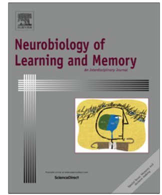
ARTICLE IN PRESS

Neurobiology of Learning and Memory xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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



Cognitive dysfunction in the dystrophin-deficient mouse model of Duchenne muscular dystrophy: A reappraisal from sensory to executive processes

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ARTICLE INFO

Article history:
Received 3 April 2015
Revised 9 July 2015
Accepted 10 July 2015
Available online xxxx

Keywords:
mdx mouse
Associative learning
Executive functions
Working memory
Spatial learning
Hearing

ABSTRACT

Duchenne muscular dystrophy (DMD) is associated with language disabilities and deficits in learning and memory, leading to intellectual disability in a patient subpopulation. Recent studies suggest the presence of broader deficits affecting information processing, short-term memory and executive functions. While the absence of the full-length dystrophin (Dp427) is a common feature in all patients, variable mutation profiles may additionally alter distinct dystrophin-gene products encoded by separate promoters. However, the nature of the cognitive dysfunctions specifically associated with the loss of distinct brain dystrophins is unclear. Here we show that the loss of the full-length brain dystrophin in *mdx* mice does not modify the perception and sensorimotor gating of auditory inputs, as assessed using auditory brainstem recordings and prepulse inhibition of startle reflex. In contrast, both acquisition and long-term retention of cued and trace fear memories were impaired in *mdx* mice, suggesting alteration in a functional circuit including the amygdala. Spatial learning in the water maze revealed reduced path efficiency, suggesting qualitative alteration in *mdx* mice learning strategy. However, spatial working memory performance and cognitive flexibility challenged in various behavioral paradigms in water and radial-arm mazes were unimpaired. The full-length brain dystrophin therefore appears to play a role during acquisition of associative learning as well as in general processes involved in memory consolidation, but no overt involvement in working memory and/or executive functions could be demonstrated in spatial learning tasks.

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1. Introduction

Duchenne muscular dystrophy (DMD) is a common X-linked neuromuscular disorder caused by mutations in the *dmd* gene that prevent expression of dystrophin (Dp427, dystrophin protein of 427 kDa), a cytoskeletal protein normally expressed in both muscles and brain. This syndrome is associated with non-progressive cognitive and behavioral disabilities leading to compromised academic achievements and intellectual disabilities (ID) in a patient subpopulation. The etiology of the central defects is complex due to the variable location of mutations within the *dmd* gene, which may affect several shorter C-terminal brain dystrophins normally produced from independent promoters (reviewed in Perronnet & Vaillend, 2010; Waite, Tinsley, Locke, & Blake, 2009). While genotype–phenotype studies unveiled that the severity of ID increases with the cumulative loss of different dystrophins (Desguerre et al., 2009), studies of heterogeneous cohorts of patients failed to characterize the precise nature of the cognitive dysfunctions specifically associated with the loss of distinct *dmd*-gene products.

Beyond global measures of the intelligence quotient (IQ), cognitive functioning in DMD includes deficits in language abilities, in reading and visuospatial learning and in both short- and long-term memories (Snow, Anderson, & Jakobson, 2013 for a review; Piccini et al., 2014). Impairments in working or short-term memory and limited verbal/auditory span have been highlighted as cardinal features of the neurocognitive profile in DMD, even in individuals with an IQ within the normal range, suggesting a link with the full-length brain dystrophin commonly lost in all patients (Hinton, De Vivo, Nereo, Goldstein, & Stern, 2000; Hinton, De Vivo, Nereo, Goldstein, & Stern, 2001). Recent studies,

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http://dx.doi.org/10.1016/j.nlm.2015.07.006
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however, suggest the presence of a broader deficit in higher-order cognitive processing capabilities, specifically in executive function, which might constitute a core endophenotype leading to cognitive deficits in DMD (Donders & Taneja, 2009; Mento, Tarantino, & Bisiacchi, 2011; Wicksell, Kihlgren, Melin, & Eeg-Olofsson, 2004). Executive functions refer to a set of control processes that optimize performance in cognitive tasks, mediate adapted and flexible behaviors and contribute to the temporal encoding and effortful processing in working memory required for response selection. Deficits in both memory processes and executive functions are likely related to the absence of brain dystrophin in central synapses of hippocampus, cerebellum and cortical areas including prefrontal cortex regions (Lidov, Byers, & Kunkel, 1993). Putative sensory processing disorders have also been suggested but largely overlooked (Poysky & Behavior in DMD Study Group, 2007), yet they might contribute to deficits in information processing and would be in line with the expression of dystrophin in sensory organs (Dodson, Piper, Clarke, Quinlivan, & Dickson, 1995; Wersinger et al., 2011). Previous behavioral studies in the dystrophin-deficient *mdx* mouse revealed deficits in diverse forms of long-term memory, associated with altered synapse plasticity and morphology (Miranda et al., 2009; Vaillend, Billard, & Laroche, 2004; Vaillend, Rendon, Misslin, & Ungerer, 1995). However, the possibility that brain dystrophin might play a broader role in perception and information processing and/or in executive functions has not been thoroughly investigated.

In the present study, we underwent a large-scale behavioral study in *mdx* and littermate control mice to first explore perception and gating of auditory stimuli, and the capacity to integrate relevant auditory stimuli during acquisition of associative learning. We then investigated working memory and its sensitivity to proactive interference, using both win-stay and win-shift paradigms in spatial learning tasks in which newly acquired information needs to be updated on a moment-to-moment basis to guide adapted behavior. To further address distinct properties of executive functions, we also analyzed cognitive flexibility in spatial tasks involving adaptation of behavioral strategies and performance during reversal learning.

