Experimental Neurology 279 (2016) 40–56

Contents lists available at ScienceDirect

Experimental Neurology

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

Research Paper

Longitudinal measures of cognition in the Ts65Dn mouse: Refining windows and defining modalities for therapeutic intervention in Down syndrome

J. Luis Olmos-Serrano [a], William A. Tyler [a], Howard J. Cabral [b], Tarik F. Haydar [a,*]

[a] Department of Anatomy and Neurobiology, Boston University School of Medicine, 72 East Concord Street, L-1004, Boston, MA 02118, United States
[b] Department of Biostatistics, Boston University School of Public Health, 801 Massachusetts Avenue, Boston, MA 02118, United States

ARTICLE INFO

Article history:
Received 9 October 2015
Received in revised form 2 February 2016
Accepted 4 February 2016
Available online 24 February 2016

Keywords:
Down syndrome
Ts65Dn
Mouse model
Cognition
Aging
Reversal learning
Mouse behavior
Developmental disorder
Developmental milestones
Alzheimer Disease

ABSTRACT

Mouse models have provided insights into adult changes in learning and memory in Down syndrome, but an in-depth assessment of how these abnormalities develop over time has never been conducted. To address this shortcoming, we conducted a longitudinal behavioral study from birth until late adulthood in the Ts65Dn mouse model to measure the emergence and continuity of learning and memory deficits in individuals with a broad array of tests. Our results demonstrate for the first time that the pace at which neonatal and perinatal milestones are acquired is correlated with later cognitive performance as an adult. In addition, we find that life-long behavioral indexing stratifies mice within each genotype. Our expanded assessment reveals that diminished cognitive flexibility, as measured by reversal learning, is the most robust learning and memory impairment in both young and old Ts65Dn mice. Moreover, we find that reversal learning degrades with age and is therefore a useful biomarker for studying age-related decline in cognitive ability. Altogether, our results indicate that preclinical studies aiming to restore cognitive function in Ts65Dn should target both neonatal milestones and reversal learning in adulthood. Here we provide the quantitative framework for this type of approach.

1. Introduction

Down syndrome (DS), the leading genetic cause of intellectual disability afflicting 1 in every 691 live births, is characterized by a constellation of phenotypes affecting many organ systems (Parker et al., 2010). Abnormalities in the nervous system include microcephaly, motor impairment and cognitive deficits (Cocchi et al., 2010; Parker et al., 2010). Intellectual disability is characterized by deficits in problem solving, reasoning, and learning and memory, as well as deficiencies in conceptual, social and practical skills. It is a universal and life-long condition for individuals with DS and its severity varies widely, ranging from mild to moderate impairment on intelligence quotient tests (Patterson et al., 2013). Reversal learning, an adaptive behavior in which a learned response must be extinguished and replaced by an alternate response, is commonly impaired in DS (Campbell et al., 2013). Proper modeling of these neuropsychological disabilities will enable an understanding of their etiology and their individual contribution to intellectual disability in DS. This will facilitate the development of tailored behavioral and medical therapies.

Due to their genetic similarities to humans, mouse models are used to replicate the behavioral landscape of DS. The trisomic Ts65Dn mouse is currently the most commonly employed model of DS, mainly because it displays several phenotypic abnormalities which parallel those found in humans, including delays in brain development, hyperactivity, and motor dysfunction (Davisson et al., 1993; Costa et al., 1999; Chakrabarti et al., 2007; Sanders et al., 2009). In addition, several studies have reported cognitive impairment in Ts65Dn mice using a variety of behavioral paradigms, primarily examining spatial learning and memory (L/M) (Das and Reeves, 2011; Ruparelia et al., 2013). While these studies provide a framework of the L/M impairments in Ts65Dn, key questions about the onset and magnitude of these deficits as well as in-depth assessment over an extended period of time remain unanswered. This is particularly important when defining the optimal therapeutic window to treat intellectual disability in humans with DS since cognitive deficits appear early in life and can be varied within the population. Importantly, increasing lines of evidence suggest that failure to reach key developmental milestones, even during the first year of life, may at least partially predict cognitive challenges that manifest in adult

