. Evaluation of spatial learning and memory after chronic administration of fluoxetine

I initially examined the effects of a pharmacological treatment with fluoxetine on the performance of Ts65Dn mice in MWM test. The analysis of swimming paths showed that the latency to find the hidden platform on the ninth day of training in non-treated WT mice (WT-NT, 10.15 ± 3.04 sec; n = 5) was statistically shorter with respect to both non-treated Ts65Dn

(Ts65Dn-NT, 35.07 ± 5,88 s; n = 8) and treated Ts65Dn mice (Ts65Dn-FLX, 44 ± 5.86 s; n = 8). While these data confirm a spatial learning deficit in adult trisomic mice, there was no statistical difference in the performance between treated and non-treated Ts65Dn mice. Performance of control group of WT mice treated with fluoxetine (WT-FLX, 23.55 ± 9.33 s; n = 4) did not statistically differ from that of non-treated WT mice (Figure 7).

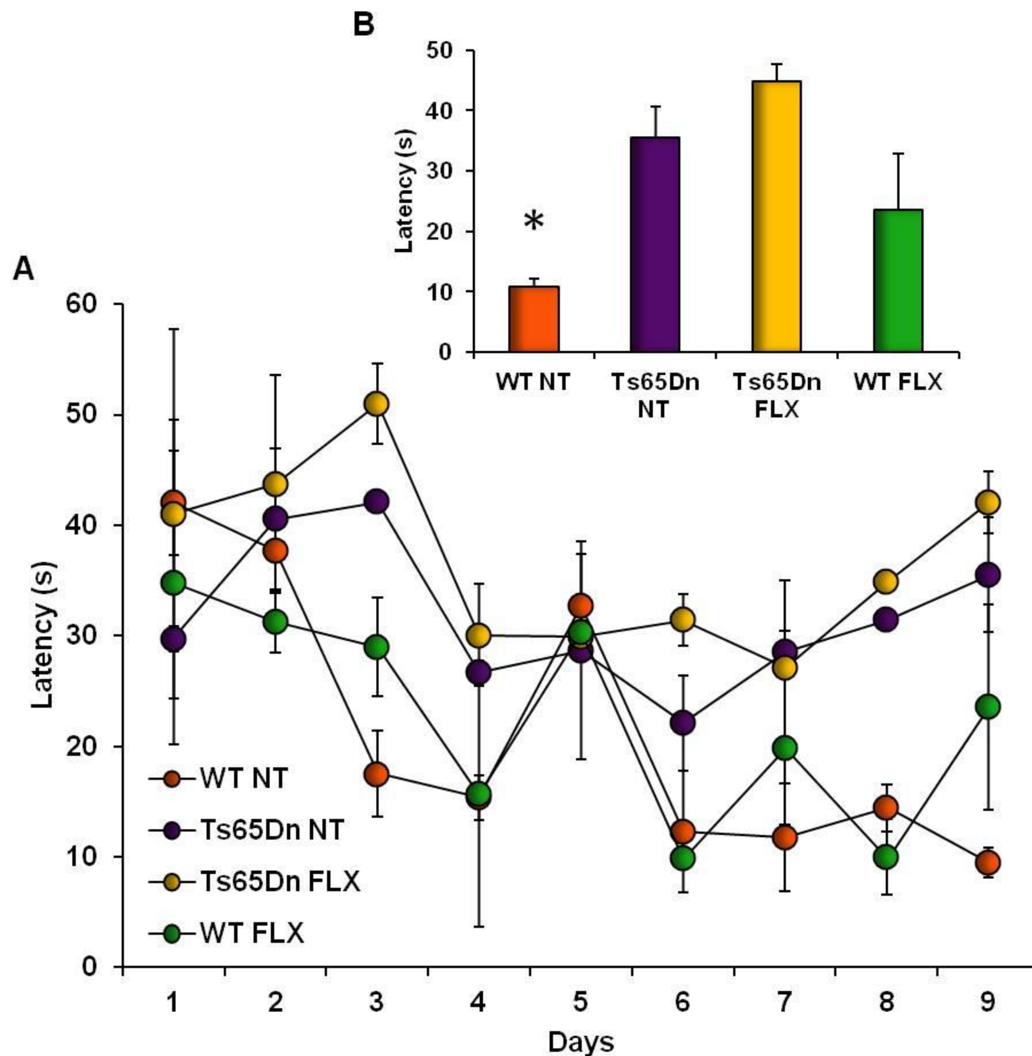


Figure 7. Effects of fluoxetine administration on spatial learning in Ts65Dn mice. (**A**) Learning curves for WT-NT (orange), Ts65Dn-NT (violet), Ts65Dn-FLX (yellow) and WT-FLX mice (green). (**B**) The histogram shows latency to locate the submerged platform on the last day of training for the four groups. Two Way RM ANOVA revealed a statistically significant interaction between the genotype group and the days of training ($p < 0.05$). A Holm-Sidak multiple comparison procedure demonstrated a statistical difference at ninth training day between WT-NT and Ts65Dn-NT and between WT-NT and Ts65Dn-FLX ($p < 0.05$), but not between other experimental groups. *, statistical significance; error bars, s.e.m.

In line with data from literature, Ts65Dn mice demonstrated poor spatial memory in the probe trial. While WT-NT mice spent significantly longer time in the quadrant where the platform was placed during the period of training, Ts65Dn-NT mice showed no preference for the target quadrant, indicating that they did not remember the position of the hidden platform. On the other hand, Ts65Dn-FLX mice spent more time in the target of interest than in other quadrants (Figure 8). Even if this tendency was not statistically different from notreated Ts65Dn mice, it suggests a positive trend towards improvement of cognitive functions.

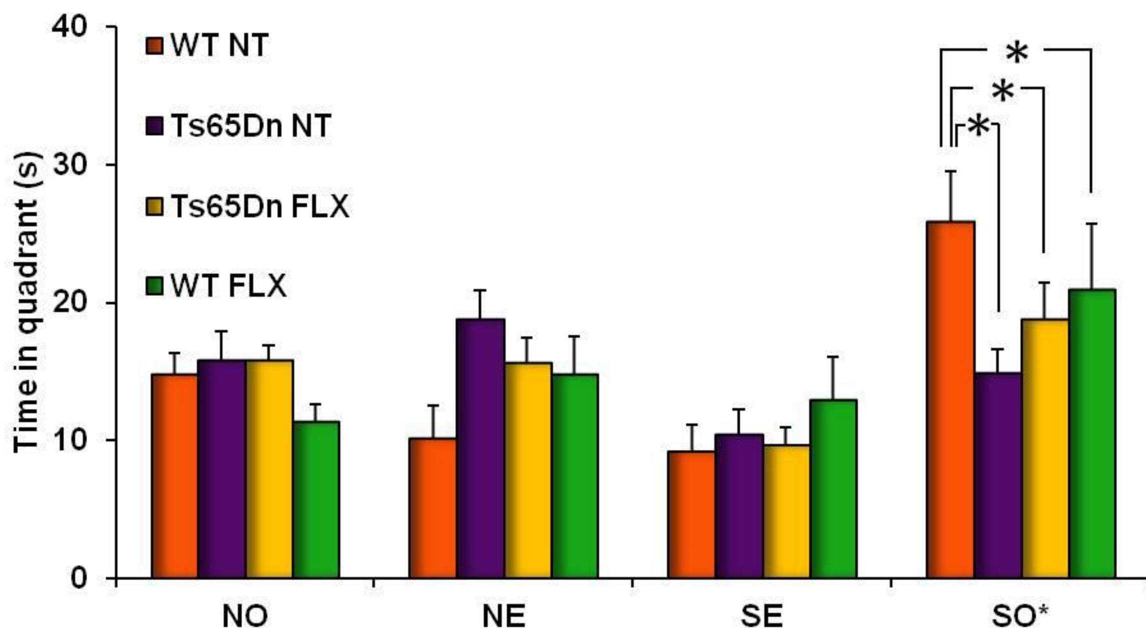


Figure 8. Effects of fluoxetine administration on spatial memory in Ts65Dn mice. Probe trial for WT-NT (orange), Ts65Dn-NT (violet), Ts65Dn-FLX (yellow) and WT-FLX mice (green). Two Way RM ANOVA revealed a statistically significant difference between the four experimental groups for the factor of quadrant ($p < 0.001$). A Holm-Sidak multiple comparison procedure revealed that while Ts65Dn-NT mice didn't show any preference for the target quadrant (SO), WT mice spent significantly more time in the SO quadrant than in the other quadrants. Moreover, the time spent in the target quadrant was significantly longer in WT-NT mice than in the other three groups ($p < 0.05$), which instead did not statistically differ between each others. *, statistical significance; error bars, s.e.m.

5.1.7. Chronic treatment with fluoxetine improves short-term spatial memory impairment in Ts65Dn mice

I also assessed short-term spatial memory abilities in the NPR task, which tests the animal ability to discriminate an old versus a novel position of a familiar object. An intact performance in the NPR relies on correct hippocampal functions (Mumby et al., 2002), and it has been recently reported that Ts65Dn mice display a robust deficit in this task (Kleschevnikov et al., 2012).

During the acquisition phase, all experimental groups explored both objects equally, with a discrimination index close to the expected 50% (WT-NT, 54.87 ± 3.5%, n = 5; Ts65Dn-NT, 47.82 ± 4.45%, n = 4; Ts65Dn-FLX, 53.49 ± 1.62%, n = 8; WT-FLX, 55.22 ± 3.26%, n = 4). In the test phase, while WT-NT mice spent more time exploring the moved object (discrimination index: 76.50 ± 3.48%), an impaired performance was found in Ts65Dn-NT mice, which spent even less time exploring the moved object with respect to the unmoved one, thus resulting in a low discrimination index (37.20 ± 7.58%). Fluoxetine appeared to fully rescue memory abilities in trisomic mice, which displayed a clear preference toward the moved object (discrimination index: 64.04 ± 5.65%). Treatment with fluoxetine did not significantly affect the outcome of euploid mice in the NPR test (64.52 ± 7.41%) (Figure 9).

Thus, chronic treatment with fluoxetine was able to rescue short-term spatial memory abilities in adult Ts65Dn mice tested in the NPR task.

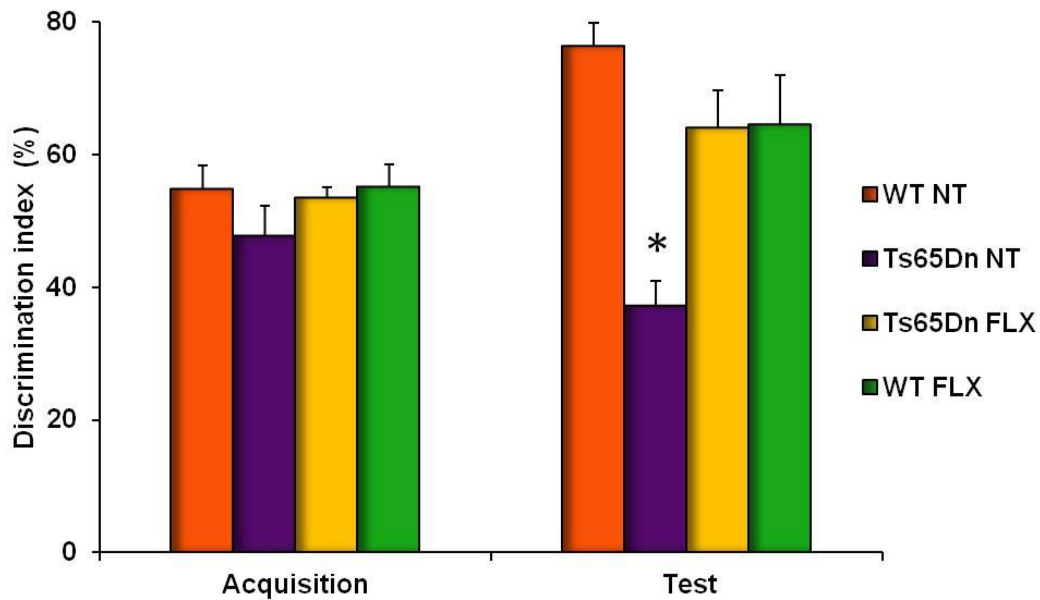


Figure 9. Spatial short-term memory improvement in Ts65Dn mice after chronic fluoxetine treatment. Discrimination indexes for the acquisition and test phase of WT-NT (orange), Ts65Dn-NT (violet), Ts65Dn-FLX (yellow) and WT-FLX (green) mice. While there was no difference among the exploration indexes of the four experimental groups of mice during the acquisition phase (One way ANOVA, Holm-Sidak method, $p = 0.377$), a significantly lower exploration index was found in Ts65Dn-NT mice compared to WT-NT animals in the testing phase (One way ANOVA, Holm-Sidak method, $p < 0.01$). In contrast, Ts65Dn-FLX mice displayed an exploration index statistically higher than that of Ts-65Dn-NT animals, and not significantly different from that of WT-NT mice (One way ANOVA, Holm-Sidak method, $p < 0.01$; $p = 0.112$, respectively). Treatment with fluoxetine did not significantly affect the testing outcome of euploid mice ($p = 0.188$) .*, statistical significance; error bars, s.e.m.

5.1.8. Effects of chronic fluoxetine administration on hippocampal synaptic plasticity at the CA3-CA1 synapse

The spatial memory impairment displayed by Ts65Dn mice has been repeatedly related to synaptic plasticity deficits in the hippocampus, thus I studied LTP in CA1 in response to TBS of the Schaffer collateral pathway, a form of long-term synaptic plasticity impaired in the Ts65Dn mouse brain (Siarey et al., 1997, Kleschevnikov, 2004, Costa and Grybko, 2005).

I found a significant potentiation of the response after TBS with respect to the

baseline level in both WT-NT (167.91 ± 8.64%, n = 7 slices and 4 animals) and Ts65Dn-NT (134.68 ± 11.14%, n = 9 slices and 4 animals) mice. However, the level of potentiation was significantly lower in Ts65Dn-NT than in WT-NT mice, revealing a partial occlusion of synaptic plasticity in trisomic animals (Figure 10). A robust rescue of LTP was instead evident in Ts65Dn-FLX mice (167.31 ± 10.71%, n = 9 slices and 4 animals) (Figure 10). No significant change in LTP levels was found between WT-FLX (183.87 ± 12.40%, n = 8 slices and 5) and WT-NT animals.

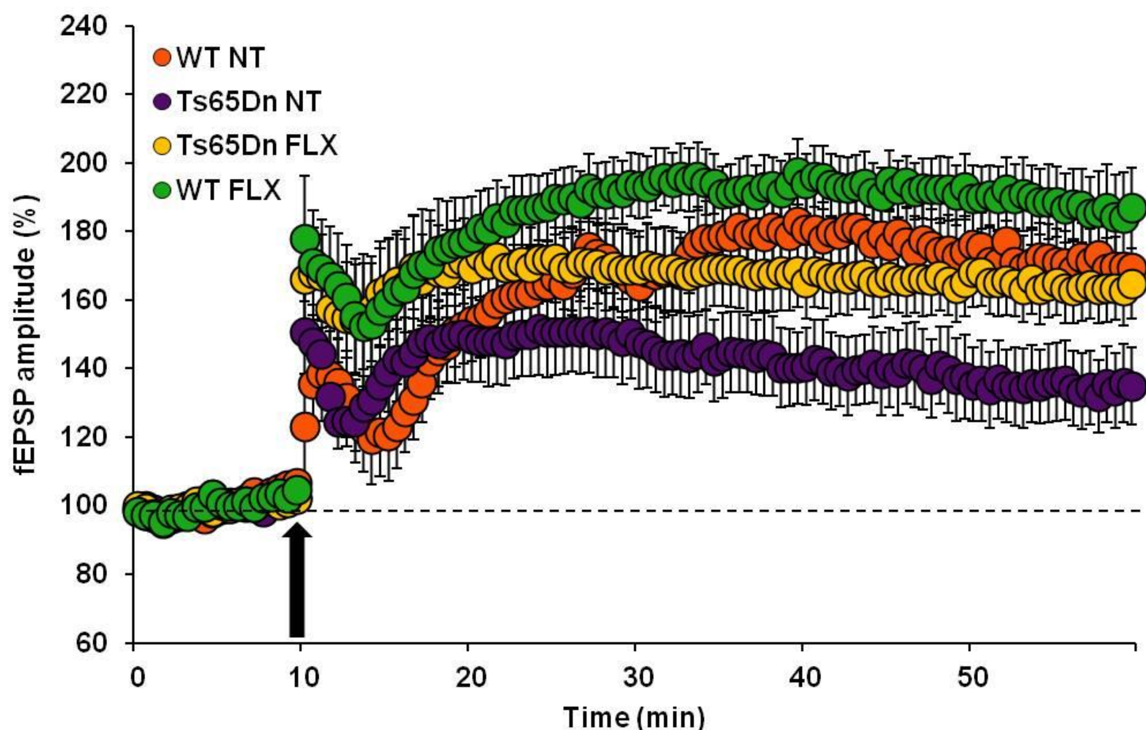


Figure 10. Recovery of LTP at Schaffer collateral – CA1 synapses in Ts65Dn mice treated with fluoxetine. Averaged data for LTP induced in WT-NT (orange), Ts65Dn-NT (violet), Ts65Dn-FLX (yellow) and WT-FLX (green) mice. While slices from all groups showed potentiation of the response after theta burst stimulation (TBS) (Two Way ANOVA, baseline vs. the last 20 min post-TBS, post-hoc Tukey test, $p < 0.05$), levels of LTP were significantly lower in Ts65Dn-NT than in WT-NT mice (Two Way ANOVA, baseline vs. the last 20 min post-TBS, pos-thoc Tukey test, $p < 0.05$). Conversely, LTP levels of Ts65Dn-FLX mice were significantly higher than those of Ts65Dn-NT animals, but did not differ from WT-NT animals (Two-Way ANOVA, pos-thoc Tukey test, $p < 0.05$ and $p = 1.0$, respectively). No significant changes were present between LTP levels of WT-NT and WT-FLX mice ($p = 0.510$). The arrow indicates TBS; error bars, s.e.m..

No significant changes were found in the I/O curves of the four experimental groups, indicating that Schaffer collaterals – CA1 synapse excitability was not affected by fluoxetine administration (Figure 11).

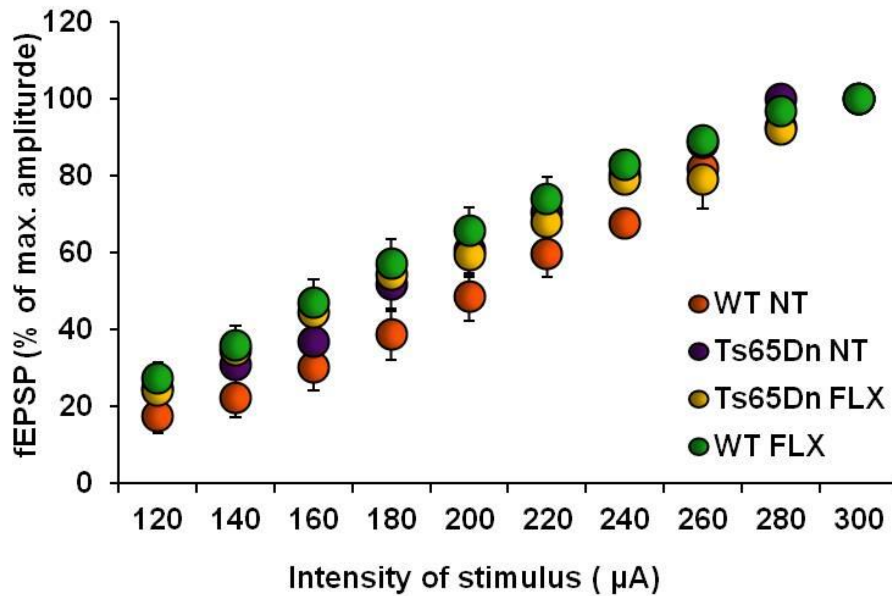


Figure 11. Effects of chronic fluoxetine administration on Schaffer collaterals – CA1 synaptic excitability in Ts65Dn mice. Amplitude of f-EPSPs normalized to saturation level as a function of stimulation intensity in WT-NT (orange), Ts65Dn-NT (violet), Ts65Dn-FLX (yellow) and WT-FLX (green) mice. Two-way RM ANOVA revealed no changes among the different genotypes and treatments ($p = 0.339$). error bars, s.e.m..

5.1.9. Chronic fluoxetine administration normalizes excessive GABA release in the hippocampus of Ts65Dn mice

As discussed before, substantial evidence suggests that excessive inhibition is critically involved in the cognitive deficits displayed by Ts65Dn mice (Siarey et al., 1997, Siarey et al., 1999, Kleschevnikov, 2004, Fernandez et al., 2007, Belichenko et al., 2009b). Thus, I investigated whether restoration of spatial memory abilities and long-term synaptic plasticity in the hippocampus elicited by fluoxetine was accompanied by a reduction of GABA release in the hippocampus. Specifically, KCl-evoked release of [^{3}H]GABA from synaptosomes in

superfusion was measured in the hippocampus of WT-NT (n = 10), Ts65Dn-NT (n = 16), Ts65Dn-FLX (n = 12) and WT-FLX mice (n = 13) (Figure 12).

I found that the stimulus-evoked release of GABA was increased in the hippocampus of Ts65Dn-NT mice (4.23 ± 0.21%) compared to WT-NT animals (3.11 ± 0.34%), thus confirming the previously established overinhibition effect. In contrast, the depolarization-evoked overflow of GABA was decreased in Ts65Dn-FLX animals (3.48 ± 0.28%), which displayed GABA release levels very similar to those found in WT-NT mice. Fluoxetine treatment did not affect GABA release in WT mice (3.62 ± 0.32%).

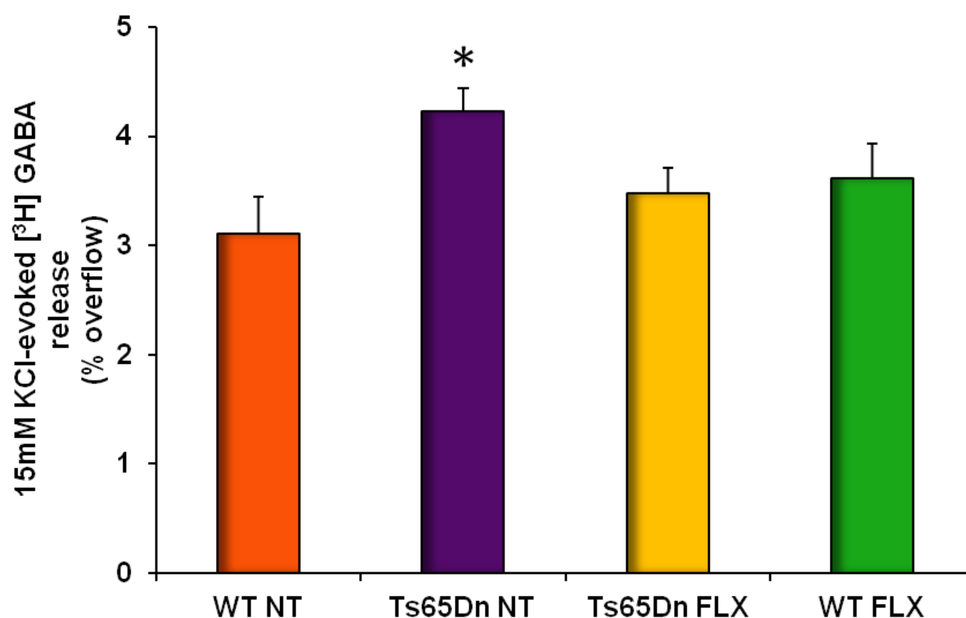


Figure 12. Fluoxetine normalizes GABA release in hippocampal synaptosomes of Ts65Dn mice. The graph reports quantification of 15 mM KCl evoked GABA release from hippocampal synaptosomes of WT-NT (orange), Ts65Dn-NT (violet), Ts65Dn-FLX (yellow) and WT-FLX (green) mice. One way ANOVA showed that GABA release in the four groups significantly differed among different experimental groups ($p < 0.05$). GABA levels were increased in the hippocampus of Ts65Dn-NT mice compared to WT-NT animals (multiple comparison procedure with Holm-Sidak method, $p < 0.05$). In contrast, GABA overflow was decreased in Ts65Dn-FLX animals, which displayed GABA levels very similar to those found in WT-NT mice and significantly lower than those of Ts65Dn-NT animals (multiple comparison procedure with Holm-Sidak method, $p = 0.367$ and $p < 0.5$, respectively). Fluoxetine treatment did not affect GABA release in WT-FLX mice. *, statistical significance; error bars, s.e.m.

