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## Abnormal Expression of Synaptic Proteins and Neurotrophin-3 in the Down Syndrome Mouse Model Ts65Dn

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### Abstract

Down Syndrome (DS) results from triplication of the whole or distal part of human chromosome 21. DS subjects suffer from deficits in learning and memory and cognitive functions in general, and, starting from early development, their brains show dendritic and spine structural alterations and cell loss. These defects concern many cortical brain regions as well as the hippocampus, which is known to play a critical role in memory and cognition. Most of these abnormalities are reproduced in the mouse model Ts65Dn, which is partially trisomic for the mouse chromosome 16 that is homologous to a portion of human chromosome 21. Thus, Ts65Dn is widely utilized as an animal model of DS. To better understand the molecular defects underlying the cognitive and particularly the memory impairments of DS, we investigated whether the expression of several molecules known to play critical roles in long-term synaptic plasticity and long-term memory in a variety of species is dysregulated in either the neonatal brain or adult hippocampus of Ts65Dn mice. We found abnormal expression of the synaptic proteins synaptophysin, MAP2 and CDK5 and of the neurotrophin NT-3. Both the neonatal brain and adult hippocampus revealed significant abnormalities. These results suggest that a dysregulation in the expression of neurotrophins as well as proteins involved in synaptic development and plasticity may play a potential role in the neural pathology of DS in humans.

### Keywords

Down Syndrome; Ts65Dn; memory; plasticity; synaptic protein; neurotrophin

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Down syndrome (DS), the most common genetically based mental retardation disorder, is caused by the presence of an extra copy of chromosome 21 (trisomy 21). DS is characterized by a variety of deficits that affect many systems including skeletal, skin, immune and nervous. Although a high degree of variability among individuals has been described, the nervous system

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dysfunctions include severe impairments in learning, memory, speech and motor behavior (Roubertoux and Kerdelhue, 2006; Vicari, 2006; Raz et al., 1995).

Autopsy studies have revealed that the brains of DS subjects both at early developmental ages as well as in adults carry several synaptic abnormalities including dendritic and spine structural alterations and loss of choline acetyltransferase in several cortical regions including the hippocampus (Purpura 1973; Purpura 1975; Wisniewski 1990; Perry et al., 1986). Moreover, DS subjects have reduced hippocampal and cerebellar volumes (Wisniewski et al., 1984; Kaufmann and Moser, 2000; Fiala et al., 2002; Pinter et al., 2001a, b). These findings imply that DS pathology is associated with abnormal synaptic function, and consequently, abnormal cognition, which may result from alterations in biochemical substrates of plasticity either during development or in adult brain regions involved in cognitive processes. One of these regions is the hippocampus (Pinter et al., 2001a; Sylvester, 1983; Krasuski et al., 2002), which is known to play a critical role in memory formation (Eichenbaum, 2004). In agreement, high-resolution magnetic resonance imaging (MRI) has confirmed that subjects with DS have smaller overall brain volumes and, in particular, disproportionately smaller hippocampal regions (Pealson et al., 1998). The presence of these abnormalities from an early age suggests that fetal or early postnatal developmental differences may contribute to the morphological, cognitive and developmental deficits of DS (Raz et al., 1995; Pinter et al., 2001a, b). Despite the fact that the morphometric and histological studies show clear and progressive deficits in the brains of DS subjects, little is known about the molecular alterations that underlie these defects.

Because of difficulties in obtaining human samples to study DS, many molecular and behavioral studies have used genetic mouse models of trisomy 21 that reflect some critical phenotypic aspects of the human disorder. One of the most widely utilized models is the Ts65Dn mouse, which carries a partial trisomy of the mouse chromosome 16 (Davisson et al., 1990; Reeves et al., 1995) that is homologous to a portion of the human chromosome 21. This mouse model shows several histological defects of DS, including decreased cell number in subregions of the hippocampus (Insausti et al., 1998; Lorenzi and Reeves, 2006), reduction in asymmetric synapses in the temporal cortex (Kurt et al., 2000), reduced volume and neuronal density in the cerebellum (Baxter et al., 2000), age-related degeneration of basal forebrain cholinergic neurons (Cooper et al., 2001) and diminished responsiveness of acetylcholine (ACh) release in the hippocampus to behavioral testing (Chang and Gold, 2007). Moreover, Ts65Dn mice, like DS subjects, are cognitively impaired, especially in tasks that involve the hippocampus (Reeves et al., 1995; Escorihuela et al., 1995; Demas et al., 1998; Seregaza et al., 2006; Fernandez et al., 2007). Ts65Dn mice also exhibit a variety of hippocampal, electrophysiological and synaptic dysfunctions (Hanson et al., 2007; Kurt et al., 2000; Belichenko et al., 2004).