2. Materials and methods

2.1. Animals

Mice of the C57BL/10ScSn-*Dmd*^{mdx}/J (*mdx*) mutant and C57BL/10ScSnOlaHsd wild-type control strains were purchased from Charles River Laboratories (Saint-Germain sur l'Arbresle, France) and Harlan Laboratories (U.K), respectively. As the *mdx* mutation is recessive and located in the *dmd* gene on the X chromosome, heterozygous females (*mdx*/+) were mated with wild-type inbred males (+/Y) to produce male mutant (*mdx*/Y) and wild-type littermate mice (+/Y). The genotype was determined by PCR as described (Perronnet et al., 2012). Male siblings were kept in the same cage (two to five mice per cage) under a 12-h light–dark cycle (light on: 7.00 a.m.) with food and water *ad libitum*. Independent groups of 10–16 weeks male siblings were used for each test. Test mice were placed in individual caging at least 8 days before testing. All experiments were conducted blind to the genotype and in accordance with the guidelines established by the European Communities Council Directive (2010/63/EU Council Directive Decree). The protocol was approved by the ethical committee Paris-Sud and Centre (CEEA N°59).

2.2. Immunofluorescence

Brains dissected out following cervical dislocation were fresh-frozen in powdered dry-ice and stored at −80 °C. Brains sections (30 μm thick) were cut at −12 °C on a cryostat and collected on Superfrost+ glass slides (Roth, France). For immunofluorescence analysis, the slides were first thawed for 1 min at RT, immersed in an acetone/methanol fixative solution (1:1) for 5 min at −20 °C and washed 3 times in 0.1 M phosphate-buffered saline (PBS). Slides were then incubated first in a blocking solution for 45 min (10% normal goat serum, 0.3% Triton X-100, 1% bovine serum albumin), then overnight at 4 °C in the 5G5 primary monoclonal antibody directed against the N-terminus of full-length dystrophin (dilution: neat; gift from D. Mornet, Montpellier), then washed and incubated with secondary antibody conjugated to Cy3 (Jackson Immunoresearch, USA) diluted 1:400 in PBS 0.1 M with 5% NGS and 1% BSA for 1 h at RT. No specific dystrophin staining was observed in sections processed from *mdx* mice and in control sections from WT mice when prepared by omitting the primary antibody. Images were collected using a laser scanning confocal microscope LSM 700 (Zeiss) at 555 nm. Stacks of 13 images (320 × 320 μm) spaced by 500 nm were imported using an EC Plan-Neofluar 40×/1.30 Oil M27 at a resolution of 310 nm/pixel. Stacks were processed with the ImageJ imaging system (http://rsb.info.nih.gov/ij) using a maximum intensity projection of all the z-stack images. Brain regions expressing dystrophin were identified using the mouse brain Atlas (Paxinos & Franklin, 2001).

2.3. Sensory processes

2.3.1. Auditory brainstem responses

The auditory brainstem responses (ABRs) were recorded in response to acoustic stimuli under deep anesthesia (95 mg/kg ketamine, 24 mg/kg xylazine, I.P.). Mice were placed on a heating blanket to avoid hypothermia. Auditory stimuli were presented monaurally using an insert earphone (Etymotic Research ER-1, USA) ending with a 17 mm polyethylene tubing inserted into external auditory meatus to avoid sound reverberation on ear lobe. ABRs were recorded using two subcutaneous electrodes (SC25, Neuro-Services, France) located just above the tympanic bulla and skull dorsal midline and one ground electrode placed in the thigh. Insertion of subcutaneous electrodes was not associated with any sign of discomfort. ABRs were collected by a Centor-USB interface (DeltaMed, France). A session lasted 40–50 min. Each session started with click presentations (100 μs) which contained a broad range of frequencies (2–32 kHz). This was followed by pure tone bursts (duration: 10 ms; rise-fall time: 2 ms) delivered at specific frequencies (8, 2, 16, 24, 4, and 32 kHz) and the session ended with click presentations to control that the experiment did not induce acoustic trauma. Click and pure tone bursts were presented at 70, 50, 40, 30, 20, 10, 5 and at 0 dB SPL (decibel in sound pressure level), to quantify the ABR wave amplitudes across decreasing stimulus intensities. Stimuli were presented at 15 Hz, which enabled recording of stable peak latencies. An artifact rejection procedure was used to avoid respiratory and cardiac interferences (response amplitudes > 40 μV). The signal was filtered at 0.2–3.2 kHz with a sampling rate of 100 kHz and waveforms were averaged (500–1000 waveforms depending on the stimulus intensity) and stored for off-line analyses. ABR latency was taken at the positive amplitude peak of the waveform (Fig. 2B). Amplitude was calculated as the difference between the positive and negative peaks of the waveform (I to I′ and III to III′, Fig. 2B). All sound intensities are expressed in dB SPL.

2.3.2. Auditory gating

The acoustic startle reflex (ASR) was induced by a high intensity tone (pulse) and its amplitude was modulated by presentation of non-startling low-intensity prepulses to evaluate auditory gating.

Apparatus – Testing was conducted in a conditioning chamber (19 × 25 × 19 cm) placed on an anti-vibration table inside a dark

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