5.2. Impact of environmental enrichment on visual system development in Ts65Dn mice

In the second part of my Thesis I investigated whether exposure to EE from birth elicits beneficial effects on the developing visual system of Ts65Dn mice. I performed a quantitative study of maternal behaviour, a fundamental mediator of the effects of EE during the earliest postnatal period (Sale et al., 2004), expressed by Ts65Dn females in different environmental conditions and found that enriched pups received higher levels of maternal care when compared with standard-reared pups. Moreover, my results suggest that pre-weaning onset of EE results in an acceleration of the visual system maturation in enriched Ts65Dn offspring.

5.2.1. Maternal effects of environmental enrichment in Ts65Dn mice

It has been demonstrated that maternal care exerts an essential role in the postnatal development of newborn rodents (Liu et al., 2000) and that an enhancement in the quantity and quality of maternal care induced by EE is able to accelerate the maturation of CNS (Sale et al., 2004). However, it is not known whether EE exerts the same positive effects on maternal behaviour in Ts65Dn females and whether this form of precocious EE may be used as a strategy to increase stimulation of trisomic offspring during early phases of life. Thus, I performed a detail observation of postpartum behaviour of Ts65Dn females reared in SC (n = 6; n refers to a number of observed litters) and EE (n = 5) cages focusing on the presence of mother/helper female in the nest (passive stimulation), licking, stepping, nest building and arched-back nursing (Figure 13A-E).

Mother/helper female in the nest (passive stimulation)

Tactile stimulation is an important factor of postnatal care received by the pups as it

regulates the expression of hormones important for development (Kuhn and Schanberg, 1998, Schanberg et al., 2003), promotes recovery from perinatal cortical lesions, and affects adult pattern of cortical cell dendritic fields (Kolb and Gibb, 2010). The presence of mother in the nest during the early postnatal period and physical contact with pups represents a passive form of tactile stimulation. In the present study, the time that Ts65Dn females spent in the nest in SC (72.45 ± 3.38%) was significantly lower respect to EE (91.66 ± 1.38%) (Figure 13A), indicating that enriched pups experienced higher levels of passive stimulation.

Stepping

Active forms of maternal tactile stimulation are also critically involved in structural and functional processes of cerebral development in newborns (Weaver et al., 2004, Champagne and Curley, 2009). Evaluation of stepping, maternal behaviour resembling human massage, experienced by pups revealed the increased levels of these form of active maternal stimulation in EE respect to SC (EE: 48.77 ± 4.97%; SC: 19.92 ± 2.69%) (Figure 13B).

Licking

Licking has been associated with hormonal regulation of growth and maturation of neuroendocrine function and behavioural responses to stress (Liu et al., 2000). Similarly to results reported for stepping, I found that pups raised in EE received higher levels of licking (62.89 ± 3.82%) than pups reared in SC (33.65 ± 2.15%) (Figure 13C), indicating that exposure to EE also favoured this form of active tactile stimulation of the offspring.

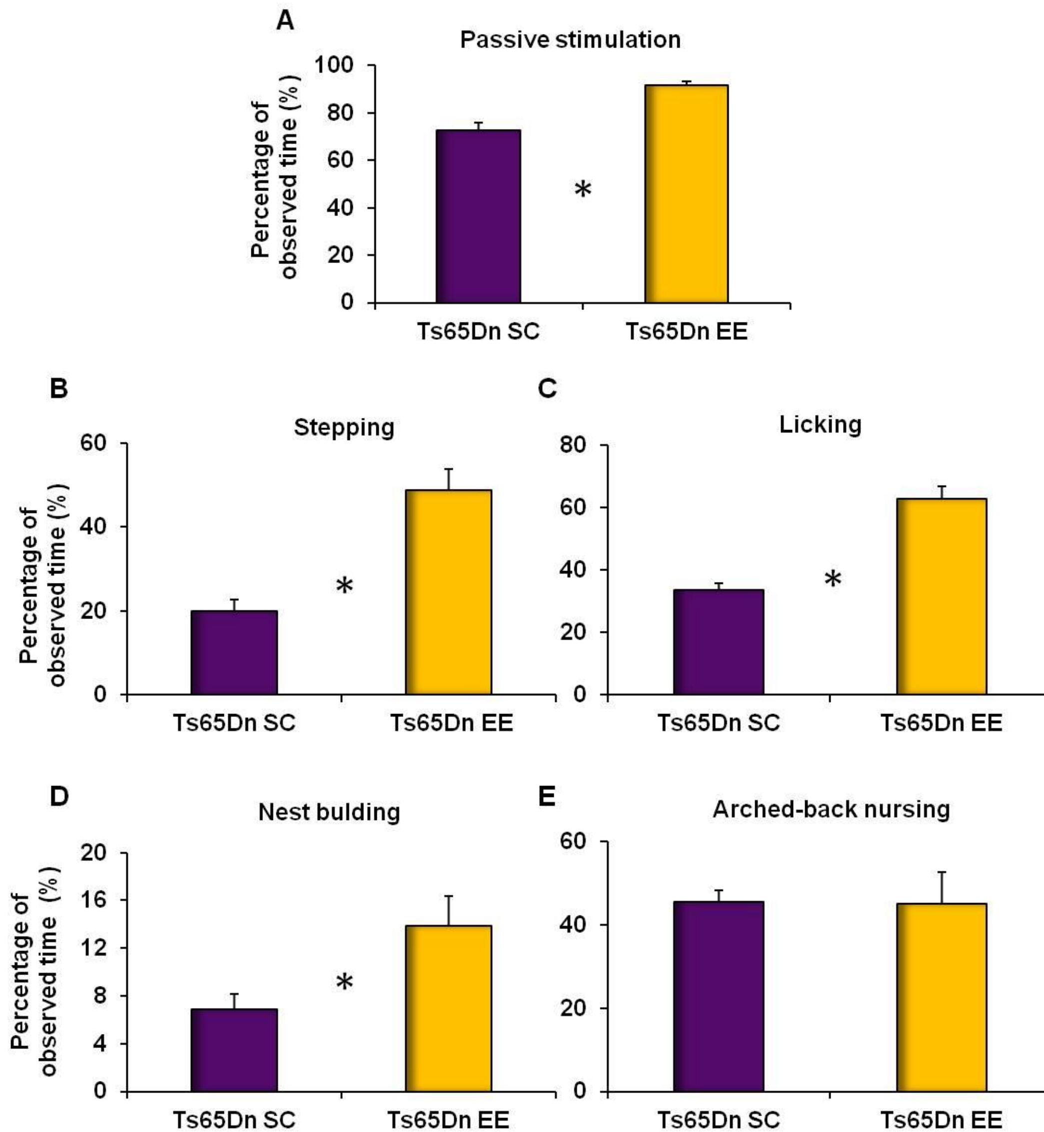
Nest building

For mice, the nest is an important factor in heat and humidity conservation, reproduction and protection from external stimuli. Therefore, the structure of the nest represents a notable

component of the quality of the maternal care. I found that enriched females spent more time in activities aimed at nest consolidation respect to females reared in SC (EE: 13.89 ± 2.44%; SC: 6.85 ± 1.28%) (Figure 13D).

Arched-back nursing

The amount of milk received by pups not only directly affects their early physical development, but may also have long-term effects by influencing adult weight and, in male mice, aggressiveness and their ability to become dominant adults (Latham and Mason, 2004). The availability of milk relies on nursing postures adopted by the mother, with an active form of nursing, such as the so-called arched-back nursing, providing better nourishment for the offspring than more passive forms, like supine and blanket nursing. Quantification of the time that Ts65Dn females spent in arched-back nursing demonstrated no difference between EE (45.22 ± 7.50%) and SC (45.55 ± 2.73%) (figure 13E), indicating that the offspring in both experimental conditions were equally nourished.



13. Environmental enrichment promotes postnatal care in Ts65Dn females. Cumulative frequencies of target behaviour recordings of Ts65Dn females during the first 11 postpartum days raised in SC (violet) and EE (yellow). T-test revealed a significant statistical difference between SC and EE for the following behaviours: passive stimulation ($p < 0.001$) (**A**), stepping ($p < 0.001$) (**B**), licking ($p <0.001$) (**C**) and nest building ($p < 0.05$) (**D**). There was not a difference between the two groups in the time spent in arched-back nursing ($p = 0.965$) (**E**). *, statistical significance; error bars, s.e.m.

5.2.2. Acceleration of the timing of eye-opening in the offspring raised in environmental enrichment

Eye opening is considered to be an important event in the maturation of visual system. Early visual experience influences the formation and strength of visual cortical synapses (Lu and

Constantine-Paton, 2004, Maffei et al., 2004, Wallace and Bear, 2004), the expression of immediate early gene (Tagawa et al., 2005) and the emergence of direction selectivity (Li et al., 2006) in the developing visual system. Therefore, I evaluated a timing of eye opening and found a significant delay in Ts65Dn offspring raised in SC (Ts65Dn-SC, 14.38 ± 0.3, n = 16) compared to WT littermates (WT-SC, 13.65 ± 0.17, n = 26); rearing in EE induced an acceleration of eye opening in both diploid and trisomic offspring, as illustrated by the precocious timing in WT (WT-EE, 12.68 ± 0.17, n = 22) and the compensation of the delay observed in Ts65Dn (Ts65Dn-EE, 13 ± 0.23, n = 16) (Figure 14).

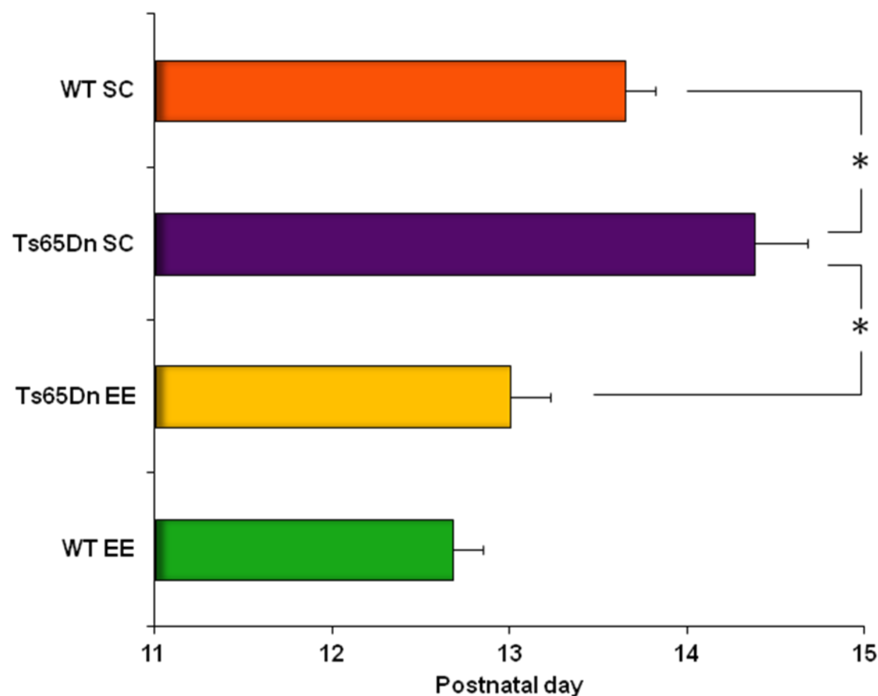


Figure 14. Accelerated timing of eye-opening in pups raised in environmental enrichment. Timing of eye opening in WT-SC (orange), Ts65Dn-SC (violet), Ts65Dn-EE (yellow) and WT-EE (green) offspring . One way ANOVA revealed a statistical difference between the four experimental groups ($p < 0.001$); a multiple comparison procedure (Holm-Sidak method) demonstrated a statistical difference between Ts65Dn-EE and Ts65Dn-SC, Ts65Dn-SC and WT-SC, and WT-SC and WT-EE ($p < 0.05$), but not between WT-SC and Ts65Dn-EE, and Ts65Dn-EE and WT-EE. *, statistical significance; error bars, s.e.m.

5.2.3. Assessment of visual acuity development in Ts65Dn mice

In line with recent data from literature (Scott-McKean et al., 2010), I confirmed that adult Ts65Dn mice display a deficit in VA similar to that observed among individuals with DS; importantly, this deficit was rescued after exposure to EE for six weeks. However, it is not known if this impairment of visual functions represents a consequence of disrupted neurodevelopmental mechanisms, or instead a sign of precocious neurodegenerative processes.

Therefore, I performed an electrophysiological assessment of VA maturation in Ts65Dn and WT offspring reared in both SC and EE at three different time points, P28, P35 and P60. Analysis of electrophysiological recordings of VEPs from V1 revealed an impairment of VA at P35 in Ts65Dn-SC mice respect to WT-SC (P28: WT-SC 0.43 ± 0.01 c/deg, n = 5; Ts65Dn-SC 0.35 ± 0.03 c/deg, n = 5; P35: WT-SC 0.45 ± 0.02 c/deg, n = 8; Ts65Dn-SC 0.3 ± 0.03 c/deg, n = 6; P60: WT-SC 0.58 ± 0.02 c/deg, n = 13; Ts65Dn-SC 0.5 ± 0.03 c/deg, n = 5). VA of enriched trisomic offspring (P28: Ts65Dn-EE 44 ± 0.03 c/deg, n = 9;P35: Ts65Dn-EE 0.44 ± 0.03 c/deg, n = 4; P60: Ts65Dn-EE 0.55 ± 0.13 c/deg, n = 6) did not differ from that of control WT-SC mice at any time point, indicating that EE from birth was able to normalize maturation of VA in Ts65Dn offspring. Euploid mice raised in EE did not differ as well from WT-SC mice at any time point (P28: WT-EE 0.53 ± 0.01, n = 6; P35: WT-EE 0.49 ± 0.02, n = 6; P60: WT-EE 0.52 ± 0.01, n = 9) (Figure 15).

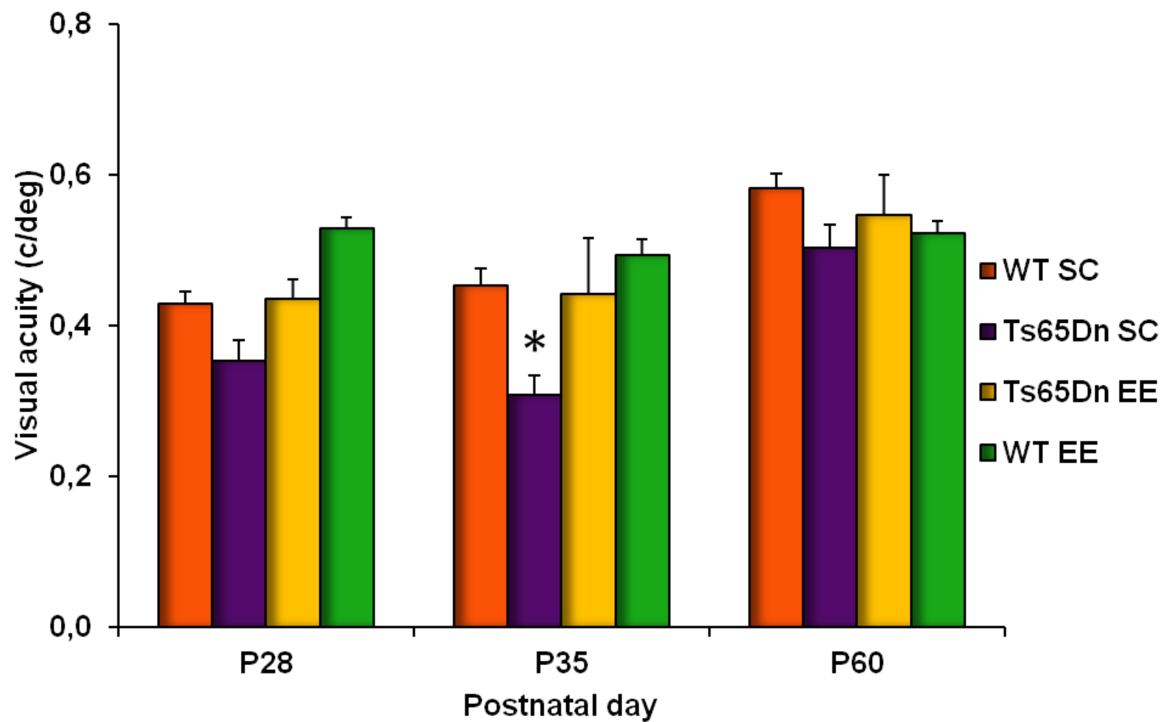


Figure 15. Environmental enrichment from birth prevents the delay in visual acuity maturation in Ts65Dn mice. Evaluation of VA by electrophysiological recordings of VEPs from V1 at P28, P35 and P60 in WT-SC (orange), Ts65Dn-SC (violet), Ts65Dn-EE (yellow) and WT-EE (green) mice. Two way ANOVA revealed a statistical difference between the four experimental groups ($p < 0.001$); a multiple comparison procedure (Tukey test) demonstrated an age-dependent statistical difference: at P35 Ts65Dn-SC mice differed from all other experimental group ($p < 0.05$). *, statistical significance; error bars, s.e.m

CHAPTER 6

DISCUSSION

6.1. Environmental enrichment and enviromimetics: amelioration of neurological phenotypes associated with Down syndrome in adulthood

It has been believed for a long time that the consequences of developmental alterations in neurogenesis, cell migration and neuronal connectivity caused by T21 are irreversible in adulthood and that adult-onset treatment of neurological deficits associated with DS may bring a little benefit for the patients. Recent findings in Ts65Dn mice, a well established mouse model of DS, have suggested that it is possible to rescue DS-related cognitive dysfunction even in adulthood (Ehninger et al., 2008, Silva and Ehninger, 2009, Bartesaghi et al., 2011). I provide new evidence that two therapeutic strategies, EE and pharmacologic treatment with fluoxetine, may be successfully employed to reverse neurophysiological deficits in adult Ts65Dn mice.

6. 1. 1. Effects of environmental enrichment on adult Ts65Dn mice

My results show that EE can be successfully employed to favor recovery from cognitive impairment, synaptic plasticity failure, and visual deficits in adult Ts65Dn mice. Moreover, I also report that levels of GABA release are markedly increased in the hippocampus and in the visual cortex of Ts65Dn mice compared to WT animals, and that exposure to EE reduces the inhibitory transmission, bringing GABA release in the synaptosomes of trisomic mice back to WT control levels. Therefore, my findings show that EE may be particularly well suited to restore healthy levels of neural activity in brain disorders characterized by excessive levels of

inhibition, such as the DS.

It has been demonstrated that a shift in the balance between excitatory and inhibitory neurotransmission to favor the latter compromises the ability of hippocampal circuits to undergo LTP (Kleschevnikov, 2004) and contributes to cognitive deficits in Ts65Dn mice (Fernandez and Garner, 2007, Fernandez et al., 2007). I found that six weeks of EE were sufficient to rescue both LTP in the DG in response to HFS of the perforant path and spatial memory abilities in the MWM test. This is the first evidence that EE in adult Ts65Dn mice can restore long-term synaptic plasticity in a neural circuit critically involved in spatial learning and memory. It has been previously reported that enriched Ts65Dn mice perform better in the MWM task than trisomic mice reared in standard conditions, but this improvement was female-specific (Martinez-Cue et al., 2002). In this previous study, Ts65Dn mice of both sexes were exposed to EE in the form of large social groups, 8-10 mice per cage. Considering that Ts65Dn male mice have a tendency toward social subordination (Martinez-Cue et al., 2006), it is possible that EE with this type of social configuration interfered with learning process in trisomic males. In agreement with this hypothesis, modifying the social component by exposing only two-three Ts65Dn littermates per cage to EE led to an improvement in spatial learning and memory in MWM also in males.

The Ts65Dn model recapitulates not only the cognitive but also the sensory deficits of DS, offering an opportunity to evaluate the effectiveness of the same treatment on sensory functions. In the present work I focused on the visual system and found, in agreement with data published by Scott-McKean et al. (2010), a lower VA in Ts65Dn mice with respect to age-matched WT controls. Moreover, I also discovered, for the first time in this model, a robust increase in VEP latencies and an alteration of OD in the V1 expressed by the C/I VEP ratio. This OD alteration is reminiscent of the effects elicited by ambyopia (Holmes and Clarke, 2006), a condition frequently reported in people with DS (Tsiaras et al., 1999),

indicating that dysfunction of V1 may contribute to the poor vision in DS. This is in line with clinical data showing that the use of corrective lenses in people with DS does not eliminate major part of their visual deficits (John et al., 2004), suggesting that, in addition to anatomical eye abnormalities leading to refractive errors (Shapiro and France, 1985, Liyanage and Barnes, 2008), more subtle functional changes are present at the level of neural connections in the V1. I report a complete reversal of all visual deficits in Ts65Dn mice after adult-onset exposure to EE, providing the first evidence that, in addition to the hippocampus, the beneficial effects of EE are evident also in the visual system of trisomic mice.

To investigate the cellular mechanisms of the observed functional improvements elicited by EE, I analyzed GABA release from hippocampal and visual cortex synaptosomes. I found increased levels of depolarization-evoked overflow of GABA in both the hippocampus and visual cortex of Ts65D mice respect to WT mice. Enhanced levels of inhibition in Ts65Dn mice was first documented in the hippocampus by use of electrophysiological techniques (Kleschevnikov, 2004). Subsequent studies provided also morphological evidence of an imbalance in excitation/inhibition ratio that favors the latter, by showing that immunoreactivity of several proteins associated with GABAergic synapses, such as $GABA_A$ receptor-associated protein (GABARAP), neuroligin 2, and vesicular GABA transporter, was increased in Ts65Dn mice (Belichenko et al., 2009b). These changes were accompanied by changes in the microcircuitry of inhibitory synaptic connections in the DG of Ts65Dn mice (Belichenko et al., 2004). Alterations of excitation/inhibition ratio are also present in the primary somatosensory cortex of Ts65Dn mice, where an increment of GAD67 immunoreactivity was reported (Pérez-Cremades et al., 2010). My data provide new biochemical evidence of excessive GABAergic signaling in the hippocampus of Ts65Dn mice and demonstrate for the first time that an imbalance between inhibition and excitation is also evident in the V1 of trisomic mice, supporting the idea that excessive GABAergic

transmission is a general property of the Ts65Dn brain. EE-induced lowering of GABAergic transmission in the hippocampus could directly contribute to the rescue of LTP in enriched Ts65Dn mice, as a pharmacological reduction of the inhibitory tone was found to reverse synaptic plasticity deficits in the DG of trisomic mice (Kleschevnikov, 2004, Fernandez et al., 2007).

Together with a reduction in GABA signaling, other mechanisms could contribute to the EE-induced amelioration of cognitive and sensory deficits in Ts65Dn mice. Decreased neural stem cell proliferation rate and reduced neurogenesis during Ts65Dn forebrain development are thought to be among the key mechanisms at the basis of cognitive deficits in this mouse (Chakrabarti et al., 2007). Emerging evidence indicates that altered adult neurogenesis could also contribute to poor cognitive performance in Ts65Dn mice (Clark et al., 2006, Bianchi et al., 2010a, Bianchi et al., 2010b, Chakrabarti et al., 2011). EE paradigm has been shown to enhance adult hippocampal neurogenesis and learning ability in healthy rodents (Kempermann et al., 1998, Llorens-Martin et al., 2007) and recently the same effect of EE on postnatal neurogenesis has been reported in Ts65Dn mice (Chakrabarti et al., 2011). Therefore, I cannot rule out the possibility that the normalization of hippocampal function that I found in enriched Ts65Dn mice was facilitated by enhancement of adult neurogenesis.