To better understand the molecular defects underlying the cognitive impairments of DS, in this study, we determined whether the expression of molecules known to play critical roles in long-term synaptic plasticity and long-term memory is dysregulated in either the developing brain and/or the hippocampus of adult Ts65Dn mice. The classes of proteins investigated include pre- and post-synaptic proteins, neurotrophins, transcription factors and membrane receptors, which were all previously shown to be critically implicated in long-term synaptic plasticity and long-term memory.

Specifically, we studied: 1) the synaptic proteins synaptophysin, synapsin, MAP2, PSD95, spinophilin, gephyrin and the cyclin-dependent kinase 5 (CDK5) and its cofactors p35 and p25, 2) the neurotrophins neurotrophin 3 (NT-3) and brain-derived neurotrophic factor (BDNF), 3) the transcription factors phosphorylated cAMP response element binding protein (pCREB) and CCAAT enhancer binding protein  $\beta$  (C/EBP $\beta$ ) and 4) the membrane receptors muscle-

specific tyrosine kinase receptor (MuSK) and subunits of the glutamate receptors NMDA and AMPA.

## Experimental Procedures

### Mouse brain and hippocampal samples

Ts65Dn mice and littermate (Lm) controls were offspring of Ts65Dn females (stock # 001924) and B6EiC3SnF1 males (stock # 001875), purchased from the Jackson Laboratory. Each breeding pair was housed together for the duration of breeding in 28.5 × 23.8 × 12.5 cm polycarbonate cages. The offspring were weaned at 21–28 days of age, and housed by gender and litter in groups of four mice. Tail samples from each offspring were genotyped by PCR for partial trisomy (Liu et al., 2003). The mice for the adult group were ear-marked at the time the tail samples were taken under brief isoflurane anesthesia. All cages were located in ventilated Thoren racks. The bottom of each cage was lined with Alpha-dri bedding (Shephard Specialty Papers), which was changed weekly. Food (Lab Diet Mouse Chow 20 #5008) and autoclaved water were always available. The mouse room was kept at 71 ± 3° F, and on a 12 hour lights on (at 6 am)/off cycle. The brains were extracted from the skull without perfusion under pentobarbital (50 mg/kg) anesthesia and kept on dry ice. Animal breeding, maintenance and brain extraction procedures were approved by the Animal Care and Use Committee of the Institute for Basic Research. Total brain extracts were obtained for 1-day-old mice and hippocampal extracts were obtained for 4-month-old mice.

