Neurobiology of Disease 106 (2017) 89–100

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Neuroanatomical alterations and synaptic plasticity impairment in the perirhinal cortex of the Ts65Dn mouse model of Down syndrome

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ARTICLE INFO

Article history:
Received 10 March 2017
Revised 21 June 2017
Accepted 22 June 2017
Available online 23 June 2017

Keywords:
Down syndrome
Memory impairment
Ts65Dn model
Perirhinal cortex
Synaptic function
GIRK2

ABSTRACT

Down syndrome (DS), a genetic condition due to triplication of Chromosome 21, is characterized by numerous neurodevelopmental alterations and intellectual disability. Individuals with DS and DS mouse models are impaired in several memory domains, including hippocampus-dependent declarative (spatial, in rodents) memory and visual recognition memory, a form of memory in which the perirhinal cortex (PRC) plays a fundamental role. The anatomo-functional substrates of hippocampus-dependent memory impairment have been largely elucidated in the Ts65Dn mouse model of DS. In contrast, there is a lack of corresponding information regarding visual recognition memory. Therefore, we deemed it of interest to examine at both an anatomical and functional level the PRC of Ts65Dn mice. We found that the PRC of adult (1.5–3.5 month-old) Ts65Dn mice exhibited diffused hypocellularity and neurons with a reduced spine density. No difference between Ts65Dn and euploid mice was detected in the abundance of glutamatergic and GABAergic terminals. We examined brain slices for long-term potentiation (LTP), a form of synaptic plasticity involved in long-term memory. Theta burst stimulation of intracortical fibers was used in order to elicit LTP in the superficial layers of the PRC. We found that in trisomic slices LTP had a similar time-course but a reduced magnitude in comparison with euploid slices. While exposure to the $GABA_A$ receptor antagonist picrotoxin had no effect on LTP magnitude, exposure to the $GABA_B$ receptor antagonist CGP55845 caused an increase in LTP magnitude that became even larger than in euploid slices. Western blot analysis showed increased levels of the G-protein-activated inwardly rectifying K^+ channel 2 (GIRK2) in the PRC of Ts65Dn mice, consistent with triplication of the gene coding for GIRK2. This suggests that the reduced magnitude of LTP may be caused by GIRK2-dependent exaggerated $GABA_B$ receptor-mediated inhibition. Results provide novel evidence for anatomo-functional alterations in the PRC of Ts65Dn mice. These alterations may underlie trisomy-due impairment in visual recognition memory.

1. Introduction

Down syndrome (DS) is a genetic condition due to triplication of Chromosome 21. The clinical presentation of DS is complex and variable. A few features occur to some degree in every individual with trisomy 21, including characteristic facial dysmorphology, a small and hypocellular brain, and the histopathology of Alzheimer's disease, which is present by the fourth decade. The neurological profile of individuals with DS, including intellectual disability, has been associated with brain hypotrophy and hypocellularity. Although the brains of DS people are smaller overall, some brain areas such as the hippocampus, the cerebellum and the frontal lobe are particularly affected (Kesslak et al., 1994; Raz et al., 1995; Aylward et al., 1997; Aylward et al., 1999; Pinter et al., 2001; Guidi et al., 2008; Guidi et al., 2011).

Down syndrome children exhibit incomplete, delayed acquisition, and atypical utilization of cognitive, motor and adaptive functions. The IQ of children with DS (chronological age = 6.5–8 years) ranges between 45 and 71 and declines in adolescents and young adults with DS (chronological age = 12.2–25 years) to a range from 28 to 47 (Chapman and Hesketh, 2000; Vicari, 2004, 2006). Children with DS have normal learning abilities for tasks requiring implicit memory but exhibit selective impairment of explicit memory, with poor information encoding, impaired retrieval abilities and attention deficits (Carlesimo et al., 1997; Vicari et

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Available online on ScienceDirect (www.sciencedirect.com).

http://dx.doi.org/10.1016/j.nbd.2017.06.017
0969-9961/

al., 2000). When tested for learning tasks that specifically assess the state of function of the hippocampal and prefrontal systems, infants and adults with DS show a severe impairment (Nadel, 2003).

Mouse models of DS have been created in order to understand the mechanisms underlying brain dysfunction in DS and to devise possible pharmacotherapy (Reeves, 2006). Among these models, the Ts65Dn mouse is the most widely used because it recapitulates numerous neuroanatomical and functional alterations of DS (Reeves, 1995). Ts65Dn mice display notably reduced levels of performance in tasks that require the integrity of the hippocampal system, such as spontaneous alternation (T-maze task), contextual memory (fear-conditioning test) and spatial memory (radial arm maze test and Morris Water Maze test) (Demas et al., 1996; Belichenko et al., 2007; Salehi et al., 2009; Bianchi et al., 2010). Consistent with these behavioral deficits, Ts65Dn mice exhibit impairment of long-term potentiation (LTP), a form of synaptic plasticity that is considered an electrophysiological correlate of learning and memory (Gruart et al., 2015), in the dentate gyrus and hippocampus (Siarey et al., 1997; Kleschevnikov et al., 2004; Costa and Grybko, 2005; Kleschevnikov et al., 2012a; Begenisic et al., 2014). Impairment of LTP appears to be due to excessive inhibition, because pharmacological reduction of inhibition restores LTP (Kleschevnikov et al., 2004; Costa and Grybko, 2005; Kleschevnikov et al., 2012a). This evidence in a mouse model may explain, in part, the impairment in hippocampus-dependent declarative memory documented in children and adults with DS.