In adult rodents, EE is also able to enhance the synthesis and secretion of BDNF (Sale et al., 2007b, Sale et al., 2009, Spolidoro et al., 2009, Sale et al., 2010), a key regulator of neural circuit development and function (Park and Poo, 2013). The expression, secretion and actions of BDNF are directly influenced by neural activity, while secreted BDNF is capable of mediating many activity-dependent processes in the mammalian brain, including synapse plasticity and higher cognitive functions (Park and Poo, 2013). Ts65Dn mice have defective expression of BDNF in the hippocampus (Bianchi et al., 2010b, Fukuda et al., 2010) and the frontal cortex (Bimonte-Nelson et al., 2003) that could contribute to cognitive dysfunction in

this DS model. Thus, it is possible that EE in my study also restored BDNF levels, facilitating plastic brain mechanisms and promoting the reorganization of affected neural circuits in the mature Ts65Dn brain.

EE also increases the expression of NGF in WT rodents (Torasdotter et al., 1998, Pham et al., 1999b, Ickes et al., 2000). NGF is a neurotrophic factor that enhances the survival, differentiation and function of specific neurons of the peripheral and central nervous system during development, adult life and aging (Sofroniew et al., 2001). Moreover, it plays a role in the events of degeneration and repair of the nervous system in diseases with different etiologies, such as neurodegenerative disorders (Tirassa et al., 2002). In adult Ts65Dn mice, an abnormal retrograde transport of NGF has been associated with a progressive age-dependent degeneration of BFCNs and a deterioration of learning and memory processes (Cooper et al., 2001, Hyde and Crnic, 2001, Hunter et al., 2003). Recently it has been shown that there is a deficit in the hippocampal NGF expression also in the early postnatal period (Bianchi et al., 2010b), that may contribute to a poor cognitive performance of young adult trisomic mice. Therefore, I could expect that the improvement of the hippocampal function and cognitive abilities that I found in young adult Ts65Dn mice after 6 weeks of EE was accompanied by the normalization of the NGF levels.

It remains unclear to what extent every single component of EE contributes to its positive behavioral outcomes observed in this model of DS. A recently published paper has provided some insights into this area reporting that Ts65Dn females take advantage of sustained wheel running (Kida et al., 2013). However, in this study cognitive functions were improved only in mice that started physical activity early in life at weaning, while a cohort with free access to the wheel from 7 months of age did not display any cognitive benefits.

6. 1. 2. Effects of fluoxetine treatment on adult Ts65Dn mice

In addition to EE, the antidepressant fluoxetine has been also shown to enhance neuronal plasticity in adult rodents. My findings show that fluoxetine can be safely and successfully employed to favor recovery from cognitive impairment and synaptic plasticity failure in adult Ts65Dn mice. Importantly, these beneficial effects were accompanied by normalization of GABA signaling, indicating that fluoxetine administration could be a good pharmacological alternative to cope with excessive levels of inhibition that are believed to be at the basis of DS associated neurological phenotypes (Fernandez and Garner, 2007).

A recent study reported that a high-dose fluoxetine treatment did not rescue the behavioral impairments of Ts65Dn mice, but elicited severe side effects, including seizures and death (Heinen et al., 2012). Seizures are not among frequent side effects of fluoxetine administration in general clinical populations, but they could be observed in the case of fluoxetine intoxication (Prasher, 1993, Rosenstein et al., 1993, Oke et al., 2001, Suchard, 2008). The fluoxetine dosing regimen followed by Heinen et al. (2012) corresponds to about 80 mg/kg/day, i.e. a dose even two time higher than that at which I found a significant number of death and seizure cases. While this high-dosing scheme has proved safe and successful to promote neural plasticity in the rat (Maya Vetencourt et al., 2008), it could lower seizure threshold in Ts65Dn mice, that are more prone to develop epileptic attacks (Westmark et al., 2010).

Before the onset of treatment, I reported a dose-response relationship of fluoxetine administration with respect to the adverse effects in Ts65Dn mice and found that death and convulsions could be completely avoided when the dosage was significantly decreased. Therefore, my data suggest that fluoxetine side effects are dose-dependent and provide evidence that assumption of ~10 mg/kg/day is an optimal dose to achieve a good benefit/risk

balance in Ts65Dn mice. In agreement with my data, it has been recently reported that the same low dose of fluoxetine increases neural plasticity in the mouse neural circuit involved in fear memory (Karpova et al., 2011). Since it has been previously reported that this protocol of chronic fluoxetine administration results in fluoxetine plasma levels within the therapeutic range in humans (Raju et al., 1999, Karpova et al., 2011), together these results encourage further studies aimed at investigating the applicability of fluoxetine to people with DS.

I started the evaluation of fluoxetine treatment on adult Ts65Dn mice with the MWM test. My results confirmed a well established impairment of spatial learning and memory in non treated Ts65Dn mice. In line with data from the literature, this deficit was associated with abnormal LTP expression at the CA3-CA1 synapses. While there was no evident improvement in MWM performance in fluoxetine treated Ts65Dn animals, LTP expression was completely restored, indicating that fluoxetine treatment favoured synaptic plasticity in trisomic mice. Evidence exists that MWM performance can be impaired when LTP is preserved, and it has been also shown that a normal MWM performance may be possible even in animals in which LTP processes have been blocked (Cain et al., 1997, D'Hooge and De Deyn, 2001). It is important to note that the MWM is a challenging task for rodents, employing a variety of complex mnemonic processes. These processes encompass the acquisition and spatial localization of relevant visual cues that are subsequently processed, consolidated, retained, and then retrieved in order to successfully navigate and thereby locate a hidden platform to escape the water (Morris, 1984, McNamara and Skelton, 1993, Terry, 2009).

The hippocampus has been implicated as an essential structure for place learning in MWM, but other brain structures like basal forebrain, striatum, cerebellum and cerebral cortex were shown to be involved in MWM performance as well (D'Hooge and De Deyn, 2001). Moreover, spatial learning in general and MWM performance in particular seem to be

dependent on the coordinate action of these different brain regions. Thus, dysfunction or disconnection of any brain structure that contribute to successful spatial navigation may compromise the ability of animals to complete the MWM task. Importantly, in DS and Ts65Dn mice almost all neural regions involved in spatial learning and memory are affected by trisomy. Altered neural development results in aberrant formation of hippocampus (Lorenzi and Reeves, 2006, Bianchi et al., 2010b) and cerebellum (Roper et al., 2006), which probably leads to poor MWM performance in young adult Ts65Dn mice, while precocious basal forebrain neuronal degeneration typically occurring in this model may accelerate age-dependent decline of performance in this task. Additionally, clinical data from DS patients indicated altered prefrontal information processing (Rowe et al., 2006). It could be expected that a similar prefrontal dysfunction provoked by trisomy in Ts65Dn mice contribute to their poor MWM performance, as observed in rats with lesions of the prefrontal cortex that display impaired hidden-platform acquisition (Mogensen et al., 1995). Thus, my results suggest that the beneficial effects of fluoxetine on hippocampal function, here illustrated by rescue of LTP at CA3-CA1 synapses, are not powerful enough to compensate for all structural and functional deficits that may lead to ineffective spatial navigation and impaired MWM performance in this model of DS.

On the other hand, I found that chronic fluoxetine treatment had beneficial effects on performance of Ts65Dn mice in a novel place recognition test, a hippocampus-based cognitive task less challenging than MWM. This cognitive paradigm evaluates the ability of mice to discriminate an old versus a novel position of the familiar object. While the role of the hippocampus in recognition memory is still controversial (Bussey et al., 2000, Clark et al., 2000, Clark et al., 2001, Mumby et al., 2002, Winters et al., 2004, Forwood et al., 2005, Good et al., 2007, Langston and Wood, 2010), the hippocampus appears to be critical under conditions in which recognition memory relies on spatial orientation during the task (Kesner

and Novak, 1982, Chiba et al., 1994, Bussey et al., 2000, Fortin et al., 2002).). Since it has been demonstrated that dependence from the hippocampus in the novel place recognition test is particularly strong for short retention periods (Jessberger et al., 2009), I evaluated the effects of chronic fluoxetine treatment on short-term memory for place recognition in Ts65Dn mice. While I observed a total inability of Ts65Dn mice to distinguish an old versus a novel position of familiarized object, fluoxetine treated Ts65Dn mice performed as well as WT control group, demonstrating that 8 weeks of fluoxetine administration were sufficient to improve spatial orientation and to rescue the short-term recognition memory deficit of Ts65Dn mice in a task critically dependent on the hippocampal integrity.

I also report that GABA release, markedly increased in the hippocampus of Ts65Dn mice compared to euploid animals, was brought back to normal WT control levels after 8 weeks of chronic treatment with fluoxetine. Costa and Grybco (2005) showed that LTP deficits in CA3-CA1 hippocampal circuits can be rescued by bath application of picrotoxin, a noncompetitive antagonist for $GABA_A$ receptor chloride channels, indicating that increased $GABA_A$-mediated inhibition in Ts65Dn mice may underlie the observed deficits. Thus, it is possible that fluoxetine-induced reduction of excessive GABAergic inhibition in the hippocampus of Ts65Dn mice enables normal excitatory/inhibitory ratio and abolishes LTP abnormalities at CA3-CA1 synapses. This is in line with the assumption that LTP mechanisms are actually intact in Ts65Dn mice, but that LTP is impaired by a shift in the excitation–inhibition ratio of the synaptic transmission evoked during electrical stimulation (Kleschevnikov, 2004, Costa and Grybko, 2005, Hanson et al., 2007).

Fluoxetine might elicit a reduction of GABA release by increasing brain 5-HT levels. Consistent with this hypothesis, it has been reported that 5-HT inhibits GABA release in interneuron subtypes of several brain regions via a presynaptic mechanism mediated by 5-HT1/2 receptor families (Koyama et al., 1999, Xiang and Prince, 2003), probably regulating

the availability of transmitter vesicles (Wang and Zucker, 1998). These results are in good agreement with data obtained from adult rats exposed to either EE conditions or to fluoxetine administration. In both models, it has been shown that an increase in 5-HT levels is accompanied by a reduced brain GABA extracellular release (Sale et al., 2007b, Maya Vetencourt et al., 2008, Baroncelli et al., 2010b).

Even if I focused on GABA release, I cannot exclude the possibility that fluoxetine might improve memory abilities and synaptic plasticity in Ts65Dn mice also via other mechanisms. Similarly to EE, chronic fluoxetine treatment increases the expression of the neurotrophin BDNF in limbic structures, most notably in the hippocampus (Nibuya et al., 1995, D'Sa and Duman, 2002). It is important to note that BDNF plays an important role in synaptic plasticity as it activates distinct mechanisms to regulate the induction, early maintenance, and late maintenance phases of LTP (Bramham and Messaoudi, 2005). For example, genetic deletion of BDNF in mice disrupted normal induction of LTP in the CA1 region of the hippocampus (Korte et al., 1995, Xie et al., 2000), and this defect was rescued by reintroducing BDNF (Korte et al., 1996, Patterson et al., 1996). Moreover, chelation of endogenously secreted BDNF with TrkB–IgG or using antibodies against BDNF reduces LTP (Figurov et al., 1996, Kang et al., 1997). As mentioned in the previous chapter, Ts65Dn mice have defective expression of BDNF in the hippocampus (Bianchi et al., 2010b, Fukuda et al., 2010). Bianchi et al. (2010) showed that, in young Ts65Dn mice, this deficit could be successfully treated by fluoxetine administration, bringing back BDNF to normal WT control levels. Therefore, it is likely that fluoxetine results in a similar upregulation of BDNF expression also in adult Ts65Dn mice, facilitating LTP induction at CA3-CA1 synapses.

6.2. Developmental impact of environmental enrichment in Ts65Dn mice

Studies in mouse models of DS have enabled a better understanding of aberrant molecular and cellular mechanisms underlying deficits in the mature and aging nervous system. However, it is still unclear to what extent developmental alterations induced by trisomy contribute to these deficits observed in adulthood. In order to address this question, I focused on the developing visual system in Ts65Dn mice. Understanding this system is of particular relevance to individuals with DS who, in addition to having ocular abnormalities including strabismus, astigmatism, anisometropia and inaccurate accommodation (da Cunha and Moreira, 1996, Wong and Ho, 1997, Woodhouse et al., 1997, Cregg et al., 2001, Haugen and Hovding, 2001), have also lower VA and contrast sensitivity (John et al., 2004, Suttle and Turner, 2004). Moreover, the maturation of visual system is a good model to evaluate the impact of enhanced environmental stimulation on central nervous system development. It has been shown in normally developing mice that rearing from birth in EE induces functional and anatomical changes leading to an acceleration of visual system development that is evident at the behavioral, electrophysiological and molecular level (Cancedda et al., 2004, Sale et al., 2004, Landi et al., 2007a). My findings provide initial evidence that increased multisensory stimulation provided by pre-weaning EE could be employed as a therapeutic strategy to counteract aberrant development of CNS in Ts65Dn mice.

6.2.1. Maternal effects of environmental enrichment in Ts65Dn mice

Maternal care is one of the most important sources of sensory experience during the early postnatal period (Hofer, 1984, Ronca et al., 1993, Liu et al., 2000) and it plays an important role in the regulation of nervous system development, with long-term consequences on brain functioning in adulthood (Fleming et al., 1997, Meaney and Szyf, 2005, Champagne et al.,

2008). Here I provide the first evidence that EE is able to enhance maternal care in Ts65Dn mothers, leading to increased sensory stimulation of trisomic pups during this early phase of development.

Ts65Dn females have been characterized as poor mothers since they express low levels of maternal care, frequently abandoning their litters (Moore et al., 2010). In the rodent maternal care model, naturally-occurring variations in the amount of maternal licking and grooming during the first days of life have been associated with individual differences in cognitive functioning, emotionality and stress resilience in adult offspring (Liu et al., 1997, Liu et al., 2000, Bredy et al., 2003, Weaver et al., 2005). Pups that experience a high level of maternal care exhibit greater spatial learning and memory performances in adulthood (Liu et al., 2000, Bredy et al., 2003), and reduced anxiety and stress responsiveness (Liu et al., 1997). On the other hand, the offspring of mothers who exhibit reduced maternal care are more susceptible to stress-related disease (Liu et al., 1997), enter puberty earlier and show altered sexual behavior (Cameron et al., 2008). Therefore, it could be hypothesized that poor maternal care displayed by Ts65Dn females could additionally compromise DS associated phenotypes in pups carrying trisomy.

Rearing in EE was able to optimize levels of maternal stimulation received by pups, resulting in higher levels of maternal care expressed by enriched Ts65Dn females compared to standard-reared animals. I found increased levels of licking and stepping received by enriched pups during the first eleven postnatal days. The enhancement of these two active forms of sensory stimulation was associated with an increased presence of the mother or other females in the nest, resulting in a strong tactile stimulation in enriched conditions. Compared to standard-reared trisomic mothers, enriched Ts65Dn females also spent more time in activities aimed at nest building and nest consolidation, which can lead to a more protective ambient for the growth and development of the offspring. Interestingly, I observed

no difference in the time spent in arched-back nursing between EE and SC, indicating that the offspring in both experimental conditions received similar amounts of milk.

My findings support the hypothesis that increased maternal care is a key mediator of the effects of EE on developing pups (Sale et al., 2004). Living in an EE setting provides intense maternal touch as an alternative source of environmental stimulation during the first days of rodent life, the period characterized with a lack of direct interaction with the surroundings. The effects of EE on the offspring development were reproduced in standard-reared rat pups that were exposed to a daily massage, a procedure that mimics active forms of maternal tactile stimulation, during their first ten postnatal days of life (Guzzetta et al., 2009). Importantly, it has been previously shown that this protocol is able to compensate for the deleterious effects of long-lasting maternal deprivation (Schanberg and Field, 1987, Burton et al., 2007, Chatterjee et al., 2007).

It would be important to evaluate in coming studies the extent to which different environmental conditions affect the physiology of pregnant Ts65Dn females. Placental exchanges from the mother to the fetus provide the offspring with respiratory gases, nutrients and hormones necessary for intrauterine fetal growth and development (Anthony et al., 1995). It is expected that any modification of maternal physiology induced by environmental factors would alter the capacity of placenta to deliver supplies to fetus. Indeed, in both humans and rodents, exposure of pregnant females to stress induces unfavourable physiological response to pregnancy, leading to physical and mental health problems in the offspring that could be manifested at different stages of life (Beydoun and Saftlas, 2008). On the other hand, it has been shown that exposure of female rats to EE for the entire length of gestation is able to modulate dynamics of IGF-1 (Sale et al., 2007a). IGF-1 is a circulating polypeptide structurally related to insulin that mediates many of the effects of growth hormone (GH) in the body (Jones and Clemmons, 1995) and plays also an important role in

the developing brain (Anlar et al., 1999). Sale et al. (2007a) proposed an interesting model in which sustained physical exercise during pregnancy increases IGF-I in the mother, promoting placental transfer of nutrients to the fetus; this would in turn lead to increased amounts of IGF-I autonomously produced by the fetus, resulting in the accelerated development of nervous system. Therefore, it is possible that exposing Ts65Dn females to EE during pregnancy may influence the development of trisomic fetuses in a similar manner.

6.2.2. Influence of pre-weaning onset of environmental enrichment on visual system development in Ts65Dn mice

It has been shown that EE exerts positive effects on experience-dependent plasticity processes involved in neural wiring and refinement of the visual cortex during critical periods in normally developing pups (Cancedda et al., 2004, Landi et al., 2007a). Here I report that pre-weaning EE is able to influence neural development also under pathological conditions, promoting visual system maturation in enriched Ts65Dn offspring.

In normally developing mice, eye opening occurs around P10 – P13 (Smith and Trachtenberg, 2007). In my study diploid control mice reared in SC opened their eyes within this expected time window, while their trisomic littermates displayed a delay in eye opening. Given that the timing of eye opening corresponds closely to that of the development of other epidermal structures in the body (Maconnachie, 1979, Findlater et al., 1993), its delay in Ts65Dn pups is an indicator of the more general retardation in their physical growth and maturation. This deficit is in line with previously reported delays in the achievement of certain physical and sensorimotor milestones observed in both Ts65Dn pups (Holtzman et al., 1996) and infants and children with DS (Hayes and Batshaw, 1993). It is important to note that before eye-opening neuronal activities within the visual cortex are generated mainly

intrinsically, while after it sensory inputs from eyes reach the visual cortex and modulate the pattern of network activity (Rochefort et al., 2009) . Therefore, a delay in eye opening can negatively influence the maturation of the visual cortex.

The progressive increase of VA is a sensitive parameter of visual system maturation. For the first time in literature my data suggest that this process is altered in Ts65Dn pups. While WT mice reared in SC showed linear progression of VA with time, this was not the case in standard-reared Ts65Dn offspring, which even displayed a drop of function at P35. Importantly, at P60 Ts65Dn mice reached adult-like VA value typical of WT controls. Therefore, despite a non linear maturation of VA, it seems that the visual system of trisomic mice is able to compensate this deficit at the end of developmental period. In agreement with findings reported by Scott-McKean et al. (2010), my data obtained from adult Ts65Dn mice demonstrate that VA impairment emerges again later in life, at about 4 months of age. Although these findings may appear discrepant at first sight, they actually suggest the possibility that the VA deficit observed in older adult animals could be rather a sign of precocious neurodegenerative processes than the direct outcome of aberrant neural development.

I demonstrated that rearing in EE promotes visual system maturation in both Ts65Dn and WT mice. EE from birth was able to compensate the delay of eye opening in Ts65Dn offspring. Moreover, VA of enriched trisomic mice was comparable with that of standard-reared WT littermates at all investigated developmental stages. In line with data form literature (Cancedda et al., 2004, Sale et al., 2004), in my study enrichment exerted profound effects on WT animals as well, leading to a precocious eye opening and anticipated maturation of VA.

Molecular and cellular mechanisms at the basis of the positive effects of EE on visual system development in Ts65Dn mice remain to be elucidated. Some of the mechanisms

underlying the accelerated maturation of visual system in enriched euploid animals (Cancedda et al., 2004, Sale et al., 2004) could be activated as well by EE during early developmental phase in trisomic mice. One of the key molecules involved in the control of visual cortical development during a critical period early in life is the neurotrophin BDNF (Berardi et al., 2003). Reduction of BDNF levels have been already reported in the hippocampus of Ts65Dn mice at P15 (Bianchi et al., 2010b), thus it is possible that a similar deficit exist also in the visual cortex. Given that acceleration of visual system development in WT mice is accompanied by increased levels of BDNF (Cancedda et al., 2004, Sale et al., 2004), a similar BDNF response to EE could be expected also in Ts65Dn mice. Interestingly, the enrichment-induced enhancement of BDNF in WT animals is evident already at P7, i.e. even before eye opening (Cancedda et al., 2004, Sale et al., 2004), supporting the hypothesis that different levels of maternal care in enriched condition could induce the acceleration of eye opening in Ts65Dn mice.

Another molecule that has a key role in visual development and responds to EE paradigm is IGF-I. It has been shown that IGF-I expression is increased at P18 in the visual cortex of rats raised in EE compared to standard-reared animals. Moreover, exogenous IGF-I supply mimics, whereas blocking IGF-I action prevents, the effects of EE on VA maturation (Ciucci et al., 2007). Importantly, initial clinical evidence exists that IGF-1 could also be involved in the effects of early EE on human brain. Guzzetta et al. (2009) reported that accelerated VA maturation in healthy pre-term infants exposed to massage therapy, a form of early EE, was associated with increased levels of plasma IGF-1. So far, the role of IGF-1 in the pathophysiology of DS has been studied only in the context of growth retardation. Growth retardation is a typical feature of DS and it becomes evident in infants from the age of 6 months, when GH starts to regulate growth (Anneren et al., 1990). While serum levels of GH in DS children were normal, serum IGF-1 levels did not rise during childhood, as occurs

in normally developing children, and remained at a low level throughout life (Anneren et al., 1990). Considering the relevance of IGF-1 for CNS development (Anlar et al., 1999) and the wide spectrum of its effects on the adult brain (Torres-Aleman, 1999), it would be important to investigate whether and to what extent this deficit in circulating levels of IGF-1 influence DS neural phenotypes during different stages of life.

6.3. Conclusions

Major findings from animal models suggest that a number of behavioral, cognitive and neurological symptoms associated with neurodevelopmental and neurodegenerative disorders may be ameliorated or reduced with exposure to enriched environments, likely via mechanisms of experience dependent neural plasticity

One may argue that today individuals with DS are already highly stimulated. Indeed, in developed countries children with DS almost always undergo a sensory integration therapy that, conceptualized as an active process in which the child engages with the physical environment to promote neurological change, shares several key principles of EE (Reynolds et al., 2010). However, in the past few decades improved medical assistance caused a marked prolongation of life expectancy in people with DS, who now may also outlive their parents, leading to an emerging risk of social and cognitive impoverishment of this population in older age. Moreover, there is a high prevalence of depression in young adult DS individuals that may lead to additional decline in adaptive behavior and cognitive performance during mid and late adulthood (Burt et al., 1992). Therefore, intervention paradigms based on exposure of adult subjects to novel stimuli and learning experiences alone, or in a combination with antidepressants such as fluoxetine, may be well suited to prevent additional cognitive deterioration in aging individuals with DS.