### Western blot analysis

Quantitative western blot analyses were carried out as previously described (Garcia-Osta et al., 2006; Taubenfeld et al., 2002). Briefly, tissues were homogenized in lysis buffer (0.2M NaCl, 5mM EDTA, 10% glycerol, 100mM HEPES, 2mM sodium phosphate) containing a protease inhibitor cocktail (Sigma, Saint Louis, Missouri, used as recommended by the manufacturer), 0.5 mM phenylmethylsulphonyl fluoride (PMSF), 2mM dithiothreitol (DTT), the phosphatase inhibitors contained in phosphatase inhibitor cocktail 1 (Sigma, used as recommended by the manufacturer), 2mM NaF, 1µM microcystin LR and 1 mM sodium orthovanadate (SOV). Protein concentration was determined using the BioRad protein assay (BioRad Laboratories, Hercules, California). Twenty µg/lane of total protein extract was resolved on 7.5%, 10%, or 15% polyacrylamide gels, according to each marker's molecular weight, and then transferred to Immobilon-P membranes (Millipore, Billerica, Massachusetts). Membranes were incubated with primary antibodies in Tris-buffered saline overnight at 4°C. Primary antibodies: anti-synaptophysin (1:1,000), anti-MAP2 (1:1,000), anti-synapsin (1:1,000), anti-BDNF (1:500); anti-GluR2 (1:1,000); anti-PSD95 (1:50,000) and anti-pCREB (1:1,000) (Millipore, Billerica, Massachusetts); anti-MuSK (1: 300) (R&D Systems, Minneapolis, Minnesota); anti-GluR1 (1:1,000), anti-GluR3 (1:2,000), anti-NT-3 (1:1,000), anti-C/EBPβ (1:5,000), anti-CDK5 (1:1,500) and anti-p35 (1: 1,000) (Santa Cruz Biotechnology, Santa Cruz, California); anti-NR1 (1:500) (a generous gift from Dr. Wolfe, Luo et al., 1997); anti-spinophilin (1:1,000) (a generous gift from Drs. Greengard, Allen and Morrison, Allen et al., 1997); anti-gephyrin (1:250) (BD Biosciences, San Jose, California). After the primary antibody incubation, the membranes were washed and treated with a secondary HRP-labeled goat anti-rabbit (1:4,000), goat anti-mouse (1:4,000) (Amersham, Arlington Heights, Illinois), or donkey anti-goat (1: 5,000) (Santa Cruz Biotechnology), as required, for 1 hour at room temperature. Antibodies against actin (1:5,000) (Santa Cruz Biotechnology) or nuclear pore complex proteins (1:5,000) (Covance Innovative Antibodies, Princeton, NJ) were used to account for loading variation. Membranes were washed and incubated with enhanced chemiluminescence (ECL) detection reagents (Amersham) and exposed to HyBlot CL Autoradiography Film (Denville Scientific, Inc., Metuchen, NJ).

Quantitative densitometric analysis was performed using NIH Image software. Statistical significance analysis was calculated using a student *t*-test.

## Results

Quantitative western blot analysis was employed to determine the expression level of several proteins known to play an important role in long-term synaptic plasticity and memory in the brains of Ts65Dn and littermate (Lm) control mice. We investigated two distinct brain preparations at two different ages: neonatal brain and adult hippocampus. Thus, 1-day-old whole brain extracts (Lm: *n* = 11, Ts65Dn: *n* = 6) and 4-month old hippocampal extracts (Lm and Ts65Dn: *n* = 8 each) were used. Whole neonatal brain extracts were used to test potential developmental dysregulations, whereas adult hippocampal extracts were employed to investigate potential alterations critically involved in learning and memory.

### MAP2 and NT-3 are significantly increased in the neonate brain of Ts65Dn mice

We investigated five classes of proteins. First, we determined the expression of synaptic proteins known to critically participate to synaptic plasticity and learning and memory (Gong et al. 2006; Tartaglia et al. 2001; Sarrouilhe et al. 2006; Li et al. 20004; Chhatwal et al. 2005). These included: i) synaptophysin, a pre-synaptic vesicle membrane protein known to mediate vesicle release (Valtorta et al., 2004), ii) synapsin, a pre-synaptic protein which binds synaptic vesicles to the cytoskeleton and regulates synaptic vesicle release (Hilfiker et al., 1999), iii) MAP2, a microtubule-associated protein highly concentrated in the neuronal soma and dendrites that participates in several morphological and regulatory changes (Dehmelt and Halpain, 2005), iv) PSD95, a scaffolding protein present in the post-synaptic density implicated in glutamatergic synaptic functions (Elias and Nicoll, 2007), v) spinophilin, a multifunctional scaffold protein that regulates both membrane and cytoskeletal functions and is involved in spine morphology and density regulation, synaptic plasticity and neuronal migration (Sarrouilhe et al., 2006) and vi) gephyrin, a scaffolding protein which is essential for synaptic clustering of inhibitory neurotransmitter receptors (Fritschy et al., 2003).

As shown in Table 1, in Ts65Dn neonate brains, the levels of synaptophysin, synapsin, PSD95, spinophilin and gephyrin were found to be similar to those of littermate controls. In contrast, as shown in both Table 1 and Fig. 1a, MAP2 levels were found to be significantly increased ( $p < 0.05$ ) in the Ts65Dn brains compared to those of littermate controls.