* Corresponding author at: Laboratory of Neural Development and Intellectual Disorders, Department of Anatomy and Neurobiology, 72 East Concord Street, L-813, Boston University School of Medicine, Boston, MA, 02118, United States.
E-mail addresses: lolmos@bu.edu (J.L. Olmos-Serrano), wtyler@bu.edu (W.A. Tyler), hjcab@bu.edu (H.J. Cabral), thaydar@bu.edu (T.F. Haydar).

http://dx.doi.org/10.1016/j.expneurol.2016.02.005
0014-4886/

life (Schillace, 1964; von Wendt et al., 1984; Taanila et al., 2005; Murray et al., 2006). Thus, identifying the severity and particular cognitive impairments on an individual basis at an early stage of life could facilitate targeted treatments. In addition, the high incidence of Alzheimer's disease (AD) pathology and dementia after 40 years of age is one of the most debilitating consequences of aging for people with DS (Zigman, 2013) and there is great interest in discovering whether biological changes in the DS brain precede the later dementia. A protracted study may therefore elucidate the biological basis for the association between trisomy 21 and AD, and could thereby improve outcomes for all individuals who develop AD pathology and dementia.

In this study, we performed a systematic behavioral analysis of Ts65Dn and control mice from birth to adulthood. Each animal was evaluated in a battery of developmental milestone tests, followed by several cognitive and innate behavioral tasks conducted during adolescence and then repeated after advanced aging. This allowed us to elucidate key correlations between development and adult cognitive function. Our results show that young Ts65Dn mice acquire spatial learning at the same pace as euploid mice, but exhibit a pronounced reversal learning impairment at both young and old ages. Unsupervised principal component (PCA) and hierarchical clustering analyses (HCA) demonstrate that Ts65Dn mice can be clearly differentiated from euploid mice and identify variable phenotypes between individuals within each genotype. Our longitudinal study also shows that the developmental milestone performance of individual mice parallels their cognitive scores in adulthood. For the first time, these tests also uncover an age-dependent decline in learning in both Ts65Dn and euploid mice. Taken together, our results provide a detailed analysis of the timing and specificity of cognitive deficits expressed in Ts65Dn which serve as a novel and sensitive platform to assess treatment strategies aimed at improving cognitive function in DS.

2. Material & methods

2.1. Animals

Ts65Dn and euploid fetuses from 20 litters were generated by mating Ts65Dn females (Stock No. 005252 without the mutant Pde6b allele impacting retinal degeneration) with C57BL/6JEi × C3SnHeSnJ (B6EiC3) F1 hybrid males (Stock No. 003647), both obtained from The Jackson Laboratory, Bar Harbor, ME). Seventeen euploids and fifteen Ts65Dn males were used for developmental milestone tests. From these animals, fourteen euploids and fifteen Ts65Dn mice were chosen for their proximity in birthdates for adult behavior assessment. Weaning was performed at postnatal day (PND) 21 and sibling males were housed regardless of genotype. Animals were kept in the same cage until two weeks prior to the onset of adult behavioral assessments. Between the end of the behavioral assessment at 2 months of age and the completion of the behavioral assessment at 11 months of age, 5 Ts65Dn died. PCR genotyping was performed on genomic DNA extracted from tail tips. Genotyping was performed on the day of birth. For genotyping, we used primers previously described (Reinholdt et al., 2011). Mutant primers: Chr17fwd-5′-GTGGCAAGAGACTCAAATTCAAC-3′ and Chr16rev-5′-TGGCTTATTATTATCAGGGCATTT-3′. These primers amplify a 275 bp product. Positive control primers: IMR8545_5′-AAAG TCGCTCTGAGTTGTTAT-3′ and IMR8546_5'GGAGCGGGAGAAATGGAT ATG-3′. These primers amplify a 600 bp product from the Rosa locus. The PCR cycling conditions were: Step 1: 95 °C for 2 min; Step 2: 95 °C for 20 s; Step 3: 55 °C for 30 s; Step 4: 72 °C for 45 s (Steps 2–4, 40 cycles); Step 5: 72 °C 5 min, followed by a final 5 min extension at 72 °C and hold at 4 °C. PCR products were separated on a 1% agarose gel.