On the other hand, the early intervention programs of newborns with DS could also benefit from translational research. In parallel to the effects obtained in massaged rats, it has been reported that enriching the environment in terms of body massage during the first months of life accelerates development of some brain functions, such as faster reduction in the latency of VEPs and an increase in behavioral visual acuity, in healthy pre-term infants (Guzzetta et al., 2009). An ongoing clinical study undertaken by our collaborators at the Stella Maris Scientific Institute (Calambrone, Pisa, Italy) evaluates the effects of a similar protocol of infant massage on brain development in DS neonates.

REFERENCES

Acler M, Robol E, Fiaschi A, Manganotti P (2009) A double blind placebo RCT to investigate the effects of serotonergic modulation on brain excitability and motor recovery in stroke patients. Journal of neurology 256:1152-1158.

Altafaj X, Dierssen M, Baamonde C, Marti E, Visa J, Guimera J, Oset M, Gonzalez JR, Florez J, Fillat C, Estivill X (2001) Neurodevelopmental delay, motor abnormalities and cognitive deficits in transgenic mice overexpressing Dyrk1A (minibrain), a murine model of Down's syndrome. Hum Mol Genet 10:1915-1923.

Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY (1999) Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nature genetics 23:185-188.

Anagnostaras SG, Maren S, Sage JR, Goodrich S, Fanselow MS (1999) Scopolamine and Pavlovian fear conditioning in rats: dose-effect analysis. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 21:731-744.

Anlar B, Sullivan KA, Feldman EL (1999) Insulin-like growth factor-I and central nervous system development. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 31:120-125.

Anneren G, Gustavson KH, Sara VR, Tuvemo T (1990) Growth retardation in Down syndrome in relation to insulin-like growth factors and growth hormone. American journal of medical genetics Supplement 7:59-62.

Anthony RV, Pratt SL, Liang R, Holland MD (1995) Placental-fetal hormonal interactions: impact on fetal growth. Journal of animal science 73:1861-1871.

Antonarakis SE, Epstein CJ (2006) The challenge of Down syndrome. Trends in Molecular Medicine 12:473-479.

Antonarakis SE, Lyle R, Chrast R, Scott HS (2001) Differential gene expression studies to explore the molecular pathophysiology of Down syndrome. Brain research Brain research reviews 36:265-274.

Antonarakis SE, Lyle R, Dermitzakis ET, Reymond A, Deutsch S (2004) Chromosome 21 and down syndrome: from genomics to pathophysiology. Nature reviews Genetics 5:725-738.

Arendash GW, Garcia MF, Costa DA, Cracchiolo JR, Wefes IM, Potter H (2004) Environmental enrichment improves cognition in aged Alzheimer's transgenic mice despite stable beta-amyloid deposition. Neuroreport 15:1751-1754.

Asaka Y, Jugloff DG, Zhang L, Eubanks JH, Fitzsimonds RM (2006) Hippocampal synaptic plasticity is impaired in the Mecp2-null mouse model of Rett syndrome. Neurobiol Dis 21:217-227.

Asberg M, Thoren P, Traskman L, Bertilsson L, Ringberger V (1976) "Serotonin depression"--a biochemical subgroup within the affective disorders? Science 191:478-480.

Aylward EH, Li Q, Honeycutt NA, Warren AC, Pulsifer MB, Barta PE, Chan MD, Smith PD, Jerram M, Pearlson GD (1999) MRI volumes of the hippocampus and amygdala in adults with Down's syndrome with and without dementia. The American journal of psychiatry 156:564-568.

Bagetta V, Ghiglieri V, Sgobio C, Calabresi P, Picconi B (2010) Synaptic dysfunction in Parkinson's disease. Biochemical Society transactions 38:493-497.

Ball MJ, Nuttall K (1980) Neurofibrillary tangles, granulovacuolar degeneration, and neuron loss in Down Syndrome: quantitative comparison with Alzheimer dementia. Annals of neurology 7:462-465.

Ball SL, Holland AJ, Hon J, Huppert FA, Treppner P, Watson PC (2006) Personality and behaviour changes mark the early stages of Alzheimer's disease in adults with Down's syndrome: findings from a prospective population-based study. International journal of geriatric psychiatry 21:661-673.

Ball SL, Holland AJ, Treppner P, Watson PC, Huppert FA (2008) Executive dysfunction and its association with personality and behaviour changes in the development of Alzheimer's disease in adults with Down syndrome and mild to moderate learning disabilities. The British journal of clinical psychology / the British Psychological Society 47:1-29.

Baloyannis SJ (2009) Dendritic pathology in Alzheimer's disease. Journal of the neurological sciences 283:153-157.

Bar-Peled O, Gross-Isseroff R, Ben-Hur H, Hoskins I, Groner Y, Biegon A (1991) Fetal human brain exhibits a prenatal peak in the density of serotonin 5-HT1A receptors. Neurosci Lett 127:173-176.

Baroncelli L, Braschi C, Spolidoro M, Begenisic T, Maffei L, Sale A (2011) Brain plasticity and disease: a matter of inhibition. Neural Plast 2011:286073.

Baroncelli L, Braschi C, Spolidoro M, Begenisic T, Sale A, Maffei L (2010a) Nurturing brain plasticity: impact of environmental enrichment. Cell death and differentiation 17:1092-1103.

Baroncelli L, Sale A, Viegi A, Maya Vetencourt JF, De Pasquale R, Baldini S, Maffei L (2010b) Experience-dependent reactivation of ocular dominance plasticity in the adult visual cortex. Exp Neurol 226:100-109.

Bartesaghi R, Guidi S, Ciani E (2011) Is it possible to improve neurodevelopmental abnormalities in Down syndrome? Reviews in the Neurosciences 22.

Bartoletti A, Medini P, Berardi N, Maffei L (2004) Environmental enrichment prevents effects of dark-rearing in the rat visual cortex. Nat Neurosci 7:215-216.

Battaglia F, Quartarone A, Rizzo V, Ghilardi MF, Di Rocco A, Tortorella G, Girlanda P (2008) Early impairment of synaptic plasticity in patients with Down's syndrome. Neurobiol Aging 29:1272-1275.

Battaglia F, Wang HY, Ghilardi MF, Gashi E, Quartarone A, Friedman E, Nixon RA (2007) Cortical plasticity in Alzheimer's disease in humans and rodents. Biol Psychiatry 62:1405-1412.

Baxter LL, Moran TH, Richtsmeier JT, Troncoso J, Reeves RH (2000) Discovery and genetic localization of Down syndrome cerebellar phenotypes using the Ts65Dn mouse. Hum Mol Genet 9:195-202.

Becker L, Mito T, Takashima S, Onodera K (1991) Growth and development of the brain in Down syndrome. Progress in clinical and biological research 373:133-152.

Becker LE, Armstrong DL, Chan F (1986) Dendritic atrophy in children with Down's syndrome. Annals of neurology 20:520-526.

Belichenko NP, Belichenko PV, Kleschevnikov AM, Salehi A, Reeves RH, Mobley WC (2009a) The "Down Syndrome Critical Region" Is Sufficient in the Mouse Model to Confer Behavioral, Neurophysiological, and Synaptic Phenotypes Characteristic of Down Syndrome. Journal of Neuroscience 29:5938-5948.

Belichenko PV, Kleschevnikov AM, Masliah E, Wu C, Takimoto-Kimura R, Salehi A, Mobley WC (2009b) Excitatory-inhibitory relationship in the fascia dentata in the Ts65Dn mouse model of down syndrome. The Journal of Comparative Neurology 512:453-466.

Belichenko PV, Kleschevnikov AM, Salehi A, Epstein CJ, Mobley WC (2007) Synaptic and cognitive abnormalities in mouse models of down syndrome: Exploring genotype-phenotype relationships. The Journal of Comparative Neurology 504:329-345.

Belichenko PV, Masliah E, Kleschevnikov AM, Villar AJ, Epstein CJ, Salehi A, Mobley WC (2004) Synaptic structural abnormalities in the Ts65Dn mouse model of Down Syndrome. J Comp Neurol 480:281-298.

Benevento LA, Bakkum BW, Port JD, Cohen RS (1992) The effects of dark-rearing on the electrophysiology of the rat visual cortex. Brain Res 572:198-207.

Bennett JC, McRae PA, Levy LJ, Frick KM (2006) Long-term continuous, but not daily, environmental enrichment reduces spatial memory decline in aged male mice. Neurobiology of learning and memory 85:139-152.

Berardi N, Pizzorusso T, Maffei L (2000) Critical periods during sensory development. Curr Opin Neurobiol 10:138-145.

Berardi N, Pizzorusso T, Ratto GM, Maffei L (2003) Molecular basis of plasticity in the visual cortex. Trends Neurosci 26:369-378.

Best TK, Cramer NP, Chakrabarti L, Haydar TF, Galdzicki Z (2012) Dysfunctional hippocampal inhibition in the Ts65Dn mouse model of Down syndrome. Experimental Neurology 233:749-757.

Best TK, Siarey RJ, Galdzicki Z (2007) Ts65Dn, a Mouse Model of Down Syndrome, Exhibits Increased GABAB-Induced Potassium Current. Journal of Neurophysiology 97:892-900.

Beydoun H, Saftlas AF (2008) Physical and mental health outcomes of prenatal maternal stress in human and animal studies: a review of recent evidence. Paediatric and perinatal epidemiology 22:438-466.

Bhattacharyya A, McMillan E, Chen SI, Wallace K, Svendsen CN (2009) A critical period in cortical interneuron neurogenesis in down syndrome revealed by human neural progenitor cells. Dev Neurosci 31:497-510.

Bianchi P, Ciani E, Contestabile A, Guidi S, Bartesaghi R (2010a) Lithium Restores Neurogenesis in the Subventricular Zone of the Ts65Dn Mouse, a Model for Down Syndrome. Brain Pathology 20:106-118.

Bianchi P, Ciani E, Guidi S, Trazzi S, Felice D, Grossi G, Fernandez M, Giuliani A, Calza L, Bartesaghi R (2010b) Early pharmacotherapy restores neurogenesis and cognitive

performance in the Ts65Dn mouse model for Down syndrome. The Journal of neuroscience : the official journal of the Society for Neuroscience 30:8769-8779.

Bick-Sander A, Steiner B, Wolf SA, Babu H, Kempermann G (2006) Running in pregnancy transiently increases postnatal hippocampal neurogenesis in the offspring. Proceedings of the National Academy of Sciences of the United States of America 103:3852-3857.

Bimonte-Nelson HA, Hunter CL, Nelson ME, Granholm A-CE (2003) Frontal cortex BDNF levels correlate with working memory in an animal model of Down syndrome. Behavioural Brain Research 139:47-57.

Bittles AH, Bower C, Hussain R, Glasson EJ (2007) The four ages of Down syndrome. European journal of public health 17:221-225.

Bittles AH, Glasson EJ (2004) Clinical, social, and ethical implications of changing life expectancy in Down syndrome. Developmental medicine and child neurology 46:282-286.

Blessed G, Tomlinson BE, Roth M (1968) The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects. The British journal of psychiatry : the journal of mental science 114:797-811.

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31-39.

Blue ME, Parnavelas JG (1983) The formation and maturation of synapses in the visual cortex of the rat. I. Qualitative analysis. Journal of neurocytology 12:599-616.

Bowes C, Li T, Frankel WN, Danciger M, Coffin JM, Applebury ML, Farber DB (1993) Localization of a retroviral element within the rd gene coding for the beta subunit of cGMP phosphodiesterase. Proceedings of the National Academy of Sciences of the United States of America 90:2955-2959.

Braak H, Braak E (1997) Frequency of stages of Alzheimer-related lesions in different age categories. Neurobiol Aging 18:351-357.

Bradford HF (1995) Glutamate, GABA and epilepsy. Prog Neurobiol 47:477-511.

Bramham CR, Messaoudi E (2005) BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. Prog Neurobiol 76:99-125.

Bredy TW, Humpartzoomian RA, Cain DP, Meaney MJ (2003) Partial reversal of the effect of maternal care on cognitive function through environmental enrichment. Neuroscience 118:571-576.

Brown JH, Johnson MH, Paterson SJ, Gilmore R, Longhi E, Karmiloff-Smith A (2003) Spatial representation and attention in toddlers with Williams syndrome and Down syndrome. Neuropsychologia 41:1037-1046.

Buchanan LH (1990) Early onset of presbyacusis in Down syndrome. Scandinavian audiology 19:103-110.

Buhot MC (1997) Serotonin receptors in cognitive behaviors. Curr Opin Neurobiol 7:243-254.

Buhot MC, Martin S, Segu L (2000) Role of serotonin in memory impairment. Annals of medicine 32:210-221.

Burguiere E, Arabo A, Jarlier F, De Zeeuw CI, Rondi-Reig L (2010) Role of the cerebellar cortex in conditioned goal-directed behavior. The Journal of neuroscience : the official journal of the Society for Neuroscience 30:13265-13271.

Burt DB, Loveland KA, Lewis KR (1992) Depression and the onset of dementia in adults with mental retardation. American journal of mental retardation : AJMR 96:502-511.

Burton CL, Chatterjee D, Chatterjee-Chakraborty M, Lovic V, Grella SL, Steiner M, Fleming AS (2007) Prenatal restraint stress and motherless rearing disrupts expression of plasticity markers and stress-induced corticosterone release in adult female Sprague-Dawley rats. Brain Res 1158:28-38.

Bussey TJ, Duck J, Muir JL, Aggleton JP (2000) Distinct patterns of behavioural impairments resulting from fornix transection or neurotoxic lesions of the perirhinal and postrhinal cortices in the rat. Behav Brain Res 111:187-202.

Cai R, Guo F, Zhang J, Xu J, Cui Y, Sun X (2009) Environmental enrichment improves behavioral performance and auditory spatial representation of primary auditory cortical neurons in rat. Neurobiology of learning and memory 91:366-376.

Calamandrei G, Alleva E, Cirulli F, Queyras A, Volterra V, Capirci O, Vicari S, Giannotti A, Turrini P, Aloe L (2000) Serum NGF levels in children and adolescents with either Williams syndrome or Down syndrome. Dev Med Child Neurol 42:746 –750.

Cain DP, Saucier D, Boon F (1997) Testing hypotheses of spatial learning: the role of NMDA receptors and NMDA-mediated long-term potentiation. Behav Brain Res 84:179-193.

Cameron N, Del Corpo A, Diorio J, McAllister K, Sharma S, Meaney MJ (2008) Maternal programming of sexual behavior and hypothalamic-pituitary-gonadal function in the female rat. PLoS One 3:e2210.

Cancedda L, Putignano E, Sale A, Viegi A, Berardi N, Maffei L (2004) Acceleration of visual system development by environmental enrichment. The Journal of neuroscience : the official journal of the Society for Neuroscience 24:4840-4848.

Carlesimo GA, Marotta L, Vicari S (1997) Long-term memory in mental retardation: evidence for a specific impairment in subjects with Down's syndrome. Neuropsychologia 35:71-79.

Carrillo M, Ricci LA, Coppersmith GA, Melloni RH, Jr. (2009) The effect of increased serotonergic neurotransmission on aggression: a critical meta-analytical review of preclinical studies. Psychopharmacology 205:349-368.

Casanova MF, Walker LC, Whitehouse PJ, Price DL (1985) Abnormalities of the nucleus basalis in Down's syndrome. Annals of neurology 18:310-313.

Cassel S, Carouge D, Gensburger C, Anglard P, Burgun C, Dietrich JB, Aunis D, Zwiller J (2006) Fluoxetine and cocaine induce the epigenetic factors MeCP2 and MBD1 in adult rat brain. Molecular pharmacology 70:487-492.

Castren E, Elgersma Y, Maffei L, Hagerman R (2012) Treatment of neurodevelopmental disorders in adulthood. The Journal of neuroscience : the official journal of the Society for Neuroscience 32:14074-14079.

Cataldo AM, Petanceska S, Peterhoff CM, Terio NB, Epstein CJ, Villar A, Carlson EJ, Staufenbiel M, Nixon RA (2003) App gene dosage modulates endosomal abnormalities of Alzheimer's disease in a segmental trisomy 16 mouse model of down syndrome. The Journal of neuroscience : the official journal of the Society for Neuroscience 23:6788-6792.

Chahrour M, Jung SY, Shaw C, Zhou X, Wong ST, Qin J, Zoghbi HY (2008) MeCP2, a key contributor to neurological disease, activates and represses transcription. Science 320:1224-1229.

Chakrabarti L, Best TK, Cramer NP, Carney RSE, Isaac JTR, Galdzicki Z, Haydar TF (2010) Olig1 and Olig2 triplication causes developmental brain defects in Down syndrome. Nature Neuroscience 13:927-934.

Chakrabarti L, Galdzicki Z, Haydar TF (2007) Defects in Embryonic Neurogenesis and Initial Synapse Formation in the Forebrain of the Ts65Dn Mouse Model of Down Syndrome. Journal of Neuroscience 27:11483-11495.

Chakrabarti L, Scafidi J, Gallo V, Haydar TF (2011) Environmental Enrichment Rescues Postnatal Neurogenesis Defect in the Male and Female Ts65Dn Mouse Model of Down Syndrome. Developmental Neuroscience 33:428-441.

Champagne DL, Bagot RC, van Hasselt F, Ramakers G, Meaney MJ, de Kloet ER, Joels M, Krugers H (2008) Maternal care and hippocampal plasticity: evidence for experience-dependent structural plasticity, altered synaptic functioning, and differential responsiveness to glucocorticoids and stress. The Journal of neuroscience : the official journal of the Society for Neuroscience 28:6037-6045.

Champagne FA, Curley JP (2009) Epigenetic mechanisms mediating the long-term effects of maternal care on development. Neuroscience and biobehavioral reviews 33:593-600.

Chan JS, Olivier B, de Jong TR, Snoeren EM, Kooijman E, van Hasselt FN, Limpens JH, Kas MJ, Waldinger MD, Oosting RS (2008) Translational research into sexual disorders: pharmacology and genomics. European journal of pharmacology 585:426-435.

Chang Q, Gold PE (2008) Age-related changes in memory and in acetylcholine functions in the hippocampus in the Ts65Dn mouse, a model of Down syndrome. Neurobiology of learning and memory 89:167-177.

Chao MV, Hempstead BL (1995) p75 and Trk: a two-receptor system. Trends Neurosci 18:321-326.

Chapillon P, Patin V, Roy V, Vincent A, Caston J (2002) Effects of pre- and postnatal stimulation on developmental, emotional, and cognitive aspects in rodents: a review. Developmental psychobiology 41:373-387.

Chapman PF, White GL, Jones MW, Cooper-Blacketer D, Marshall VJ, Irizarry M, Younkin L, Good MA, Bliss TV, Hyman BT, Younkin SG, Hsiao KK (1999) Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. Nat Neurosci 2:271-276.

Chatterjee D, Chatterjee-Chakraborty M, Rees S, Cauchi J, de Medeiros CB, Fleming AS (2007) Maternal isolation alters the expression of neural proteins during development: 'Stroking' stimulation reverses these effects. Brain Res 1158:11-27.

Chattopadhyaya B, Cristo GD (2012) GABAergic circuit dysfunctions in neurodevelopmental disorders. Frontiers in psychiatry 3:51.

Chen JL, Lin WC, Cha JW, So PT, Kubota Y, Nedivi E (2011) Structural basis for the role of inhibition in facilitating adult brain plasticity. Nat Neurosci 14:587-594.

Chen Y, Dyakin VV, Branch CA, Ardekani B, Yang D, Guilfoyle DN, Peterson J, Peterhoff C, Ginsberg SD, Cataldo AM, Nixon RA (2009) In vivo MRI identifies cholinergic circuitry deficits in a Down syndrome model. Neurobiol Aging 30:1453-1465.

Chen YJ, Fang PC (2005) Sensory evoked potentials in infants with Down syndrome. Acta paediatrica 94:1615-1618.

Cheng A, Haydar TF, Yarowsky PJ, Krueger BK (2004) Concurrent generation of subplate and cortical plate neurons in developing trisomy 16 mouse cortex. Dev Neurosci 26:255-265.

Chiba AA, Kesner RP, Reynolds AM (1994) Memory for spatial location as a function of temporal lag in rats: role of hippocampus and medial prefrontal cortex. Behavioral and neural biology 61:123-131.

Chollet F, Tardy J, Albucher JF, Thalamas C, Berard E, Lamy C, Bejot Y, Deltour S, Jaillard A, Niclot P, Guillon B, Moulin T, Marque P, Pariente J, Arnaud C, Loubinoux I (2011) Fluoxetine for motor recovery after acute ischaemic stroke (FLAME): a randomised placebo-controlled trial. Lancet neurology 10:123-130.

Ciucci F, Putignano E, Baroncelli L, Landi S, Berardi N, Maffei L (2007) Insulin-like growth factor 1 (IGF-1) mediates the effects of enriched environment (EE) on visual cortical development. PLoS One 2:e475.

Clark RE, West AN, Zola SM, Squire LR (2001) Rats with lesions of the hippocampus are impaired on the delayed nonmatching-to-sample task. Hippocampus 11:176-186.

Clark RE, Zola SM, Squire LR (2000) Impaired recognition memory in rats after damage to the hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience 20:8853-8860.

Clark S, Schwalbe J, Stasko MR, Yarowsky PJ, Costa ACS (2006) Fluoxetine rescues deficient neurogenesis in hippocampus of the Ts65Dn mouse model for Down syndrome. Experimental Neurology 200:256-261.

Collacott RA, Cooper SA, McGrother C (1992) Differential rates of psychiatric disorders in adults with Down's syndrome compared with other mentally handicapped adults. The British journal of psychiatry : the journal of mental science 161:671-674.

Contestabile A, Benfenati F, Gasparini L (2010) Communication breaks-Down: From neurodevelopment defects to cognitive disabilities in Down syndrome. Progress in Neurobiology 91:1-22.

Contestabile A, Ciani E, Contestabile A (2008) The place of choline acetyltransferase activity measurement in the "cholinergic hypothesis" of neurodegenerative diseases. Neurochemical research 33:318-327.

Contestabile A, Fila T, Bartesaghi R, Contestabile A, Ciani E (2006) Choline acetyltransferase activity at different ages in brain of Ts65Dn mice, an animal model for Down's syndrome and related neurodegenerative diseases. J Neurochem 97:515-526.

Contestabile A, Fila T, Ceccarelli C, Bonasoni P, Bonapace L, Santini D, Bartesaghi R, Ciani E (2007) Cell cycle alteration and decreased cell proliferation in the hippocampal dentate gyrus and in the neocortical germinal matrix of fetuses with down syndrome and in Ts65Dn mice. Hippocampus 17:665-678.

Conti L, Cattaneo E (2005) Controlling neural stem cell division within the adult subventricular zone: an APPealing job. Trends Neurosci 28:57-59.

Cooper JD, Salehi A, Delcroix JD, Howe CL, Belichenko PV, Chua-Couzens J, Kilbridge JF, Carlson EJ, Epstein CJ, Mobley WC (2001) Failed retrograde transport of NGF in a mouse model of Down's syndrome: Reversal of cholinergic neurodegenerative phenotypes following NGF infusion. Proceedings of the National Academy of Sciences 98:10439-10444.

Cornwell P, Overman W (1981) Behavioral effects of early rearing conditions and neonatal lesions of the visual cortex in kittens. Journal of comparative and physiological psychology 95:848-862.

Costa AC, Grybko MJ (2005) Deficits in hippocampal CA1 LTP induced by TBS but not HFS in the Ts65Dn mouse: a model of Down syndrome. Neurosci Lett 382:317-322.

Costa AC, Scott-McKean JJ, Stasko MR (2008) Acute injections of the NMDA receptor antagonist memantine rescue performance deficits of the Ts65Dn mouse model of Down syndrome on a fear conditioning test. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 33:1624-1632.

Costa AC, Stasko MR, Schmidt C, Davisson MT (2010) Behavioral validation of the Ts65Dn mouse model for Down syndrome of a genetic background free of the retinal degeneration mutation Pde6b(rd1). Behav Brain Res 206:52-62.

Costa AC, Walsh K, Davisson MT (1999) Motor dysfunction in a mouse model for Down syndrome. Physiology & behavior 68:211-220.