The second class of protein investigated included the neurotrophins neurotrophin-3 (NT-3) and brain derived neurotrophic factor (BDNF), two factors known to support the survival of existing neurons, the growth and differentiation of new neurons and synapses and found implicated in synaptic plasticity and memory (Chao, 2000; Shimazu et al. 2006; Yamada et al. 2002). As shown in Table 1 and Fig. 1b, the levels of NT-3 resulted significantly increased in brain extracts of neonate Ts65Dn compared to their littermates ( $p < 0.001$ ). On the other hand, BDNF levels did not any significant change (Table 1).

Third, the expression levels of synaptic kinase CDK5 and relative co-factors p35 and p25 were examined. CDK5 is a key regulator of neuronal function. It modulates cell adhesion and cytoskeletal dynamics, processes that are essential during development and in the adult nervous system. CDK5 is activated by p35 or p39, both of which may be cleaved to more stable and potent fragments, p25 and p29 (Cruz and Tsai, 2004). As shown in Table 1, in the neonate brains of Ts65Dn mice, the levels of CDK5, p35 and p25 were unchanged compared to those of littermate control brains.

In the fourth group of markers, we quantified the expression levels of two transcription factors known to play an evolutionarily critical role in long-term synaptic plasticity and memory

formation: the phosphorylated form of CREB (Bailey et al., 1996) and C/EBP $\beta$ , a transcription factor whose expression is regulated downstream of CREB (Alberini, 1999; Alberini et al., 2005). As depicted in Table 1, we found that both pCREB and C/EBP $\beta$  were expressed at similar levels compared to their respective controls.

Finally, the fifth group of proteins that were quantified included the membrane receptor MuSK and the subunits of NMDA and AMPA receptors. MuSK has been found to play a critical role in neuromuscular junction formation (Burden, 2002) and was recently reported to be essential in the hippocampus during memory consolidation (Garcia-Osta et al., 2006). Subunits of both the NMDA and AMPA ionotropic glutamate receptors are known to be critically involved in synaptic plasticity and fast synaptic transmission, respectively (Riedel et al., 2003). As shown in Table 1, in the neonate brains of Ts65Dn, the levels of MuSK, NMDA receptor subunit NR1, GluR1 and GluR2 were all similar to those of their littermate controls. Only a trend towards down-regulation, although not statistically significant, was found for GluR3.

### **In the hippocampus of adult Ts65Dn, the expression of synaptophysin is significantly decreased, whereas the expressions of NT-3 and CDK5 are significantly increased**

In the hippocampal extracts of adult Ts65Dn, the quantitative western blot analyses of the five classes of proteins revealed the following: the first class, that is, synaptic proteins, showed that whereas synapsin, MAP2, PSD95, spinophilin and gephyrin were all expressed at levels similar to those of littermate controls, the expression levels of synaptophysin were significantly decreased ( $p < 0.05$ , Fig. 2a and Table 2).

The second class of proteins investigated revealed that, similarly to what was found in the neonatal brains, the expression of NT-3 was significantly increased in the hippocampal extracts of the adult Ts65Dn ( $p < 0.05$ , Table 2 and Fig. 2b). These results suggest that an increase in NT-3 might represent a general defect throughout the lifespan. On the other hand, no significant change was found in the expression levels of BDNF.

In the third class of proteins, we found a statistically significant up-regulation of CDK5 in adult Ts65Dn hippocampus compared to controls (Table 2 and Fig. 2c). In agreement with this observation, there was a trend towards decreased expression of p35 and increased expression of p25 (Table 2), although the differences were not statistically significant.

Moreover, with the fourth class of proteins, like in the neonatal brain, the expression levels of the transcription factors pCREB and C/EBP $\beta$  were found expressed at levels comparable to those of littermate controls.

Finally, again in agreement with the results found in the neonatal brains, the investigation of the fifth class of proteins showed that MuSK, the NMDA receptor subunit NR1, GluR1 and GluR2 were found expressed at levels similar to those of their littermate controls, whereas a trend towards a down-regulation, although not statistically significant, was found for GluR3 (Table 2).

## **Discussion**

Our study revealed that the expression levels of both MAP2 and NT-3 are significantly increased in the neonatal brain of Ts65Dn compared to littermate controls. Furthermore, we found that synaptophysin is significantly decreased whereas both NT-3 and CDK5 are significantly increased in the hippocampus of adult Ts65Dn compared to that of littermate controls. These results suggest that a dysregulation in the expression of synaptic proteins and neurotrophins in the brain at early developmental ages as well as in the adult hippocampus is associated with DS.

