** EBRAINS Data Descriptor**

*The HBP Data Descriptor follows closely the data descriptor template of the journal Scientific Data (ISSN 2052-4463, https://www.nature.com/sdata/). Note that sections marked with an asterisk are obligatory.*

**TITLE\***

*110 characters maximum, including spaces, no colons and parentheses*

*The title should be descriptive for the presented data. The use of acronyms and abbreviations should be avoided where possible.*

Behavioral phenotyping of *mdx52* mouse model compared to the original *mdx* model

**AUTHORS\***

*Forename Surname 1, Forename Surname 1, and Forename Surname 2*

Amel Saoudi1,2, Faouzi Zarrouki1, Charlotte Izabelle1, Aurélie Goyenvalle2, Cyrille Vaillend1\*.

**AFFILIATIONS\***

*1. Institution*

*2. Institution*

*corresponding author(s): Forename Surname (email@address), Forename Surname (email@address)*

1 Université Paris-Saclay, CNRS, Institut des Neurosciences Paris-Saclay, 91400, Saclay, France

2 Université Paris-Saclay, UVSQ, Inserm, END-ICAP, 78000 Versailles, France

**Corresponding author:**

Cyrille Vaillend, Neuroscience Paris-Saclay Institute (Neuro-PSI), cyrille.vaillend@universite-paris-saclay.fr

**ABSTRACT\***

*170 words maximum, no references*

*The abstract should concisely describe the study, the assay(s) performed, the resulting data, and their reuse potential, but should not make any claims regarding new scientific findings.*

The exon-52-deleted *mdx52* mouse has emerged as a critical model of Duchenne muscular dystrophy (DMD), as it holds a deletion in a hotspot region of the *DMD* gene frequently mutated in patients and is eligible for preclinical studies based on exon-skipping treatment strategies. Deletion of exon 52 impedes expression of several brain dystrophins expressed from distinct promoters (Dp427, Dp260, Dp140), thus providing a key model to study the basis of the cognitive impairment associated with DMD and test therapeutic tools aimed to rescue central deficits. However, behavioral and neurobiological studies in *mdx52* mice are currently lacking. Here, we investigated emotional behavior and fear learning performance of *mdx52* mice compared to *mdx* mice that only lack Dp427, to focus on behavioral phenotypes that could be used in future comparative preclinical studies. *Mdx52* mice displayed enhanced anxiety in several dedicated tests and a severe impairment in learning an amygdala-dependent Pavlovian association. These replicable behavioral outcome measures are reminiscent of the internalizing problems reported in a quarter of DMD patients.

**BACKGROUND & SUMMARY**

*700 words maximum, optional section*

*This section should provide a more detailed overview of the study, the assay(s) performed, and the resulting data as well as referencing previous work and related literature to put the study into context. This section should also briefly outline the broader goals that motivated collection of the data, as well as their potential reuse value.*

X-linked Duchenne Muscular Dystrophy (DMD) is a recessive neuromuscular syndrome frequently associated with non-progressive cognitive deficits and comorbid behavioral and neuropsychiatric disturbances 1–3.DMD occurs because of mutations in the *DMD* gene, composed of 79 exons with multiple independent internal promoters that control the expression of different dystrophin-gene products known as dystrophin proteins (Dp) in a tissue and/or cell-specific manner. The distinct dystrophins thus differ by their molecular weight, their tissue and cellular distribution and their roles, but have the common property to be associated with scaffolds of transmembrane and cytosolic proteins involved in the clustering of various membrane ion channels and receptors. The full-length dystrophin, Dp427 (427kDa), is expressed in both muscle and brain, Dp260 (260kDa) is only detected in retina, while Dp140 (140kDa) and Dp71 (71kDa) are detected in brain. The position of a mutation or deletion within the *DMD* gene may therefore lead to the loss of different brain dystrophins 4, leading to complex phenotype-genotype relationships and various degrees of cognitive and behavioral disturbances. Proximal mutations that selectively affect expression of Dp427 are associated with mild central alterations, while distal mutations causing a cumulative loss of Dp427 and one or several of the shorter brain dystrophins, Dp140 and Dp71, are frequently linked to severe cognitive deficits 2,5–8.

Structure-function and functional studies of different mouse models of DMD may provide an essential contribution to our understanding of the affected brain mechanisms depending on the position of the mutation and loss of different dystrophins. The original Dp427-deficient *mdx* mouse model of DMD holds a nonsense mutation in exon 23 of the gene, leading to the loss of both muscle and brain Dp427. The *mdx* mouse has been extensively studied and it was shown that the loss of brain Dp427 alters brain GABAergic inhibition and is associated with emotional disturbances and mild cognitive deficits 9–12. The loss of brain Dp427 particularly alters the functionality of the neuronal network involved in the processing of fear responses, as demonstrated in *mdx* mice by enhanced fearfulness in response to mild stress and delayed fear learning and memory12–15. However, there is so far very limited behavioral studies of mouse models with distal mutations leading to cumulative loss of several dystrophins 16,17. In particular no behavioral studies have been conducted in mice holding a mutation in the “hot spot” central region of the *DMD* gene, which is frequently mutated in DMD patients (63%) and leads to a cumulative loss of Dp427 and Dp140, despite its association with more severe cognitive impairments 2,5,18,6–8,3.