Visual recognition memory is the ability to judge the prior occurrence of stimuli and is fundamental to our ability to record events and to guide prospective behavior. Studies in humans and animals indicate that recognition memory involves two separable processes, familiarity discrimination and recollection. The perirhinal cortex (PRC) is particularly involved in familiarity discrimination for individual items, while more complex aspects of recognition memory, including contextual, associative and spatial aspects of recognition memory, depend on the hippocampus (Brown and Banks, 2015). The PRC, that comprises two narrow strips of cortex, areas 35 and 36, bordering the rhinal fissure, is strongly interconnected with the hippocampal formation via the entorhinal cortex (Suzuki, 1996). Approximately 40% of the direct input to the entorhinal cortex arises from the adjacent PRC and the entorhinal cortex sends a robust return projection to the PRC itself. The PRC receives prominent inputs from various unimodal and polymodal association cortices, including the temporal neocortex. Due to its pattern of connectivity, the PRC is in a unique position to synthesize sensory information and to interact with other memory-related structures, including the hippocampus.

Assessment of non-spatial memory with the novel object recognition (NOR) test shows that Ts65Dn mice can form contextual representations of objects over the course of a few hours, but fail to discriminate object novelty over a more prolonged period of 24 h, indicating difficulties in tasks requiring long-term recognition memory (Fernandez and Garner, 2008). These defects are reminiscent of those characterizing individuals with DS (Carlesimo et al., 1997; Vicari et al., 2000; Vicari, 2004; Vicari et al., 2005; Visu-Petra et al., 2007). This behavioral evidence suggests that altered circuit organization and altered signal processing in the PRC may contribute to poor performance in long-term recognition memory in individuals with DS and DS mouse models. While various studies have examined the anatomy and physiology of the hippocampal circuits in mouse models of DS, there is a surprising lack of data regarding the PRC. We provide here novel evidence that the PRC of Ts65Dn mice exhibits neuroanatomical defects and impairment of LTP, similarly to the hippocampus, and propose that visual recognition memory alterations may be related to an altered organization of the PRC.

2. Methods

2.1. Colony

In order to obtain Ts65Dn mice, female carriers of the 17^{16} chromosome (B6EiC3Sn a/ATs(17⟨16⟩)65Dn) were mated with C57BL/6JEiJ × C3H/HeSnJ (B6EiC3Sn) F1 hybrid males provided by Jackson Laboratories (Bar Harbor, ME, USA). The genotyping of the animals was carried out using ear tissue as previously described (Reinholdt et al., 2011). Ts65Dn and euploid mice were housed 1–4 per cage. The cages contained nesting material. The animals' health and comfort were controlled by the veterinary service. The animals had access to water and food ad libitum and lived in a room with a 12:12 h dark/light cycle. Experiments were performed in accordance with the Italian and European Community law for the use of experimental animals and were approved by Bologna Veterinary Service and by the Italian Ministry of Health (approval no. 524/2015.PR).

2.2. Experimental protocol

Experiments were carried out in a total of 49 euploid and 45 Ts65Dn mice aged 1.5–5.0 months. Analysis of cellularity and spine density was carried out in mice of both sexes. Western blot analysis and electrophysiological recordings were carried out in male mice. The number, sex and age of mice used for analysis of cellularity and spine density and for western blot analysis is reported in Supplementary Table 1. The number, sex and age of mice used for electrophysiological recordings is reported in Supplementary Table 2.

2.3. Histological procedures

2.3.1. Hoechst-staining

The brain was cut with a freezing microtome into 30-μm-thick coronal sections that were serially collected in anti-freezing solution (30% glycerol; 30% ethylen-glycol; 10% PBS10X; 0.02% sodium azide; MilliQ to volume). Free-floating sections were stained with the Hoechst dye 33,342, in order to label cell nuclei.

2.3.2. Golgi staining

The FD Rapid Golgi Stain TM Kit (FD NeuroTechnologies, Inc.) was used for Golgi staining. Brain was removed, cut into two halves immersed in the impregnation solution containing mercuric chloride, potassium dichromate and potassium chromate and stored at room temperature in darkness for 3 weeks. The left hemisphere was cut with a microtome in 90-μm-thick coronal sections that were mounted on gelatin-coated slides and were air dried at room temperature in the dark for one day. After drying, sections were rinsed with distilled water and subsequently stained in a developing solution (FD Rapid Golgi Stain Kit).

2.4. Measurements

2.4.1. Image acquisition

A light microscope (Leitz) equipped with a motorized stage and focus control system and a color digital camera (Coolsnap-Pro; Media Cybernetics, Silver Spring, MD, USA) were used to take bright field images. Immunofluorescence images were taken with a Nikon Eclipse TE 2000-S inverted microscope (Nikon Corp., Kawasaki, Japan), equipped with a Nikon digital camera DS 2MBWc. Measurements were carried out using Image Pro Plus software (Media Cybernetics, Silver Spring, MD 20910, USA). For cell density and spine density evaluation sections were taken approximately at the level of the caudal one half of the hippocampal formation (Fig. 1A). This region is enclosed between Paxino's coordinates: bregma −2.46 mm and bregma −3.64 mm (Paxinos et al., 2007).

2.4.2. Cell density

We evaluated cell density in the different layers of the PRC in one out of six Hoechst-stained sections (3–6 sections per mouse). In view of the small size of the PRC we did not use dissectors, but evaluated cell density in manually traced areas comprising layers II–III, layer V and layer VI, separately, in images acquired with a ×40 objective. All cell profiles