Our results are in agreement with, and biochemically extend, the observation that morphological and functional synaptic deficits are characteristic landmarks of DS. Indeed, a number of developmental synaptic abnormalities have been described in both brains of individuals with DS and mouse models of DS. By 18 months, DS subjects have dendritic spine deficits (Kaufmann and Moser, 2000), and mice models of DS show an increase in spine size throughout the brain and a decrease in spine density in the hippocampus (Belichenko et al., 2004). Our results provide biochemical support for these observations by showing that the expression of synaptic proteins that are critical for normal spine development, synapse formation and synaptic function, in both the developing brain and the adult hippocampus, is significantly dysregulated in Ts65Dn mice.

Specifically, we found that there was a significant increase in both MAP2 and NT-3 in the neonate brain of Ts65Dn. A significant increase in NT-3 was also observed in the hippocampus of adult Ts65Dn. The increase in MAP2 in the neonatal brain might reflect a compensatory overexpression that attempts to regulate the synaptic defects found at early post-natal developmental stages. Moreover, since MAP2 seems to play a critical role in neuromorphogenic processes, such as neurite initiation and recruitment of signaling proteins that may regulate microtubule mediated transport (Dehmelt and Halpain, 2005), an excess of MAP2 may underlie defective neurite/synaptic development.

A significant increase of NT-3, but not BDNF, found in both neonatal brains and hippocampi of adult Ts65Dn mice compared to littermates, suggests that this alteration is maintained from development through adulthood. Unaltered levels of hippocampal BDNF were also reported by previous studies on trisomic mice, which, however, also reported a significant decrease of BDNF expression in the frontal cortex (Bimonte-Nelson et al., 2003).

The expression of NT-3, like that of BDNF, is known to be widespread throughout the adult brain (Maisonpierre et al., 1990; Katoh-Semba et al., 1996) and both factors appear to play similar roles in brain development, survival and differentiation of neuronal progenitor cells. We speculate that the significant increase of NT-3 in the Ts65Dn mouse model represents a protective response to the synaptic and neuronal loss known to occur in DS throughout development (Wisniewski et al., 1984; Kaufmann and Moser, 2000; Becker et al., 1991). An additional intriguing hypothesis is that an increase in NT-3 might reflect an attempt to restore the cholinergic deficit described in DS (Cooper et al., 2001; Chang and Gold, 2007). In fact, previous data reported that the expression of NT-3 by cortical neurons serves to attract basal forebrain cholinergic projections to their target cells in the cerebral cortex (Robertson et al., 2006). Hence, a cholinergic deficit could indeed stimulate an increase in NT-3 expression.

Interestingly, another neurotrophin, the nerve growth factor (NGF) has been previously found to be augmented in the hippocampi of adult Ts65Dn mice. Despite the increase in concentration, the retrograde transport of this factor from the hippocampus to the basal forebrain was found to be significantly impaired (Cooper et al., 2001). In conclusion, several lines of evidence, including ours, show altered neurotrophin expression. Thus, it is tempting to speculate that, although the direction of the change (increase or decrease) may reflect different impairments (direct or compensatory) in different brain regions, the DS pathology is associated with, and perhaps in part caused by, a dysregulation in neurotrophin expression. This is also supported by the compelling evidence that neurotrophins like NGF are able to reverse pathological changes in DS mouse models (Cooper et al., 2001). Given the fact that neurotrophins may be functionally overlapping, it is also possible that, in DS, the increase of some neurotrophins in certain brain regions, such as the hippocampus, may represent a compensatory response to a decreased availability (expression or transport) of other neurotrophins in the same or other brain regions.

A significant decrease in synaptophysin and a significant increase of CDK5 were found in the hippocampi of adult Ts65Dn. The decrease in synaptophysin is in agreement with the findings that DS is characterized by a deficit in synapse number and morphology, particularly in the hippocampus (Kaufmann and Moser, 2000; Pinter et al., 2001a, b). Hence, the deficit in synaptophysin expression likely reflects a lower overall density of synapses.