2.2. Behavioral testing

All experiments involving animals were performed in accordance with institutional and federal guidelines. Standard rodent chow and water were available *ad libitum*. In addition to standard bedding, a Nestlet square was provided in each cage. The colony room was maintained on a 12:12 light/dark cycle, with lights on at 7:00 AM. All experiments were conducted in the light phase, between 8:00 AM and 1:00 PM. To minimize olfactory cues from previous trials, each apparatus was thoroughly cleaned with 10% ethanol after each animal. Each day of testing, mice were left in their home cages in the room used for the experiment at least 1 h prior to the onset of the study for habituation. All behavioral tests were performed blindly without prior knowledge of genotype. Water T-maze and Morris watermaze tasks were the last experiments in the series.

2.3. Developmental milestones

Ts65Dn pups and euploid littermate males were tested according to previous studies (Fox, 1965; Hill et al., 2008). A comprehensive set of neonatal behavioral tests to measure different sensory and motor development parameters were used including 1) body righting and coordination (surface righting, air righting and negative geotaxis), 2) motor strength (cliff aversion and forelimb grasp), 3) sensory system maturation (rooting, auditory startle, ear twitch and eye opening), and 4) extinction of rotatory behavior (open field). Briefly, the dam was temporarily transferred to a clean cage and then pups were placed with nesting material in a small bowl positioned on a heating pad at 37 °C. One pup was assessed at a time and was placed back into the home cage nest once finished its assessment. The time each pup was out of their home nest ranged from 3 min to 12 min. To mark the pups individually, we tattooed forelimbs and/or hindlimbs on PND0 using a sterile needle filled with non-toxic green ink. Pups were evaluated each day from PND0 to 21. Only weight data was collected and no other tests were performed on PND0. The amount of time (latency) or the presence/absence of reflex was recorded and analyzed by a single experimenter. To calculate a developmental milestones (DM) score, we normalized the data to a 1 to 10 scale using the following formula; $y = 1 + (x - A) * (10 - 1) / B - A$ where x = data points, A = lowest score, B = highest score.

2.4. Nest building

Mice were individually housed for at least 48 h in clean plastic cages with approximately 1 cm of bedding lining the floor and identification cards coded to render the experimenter blind to genotype of each subject. Around 6 pm, 1 h prior to the dark phase of the lighting cycle, animals were placed in new individual cage supplied with 3 g of Nestlet material (pressed cotton squares). The next morning (15 h later) cages were assessed for nest construction. Nest construction was scored using a slightly modified version of Deacon's scoring system (Deacon, 2006). Briefly, in this 6 point scale, 0 = Nestlet intact, 1 = >90% intact Nestlet, 2 = Nestlet partially torn up, 3 = Nestlet mostly shredded but no identifiable nest site, 4 = an identifiable but flat nest, 5 = Nestlet torn >90% and a clear nest crater.

2.5. Spontaneous alternation

The natural tendency to explore was assessed using the continuous variant of the spontaneous alternation procedure, as described previously (O'Tuathaigh et al., 2007; Desbonnet et al., 2012). Briefly, without prior habituation, animals were placed individually in the center of the maze and allowed to explore freely for 6 min. A video camera, mounted centrally above the maze, recorded each session. Alternation between arms, the number of arm entries, distance moved and velocity of movement were analyzed using Ethovision videotracking (Ethovision®, Noldus). The maze was made of opaque acrylic glass (Plastic-Craft Product Corp., New York, USA). An arm entry was scored when four limbs were inside the arm. Spontaneous alternation was defined as successive entries into the three arms of the Y-maze, in overlapping triplet sets, with arm choices differing from the previous two choices expressed as