Costa DA, Cracchiolo JR, Bachstetter AD, Hughes TF, Bales KR, Paul SM, Mervis RF, Arendash GW, Potter H (2007) Enrichment improves cognition in AD mice by amyloid-related and unrelated mechanisms. Neurobiol Aging 28:831-844.

Costa RM, Federov NB, Kogan JH, Murphy GG, Stern J, Ohno M, Kucherlapati R, Jacks T, Silva AJ (2002) Mechanism for the learning deficits in a mouse model of neurofibromatosis type 1. Nature 415:526-530.

Courage ML, Adams RJ, Reyno S, Kwa PG (1994) Visual acuity in infants and children with Down syndrome. Developmental medicine and child neurology 36:586-593.

Coussons-Read ME, Crnic LS (1996) Behavioral assessment of the Ts65Dn mouse, a model for Down syndrome: altered behavior in the elevated plus maze and open field. Behav Genet 26:7-13.

Coyle JT, Oster-Granite ML, Gearhart JD (1986) The neurobiologic consequences of Down syndrome. Brain research bulletin 16:773-787.

Cracchiolo JR, Mori T, Nazian SJ, Tan J, Potter H, Arendash GW (2007) Enhanced cognitive activity--over and above social or physical activity--is required to protect Alzheimer's mice against cognitive impairment, reduce Abeta deposition, and increase synaptic immunoreactivity. Neurobiology of learning and memory 88:277-294.

Cramer N, Galdzicki Z (2012) From abnormal hippocampal synaptic plasticity in down syndrome mouse models to cognitive disability in down syndrome. Neural Plast 2012:101542.

Cregg M, Woodhouse JM, Pakeman VH, Saunders KJ, Gunter HL, Parker M, Fraser WI, Sastry P (2001) Accommodation and refractive error in children with Down syndrome: cross-sectional and longitudinal studies. Investigative ophthalmology & visual science 42:55-63.

Crowley JC, Katz LC (2002) Ocular dominance development revisited. Curr Opin Neurobiol 12:104-109.

Cuskelly M, Dadds M (1992) Behavioural problems in children with Down's syndrome and their siblings. Journal of child psychology and psychiatry, and allied disciplines 33:749-761.

D'Hooge R, De Deyn PP (2001) Applications of the Morris water maze in the study of learning and memory. Brain research Brain research reviews 36:60-90.

D'Sa C, Duman RS (2002) Antidepressants and neuroplasticity. Bipolar disorders 4:183-194.

da Cunha RP, Moreira JB (1996) Ocular findings in Down's syndrome. American journal of ophthalmology 122:236-244.

Dan Y, Poo MM (2006) Spike timing-dependent plasticity: from synapse to perception. Physiological reviews 86:1033-1048.

Das I, Reeves RH (2011) The use of mouse models to understand and improve cognitive deficits in Down syndrome. Disease Models & Mechanisms 4:596-606.

Davis GW (2006) Homeostatic control of neural activity: from phenomenology to molecular design. Annual review of neuroscience 29:307-323.

Davis WE, Sinning WE (1987) Muscle stiffness in down syndrome and other mentally handicapped subjects. Journal of motor behavior 19:130-144.

Davisson MT, Schmidt C, Reeves RH, Irving NG, Akeson EC, Harris BS, Bronson RT (1993) Segmental trisomy as a mouse model for Down syndrome. Progress in clinical and biological research 384:117-133.

Deb S, Hare M, Prior L, Bhaumik S (2007) Dementia screening questionnaire for individuals with intellectual disabilities. The British journal of psychiatry : the journal of mental science 190:440-444.

Demas GE, Nelson RJ, Krueger BK, Yarowsky PJ (1996) Spatial memory deficits in segmental trisomic Ts65Dn mice. Behav Brain Res 82:85-92.

Devenny DA, Silverman WP, Hill AL, Jenkins E, Sersen EA, Wisniewski KE (1996) Normal ageing in adults with Down's syndrome: a longitudinal study. Journal of intellectual disability research : JIDR 40 (Pt 3):208-221.

Devenny DA, Wegiel J, Schupf N, Jenkins E, Zigman W, Krinsky-McHale SJ, Silverman WP (2005) Dementia of the Alzheimer's type and accelerated aging in Down syndrome. Science of aging knowledge environment : SAGE KE 2005:dn1.

Diaz F, Zuron M (1995) Auditory evoked potentials in Down's syndrome. Electroencephalography and clinical neurophysiology 96:526-537.

Dierssen M, Benavides-Piccione R, Martinez-Cue C, Estivill X, Florez J, Elston GN, DeFelipe J (2003) Alterations of neocortical pyramidal cell phenotype in the Ts65Dn mouse model of Down syndrome: effects of environmental enrichment. Cerebral cortex 13:758-764.

Dierssen M, Ramakers GJ (2006) Dendritic pathology in mental retardation: from molecular genetics to neurobiology. Genes, brain, and behavior 5 Suppl 2:48-60.

Dierssen M, Vallina IF, Baamonde C, Garcia-Calatayud S, Lumbreras MA, Florez J (1997) Alterations of central noradrenergic transmission in Ts65Dn mouse, a model for Down syndrome. Brain Res 749:238-244.

Dogliotti G, Galliera E, Licastro F, Corsi MM (2010) Age-related changes in plasma levels of BDNF in Down syndrome patients. Immunity & ageing : I & A 7:2.

Duman RS (2002) Synaptic plasticity and mood disorders. Molecular psychiatry 7 Suppl 1:S29-34.

Duman RS, Malberg J, Nakagawa S, D'Sa C (2000) Neuronal plasticity and survival in mood disorders. Biol Psychiatry 48:732-739.

Duman RS, Monteggia LM (2006) A neurotrophic model for stress-related mood disorders. Biol Psychiatry 59:1116-1127.

Dykens EM, Shah B, Sagun J, Beck T, King BH (2002) Maladaptive behaviour in children and adolescents with Down's syndrome. Journal of intellectual disability research : JIDR 46:484-492.

Edgin JO, Pennington BF, Mervis CB (2010) Neuropsychological components of intellectual disability: the contributions of immediate, working, and associative memory. Journal of intellectual disability research : JIDR 54:406-417.

Ehninger D, Li W, Fox K, Stryker MP, Silva AJ (2008) Reversing neurodevelopmental disorders in adults. Neuron 60:950-960.

Ellingson RJ (1986) Development of visual evoked potentials and photic driving responses in normal full term, low risk premature, and Trisomy-21 infants during the first year of life. Electroencephalography and clinical neurophysiology 63:309-316.

Emerson VF, Chalupa LM, Thompson ID, Talbot RJ (1982) Behavioural, physiological, and anatomical consequences of monocular deprivation in the golden hamster (Mesocricetus auratus). Experimental brain research Experimentelle Hirnforschung Experimentation cerebrale 45:168-178.

Engidawork E, Lubec G (2003) Molecular changes in fetal Down syndrome brain. J Neurochem 84:895-904.

Escorihuela RM, Fernandez-Teruel A, Vallina IF, Baamonde C, Lumbreras MA, Dierssen M, Tobena A, Florez J (1995) A behavioral assessment of Ts65Dn mice: a putative Down syndrome model. Neurosci Lett 199:143-146.

Escorihuela RM, Vallina IF, Martinez-Cue C, Baamonde C, Dierssen M, Tobena A, Florez J, Fernandez-Teruel A (1998) Impaired short- and long-term memory in Ts65Dn mice, a model for Down syndrome. Neurosci Lett 247:171-174.

Fagiolini M, Hensch TK (2000) Inhibitory threshold for critical-period activation in primary visual cortex. Nature 404:183-186.

Fagiolini M, Jensen CL, Champagne FA (2009) Epigenetic influences on brain development and plasticity. Curr Opin Neurobiol 19:207-212.

Fagiolini M, Pizzorusso T, Berardi N, Domenici L, Maffei L (1994) Functional postnatal development of the rat primary visual cortex and the role of visual experience: dark rearing and monocular deprivation. Vision research 34:709-720.

Faizi M, Bader PL, Tun C, Encarnacion A, Kleschevnikov A, Belichenko P, Saw N, Priestley M, Tsien RW, Mobley WC, Shamloo M (2011) Comprehensive behavioral phenotyping of Ts65Dn mouse model of Down syndrome: activation of beta1-adrenergic receptor by xamoterol as a potential cognitive enhancer. Neurobiol Dis 43:397-413.

Ferencz C, Neill CA, Boughman JA, Rubin JD, Brenner JI, Perry LW (1989) Congenital cardiovascular malformations associated with chromosome abnormalities: an epidemiologic study. The Journal of pediatrics 114:79-86.

Fernandez-Martinez J, Vela EM, Tora-Ponsioen M, Ocana OH, Nieto MA, Galceran J (2009) Attenuation of Notch signalling by the Down-syndrome-associated kinase DYRK1A. Journal of cell science 122:1574-1583.

Fernandez F, Garner CC (2007) Over-inhibition: a model for developmental intellectual disability. Trends in Neurosciences 30:497-503.

Fernandez F, Morishita W, Zuniga E, Nguyen J, Blank M, Malenka RC, Garner CC (2007) Pharmacotherapy for cognitive impairment in a mouse model of Down syndrome. Nature Neuroscience.

Fernandez F, Trinidad JC, Blank M, Feng D-D, Burlingame AL, Garner CC (2009) Normal protein composition of synapses in Ts65Dn mice: a mouse model of Down syndrome. Journal of Neurochemistry 110:157-169.

Ferrari PF, Palanza P, Parmigiani S, de Almeida RM, Miczek KA (2005) Serotonin and aggressive behavior in rodents and nonhuman primates: predispositions and plasticity. European journal of pharmacology 526:259-273.

Ferrer I, Gullotta F (1990) Down's syndrome and Alzheimer's disease: dendritic spine counts in the hippocampus. Acta Neuropathol 79:680-685.

Fidler DJ, Hepburn S, Rogers S (2006) Early learning and adaptive behaviour in toddlers with Down syndrome: evidence for an emerging behavioural phenotype? Down's syndrome, research and practice : the journal of the Sarah Duffen Centre / University of Portsmouth 9:37-44.

Figurov A, Pozzo-Miller LD, Olafsson P, Wang T, Lu B (1996) Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. Nature 381:706-709.

Findlater GS, McDougall RD, Kaufman MH (1993) Eyelid development, fusion and subsequent reopening in the mouse. Journal of anatomy 183 (Pt 1):121-129.

Finkbeiner S, Tavazoie SF, Maloratsky A, Jacobs KM, Harris KM, Greenberg ME (1997) CREB: a major mediator of neuronal neurotrophin responses. Neuron 19:1031-1047.

Fischer-Brandies H (1988) Cephalometric comparison between children with and without Down's syndrome. European journal of orthodontics 10:255-263.

Fischer A, Sananbenesi F, Wang X, Dobbin M, Tsai LH (2007) Recovery of learning and memory is associated with chromatin remodelling. Nature 447:178-182.

Fleming AS, Steiner M, Corter C (1997) Cortisol, hedonics, and maternal responsiveness in human mothers. Hormones and behavior 32:85-98.

Folin M, Baiguera S, Conconi MT, Pati T, Grandi C, Parnigotto PP, Nussdorfer GG (2003) The impact of risk factors of Alzheimer's disease in the Down syndrome. International journal of molecular medicine 11:267-270.

Folsom RC, Widen JE, Wilson WR (1983) Auditory brain-stem responses in infants with Down's syndrome. Archives of otolaryngology 109:607-610.

Fortin NJ, Agster KL, Eichenbaum HB (2002) Critical role of the hippocampus in memory for sequences of events. Nat Neurosci 5:458-462.

Forwood SE, Winters BD, Bussey TJ (2005) Hippocampal lesions that abolish spatial maze performance spare object recognition memory at delays of up to 48 hours. Hippocampus 15:347-355.

Fregnac Y, Imbert M (1978) Early development of visual cortical cells in normal and dark-reared kittens: relationship between orientation selectivity and ocular dominance. The Journal of physiology 278:27-44.

Freret T, Billard JM, Schumann-Bard P, Dutar P, Dauphin F, Boulouard M, Bouet V (2012) Rescue of cognitive aging by long-lasting environmental enrichment exposure initiated before median lifespan. Neurobiol Aging 33:1005 e1001-1010.

Frostad WA, Cleall JF, Melosky LC (1971) Craniofacial complex in the trisomy 21 syndrome (Down's syndrome). Archives of oral biology 16:707-722.

Fukuda Y, Berry TL, Nelson M, Hunter CL, Fukuhara K, Imai H, Ito S, Granholm-Bentley AC, Kaplan AP, Mutoh T (2010) Stimulated neuronal expression of brain-derived neurotrophic factor by Neurotropin. Molecular and cellular neurosciences 45:226-233.

Galli M, Rigoldi C, Brunner R, Virji-Babul N, Giorgio A (2008) Joint stiffness and gait pattern evaluation in children with Down syndrome. Gait & posture 28:502-506.

Gao WJ, Wormington AB, Newman DE, Pallas SL (2000) Development of inhibitory circuitry in visual and auditory cortex of postnatal ferrets: immunocytochemical localization of calbindin- and parvalbumin-containing neurons. J Comp Neurol 422:140-157.

Gardiner K, Davisson M (2000) The sequence of human chromosome 21 and implications for research into Down syndrome. Genome Biol 1:REVIEWS0002.

Gasparini L, Dityatev A (2008) Beta-amyloid and glutamate receptors. Exp Neurol 212:1-4.

Gharib WH, Robinson-Rechavi M (2011) When orthologs diverge between human and mouse. Briefings in bioinformatics 12:436-441.

Glasson EJ, Sullivan SG, Hussain R, Petterson BA, Montgomery PD, Bittles AH (2002) The changing survival profile of people with Down's syndrome: implications for genetic counselling. Clinical genetics 62:390-393.

Godridge H, Reynolds GP, Czudek C, Calcutt NA, Benton M (1987) Alzheimer-like neurotransmitter deficits in adult Down's syndrome brain tissue. Journal of neurology, neurosurgery, and psychiatry 50:775-778.

Golden JA, Hyman BT (1994) Development of the superior temporal neocortex is anomalous in trisomy 21. Journal of neuropathology and experimental neurology 53:513-520.

Good MA, Barnes P, Staal V, McGregor A, Honey RC (2007) Context- but not familiarity-dependent forms of object recognition are impaired following excitotoxic hippocampal lesions in rats. Behav Neurosci 121:218-223.

Gordon JA, Stryker MP (1996) Experience-dependent plasticity of binocular responses in the primary visual cortex of the mouse. The Journal of neuroscience : the official journal of the Society for Neuroscience 16:3274-3286.

Granholm AC, Sanders LA, Crnic LS (2000) Loss of cholinergic phenotype in basal forebrain coincides with cognitive decline in a mouse model of Down's syndrome. Exp Neurol 161:647-663.

Greenwood PM (2007) Functional plasticity in cognitive aging: review and hypothesis. Neuropsychology 21:657-673.

Greenwood PM, Parasuraman R (2010) Neuronal and cognitive plasticity: a neurocognitive framework for ameliorating cognitive aging. Frontiers in aging neuroscience 2:150.

Gropp A, Kolbus U, Giers D (1975) Systematic approach to the study of trisomy in the mouse. II. Cytogenetics and cell genetics 14:42-62.

Grote HE, Bull ND, Howard ML, van Dellen A, Blakemore C, Bartlett PF, Hannan AJ (2005) Cognitive disorders and neurogenesis deficits in Huntington's disease mice are rescued by fluoxetine. The European journal of neuroscience 22:2081-2088.

Guidi S, Bonasoni P, Ceccarelli C, Santini D, Gualtieri F, Ciani E, Bartesaghi R (2008) Neurogenesis impairment and increased cell death reduce total neuron number in the hippocampal region of fetuses with Down syndrome. Brain Pathol 18:180-197.

Guidi S, Ciani E, Bonasoni P, Santini D, Bartesaghi R (2011) Widespread proliferation impairment and hypocellularity in the cerebellum of fetuses with down syndrome. Brain Pathol 21:361-373.

Guidi S, Stagni F, Bianchi P, Ciani E, Ragazzi E, Trazzi S, Grossi G, Mangano C, Calza L, Bartesaghi R (2013) Early pharmacotherapy with fluoxetine rescues dendritic pathology in the Ts65Dn mouse model of down syndrome. Brain Pathol 23:129-143.

Guihard-Costa AM, Khung S, Delbecque K, Menez F, Delezoide AL (2006) Biometry of face and brain in fetuses with trisomy 21. Pediatric research 59:33-38.

Guo Y, Kaplan IV, Cooper NG, Mower GD (1997) Expression of two forms of glutamic acid decarboxylase (GAD67 and GAD65) during postnatal development of the cat visual cortex. Brain research Developmental brain research 103:127-141.

Guy J, Gan J, Selfridge J, Cobb S, Bird A (2007) Reversal of neurological defects in a mouse model of Rett syndrome. Science 315:1143-1147.

Guzzetta A, Baldini S, Bancale A, Baroncelli L, Ciucci F, Ghirri P, Putignano E, Sale A, Viegi A, Berardi N, Boldrini A, Cioni G, Maffei L (2009) Massage accelerates brain development and the maturation of visual function. The Journal of neuroscience : the official journal of the Society for Neuroscience 29:6042-6051.

Hagberg B, Aicardi J, Dias K, Ramos O (1983) A progressive syndrome of autism, dementia, ataxia, and loss of purposeful hand use in girls: Rett's syndrome: report of 35 cases. Annals of neurology 14:471-479.

Hall B (1965) Delayed ontogenesis in human trisomy syndromes. Hereditas 52:334-344.

Hammerle B, Elizalde C, Tejedor FJ (2008) The spatio-temporal and subcellular expression of the candidate Down syndrome gene Mnb/Dyrk1A in the developing mouse brain suggests distinct sequential roles in neuronal development. The European journal of neuroscience 27:1061-1074.

Hammerle B, Vera-Samper E, Speicher S, Arencibia R, Martinez S, Tejedor FJ (2002) Mnb/Dyrk1A is transiently expressed and asymmetrically segregated in neural progenitor cells at the transition to neurogenic divisions. Developmental biology 246:259-273.

Hampton TG, Stasko MR, Kale A, Amende I, Costa AC (2004) Gait dynamics in trisomic mice: quantitative neurological traits of Down syndrome. Physiology & behavior 82:381-389.

Han F, Yu H, Zhang J, Tian C, Schmidt C, Nava C, Davisson MT, Zheng QY (2009) Otitis media in a mouse model for Down syndrome. International journal of experimental pathology 90:480-488.

Hannigan JH, Berman RF, Zajac CS (1993) Environmental enrichment and the behavioral effects of prenatal exposure to alcohol in rats. Neurotoxicology and teratology 15:261-266.

Hansen LT, Berthelsen H (2000) The effect of environmental enrichment on the behaviour of caged rabbits (Oryctolagus cuniculus). Applied animal behaviour science 68:163-178.

Hanson JE, Blank M, Valenzuela RA, Garner CC, Madison DV (2007) The functional nature of synaptic circuitry is altered in area CA3 of the hippocampus in a mouse model of Down's syndrome. The Journal of physiology 579:53-67.

Harashima C, Jacobowitz DM, Witta J, Borke RC, Best TK, Siarey RJ, Galdzicki Z (2006) Abnormal expression of the G-protein-activated inwardly rectifying potassium channel 2 (GIRK2) in hippocampus, frontal cortex, and substantia nigra of Ts65Dn mouse: A model of Down syndrome. The Journal of Comparative Neurology 494:815-833.

Harauzov A, Spolidoro M, DiCristo G, De Pasquale R, Cancedda L, Pizzorusso T, Viegi A, Berardi N, Maffei L (2010) Reducing Intracortical Inhibition in the Adult Visual Cortex Promotes Ocular Dominance Plasticity. Journal of Neuroscience 30:361-371.

Hassmann E, Skotnicka B, Midro AT, Musiatowicz M (1998) Distortion products otoacoustic emissions in diagnosis of hearing loss in Down syndrome. International journal of pediatric otorhinolaryngology 45:199-206.

Hassold T, Hunt P (2001) To err (meiotically) is human: the genesis of human aneuploidy. Nature reviews Genetics 2:280-291.

Haugen OH, Hovding G (2001) Strabismus and binocular function in children with Down syndrome. A population-based, longitudinal study. Acta ophthalmologica Scandinavica 79:133-139.

Haydar TF (2005) Advanced microscopic imaging methods to investigate cortical development and the etiology of mental retardation. Ment Retard Dev Disabil Res Rev 11:303-316.

Haydar TF, Blue ME, Molliver ME, Krueger BK, Yarowsky PJ (1996) Consequences of trisomy 16 for mouse brain development: corticogenesis in a model of Down syndrome. The Journal of neuroscience : the official journal of the Society for Neuroscience 16:6175-6182.

Haydar TF, Reeves RH (2012) Trisomy 21 and early brain development. Trends in Neurosciences 35:81-91.

Hayes A, Batshaw ML (1993) Down syndrome. Pediatric clinics of North America 40:523-535.

He HY, Hodos W, Quinlan EM (2006) Visual deprivation reactivates rapid ocular dominance plasticity in adult visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 26:2951-2955.

He HY, Ray B, Dennis K, Quinlan EM (2007) Experience-dependent recovery of vision following chronic deprivation amblyopia. Nat Neurosci 10:1134-1136.

Heinen M, Hettich MM, Ryan DP, Schnell S, Paesler K, Ehninger D (2012) Adult-onset fluoxetine treatment does not improve behavioral impairments and may have adverse effects on the Ts65Dn mouse model of Down syndrome. Neural Plast 2012:467251.

Hensch TK (2004) Critical period regulation. Annual review of neuroscience 27:549-579.

Herring A, Ambree O, Tomm M, Habermann H, Sachser N, Paulus W, Keyvani K (2009) Environmental enrichment enhances cellular plasticity in transgenic mice with Alzheimer-like pathology. Exp Neurol 216:184-192.

Hill CA, Reeves RH, Richtsmeier JT (2007) Effects of aneuploidy on skull growth in a mouse model of Down syndrome. Journal of anatomy 210:394-405.

Hockly E, Cordery PM, Woodman B, Mahal A, van Dellen A, Blakemore C, Lewis CM, Hannan AJ, Bates GP (2002) Environmental enrichment slows disease progression in R6/2 Huntington's disease mice. Annals of neurology 51:235-242.

Hodges RJ, Wallace EM (2005) Testing for Down syndrome in the older woman: a risky business? The Australian & New Zealand journal of obstetrics & gynaecology 45:486-488.

Hof PR, Bouras C, Perl DP, Sparks DL, Mehta N, Morrison JH (1995) Age-related distribution of neuropathologic changes in the cerebral cortex of patients with Down's syndrome. Quantitative regional analysis and comparison with Alzheimer's disease. Archives of neurology 52:379-391.

Hofer MA (1984) Relationships as regulators: a psychobiologic perspective on bereavement. Psychosomatic medicine 46:183-197.

Hofer SB, Bonhoeffer T (2010) Dendritic spines: the stuff that memories are made of? Current biology : CB 20:R157-159.

Holmes JM, Clarke MP (2006) Amblyopia. Lancet 367:1343-1351.

Holtzman DM, Santucci D, Kilbridge J, Chua-Couzens J, Fontana DJ, Daniels SE, Johnson RM, Chen K, Sun Y, Carlson E, Alleva E, Epstein CJ, Mobley WC (1996) Developmental abnormalities and age-related neurodegeneration in a mouse model of Down syndrome. Proceedings of the National Academy of Sciences of the United States of America 93:13333-13338.

Houeland G, Romani A, Marchetti C, Amato G, Capsoni S, Cattaneo A, Marie H (2010) Transgenic mice with chronic NGF deprivation and Alzheimer's disease-like pathology display hippocampal region-specific impairments in short- and long-term plasticities. The Journal of neuroscience : the official journal of the Society for Neuroscience 30:13089-13094.