A significant increase in CDK5 expression level in the hippocampus of adult Ts65Dn was accompanied by a trend toward a decrease and increase, respectively, of the CDK5 co-factors p35 and p25, which were, however, not significant. What could a CDK5 increase in the hippocampus of adult DS signify? CDK5 is a serine/threonine kinase with a multitude of functions, including neural development, dopaminergic function, neurodegeneration, learning and memory, adult synaptic plasticity and neurotransmitter release and endocytosis in the adult synapse (Ayala et al., 2007, Hawasli and Bibb, 2007, Cheng and Ip, 2003; Evans and Cousin, 2007; Graham et al., 2007, Fischer et al., 2003; Angelo et al., 2006). Thus, the significant CDK5 augmentation in the hippocampi of Ts65Dn may represent a compensatory mechanism in response to a developmental neuronal or synaptic alteration resulting from the trisomy. This would be in agreement with the abnormalities found in synaptophysin and MAP2.

Interestingly, CDK5 and its cofactors contribute to the pathogenesis of several neurodegenerative diseases such as Alzheimer's disease (Patrick et al., 1999), amyotrophic lateral sclerosis (Nguyen et al., 2001) and Niemann–Pick type C disease (Bu et al., 2002) as well as psychiatric illnesses such as addiction (Cruz et al., 2003; Bibb, 2003). Thus, DS seems to represent an additional pathology that manifests a CDK5 dysregulation.

The expression levels of other synaptic markers such as synapsin, PSD95, spinophilin and gephyrin as well that of neurotransmitter receptors including the glutamate receptors AMPA (subunits GluR1, GluR2, GluR3) and NMDA (subunit NR1) were unchanged in both the neonate brains as well as hippocampi of adult Ts65Dn compared to their littermate controls. If, as indicated above, synapse density is dysregulated, why are all these proteins expressed at normal levels? One possibility is that, because our quantitative expression analysis has been carried out on total extracts, only relatively large changes have been detected, while leaving smaller (e.g. local) alterations undetectable. It is also possible that the dysregulation of only certain synaptic proteins and not others occurs as a downstream effect of increased gene dose.

In conclusion, dysregulation in the expression of neurotrophins and proteins involved in synaptic development and plasticity functions at both early developmental stages and in adulthood seem to be a hallmark of the DS mouse model Ts65Dn, suggesting a potential role for these abnormalities in the neural pathology of DS in humans.

## Abbreviations

DS, Down Syndrome  
 AD, Alzheimer's disease  
 MAP2, Microtubule-associated protein 2  
 PSD95, Postsynaptic density 95  
 CDK5, Cyclin-dependent kinase 5  
 NT-3, Neurotrophin-3  
 BDNF, Brain-derived neurotrophic factor  
 pCREB, Phosphorylated cAMP response element binding protein  
 C/EBP $\beta$ , CCAAT enhancer binding protein  $\beta$   
 MuSK, Muscle-specific tyrosine kinase receptor  
 NMDA, N-methyl-D-aspartic acid  
 AMPA,  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4- propionic acid

NGF, Nerve growth factor  
 ACh, Acetylcholine  
 PCR, Polymerase chain reaction  
 HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid  
 HRP, Horseradish peroxidase  
 ECL, Enhanced chemiluminescence  
 MRI, Magnetic resonance imaging

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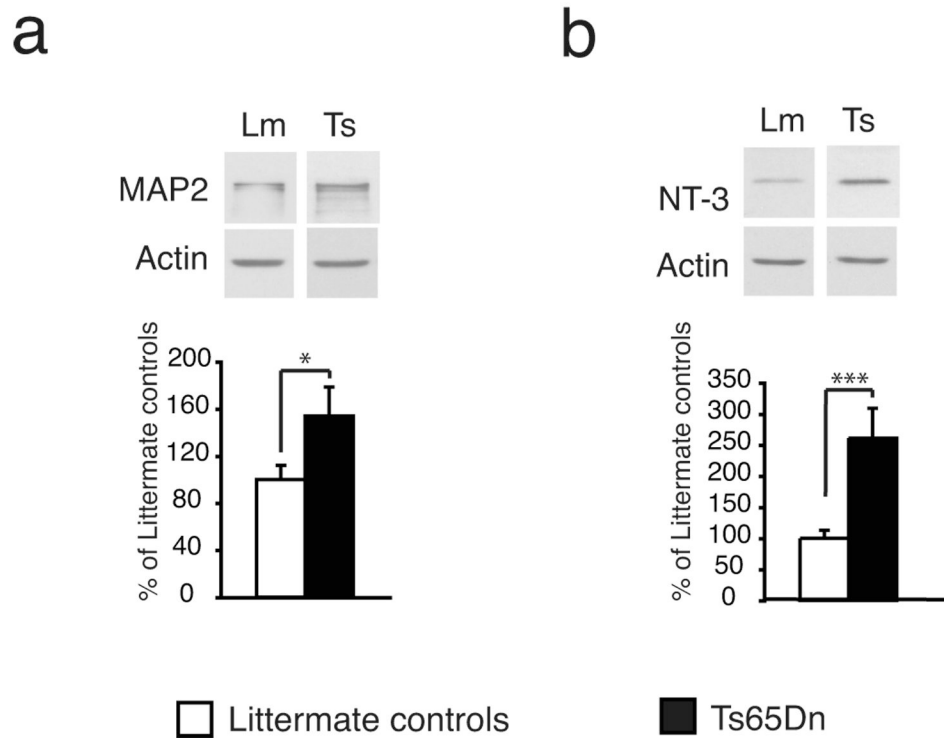


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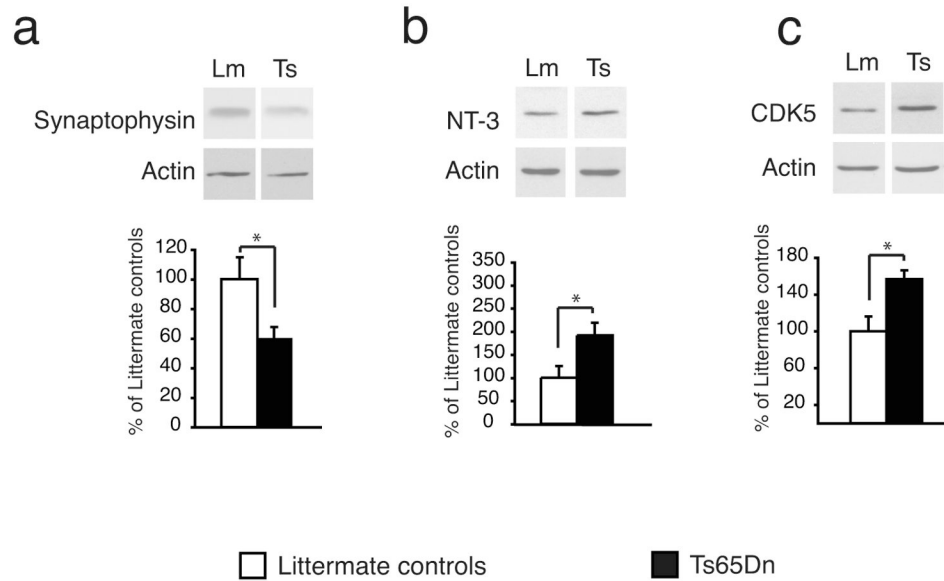
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## Neonatal brain



**Fig. 1.** Representative examples and quantitative densitometric analyses of western blot of extracts from 1-day-old (neonatal brain) Ts65Dn whole brain and littermate age-matched controls. Protein levels were normalized using actin or nuclear pore complex protein to account for differences in loading. Data are expressed as mean percentage  $\pm$ SEM of the littermate control mean values. **(a)** MAP2 is significantly increased in neonatal day 1 Ts65Dn. **(b)** NT-3 is significantly increased in neonatal day 1 Ts65Dn.

## Adult hippocampus

**Fig. 2.**

Representative examples and quantitative densitometric analysis of western blot of extracts from hippocampi of 4-month-old Ts65Dn (adult hippocampus) and those of littermate age-matched controls. Protein levels were normalized using actin to account for differences in loading. Data are expressed as mean percentage  $\pm$ SEM of the littermate control mean values. **(a)** Synaptophysin is significantly decreased in Ts65Dn hippocampi. **(b)** NT-3 is significantly increased in Ts65Dn hippocampi. **(c)** CDK5 is significantly increased in the hippocampi of Ts65Dn.