Huang EJ, Reichardt LF (2001) Neurotrophins: roles in neuronal development and function. Annual review of neuroscience 24:677-736.

Huang ZJ, Kirkwood A, Pizzorusso T, Porciatti V, Morales B, Bear MF, Maffei L, Tonegawa S (1999) BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. Cell 98:739-755.

Hubel DH, Wiesel TN (1970) The period of susceptibility to the physiological effects of unilateral eye closure in kittens. The Journal of physiology 206:419-436.

Hubel DH, Wiesel TN, LeVay S (1977) Plasticity of ocular dominance columns in monkey striate cortex. Philosophical transactions of the Royal Society of London Series B, Biological sciences 278:377-409.

Hunter CL, Bachman D, Granholm AC (2004) Minocycline prevents cholinergic loss in a mouse model of Down's syndrome. Annals of neurology 56:675-688.

Hunter CL, Bimonte HA, Granholm AC (2003) Behavioral comparison of 4 and 6 month-old Ts65Dn mice: age-related impairments in working and reference memory. Behav Brain Res 138:121-131.

Huttenlocher PR (1990) Morphometric study of human cerebral cortex development. Neuropsychologia 28:517-527.

Hyde LA, Crnic LS (2001) Age-related deficits in context discrimination learning in Ts65Dn mice that model Down syndrome and Alzheimer's disease. Behavioral Neuroscience 115:1239-1246.

Ickes BR, Pham TM, Sanders LA, Albeck DS, Mohammed AH, Granholm AC (2000) Long-term environmental enrichment leads to regional increases in neurotrophin levels in rat brain. Exp Neurol 164:45-52.

Insausti AM, Megias M, Crespo D, Cruz-Orive LM, Dierssen M, Vallina IF, Insausti R, Florez J (1998) Hippocampal volume and neuronal number in Ts65Dn mice: a murine model of Down syndrome. Neurosci Lett 253:175-178.

Issa NP, Trachtenberg JT, Chapman B, Zahs KR, Stryker MP (1999) The critical period for ocular dominance plasticity in the Ferret's visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 19:6965-6978.

Ivarsson M, Paterson LM, Hutson PH (2005) Antidepressants and REM sleep in Wistar-Kyoto and Sprague-Dawley rats. European journal of pharmacology 522:63-71.

Iwata N, Tsubuki S, Takaki Y, Shirotani K, Lu B, Gerard NP, Gerard C, Hama E, Lee HJ, Saido TC (2001) Metabolic regulation of brain Abeta by neprilysin. Science 292:1550-1552.

Jacola LM, Byars AW, Chalfonte-Evans M, Schmithorst VJ, Hickey F, Patterson B, Hotze S, Vannest J, Chiu CY, Holland SK, Schapiro MB (2011) Functional magnetic resonance imaging of cognitive processing in young adults with Down syndrome. American journal on intellectual and developmental disabilities 116:344-359.

Jamain S, Quach H, Betancur C, Rastam M, Colineaux C, Gillberg IC, Soderstrom H, Giros B, Leboyer M, Gillberg C, Bourgeron T, Paris Autism Research International Sibpair S (2003) Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. Nature genetics 34:27-29.

Jankowsky JL, Melnikova T, Fadale DJ, Xu GM, Slunt HH, Gonzales V, Younkin LH, Younkin SG, Borchelt DR, Savonenko AV (2005) Environmental enrichment mitigates cognitive deficits in a mouse model of Alzheimer's disease. The Journal of neuroscience : the official journal of the Society for Neuroscience 25:5217-5224.

Jansen J, Bolhuis JE, Schouten WG, Spruijt BM, Wiegant VM (2009) Spatial learning in pigs: effects of environmental enrichment and individual characteristics on behaviour and performance. Animal cognition 12:303-315.

Jarrold C, Baddeley AD, Phillips C (1999) Down syndrome and the phonological loop: the evidence for, and importance of, a specific verbal short-term memory deficit. Down's syndrome, research and practice : the journal of the Sarah Duffen Centre / University of Portsmouth 6:61-75.

Jellinger KA, Bancher C (1998) Neuropathology of Alzheimer's disease: a critical update. Journal of neural transmission Supplementum 54:77-95.

Jessberger S, Clark RE, Broadbent NJ, Clemenson GD, Jr., Consiglio A, Lie DC, Squire LR, Gage FH (2009) Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. Learning & memory 16:147-154.

Jiang ZD, Wu YY, Liu XY (1990) Early development of brainstem auditory evoked potentials in Down's syndrome. Early human development 23:41-51.

Jirikowic T, Olson HC, Kartin D (2008) Sensory processing, school performance, and adaptive behavior of young school-age children with fetal alcohol spectrum disorders. Physical & occupational therapy in pediatrics 28:117-136.

Jobling A (2001) Life be in it: lifestyle choices for active leisure. Down's syndrome, research and practice : the journal of the Sarah Duffen Centre / University of Portsmouth 6:117-122.

John FM, Bromham NR, Woodhouse JM, Candy TR (2004) Spatial vision deficits in infants and children with Down syndrome. Investigative ophthalmology & visual science 45:1566-1572.

Jones JI, Clemmons DR (1995) Insulin-like growth factors and their binding proteins: biological actions. Endocrine reviews 16:3-34.

Jorge RE, Acion L, Moser D, Adams HP, Jr., Robinson RG (2010) Escitalopram and enhancement of cognitive recovery following stroke. Archives of general psychiatry 67:187-196.

Jung CK, Herms J (2012) Structural Dynamics of Dendritic Spines are Influenced by an Environmental Enrichment: An In Vivo Imaging Study. Cerebral cortex.

Kahlem P, Sultan M, Herwig R, Steinfath M, Balzereit D, Eppens B, Saran NG, Pletcher MT, South ST, Stetten G, Lehrach H, Reeves RH, Yaspo ML (2004) Transcript level alterations reflect gene dosage effects across multiple tissues in a mouse model of down syndrome. Genome research 14:1258-1267.

Kang H, Welcher AA, Shelton D, Schuman EM (1997) Neurotrophins and time: different roles for TrkB signaling in hippocampal long-term potentiation. Neuron 19:653-664.

Karpova NN, Pickenhagen A, Lindholm J, Tiraboschi E, Kulesskaya N, Agustsdottir A, Antila H, Popova D, Akamine Y, Bahi A, Sullivan R, Hen R, Drew LJ, Castren E (2011) Fear erasure in mice requires synergy between antidepressant drugs and extinction training. Science 334:1731-1734.

Kasai H, Matsuzaki M, Noguchi J, Yasumatsu N, Nakahara H (2003) Structure-stability-function relationships of dendritic spines. Trends Neurosci 26:360-368.

Kaufmann WE, Moser HW (2000) Dendritic anomalies in disorders associated with mental retardation. Cerebral cortex 10:981-991.

Kempermann G, Kuhn HG, Gage FH (1998) Experience-induced neurogenesis in the senescent dentate gyrus. The Journal of neuroscience : the official journal of the Society for Neuroscience 18:3206-3212.

Kerr B, Silva PA, Walz K, Young JI (2010) Unconventional transcriptional response to environmental enrichment in a mouse model of Rett syndrome. PLoS One 5:e11534.

Kesner RP (2007) A behavioral analysis of dentate gyrus function. Progress in brain research 163:567-576.

Kesner RP, Novak JM (1982) Serial position curve in rats: role of the dorsal hippocampus. Science 218:173-175.

Kida E, Rabe A, Walus M, Albertini G, Golabek AA (2013) Long-term running alleviates some behavioral and molecular abnormalities in Down syndrome mouse model Ts65Dn. Exp Neurol 240:178-189.

Kim JJ, Fanselow MS (1992) Modality-specific retrograde amnesia of fear. Science 256:675-677.

Kiorpes L (2006) Visual processing in amblyopia: animal studies. Strabismus 14:3-10.

Kirkwood A, Lee HK, Bear MF (1995) Co-regulation of long-term potentiation and experience-dependent synaptic plasticity in visual cortex by age and experience. Nature 375:328-331.

Kish S, Karlinsky H, Becker L, Gilbert J, Rebbetoy M, Chang LJ, DiStefano L, Hornykiewicz O (1989) Down's syndrome individuals begin life with normal levels of brain cholinergic markers. J Neurochem 52:1183-1187.

Kleschevnikov AM (2004) Hippocampal Long-Term Potentiation Suppressed by Increased Inhibition in the Ts65Dn Mouse, a Genetic Model of Down Syndrome. Journal of Neuroscience 24:8153-8160.

Kleschevnikov AM, Belichenko PV, Faizi M, Jacobs LF, Htun K, Shamloo M, Mobley WC (2012) Deficits in Cognition and Synaptic Plasticity in a Mouse Model of Down Syndrome Ameliorated by GABAB Receptor Antagonists. Journal of Neuroscience 32:9217-9227.

Kodituwakku PW (2009) Neurocognitive profile in children with fetal alcohol spectrum disorders. Developmental disabilities research reviews 15:218-224.

Kolb B (1974) Social behavior of rats with chronic prefrontal lesions. Journal of comparative and physiological psychology 87:466-474.

Kolb B, Gibb R (2010) Tactile stimulation after frontal or parietal cortical injury in infant rats facilitates functional recovery and produces synaptic changes in adjacent cortex. Behav Brain Res 214:115-120.

Kondo M, Gray LJ, Pelka GJ, Christodoulou J, Tam PP, Hannan AJ (2008) Environmental enrichment ameliorates a motor coordination deficit in a mouse model of Rett syndrome--Mecp2 gene dosage effects and BDNF expression. The European journal of neuroscience 27:3342-3350.

Koo BK, Blaser S, Harwood-Nash D, Becker LE, Murphy EG (1992) Magnetic resonance imaging evaluation of delayed myelination in Down syndrome: a case report and review of the literature. Journal of child neurology 7:417-421.

Korbel JO, Tirosh-Wagner T, Urban AE, Chen XN, Kasowski M, Dai L, Grubert F, Erdman C, Gao MC, Lange K, Sobel EM, Barlow GM, Aylsworth AS, Carpenter NJ, Clark RD, Cohen MY, Doran E, Falik-Zaccai T, Lewin SO, Lott IT, McGillivray BC, Moeschler JB, Pettenati MJ, Pueschel SM, Rao KW, Shaffer LG, Shohat M, Van Riper AJ, Warburton D, Weissman S, Gerstein MB, Snyder M, Korenberg JR (2009) The genetic architecture of Down syndrome phenotypes revealed by high-resolution analysis of human segmental trisomies. Proceedings of the National Academy of Sciences of the United States of America 106:12031-12036.

Korte M, Carroll P, Wolf E, Brem G, Thoenen H, Bonhoeffer T (1995) Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. Proceedings of the National Academy of Sciences of the United States of America 92:8856-8860.

Korte M, Griesbeck O, Gravel C, Carroll P, Staiger V, Thoenen H, Bonhoeffer T (1996) Virus-mediated gene transfer into hippocampal CA1 region restores long-term potentiation in brain-derived neurotrophic factor mutant mice. Proceedings of the National Academy of Sciences of the United States of America 93:12547-12552.

Koyama S, Kubo C, Rhee JS, Akaike N (1999) Presynaptic serotonergic inhibition of GABAergic synaptic transmission in mechanically dissociated rat basolateral amygdala neurons. The Journal of physiology 518 (Pt 2):525-538.

Kozorovitskiy Y, Gross CG, Kopil C, Battaglia L, McBreen M, Stranahan AM, Gould E (2005) Experience induces structural and biochemical changes in the adult primate brain. Proceedings of the National Academy of Sciences of the United States of America 102:17478-17482.

Krasuski JS, Alexander GE, Horwitz B, Rapoport SI, Schapiro MB (2002) Relation of medial temporal lobe volumes to age and memory function in nondemented adults with Down's syndrome: implications for the prodromal phase of Alzheimer's disease. The American journal of psychiatry 159:74-81.

Krinsky-McHale SJ, Devenny DA, Kittler P, Silverman W (2008) Selective attention deficits associated with mild cognitive impairment and early stage Alzheimer's disease in adults with Down syndrome. American journal of mental retardation : AJMR 113:369-386.

Kuhn CM, Schanberg SM (1998) Responses to maternal separation: mechanisms and mediators. International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience 16:261-270.

Kuhn DE, Nuovo GJ, Terry AV, Jr., Martin MM, Malana GE, Sansom SE, Pleister AP, Beck WD, Head E, Feldman DS, Elton TS (2010) Chromosome 21-derived microRNAs provide an etiological basis for aberrant protein expression in human Down syndrome brains. The Journal of biological chemistry 285:1529-1543.

Kurt MA, Kafa MI, Dierssen M, Davies DC (2004) Deficits of neuronal density in CA1 and synaptic density in the dentate gyrus, CA3 and CA1, in a mouse model of Down syndrome. Brain Res 1022:101-109.

La Rue A (2010) Healthy brain aging: role of cognitive reserve, cognitive stimulation, and cognitive exercises. Clinics in geriatric medicine 26:99-111.

Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, Wals P, Zhang C, Finch CE, Krafft GA, Klein WL (1998) Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. Proceedings of the National Academy of Sciences of the United States of America 95:6448-6453.

Landi S, Cenni MC, Maffei L, Berardi N (2007a) Environmental enrichment effects on development of retinal ganglion cell dendritic stratification require retinal BDNF. PLoS One 2:e346.

Landi S, Ciucci F, Maffei L, Berardi N, Cenni MC (2009) Setting the pace for retinal development: environmental enrichment acts through insulin-like growth factor 1 and brain-derived neurotrophic factor. The Journal of neuroscience : the official journal of the Society for Neuroscience 29:10809-10819.

Landi S, Sale A, Berardi N, Viegi A, Maffei L, Cenni MC (2007b) Retinal functional development is sensitive to environmental enrichment: a role for BDNF. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 21:130-139.

Lanfranchi S, Cornoldi C, Vianello R (2004) Verbal and visuospatial working memory deficits in children with Down syndrome. American journal of mental retardation : AJMR 109:456-466.

Langston RF, Wood ER (2010) Associative recognition and the hippocampus: differential effects of hippocampal lesions on object-place, object-context and object-place-context memory. Hippocampus 20:1139-1153.

Larsen KB, Laursen H, Graem N, Samuelsen GB, Bogdanovic N, Pakkenberg B (2008) Reduced cell number in the neocortical part of the human fetal brain in Down syndrome. Annals of anatomy = Anatomischer Anzeiger : official organ of the Anatomische Gesellschaft 190:421-427.

Larson J, Lynch G, Games D, Seubert P (1999) Alterations in synaptic transmission and long-term potentiation in hippocampal slices from young and aged PDAPP mice. Brain Res 840:23-35.

Latham N, Mason G (2004) From house mouse to mouse house: the behavioural biology of free-living Mus musculus and its implications in the laboratory. Applied animal behaviour science 86:261-289.

Laviola G, Hannan AJ, Macri S, Solinas M, Jaber M (2008) Effects of enriched environment on animal models of neurodegenerative diseases and psychiatric disorders. Neurobiol Dis 31:159-168.

Lazarov O, Robinson J, Tang YP, Hairston IS, Korade-Mirnics Z, Lee VM, Hersh LB, Sapolsky RM, Mirnics K, Sisodia SS (2005) Environmental enrichment reduces Abeta levels and amyloid deposition in transgenic mice. Cell 120:701-713.

Lazic SE, Grote H, Armstrong RJ, Blakemore C, Hannan AJ, van Dellen A, Barker RA (2004) Decreased hippocampal cell proliferation in R6/1 Huntington's mice. Neuroreport 15:811-813.

Leamey CA, Van Wart A, Sur M (2009) Intrinsic patterning and experience-dependent mechanisms that generate eye-specific projections and binocular circuits in the visual pathway. Curr Opin Neurobiol 19:181-187.

Lee HH, Kim H, Lee JW, Kim YS, Yang HY, Chang HK, Lee TH, Shin MC, Lee MH, Shin MS, Park S, Baek S, Kim CJ (2006) Maternal swimming during pregnancy enhances short-term memory and neurogenesis in the hippocampus of rat pups. Brain & development 28:147-154.

Lepagnol-Bestel AM, Zvara A, Maussion G, Quignon F, Ngimbous B, Ramoz N, Imbeaud S, Loe-Mie Y, Benihoud K, Agier N, Salin PA, Cardona A, Khung-Savatovsky S, Kallunki P, Delabar JM, Puskas LG, Delacroix H, Aggerbeck L, Delezoide AL, Delattre O, Gorwood P, Moalic JM, Simonneau M (2009) DYRK1A interacts with

the REST/NRSF-SWI/SNF chromatin remodelling complex to deregulate gene clusters involved in the neuronal phenotypic traits of Down syndrome. Human Molecular Genetics 18:1405-1414.

Levi DM (2006) Visual processing in amblyopia: human studies. Strabismus 14:11-19.

Levy J (1991) The gastrointestinal tract in Down syndrome. Progress in clinical and biological research 373:245-256.

Lewis TL, Maurer D (2005) Multiple sensitive periods in human visual development: evidence from visually deprived children. Developmental psychobiology 46:163-183.

Li S, Hong S, Shepardson NE, Walsh DM, Shankar GM, Selkoe D (2009) Soluble oligomers of amyloid Beta protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. Neuron 62:788-801.

Li Y, Fitzpatrick D, White LE (2006) The development of direction selectivity in ferret visual cortex requires early visual experience. Nat Neurosci 9:676-681.

Liu D, Diorio J, Day JC, Francis DD, Meaney MJ (2000) Maternal care, hippocampal synaptogenesis and cognitive development in rats. Nat Neurosci 3:799-806.

Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S, Pearson D, Plotsky PM, Meaney MJ (1997) Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science 277:1659-1662.

Liyanage S, Barnes J (2008) The eye and Down's syndrome. British journal of hospital medicine 69:632-634.

Llorens-Martin MV, Rueda N, Martinez-Cue C, Torres-Aleman I, Florez J, Trejo JL (2007) Both increases in immature dentate neuron number and decreases of immobility time in the forced swim test occurred in parallel after environmental enrichment of mice. Neuroscience 147:631-638.

Lockrow J, Prakasam A, Huang P, Bimonte-Nelson H, Sambamurti K, Granholm A-C (2009) Cholinergic degeneration and memory loss delayed by vitamin E in a Down syndrome mouse model. Experimental Neurology 216:278-289.

Lonetti G, Angelucci A, Morando L, Boggio EM, Giustetto M, Pizzorusso T (2010) Early environmental enrichment moderates the behavioral and synaptic phenotype of MeCP2 null mice. Biol Psychiatry 67:657-665.

Lonze BE, Ginty DD (2002) Function and regulation of CREB family transcription factors in the nervous system. Neuron 35:605-623.

Lorenzi HA, Reeves RH (2006) Hippocampal hypocellularity in the Ts65Dn mouse originates early in development. Brain Res 1104:153-159.

Losin EA, Rivera SM, O'Hare ED, Sowell ER, Pinter JD (2009) Abnormal fMRI activation pattern during story listening in individuals with Down syndrome. American journal on intellectual and developmental disabilities 114:369-380.

Lott IT, Head E (2001) Down syndrome and Alzheimer's disease: a link between development and aging. Ment Retard Dev Disabil Res Rev 7:172-178.

Lott IT, Head E (2005) Alzheimer disease and Down syndrome: factors in pathogenesis. Neurobiology of Aging 26:383-389.

Lu W, Constantine-Paton M (2004) Eye opening rapidly induces synaptic potentiation and refinement. Neuron 43:237-249.

Lubec B, Yoo BC, Dierssen M, Balic N, Lubec G (2001) Down syndrome patients start early prenatal life with normal cholinergic, monoaminergic and serotoninergic innervation. Journal of neural transmission Supplementum 303-310.

Luhmann HJ, Prince DA (1991) Postnatal maturation of the GABAergic system in rat neocortex. J Neurophysiol 65:247-263.

Lyle R, Bena F, Gagos S, Gehrig C, Lopez G, Schinzel A, Lespinasse J, Bottani A, Dahoun S, Taine L, Doco-Fenzy M, Cornillet-Lefebvre P, Pelet A, Lyonnet S, Toutain A, Colleaux L, Horst J, Kennerknecht I, Wakamatsu N, Descartes M, Franklin JC, Florentin-Arar L, Kitsiou S, Ait Yahya-Graison E, Costantine M, Sinet PM, Delabar JM, Antonarakis SE (2009) Genotype-phenotype correlations in Down syndrome identified by array CGH in 30 cases of partial trisomy and partial monosomy chromosome 21. European journal of human genetics : EJHG 17:454-466.

Maconnachie E (1979) A study of digit fusion in the mouse embryo. Journal of embryology and experimental morphology 49:259-276.

Maenz B, Hekerman P, Vela EM, Galceran J, Becker W (2008) Characterization of the human DYRK1A promoter and its regulation by the transcription factor E2F1. BMC molecular biology 9:30.

Maffei A, Nelson SB, Turrigiano GG (2004) Selective reconfiguration of layer 4 visual cortical circuitry by visual deprivation. Nat Neurosci 7:1353-1359.

Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience 20:9104-9110.

Mann DM (1988) The pathological association between Down syndrome and Alzheimer disease. Mechanisms of ageing and development 43:99-136.

Mann DM, Yates PO, Marcyniuk B, Ravindra CR (1985) Pathological evidence for neurotransmitter deficits in Down's syndrome of middle age. Journal of mental deficiency research 29 (Pt 2):125-135.

Martinez-Cue C, Baamonde C, Lumbreras M, Paz J, Davisson MT, Schmidt C, Dierssen M, Florez J (2002) Differential effects of environmental enrichment on behavior and learning of male and female Ts65Dn mice, a model for Down syndrome. Behav Brain Res 134:185-200.

Martinez-Cue C, Rueda N, Garcia E, Davisson MT, Schmidt C, Florez J (2005) Behavioral, cognitive and biochemical responses to different environmental conditions in male Ts65Dn mice, a model of Down syndrome. Behav Brain Res 163:174-185.

Martinez-Cue C, Rueda N, Garcia E, Florez J (2006) Anxiety and panic responses to a predator in male and female Ts65Dn mice, a model for Down syndrome. Genes, brain, and behavior 5:413-422.

Martinez de Lagran M, Altafaj X, Gallego X, Marti E, Estivill X, Sahun I, Fillat C, Dierssen M (2004) Motor phenotypic alterations in TgDyrk1a transgenic mice implicate DYRK1A in Down syndrome motor dysfunction. Neurobiol Dis 15:132-142.

Martinowich K, Manji H, Lu B (2007) New insights into BDNF function in depression and anxiety. Nat Neurosci 10:1089-1093.

Maurer D, Lewis TL, Brent HP, Levin AV (1999) Rapid improvement in the acuity of infants after visual input. Science 286:108-110.

Maya Vetencourt JF, Sale A, Viegi A, Baroncelli L, De Pasquale R, O'Leary OF, Castren E, Maffei L (2008) The antidepressant fluoxetine restores plasticity in the adult visual cortex. Science 320:385-388.

Maya Vetencourt JF, Tiraboschi E, Spolidoro M, Castren E, Maffei L (2011) Serotonin triggers a transient epigenetic mechanism that reinstates adult visual cortex plasticity in rats. The European journal of neuroscience 33:49-57.

Mazarakis NK, Cybulska-Klosowicz A, Grote H, Pang T, Van Dellen A, Kossut M, Blakemore C, Hannan AJ (2005) Deficits in experience-dependent cortical plasticity and sensory-discrimination learning in presymptomatic Huntington's disease mice. The Journal of neuroscience : the official journal of the Society for Neuroscience 25:3059-3066.

Mc Donald JM, Savva GM, Brayne C, Welzel AT, Forster G, Shankar GM, Selkoe DJ, Ince PG, Walsh DM, Medical Research Council Cognitive F, Ageing S (2010) The presence of sodium dodecyl sulphate-stable Abeta dimers is strongly associated with Alzheimer-type dementia. Brain : a journal of neurology 133:1328-1341.