**Table 1**

Densitometric analyses of western blot of extracts from neonatal brain. Data are expressed as mean percentage $\pm$ SEM off the littermate controls mean value

	Littermate controls	Ts65Dn
<b>Synaptic Proteins</b>		
Synaptophysin	100.0 $\pm$ 9.3%	98.9 $\pm$ 6.0%
Synapsin	100.0 $\pm$ 8.2%	111.1 $\pm$ 13.9%
MAP2	100.0 $\pm$ 12.1%	153.9 $\pm$ 24.6% *
PSD95	100.0 $\pm$ 11.4%	86.8 $\pm$ 10.7%
Spinophilin	100.0 $\pm$ 8.9%	90.5 $\pm$ 17.7 %
Gephyrin	100.0 $\pm$ 13.3%	83.3% $\pm$ 9.5%
<b>Neurotrophins</b>		
NT3	100.0 $\pm$ 12.9 %	260.9 $\pm$ 47.9 % *
BDNF	100.0 $\pm$ 11.5 %	75.4 $\pm$ 6.6 %
<b>CDK5 and cofactors</b>		
CDK5	100.0 $\pm$ 5.3%	108.9 $\pm$ 13.1%
p35	100.0 $\pm$ 13.0%	87.3 $\pm$ 8.2 %
p25	100.0 $\pm$ 11.9 %	120.5 $\pm$ 11.9 %
<b>Transcription Factors</b>		
pCREB	100.0 $\pm$ 12.1 %	87.2 $\pm$ 7.2 %
c/EBP $\beta$	100.0 $\pm$ 11.0 %	103.9 $\pm$ 14.7 %
<b>Membrane Receptors</b>		
MuSK	100.0 $\pm$ 11.8 %	98.9 $\pm$ 11.8%
NR1	100.0 $\pm$ 9.1%	82.1 $\pm$ 12.4%
GluR1	100.0 $\pm$ 9.9%	105.4 $\pm$ 10.8%
GluR2	100.0 $\pm$ 8.2%	99.6 $\pm$ 14.4%
GluR3	100.0 $\pm$ 14.2%	83.3% $\pm$ 7.7%

\* Significant differences

**Table 2**

Densitometric analyses of western blot of extracts from adult hippocampi. Data are expressed as mean percentage  $\pm$ SEM off the littermate controls mean value

	Littermate controls	Ts65Dn
<b>Synaptic Proteins</b>		
Synaptophysin	100.0 $\pm$ 12.3%	61.0 $\pm$ 7.1% *
Synapsin	100.0 $\pm$ 5.4%	99.0 $\pm$ 8.2 %
MAP2	100.0 $\pm$ 20.0%	140.8 $\pm$ 13.9%
PSD95	100.0 $\pm$ 11.9%	71.2 $\pm$ 12.7%
Spinophilin	100.0 $\pm$ 17.3%	112.8 $\pm$ 11.8%
Gephyrin	100.0 $\pm$ 14.4%	103.8 $\pm$ 9.8%
<b>Neurotrophins</b>		
NT3	100.0 $\pm$ 24.9 %	191.4 $\pm$ 27.6 % *
BDNF	100.0 $\pm$ 7.5 %	105.4 $\pm$ 6.0 %
<b>CDK5 and cofactors</b>		
CDK5	100.0 $\pm$ 16.1%	156.5 $\pm$ 9.7 % *
p35	100.0 $\pm$ 14.1%	76.5 $\pm$ 30.3%
p25	100.0 $\pm$ 10.9%	135.5 $\pm$ 30.4%
<b>Transcription Factors</b>		
pCREB	100.0 $\pm$ 14.0 %	160.0 $\pm$ 34.6 %
c/EBP $\beta$	100.0 $\pm$ 8.8 %	91.2 $\pm$ 10.1 %
<b>Membrane Receptors</b>		
MuSK	100.0 $\pm$ 9.3 %	90.1 $\pm$ 6.0 %
NR1	100.0 $\pm$ 15.6%	99.8 $\pm$ 8.7%
GluR1	100.0 $\pm$ 19.0%	99.1 $\pm$ 13.1%
GluR2	100.0 $\pm$ 17.0%	86.0 $\pm$ 17.6
GluR3	100.0 $\pm$ 3.9%	78.3 $\pm$ 14.3

\* Significant differences