McNamara RK, Skelton RW (1993) The neuropharmacological and neurochemical basis of place learning in the Morris water maze. Brain research Brain research reviews 18:33-49.

McOmish CE, Hannan AJ (2007) Enviromimetics: exploring gene environment interactions to identify therapeutic targets for brain disorders. Expert opinion on therapeutic targets 11:899-913.

Meakin SO, Shooter EM (1992) The nerve growth factor family of receptors. Trends Neurosci 15:323-331.

Meakin SO, Suter U, Drinkwater CC, Welcher AA, Shooter EM (1992) The rat trk protooncogene product exhibits properties characteristic of the slow nerve growth factor receptor. Proceedings of the National Academy of Sciences of the United States of America 89:2374-2378.

Meaney MJ, Szyf M (2005) Maternal care as a model for experience-dependent chromatin plasticity? Trends Neurosci 28:456-463.

Medini P, Pizzorusso T (2008) Visual experience and plasticity of the visual cortex: a role for epigenetic mechanisms. Frontiers in bioscience : a journal and virtual library 13:3000-3007.

Megarbane A, Ravel A, Mircher C, Sturtz F, Grattau Y, Rethore MO, Delabar JM, Mobley WC (2009) The 50th anniversary of the discovery of trisomy 21: the past, present, and future of research and treatment of Down syndrome. Genetics in medicine : official journal of the American College of Medical Genetics 11:611-616.

Megias M, Verduga R, Dierssen M, Florez J, Insausti R, Crespo D (1997) Cholinergic, serotonergic and catecholaminergic neurons are not affected in Ts65Dn mice. Neuroreport 8:3475-3478.

Menendez M (2005) Down syndrome, Alzheimer's disease and seizures. Brain & development 27:246-252.

Meneses A (1999) 5-HT system and cognition. Neuroscience and biobehavioral reviews 23:1111-1125.

Meneses A, Liy-Salmeron G (2012) Serotonin and emotion, learning and memory. Rev Neurosci 23:543-553.

Micheva KD, Beaulieu C (1997) Development and plasticity of the inhibitory neocortical circuitry with an emphasis on the rodent barrel field cortex: a review. Canadian journal of physiology and pharmacology 75:470-478.

Mirochnic S, Wolf S, Staufenbiel M, Kempermann G (2009) Age effects on the regulation of adult hippocampal neurogenesis by physical activity and environmental enrichment in the APP23 mouse model of Alzheimer disease. Hippocampus 19:1008-1018.

Moechars D, Dewachter I, Lorent K, Reverse D, Baekelandt V, Naidu A, Tesseur I, Spittaels K, Haute CV, Checler F, Godaux E, Cordell B, Van Leuven F (1999) Early phenotypic changes in transgenic mice that overexpress different mutants of amyloid precursor protein in brain. The Journal of biological chemistry 274:6483-6492.

Mogensen J, Pedersen TK, Holm S, Bang LE (1995) Prefrontal cortical mediation of rats' place learning in a modified water maze. Brain research bulletin 38:425-434.

Mohammed AH, Zhu SW, Darmopil S, Hjerling-Leffler J, Ernfors P, Winblad B, Diamond MC, Eriksson PS, Bogdanovic N (2002) Environmental enrichment and the brain. Progress in brain research 138:109-133.

Moldrich RX, Dauphinot L, Laffaire J, Rossier J, Potier MC (2007) Down syndrome gene dosage imbalance on cerebellum development. Prog Neurobiol 82:87-94.

Mon-Williams M, Tresilian JR, Bell VE, Coppard VL, Jobling A, Carson RG (2001) The preparation of reach to grasp movements in adults with Down syndrome. Human movement science 20:587-602.

Moore CS (2006) Postnatal lethality and cardiac anomalies in the Ts65Dn Down syndrome mouse model. Mammalian genome : official journal of the International Mammalian Genome Society 17:1005-1012.

Moore CS, Hawkins C, Franca A, Lawler A, Devenney B, Das I, Reeves RH (2010) Increased male reproductive success in Ts65Dn "Down syndrome" mice. Mammalian genome : official journal of the International Mammalian Genome Society 21:543-549.

Moore CS, Roper RJ (2007) The power of comparative and developmental studies for mouse models of Down syndrome. Mammalian Genome 18:431-443.

Moretti P, Levenson JM, Battaglia F, Atkinson R, Teague R, Antalffy B, Armstrong D, Arancio O, Sweatt JD, Zoghbi HY (2006) Learning and memory and synaptic

plasticity are impaired in a mouse model of Rett syndrome. The Journal of neuroscience : the official journal of the Society for Neuroscience 26:319-327.

Morris R (1984) Developments of a water-maze procedure for studying spatial learning in the rat. Journal of neuroscience methods 11:47-60.

Moser EI, Kropff E, Moser MB (2008) Place cells, grid cells, and the brain's spatial representation system. Annual review of neuroscience 31:69-89.

Mower AF, Liao DS, Nestler EJ, Neve RL, Ramoa AS (2002) cAMP/Ca2+ response element-binding protein function is essential for ocular dominance plasticity. The Journal of neuroscience : the official journal of the Society for Neuroscience 22:2237-2245.

Mower GD, Guo Y (2001) Comparison of the expression of two forms of glutamic acid decarboxylase (GAD67 and GAD65) in the visual cortex of normal and dark-reared cats. Brain research Developmental brain research 126:65-74.

Mufson EJ, Cochran E, Benzing W, Kordower JH (1993) Galaninergic innervation of the cholinergic vertical limb of the diagonal band (Ch2) and bed nucleus of the stria terminalis in aging, Alzheimer's disease and Down's syndrome. Dementia 4:237-250.

Mufson EJ, Counts SE, Perez SE, Ginsberg SD (2008) Cholinergic system during the progression of Alzheimer's disease: therapeutic implications. Expert review of neurotherapeutics 8:1703-1718.

Mumby DG, Gaskin S, Glenn MJ, Schramek TE, Lehmann H (2002) Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts. Learning & memory 9:49-57.

Murphy KM, Beston BR, Boley PM, Jones DG (2005) Development of human visual cortex: a balance between excitatory and inhibitory plasticity mechanisms. Developmental psychobiology 46:209-221.

Murphy KP, Carter RJ, Lione LA, Mangiarini L, Mahal A, Bates GP, Dunnett SB, Morton AJ (2000) Abnormal synaptic plasticity and impaired spatial cognition in mice transgenic for exon 1 of the human Huntington's disease mutation. The Journal of neuroscience : the official journal of the Society for Neuroscience 20:5115-5123.

Myers BA, Pueschel SM (1991) Psychiatric disorders in persons with Down syndrome. The Journal of nervous and mental disease 179:609-613.

Nadel L (2003) Down's syndrome: a genetic disorder in biobehavioral perspective. Genes, brain, and behavior 2:156-166.

Nadel L, Bohbot V (2001) Consolidation of memory. Hippocampus 11:56-60.

Naka F, Shiga T, Yaguchi M, Okado N (2002) An enriched environment increases noradrenaline concentration in the mouse brain. Brain Res 924:124-126.

Nelson L, Johnson JK, Freedman M, Lott I, Groot J, Chang M, Milgram NW, Head E (2005) Learning and memory as a function of age in Down syndrome: a study using animal-based tasks. Progress in neuro-psychopharmacology & biological psychiatry 29:443-453.

Netzer WJ, Powell C, Nong Y, Blundell J, Wong L, Duff K, Flajolet M, Greengard P (2010) Lowering beta-amyloid levels rescues learning and memory in a Down syndrome mouse model. PLoS One 5:e10943.

Neufang M, Heinze HJ, Duzel E (2006) Electromagnetic correlates of recognition memory processes. Clinical EEG and neuroscience : official journal of the EEG and Clinical Neuroscience Society 37:300-308.

Newpher TM, Ehlers MD (2009) Spine microdomains for postsynaptic signaling and plasticity. Trends in cell biology 19:218-227.

Nibuya M, Morinobu S, Duman RS (1995) Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. The Journal of neuroscience : the official journal of the Society for Neuroscience 15:7539-7547.

Nibuya M, Nestler EJ, Duman RS (1996) Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience 16:2365-2372.

Nieuwenhuis-Mark RE (2009) Diagnosing Alzheimer's dementia in Down syndrome: problems and possible solutions. Research in developmental disabilities 30:827-838.

Nithianantharajah J, Barkus C, Murphy M, Hannan AJ (2008) Gene-environment interactions modulating cognitive function and molecular correlates of synaptic plasticity in Huntington's disease transgenic mice. Neurobiol Dis 29:490-504.

Nithianantharajah J, Hannan AJ (2006) Enriched environments, experience-dependent plasticity and disorders of the nervous system. Nature reviews Neuroscience 7:697-709.

O'Doherty A, Ruf S, Mulligan C, Hildreth V, Errington ML, Cooke S, Sesay A, Modino S, Vanes L, Hernandez D, Linehan JM, Sharpe PT, Brandner S, Bliss TV, Henderson

DJ, Nizetic D, Tybulewicz VL, Fisher EM (2005) An aneuploid mouse strain carrying human chromosome 21 with Down syndrome phenotypes. Science 309:2033-2037.

Oke A, Adhiyaman V, Aziz K, Ross A (2001) Dose-dependent seizure activity associated with fluoxetine therapy. QJM : monthly journal of the Association of Physicians 94:113-114.

Okui M, Ide T, Morita K, Funakoshi E, Ito F, Ogita K, Yoneda Y, Kudoh J, Shimizu N (1999) High-level expression of the Mnb/Dyrk1A gene in brain and heart during rat early development. Genomics 62:165-171.

Olson LE, Richtsmeier JT, Leszl J, Reeves RH (2004) A chromosome 21 critical region does not cause specific Down syndrome phenotypes. Science 306:687-690.

Olson LE, Roper RJ, Sengstaken CL, Peterson EA, Aquino V, Galdzicki Z, Siarey R, Pletnikov M, Moran TH, Reeves RH (2007) Trisomy for the Down syndrome 'critical region' is necessary but not sufficient for brain phenotypes of trisomic mice. Human Molecular Genetics 16:774-782.

Origlia N, Capsoni S, Domenici L, Cattaneo A (2006) Time window in cholinomimetic ability to rescue long-term potentiation in neurodegenerating anti-nerve growth factor mice. Journal of Alzheimer's disease : JAD 9:59-68.

Orth M, Schippling S, Schneider SA, Bhatia KP, Talelli P, Tabrizi SJ, Rothwell JC (2010) Abnormal motor cortex plasticity in premanifest and very early manifest Huntington disease. Journal of neurology, neurosurgery, and psychiatry 81:267-270.

Palisano RJ, Walter SD, Russell DJ, Rosenbaum PL, Gemus M, Galuppi BE, Cunningham L (2001) Gross motor function of children with down syndrome: creation of motor growth curves. Archives of physical medicine and rehabilitation 82:494-500.

Pang TY, Hannan AJ (2013) Enhancement of cognitive function in models of brain disease through environmental enrichment and physical activity. Neuropharmacology 64:515-528.

Park H, Poo MM (2013) Neurotrophin regulation of neural circuit development and function. Nature reviews Neuroscience 14:7-23.

Parker AW, Bronks R, Snyder CW, Jr. (1986) Walking patterns in Down's syndrome. Journal of mental deficiency research 30 (Pt 4):317-330.

Parker SE, Mai CT, Canfield MA, Rickard R, Wang Y, Meyer RE, Anderson P, Mason CA, Collins JS, Kirby RS, Correa A, National Birth Defects Prevention N (2010) Updated National Birth Prevalence estimates for selected birth defects in the United States,

2004-2006. Birth defects research Part A, Clinical and molecular teratology 88:1008-1016.

Patterson SL, Abel T, Deuel TA, Martin KC, Rose JC, Kandel ER (1996) Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. Neuron 16:1137-1145.

Pearlson GD, Breiter SN, Aylward EH, Warren AC, Grygorcewicz M, Frangou S, Barta PE, Pulsifer MB (1998) MRI brain changes in subjects with Down syndrome with and without dementia. Developmental medicine and child neurology 40:326-334.

Pennington BF, Moon J, Edgin J, Stedron J, Nadel L (2003) The neuropsychology of Down syndrome: evidence for hippocampal dysfunction. Child development 74:75-93.

Pereira PL, Magnol L, Sahun I, Brault V, Duchon A, Prandini P, Gruart A, Bizot JC, Chadefaux-Vekemans B, Deutsch S, Trovero F, Delgado-Garcia JM, Antonarakis SE, Dierssen M, Herault Y (2009) A new mouse model for the trisomy of the Abcg1-U2af1 region reveals the complexity of the combinatorial genetic code of down syndrome. Hum Mol Genet 18:4756-4769.

Pérez-Cremades D, Hernández S, Blasco-Ibáñez JM, Crespo C, Nacher J, Varea E (2010) Alteration of inhibitory circuits in the somatosensory cortex of Ts65Dn mice, a model for Down’s syndrome. Journal of Neural Transmission 117:445-455.

Petit TL, LeBoutillier JC, Alfano DP, Becker LE (1984) Synaptic development in the human fetus: a morphometric analysis of normal and Down's syndrome neocortex. Exp Neurol 83:13-23.

Pham TA, Graham SJ, Suzuki S, Barco A, Kandel ER, Gordon B, Lickey ME (2004) A semi-persistent adult ocular dominance plasticity in visual cortex is stabilized by activated CREB. Learning & memory 11:738-747.

Pham TA, Impey S, Storm DR, Stryker MP (1999a) CRE-mediated gene transcription in neocortical neuronal plasticity during the developmental critical period. Neuron 22:63-72.

Pham TM, Soderstrom S, Winblad B, Mohammed AH (1999b) Effects of environmental enrichment on cognitive function and hippocampal NGF in the non-handled rats. Behav Brain Res 103:63-70.

Pinter JD, Brown WE, Eliez S, Schmitt JE, Capone GT, Reiss AL (2001) Amygdala and hippocampal volumes in children with Down syndrome: a high-resolution MRI study. Neurology 56:972-974.

Pizzorusso T, Medini P, Berardi N, Chierzi S, Fawcett JW, Maffei L (2002) Reactivation of ocular dominance plasticity in the adult visual cortex. Science 298:1248-1251.

Pizzorusso T, Medini P, Landi S, Baldini S, Berardi N, Maffei L (2006) Structural and functional recovery from early monocular deprivation in adult rats. Proceedings of the National Academy of Sciences of the United States of America 103:8517-8522.

Pizzorusso T, Ratto GM, Putignano E, Maffei L (2000) Brain-derived neurotrophic factor causes cAMP response element-binding protein phosphorylation in absence of calcium increases in slices and cultured neurons from rat visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 20:2809-2816.

Pletcher MT, Wiltshire T, Cabin DE, Villanueva M, Reeves RH (2001) Use of comparative physical and sequence mapping to annotate mouse chromosome 16 and human chromosome 21. Genomics 74:45-54.

Pollonini G, Gao V, Rabe A, Palminiello S, Albertini G, Alberini CM (2008) Abnormal expression of synaptic proteins and neurotrophin-3 in the down syndrome mouse model Ts65Dn. Neuroscience 156:99-106.

Prasher VP (1993) Seizures associated with fluoxetine therapy. Seizure : the journal of the British Epilepsy Association 2:315-317.

Pritchard MA, Kola I (1999) The "gene dosage effect" hypothesis versus the "amplified developmental instability" hypothesis in Down syndrome. Journal of neural transmission Supplementum 57:293-303.

Prusky GT, Douglas RM (2003) Developmental plasticity of mouse visual acuity. The European journal of neuroscience 17:167-173.

Prusky GT, Reidel C, Douglas RM (2000) Environmental enrichment from birth enhances visual acuity but not place learning in mice. Behav Brain Res 114:11-15.

Puglia MP, Valenzuela CF (2009) AMPAR-mediated synaptic transmission in the CA1 hippocampal region of neonatal rats: unexpected resistance to repeated ethanol exposure. Alcohol 43:619-625.

Puglia MP, Valenzuela CF (2010) Repeated third trimester-equivalent ethanol exposure inhibits long-term potentiation in the hippocampal CA1 region of neonatal rats. Alcohol 44:283-290.

Putignano E, Lonetti G, Cancedda L, Ratto G, Costa M, Maffei L, Pizzorusso T (2007) Developmental downregulation of histone posttranslational modifications regulates visual cortical plasticity. Neuron 53:747-759.

Raju SS, Noor AR, Gurthu S, Giriyappanavar CR, Acharya SB, Low HC, Quah SH (1999) Effect of fluoxetine on maximal electroshock seizures in mice: acute vs chronic administration. Pharmacological research : the official journal of the Italian Pharmacological Society 39:451-454.

Rampon C, Jiang CH, Dong H, Tang YP, Lockhart DJ, Schultz PG, Tsien JZ, Hu Y (2000) Effects of environmental enrichment on gene expression in the brain. Proceedings of the National Academy of Sciences of the United States of America 97:12880-12884.

Rampon C, Tsien JZ (2000) Genetic analysis of learning behavior-induced structural plasticity. Hippocampus 10:605-609.

Rantamaki T, Hendolin P, Kankaanpaa A, Mijatovic J, Piepponen P, Domenici E, Chao MV, Mannisto PT, Castren E (2007) Pharmacologically diverse antidepressants rapidly activate brain-derived neurotrophic factor receptor TrkB and induce phospholipase-Cgamma signaling pathways in mouse brain. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 32:2152-2162.

Rasmuson S, Olsson T, Henriksson BG, Kelly PA, Holmes MC, Seckl JR, Mohammed AH (1998) Environmental enrichment selectively increases 5-HT1A receptor mRNA expression and binding in the rat hippocampus. Brain research Molecular brain research 53:285-290.

Ratto GM, Pizzorusso T (2006) A kinase with a vision: Role of ERK in the synaptic plasticity of the visual cortex. Advances in experimental medicine and biology 557:122-132.

Raz N, Torres IJ, Briggs SD, Spencer WD, Thornton AE, Loken WJ, Gunning FM, McQuain JD, Driesen NR, Acker JD (1995) Selective neuroanatomic abnormalities in Down's syndrome and their cognitive correlates: evidence from MRI morphometry. Neurology 45:356-366.

Redish AD, Touretzky DS (1998) The role of the hippocampus in solving the Morris water maze. Neural computation 10:73-111.

Reeves RH, Garner CC (2007) A year of unprecedented progress in Down syndrome basic research. Mental Retardation and Developmental Disabilities Research Reviews 13:215-220.

Reeves RH, Irving NG, Moran TH, Wohn A, Kitt C, Sisodia SS, Schmidt C, Bronson RT, Davisson MT (1995) A mouse model for Down syndrome exhibits learning and behaviour deficits. Nature genetics 11:177-184.

Represa A, Ben-Ari Y (2005) Trophic actions of GABA on neuronal development. Trends Neurosci 28:278-283.

Reynolds S, Lane SJ, Richards L (2010) Using animal models of enriched environments to inform research on sensory integration intervention for the rehabilitation of neurodevelopmental disorders. Journal of neurodevelopmental disorders 2:120-132.

Richtsmeier JT, Baxter LL, Reeves RH (2000) Parallels of craniofacial maldevelopment in Down syndrome and Ts65Dn mice. Developmental dynamics : an official publication of the American Association of Anatomists 217:137-145.

Riedel WJ, Klaassen T, Deutz NE, van Someren A, van Praag HM (1999) Tryptophan depletion in normal volunteers produces selective impairment in memory consolidation. Psychopharmacology 141:362-369.

Risser D, Lubec G, Cairns N, Herrera-Marschitz M (1997) Excitatory amino acids and monoamines in parahippocampal gyrus and frontal cortical pole of adults with Down syndrome. Life sciences 60:1231-1237.

Rissman RA, Mobley WC (2011) Implications for treatment: GABAA receptors in aging, Down syndrome and Alzheimer’s disease. Journal of Neurochemistry no-no.

Robertson RT, Baratta J, Yu J, Guthrie KM (2006) A role for neurotrophin-3 in targeting developing cholinergic axon projections to cerebral cortex. Neuroscience 143:523-539.

Rocco FJ, Cronin-Golomb A, Lai F (1997) Alzheimer-like visual deficits in Down syndrome. Alzheimer disease and associated disorders 11:88-98.

Rochefort NL, Garaschuk O, Milos RI, Narushima M, Marandi N, Pichler B, Kovalchuk Y, Konnerth A (2009) Sparsification of neuronal activity in the visual cortex at eye-opening. Proceedings of the National Academy of Sciences of the United States of America 106:15049-15054.

Roizen NJ, Patterson D (2003) Down's syndrome. Lancet 361:1281-1289.

Roizen NJ, Wolters C, Nicol T, Blondis TA (1993) Hearing loss in children with Down syndrome. The Journal of pediatrics 123:S9-12.

Ronca AE, Lamkin CA, Alberts JR (1993) Maternal contributions to sensory experience in the fetal and newborn rat (Rattus norvegicus). Journal of comparative psychology 107:61-74.

Rondi-Reig L, Burguiere E (2005) Is the cerebellum ready for navigation? Progress in brain research 148:199-212.

Roper RJ (2005) Perinatal Loss of Ts65Dn Down Syndrome Mice. Genetics 172:437-443.

Roper RJ, Baxter LL, Saran NG, Klinedinst DK, Beachy PA, Reeves RH (2006) Defective cerebellar response to mitogenic Hedgehog signaling in Down [corrected] syndrome mice. Proceedings of the National Academy of Sciences of the United States of America 103:1452-1456.

Roper RJ, Reeves RH (2006) Understanding the basis for Down syndrome phenotypes. PLoS genetics 2:e50.

Ropper AH, Williams RS (1980) Relationship between plaques, tangles, and dementia in Down syndrome. Neurology 30:639-644.

Rosenstein DL, Nelson JC, Jacobs SC (1993) Seizures associated with antidepressants: a review. The Journal of clinical psychiatry 54:289-299.

Rosenzweig MR, Bennett EL (1969) Effects of differential environments on brain weights and enzyme activities in gerbils, rats, and mice. Developmental psychobiology 2:87-95.

Rosenzweig MR, Bennett EL (1996) Psychobiology of plasticity: effects of training and experience on brain and behavior. Behav Brain Res 78:57-65.

Rosenzweig MR, Bennett EL, Hebert M, Morimoto H (1978) Social grouping cannot account for cerebral effects of enriched environments. Brain Res 153:563-576.

Rowe J, Lavender A, Turk V (2006) Cognitive executive function in Down's syndrome. The British journal of clinical psychology / the British Psychological Society 45:5-17.

Rueda N, Flórez J, Martínez-Cué C (2008) Chronic pentylenetetrazole but not donepezil treatment rescues spatial cognition in Ts65Dn mice, a model for Down syndrome. Neuroscience Letters 433:22-27.

Rueda N, Mostany R, Pazos A, Florez J, Martinez-Cue C (2005) Cell proliferation is reduced in the dentate gyrus of aged but not young Ts65Dn mice, a model of Down syndrome. Neurosci Lett 380:197-201.

Rumble B, Retallack R, Hilbich C, Simms G, Multhaup G, Martins R, Hockey A, Montgomery P, Beyreuther K, Masters CL (1989) Amyloid A4 protein and its precursor in Down's syndrome and Alzheimer's disease. The New England journal of medicine 320:1446-1452.

Russo-Neustadt A, Beard RC, Cotman CW (1999) Exercise, antidepressant medications, and enhanced brain derived neurotrophic factor expression. Neuropsychopharmacology :

official publication of the American College of Neuropsychopharmacology 21:679-682.

Sago H, Carlson EJ, Smith DJ, Kilbridge J, Rubin EM, Mobley WC, Epstein CJ, Huang TT (1998) Ts1Cje, a partial trisomy 16 mouse model for Down syndrome, exhibits learning and behavioral abnormalities. Proceedings of the National Academy of Sciences of the United States of America 95:6256-6261.

Sago H, Carlson EJ, Smith DJ, Rubin EM, Crnic LS, Huang TT, Epstein CJ (2000) Genetic dissection of region associated with behavioral abnormalities in mouse models for Down syndrome. Pediatric research 48:606-613.

Sale A, Berardi N, Maffei L (2009) Enrich the environment to empower the brain. Trends Neurosci 32:233-239.

Sale A, Berardi N, Spolidoro M, Baroncelli L, Maffei L (2010) GABAergic inhibition in visual cortical plasticity. Frontiers in cellular neuroscience 4:10.

Sale A, Cenni MC, Ciucci F, Putignano E, Chierzi S, Maffei L (2007a) Maternal enrichment during pregnancy accelerates retinal development of the fetus. PLoS One 2:e1160.

Sale A, Maya Vetencourt JF, Medini P, Cenni MC, Baroncelli L, De Pasquale R, Maffei L (2007b) Environmental enrichment in adulthood promotes amblyopia recovery through a reduction of intracortical inhibition. Nat Neurosci 10:679-681.

Sale A, Putignano E, Cancedda L, Landi S, Cirulli F, Berardi N, Maffei L (2004) Enriched environment and acceleration of visual system development. Neuropharmacology 47:649-660.

Salehi A, Delcroix J-D, Belichenko PV, Zhan K, Wu C, Valletta JS, Takimoto-Kimura R, Kleschevnikov AM, Sambamurti K, Chung PP, Xia W, Villar A, Campbell WA, Kulnane LS, Nixon RA, Lamb BT, Epstein CJ, Stokin GB, Goldstein LSB, Mobley WC (2006) Increased App Expression in a Mouse Model of Down's Syndrome Disrupts NGF Transport and Causes Cholinergic Neuron Degeneration. Neuron 51:29-42.

Salehi A, Faizi M, Colas D, Valletta J, Laguna J, Takimoto-Kimura R, Kleschevnikov A, Wagner SL, Aisen P, Shamloo M, Mobley WC (2009) Restoration of norepinephrine-modulated contextual memory in a mouse model of Down syndrome. Science translational medicine 1:7ra17.

Scali M, Begenisic T, Mainardi M, Milanese M, Bonifacino T, Bonanno G, Sale A, Maffei L (2013) Fluoxetine treatment promotes functional recovery in a rat model of cervical spinal cord injury. Scientific reports 3:2217.

Schanberg SM, Field TM (1987) Sensory deprivation stress and supplemental stimulation in the rat pup and preterm human neonate. Child development 58:1431-1447.

Schanberg SM, Ingledue VF, Lee JY, Hannun YA, Bartolome JV (2003) PKC alpha mediates maternal touch regulation of growth-related gene expression in infant rats. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 28:1026-1030.

Schliebs R, Arendt T (2011) The cholinergic system in aging and neuronal degeneration. Behav Brain Res 221:555-563.

Schmidt-Sidor B, Wisniewski KE, Shepard TH, Sersen EA (1990) Brain growth in Down syndrome subjects 15 to 22 weeks of gestational age and birth to 60 months. Clinical neuropathology 9:181-190.

Schmitt JA, Jorissen BL, Sobczak S, van Boxtel MP, Hogervorst E, Deutz NE, Riedel WJ (2000) Tryptophan depletion impairs memory consolidation but improves focussed attention in healthy young volunteers. J Psychopharmacol 14:21-29.

Schneider C, Risser D, Kirchner L, Kitzmuller E, Cairns N, Prast H, Singewald N, Lubec G (1997) Similar deficits of central histaminergic system in patients with Down syndrome and Alzheimer disease. Neurosci Lett 222:183-186.

Schulz E, Scholz B (1992) [Neurohistological findings in the parietal cortex of children with chromosome aberrations]. Journal fur Hirnforschung 33:37-62.

Scott-McKean JJ, Chang B, Hurd RE, Nusinowitz S, Schmidt C, Davisson MT, Costa AC (2010) The mouse model of Down syndrome Ts65Dn presents visual deficits as assessed by pattern visual evoked potentials. Investigative ophthalmology & visual science 51:3300-3308.

Scott-McKean JJ, Costa AC (2011) Exaggerated NMDA mediated LTD in a mouse model of Down syndrome and pharmacological rescuing by memantine. Learning & memory 18:774-778.

Seo H, Isacson O (2005) Abnormal APP, cholinergic and cognitive function in Ts65Dn Down's model mice. Experimental Neurology 193:469-480.

Sérégaza Z, Roubertoux PL, Jamon M, Soumireu-Mourat B (2006) Mouse Models of Cognitive Disorders in Trisomy 21: A Review. Behavior Genetics 36:387-404.

Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, Selkoe DJ (2008) Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nature medicine 14:837-842.

Shapiro BL (1983) Down syndrome--a disruption of homeostasis. American journal of medical genetics 14:241-269.

Shapiro BL (1997) Whither Down syndrome critical regions? Human genetics 99:421-423.

Shapiro MB, France TD (1985) The ocular features of Down's syndrome. American journal of ophthalmology 99:659-663.

Shin M, Besser LM, Kucik JE, Lu C, Siffel C, Correa A, Congenital Anomaly Multistate P, Survival C (2009) Prevalence of Down syndrome among children and adolescents in 10 regions of the United States. Pediatrics 124:1565-1571.

Shumway-Cook A, Woollacott MH (1985) Dynamics of postural control in the child with Down syndrome. Physical therapy 65:1315-1322.

Siarey RJ, Carlson EJ, Epstein CJ, Balbo A, Rapoport SI, Galdzicki Z (1999) Increased synaptic depression in the Ts65Dn mouse, a model for mental retardation in Down syndrome. Neuropharmacology 38:1917-1920.

Siarey RJ, Stoll J, Rapoport SI, Galdzicki Z (1997) Altered long-term potentiation in the young and old Ts65Dn mouse, a model for Down Syndrome. Neuropharmacology 36:1549-1554.

Silber BY, Schmitt JA (2010) Effects of tryptophan loading on human cognition, mood, and sleep. Neuroscience and biobehavioral reviews 34:387-407.

Silva AJ, Ehninger D (2009) Adult reversal of cognitive phenotypes in neurodevelopmental disorders. Journal of neurodevelopmental disorders 1:150-157.

Smith SL, Trachtenberg JT (2007) Experience-dependent binocular competition in the visual cortex begins at eye opening. Nat Neurosci 10:370-375.

Soffie M, Hahn K, Terao E, Eclancher F (1999) Behavioural and glial changes in old rats following environmental enrichment. Behav Brain Res 101:37-49.

Sofroniew MV, Howe CL, Mobley WC (2001) Nerve growth factor signaling, neuroprotection, and neural repair. Annual review of neuroscience 24:1217-1281.

Sorra KE, Harris KM (2000) Overview on the structure, composition, function, development, and plasticity of hippocampal dendritic spines. Hippocampus 10:501-511.

Spires TL, Grote HE, Varshney NK, Cordery PM, van Dellen A, Blakemore C, Hannan AJ (2004) Environmental enrichment rescues protein deficits in a mouse model of Huntington's disease, indicating a possible disease mechanism. The Journal of neuroscience : the official journal of the Society for Neuroscience 24:2270-2276.

Spolidoro M, Sale A, Berardi N, Maffei L (2009) Plasticity in the adult brain: lessons from the visual system. Experimental brain research Experimentelle Hirnforschung Experimentation cerebrale 192:335-341.

Stafstrom CE (1993) Epilepsy in Down syndrome: clinical aspects and possible mechanisms. American journal of mental retardation : AJMR 98 Suppl:12-26.

Stasko MR, Costa AC (2004) Experimental parameters affecting the Morris water maze performance of a mouse model of Down syndrome. Behav Brain Res 154:1-17.

Stearns NA, Schaevitz LR, Bowling H, Nag N, Berger UV, Berger-Sweeney J (2007) Behavioral and anatomical abnormalities in Mecp2 mutant mice: a model for Rett syndrome. Neuroscience 146:907-921.

Stewart LS, Persinger MA, Cortez MA, Snead OC, 3rd (2007) Chronobiometry of behavioral activity in the Ts65Dn model of Down syndrome. Behav Genet 37:388-398.

Stigliani S, Zappettini S, Raiteri L, Passalacqua M, Melloni E, Venturi C, Tacchetti C, Diaspro A, Usai C, Bonanno G (2006) Glia re-sealed particles freshly prepared from adult rat brain are competent for exocytotic release of glutamate. J Neurochem 96:656-668.

Suchard JR (2008) Fluoxetine overdose-induced seizure. The western journal of emergency medicine 9:154-156.

Suetsugu M, Mehraein P (1980) Spine distribution along the apical dendrites of the pyramidal neurons in Down's syndrome. A quantitative Golgi study. Acta Neuropathol 50:207-210.

Sussan TE, Yang A, Li F, Ostrowski MC, Reeves RH (2008) Trisomy represses Apc(Min)-mediated tumours in mouse models of Down's syndrome. Nature 451:73-75.

Suttle CM, Turner AM (2004) Transient pattern visual evoked potentials in children with Down's syndrome. Ophthalmic & physiological optics : the journal of the British College of Ophthalmic Opticians 24:91-99.

Tagawa Y, Kanold PO, Majdan M, Shatz CJ (2005) Multiple periods of functional ocular dominance plasticity in mouse visual cortex. Nat Neurosci 8:380-388.

Takakusaki K (2008) Forebrain control of locomotor behaviors. Brain research reviews 57:192-198.

Takashima S, Becker LE, Armstrong DL, Chan F (1981) Abnormal neuronal development in the visual cortex of the human fetus and infant with down's syndrome. A quantitative and qualitative Golgi study. Brain Res 225:1-21.

Takashima S, Ieshima A, Nakamura H, Becker LE (1989) Dendrites, dementia and the Down syndrome. Brain & development 11:131-133.

Takashima S, Iida K, Mito T, Arima M (1994) Dendritic and histochemical development and ageing in patients with Down's syndrome. Journal of intellectual disability research : JIDR 38 (Pt 3):265-273.

Tatsumi M, Groshan K, Blakely RD, Richelson E (1997) Pharmacological profile of antidepressants and related compounds at human monoamine transporters. European journal of pharmacology 340:249-258.

Tecott LH (2007) Serotonin and the orchestration of energy balance. Cell metabolism 6:352-361.

Tejedor FJ, Hammerle B (2011) MNB/DYRK1A as a multiple regulator of neuronal development. The FEBS journal 278:223-235.

Terry AV, Jr. (2009) Spatial Navigation (Water Maze) Tasks. In: Methods of Behavior Analysis in Neuroscience (Buccafusco, J. J., ed) Boca Raton (FL).

Terry RD, Davies P (1980) Dementia of the Alzheimer type. Annual review of neuroscience 3:77-95.

Thome J, Sakai N, Shin K, Steffen C, Zhang YJ, Impey S, Storm D, Duman RS (2000) cAMP response element-mediated gene transcription is upregulated by chronic antidepressant treatment. The Journal of neuroscience : the official journal of the Society for Neuroscience 20:4030-4036.

Thompson SM, Fortunato C, McKinney RA, Muller M, Gahwiler BH (1996) Mechanisms underlying the neuropathological consequences of epileptic activity in the rat hippocampus in vitro. J Comp Neurol 372:515-528.

Timney B (1983) The effects of early and late monocular deprivation on binocular depth perception in cats. Brain Res 283:235-243.

Timney B, Mitchell DE, Giffin F (1978) The development of vision in cats after extended periods of dark-rearing. Experimental brain research Experimentelle Hirnforschung Experimentation cerebrale 31:547-560.

Tirassa P, Manni L, Aloe L, Lundeberg T (2002) Cholecystokinin-8 and nerve growth factor: two endogenous molecules working for the upkeep and repair of the nervous system. Current drug targets CNS and neurological disorders 1:495-510.

Toiber D, Azkona G, Ben-Ari S, Toran N, Soreq H, Dierssen M (2010) Engineering DYRK1A overdosage yields Down syndrome-characteristic cortical splicing aberrations. Neurobiol Dis 40:348-359.

Torasdotter M, Metsis M, Henriksson BG, Winblad B, Mohammed AH (1998) Environmental enrichment results in higher levels of nerve growth factor mRNA in the rat visual cortex and hippocampus. Behav Brain Res 93:83-90.

Torres-Aleman I (1999) Insulin-like growth factors as mediators of functional plasticity in the adult brain. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 31:114-119.

Townsend M, Shankar GM, Mehta T, Walsh DM, Selkoe DJ (2006) Effects of secreted oligomers of amyloid beta-protein on hippocampal synaptic plasticity: a potent role for trimers. The Journal of physiology 572:477-492.

Trivedi MH, Rush AJ, Armitage R, Gullion CM, Grannemann BD, Orsulak PJ, Roffwarg HP (1999) Effects of fluoxetine on the polysomnogram in outpatients with major depression. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 20:447-459.

Tsiaras WG, Pueschel S, Keller C, Curran R, Giesswein S (1999) Amblyopia and visual acuity in children with Down's syndrome. The British journal of ophthalmology 83:1112-1114.

Turleau C, Vekemans M (2010) [Trisomy 21: fifty years between medicine and science]. Medecine sciences : M/S 26:267-272.

Turrigiano GG (1999) Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same. Trends Neurosci 22:221-227.

Turrigiano GG, Nelson SB (2004) Homeostatic plasticity in the developing nervous system. Nature reviews Neuroscience 5:97-107.

Ulrich J (1985) Alzheimer changes in nondemented patients younger than sixty-five: possible early stages of Alzheimer's disease and senile dementia of Alzheimer type. Annals of neurology 17:273-277.

van Dellen A, Blakemore C, Deacon R, York D, Hannan AJ (2000) Delaying the onset of Huntington's in mice. Nature 404:721-722.

van Praag H, Kempermann G, Gage FH (2000) Neural consequences of environmental enrichment. Nature reviews Neuroscience 1:191-198.

Van Sluyters RC, Stewart DL (1974) Binocular neurons of the rabbit's visual cortex: effects of monocular sensory deprivation. Experimental brain research Experimentelle Hirnforschung Experimentation cerebrale 19:196-204.

Vicari S, Bellucci S, Carlesimo GA (2000) Implicit and explicit memory: a functional dissociation in persons with Down syndrome. Neuropsychologia 38:240-251.

Vicari S, Bellucci S, Carlesimo GA (2005) Visual and spatial long-term memory: differential pattern of impairments in Williams and Down syndromes. Developmental medicine and child neurology 47:305-311.

Vicari S, Bellucci S, Carlesimo GA (2006) Evidence from two genetic syndromes for the independence of spatial and visual working memory. Developmental medicine and child neurology 48:126-131.

Vicari S, Carlesimo A, Caltagirone C (1995) Short-term memory in persons with intellectual disabilities and Down's syndrome. Journal of intellectual disability research : JIDR 39 (Pt 6):532-537.

Vicari S, Marotta L, Carlesimo GA (2004) Verbal short-term memory in Down's syndrome: an articulatory loop deficit? Journal of intellectual disability research : JIDR 48:80-92.

Vink J, Incerti M, Toso L, Roberson R, Abebe D, Spong CY (2009) Prenatal NAP+SAL prevents developmental delay in a mouse model of Down syndrome through effects on N-methyl-D-aspartic acid and gamma-aminobutyric acid receptors. American journal of obstetrics and gynecology 200:524 e521-524.

Visu-Petra L, Benga O, Tincas I, Miclea M (2007) Visual-spatial processing in children and adolescents with Down's syndrome: a computerized assessment of memory skills. Journal of intellectual disability research : JIDR 51:942-952.

Vuksic M, Petanjek Z, Rasin MR, Kostovic I (2002) Perinatal growth of prefrontal layer III pyramids in Down syndrome. Pediatric neurology 27:36-38.

Wagner RS, Caputo AR, Reynolds RD (1990) Nystagmus in Down's syndrome. Ophthalmology 97:1439-1444.

Wainwright PE, Levesque S, Krempulec L, Bulman-Fleming B, McCutcheon D (1993) Effects of environmental enrichment on cortical depth and Morris-maze performance in B6D2F2 mice exposed prenatally to ethanol. Neurotoxicology and teratology 15:11-20.

Wallace W, Bear MF (2004) A morphological correlate of synaptic scaling in visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 24:6928-6938.

Wang C, Zucker RS (1998) Regulation of synaptic vesicle recycling by calcium and serotonin. Neuron 21:155-167.

Wang HW, Pasternak JF, Kuo H, Ristic H, Lambert MP, Chromy B, Viola KL, Klein WL, Stine WB, Krafft GA, Trommer BL (2002) Soluble oligomers of beta amyloid (1-42) inhibit long-term potentiation but not long-term depression in rat dentate gyrus. Brain Res 924:133-140.

Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ (2004) Epigenetic programming by maternal behavior. Nat Neurosci 7:847-854.

Weaver IC, Champagne FA, Brown SE, Dymov S, Sharma S, Meaney MJ, Szyf M (2005) Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: altering epigenetic marking later in life. The Journal of neuroscience : the official journal of the Society for Neuroscience 25:11045-11054.

Wechsler J, Greene M, McDevitt MA, Anastasi J, Karp JE, Le Beau MM, Crispino JD (2002) Acquired mutations in GATA1 in the megakaryoblastic leukemia of Down syndrome. Nature genetics 32:148-152.

Wei F, Dubner R, Zou S, Ren K, Bai G, Wei D, Guo W (2010) Molecular depletion of descending serotonin unmasks its novel facilitatory role in the development of persistent pain. The Journal of neuroscience : the official journal of the Society for Neuroscience 30:8624-8636.

Weitzdoerfer R, Dierssen M, Fountoulakis M, Lubec G (2001) Fetal life in Down syndrome starts with normal neuronal density but impaired dendritic spines and synaptosomal structure. Journal of neural transmission Supplementum 59-70.

Werner LA, Mancl LR, Folsom RC (1996) Preliminary observations on the development of auditory sensitivity in infants with Down syndrome. Ear and hearing 17:455-468.

West AE, Greenberg ME (2011) Neuronal activity-regulated gene transcription in synapse development and cognitive function. Cold Spring Harbor perspectives in biology 3.

Westmark CJ, Westmark PR, Malter JS (2010) Alzheimer's disease and Down syndrome rodent models exhibit audiogenic seizures. Journal of Alzheimer's disease : JAD 20:1009-1013.

Wetmore DZ, Garner CC (2010) Emerging pharmacotherapies for neurodevelopmental disorders. Journal of developmental and behavioral pediatrics : JDBP 31:564-581.

Whitaker-Azmitia PM (2001) Serotonin and brain development: role in human developmental diseases. Brain research bulletin 56:479-485.

Whitehouse PJ, Struble RG, Clark AW, Price DL (1982) Alzheimer disease: plaques, tangles, and the basal forebrain. Annals of neurology 12:494.

Whittle N, Sartori SB, Dierssen M, Lubec G, Singewald N (2007) Fetal Down syndrome brains exhibit aberrant levels of neurotransmitters critical for normal brain development. Pediatrics 120:e1465-1471.

Wiesel TN, Hubel DH (1963) Single-Cell Responses in Striate Cortex of Kittens Deprived of Vision in One Eye. J Neurophysiol 26:1003-1017.

Wilcock GK, Esiri MM (1982) Plaques, tangles and dementia. A quantitative study. Journal of the neurological sciences 56:343-356.

Winter TC, Ostrovsky AA, Komarniski CA, Uhrich SB (2000) Cerebellar and frontal lobe hypoplasia in fetuses with trisomy 21: usefulness as combined US markers. Radiology 214:533-538.

Winters BD, Forwood SE, Cowell RA, Saksida LM, Bussey TJ (2004) Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: heterogeneity of function within the temporal lobe. The Journal of neuroscience : the official journal of the Society for Neuroscience 24:5901-5908.

Wishart JG (1993) The development of learning difficulties in children with Down's syndrome. Journal of intellectual disability research : JIDR 37 (Pt 4):389-403.

Wishart JG, Pitcairn TK (2000) Recognition of identity and expression in faces by children with Down syndrome. American journal of mental retardation : AJMR 105:466-479.

Wisniewski KE (1990) Down syndrome children often have brain with maturation delay, retardation of growth, and cortical dysgenesis. American journal of medical genetics Supplement 7:274-281.

Wisniewski KE, Dalton AJ, McLachlan C, Wen GY, Wisniewski HM (1985) Alzheimer's disease in Down's syndrome: clinicopathologic studies. Neurology 35:957-961.

Wolf SA, Kronenberg G, Lehmann K, Blankenship A, Overall R, Staufenbiel M, Kempermann G (2006) Cognitive and physical activity differently modulate disease

progression in the amyloid precursor protein (APP)-23 model of Alzheimer's disease. Biol Psychiatry 60:1314-1323.

Wong V, Ho D (1997) Ocular abnormalities in Down syndrome: an analysis of 140 Chinese children. Pediatric neurology 16:311-314.

Woodhouse JM, Pakeman VH, Cregg M, Saunders KJ, Parker M, Fraser WI, Sastry P, Lobo S (1997) Refractive errors in young children with Down syndrome. Optometry and vision science : official publication of the American Academy of Optometry 74:844-851.

Woodhouse JM, Pakeman VH, Saunders KJ, Parker M, Fraser WI, Lobo S, Sastry P (1996) Visual acuity and accommodation in infants and young children with Down's syndrome. Journal of intellectual disability research : JIDR 40 (Pt 1):49-55.

Wu C, Hunter DG (2006) Amblyopia: diagnostic and therapeutic options. American journal of ophthalmology 141:175-184.

Xiang Z, Prince DA (2003) Heterogeneous actions of serotonin on interneurons in rat visual cortex. J Neurophysiol 89:1278-1287.

Xie CW, Sayah D, Chen QS, Wei WZ, Smith D, Liu X (2000) Deficient long-term memory and long-lasting long-term potentiation in mice with a targeted deletion of neurotrophin-4 gene. Proceedings of the National Academy of Sciences of the United States of America 97:8116-8121.

Yang EJ, Ahn YS, Chung KC (2001) Protein kinase Dyrk1 activates cAMP response element-binding protein during neuronal differentiation in hippocampal progenitor cells. The Journal of biological chemistry 276:39819-39824.

Yates CM, Simpson J, Gordon A, Maloney AF, Allison Y, Ritchie IM, Urquhart A (1983) Catecholamines and cholinergic enzymes in pre-senile and senile Alzheimer-type dementia and Down's syndrome. Brain Res 280:119-126.

Yonelinas AP, Otten LJ, Shaw KN, Rugg MD (2005) Separating the brain regions involved in recollection and familiarity in recognition memory. The Journal of neuroscience : the official journal of the Society for Neuroscience 25:3002-3008.

Yoshimura N, Kubota S, Fukushima Y, Kudo H, Ishigaki H, Yoshida Y (1990) Down's syndrome in middle age. Topographical distribution and immunoreactivity of brain lesions in an autopsied patient. Acta pathologica japonica 40:735-743.

Yu T, Li Z, Jia Z, Clapcote SJ, Liu C, Li S, Asrar S, Pao A, Chen R, Fan N, Carattini-Rivera S, Bechard AR, Spring S, Henkelman RM, Stoica G, Matsui S, Nowak NJ, Roder JC,

Chen C, Bradley A, Yu YE (2010) A mouse model of Down syndrome trisomic for all human chromosome 21 syntenic regions. Hum Mol Genet 19:2780-2791.

Zienowicz M, Wislowska A, Lehner M, Taracha E, Skorzewska A, Maciejak P, Plaznik A (2005) The effect of fluoxetine in a model of chemically induced seizures--behavioral and immunocytochemical study. Neurosci Lett 373:226-231.

Zigman WB, Schupf N, Sersen E, Silverman W (1996) Prevalence of dementia in adults with and without Down syndrome. American journal of mental retardation : AJMR 100:403-412.

Zoghbi HY, Bear MF (2012) Synaptic dysfunction in neurodevelopmental disorders associated with autism and intellectual disabilities. Cold Spring Harbor perspectives in biology 4.