A mouse model with a deletion in exon 52 of the *DMD* gene, the *mdx52* mouse 19, is an interesting model for preclinical studies addressing both muscular and CNS dysfunctions, as it is deficient in both Dp427 and Dp140. The *mdx52* mice are expected to display at least comparable cognitive and behavioral deficits as the ones observed in the original *mdx* mouse lacking Dp427. However, the impact of the additional loss of Dp140 in *mdx52* mice is unknown and might influence the severity and/or nature of the central deficits. Importantly, it has been shown that exon-skipping strategies based on intracerebral or systemic administration of antisense sequences are efficient to skip exon 23 to restore the reading frame and partially re-express Dp427 in the brain of *mdx* mice, thereby improving GABAergic functions, synaptic plasticity and behavioral fear responses in this model 13,20,21. Recent studies demonstrated that the *mdx52* mouse is also eligible for exon-skipping strategies using antisense sequences to skip exon 51, with therapeutic potential to restore muscle Dp427 expression and motor functions 22,23. It is therefore important to determine the behavioral profile of *mdx52* mice, to identify relevant outcome measures for using this model in preclinical studies.

In the present study we have undertaken a behavioral characterization of the *mdx52* mouse compared to the original *mdx* mouse and respective WT littermate controls, to estimate the presence and severity of the deficits, with a focus on the main phenotypes previously described in the original *mdx* mouse and used in preclinical studies. We show that *mdx52* mice display emotional disturbances and a deficit in fear learning, which are more severe than in the original *mdx* mouse lacking only Dp427.

**METHODS\***

*This section should be used to describe any steps or procedures that produced the data, including full descriptions of the experimental design, data acquisition assays, and any computational processing (e.g., normalization, image feature extraction). Each method should be described under correspondingly named subheading. It is acceptable to cite previous descriptions of the methods under use, but the descriptions should be complete enough for others to understand and reproduce the methods and processing steps without reading the associated publications. There is no word limit to the length of this section.*

The methods used to produce the data have been described previously in 12

Vaillend, C., & Chaussenot, R. (2017). Relationships linking emotional, motor, cognitive and GABAergic dysfunctions in dystrophin-deficient mdx mice. *Human molecular genetics*, *26*(6), 1041–1055. doi: 10.1093/hmg/ddx013. PMID: 28087735.

 <https://academic.oup.com/hmg/article/26/6/1041/2901819?login=true>.

**Experimental design study “Behavioral analysis”**:

Only the cages containing mice of both genotypes (siblings) were selected for experiments.

**Anxiety**

Male mice of the four genotypes: *mdx* mice (n= 15, animal ID: M01-M15) and their WT littermates (C57BL10) (n=14 animal ID: W01-W14); *mdx52* mice (n= 12, animal ID Mx01 – Mx12) and their WT littermates (C57BL6) (n=10, animal ID WT01-WT 10) were tested for exploration and anxiety at the age of 2 months by being successively submitted to three behavioral tests with intervals of 24h between tests, in the following order: Elevated plus maze (EPM), light/dark choice test (LDC), open field activity (OF).

**Unconditioned Fear**

Distinct groups of mdx (n= 6, animal ID M16 – M21) and WT littermate mice (n=3 animal ID W15 – W17) (4 months old) and of mdx52 (n=4, animal ID Mx13 – Mx16) and WT littermates (n=3, animal ID WT11 – WT13) (7 months old) were used for the restraint-induced unconditioned fear response assessment.

**Fear conditioning**

A distinct group of mdx52 (n=18 animal ID Mx17 – Mx34 ) and WT littermates (n= 18 animal ID WT14 – WT31) mice aged 4 months were submitted to the auditory-cued fear conditioning.

**Anxiogenic open field**

Independent groups of naive of mdx (n=12 animal ID M22 – M33 ) and their WT littermates (n=13 animal ID W18 – W30), mdx52 (n=15 animal ID Mx35 – Mx49) and their WT littermates (n=14 animal ID WT32 – WT45) mice aged about 2 months were submitted to a second open-field session in a more anxiogenic context.

**TECHNICAL VALIDATION\***

*This section should present any procedure that is needed to support the technical quality of the data. It should justify the reliability of the presented data. This may include: experiments supporting or validating the data-collection procedure, statistical analyses of experiment errors and variation, phenotypic or genotypic assessments of biological samples, any procedure used to ensure reliable and unbiased data production, acclimatisation procedures to ensure the protection of personal data, etc.. This should not include: follow-up experiments aimed at testing/supporting an interpretation of the data, statistical hypotheses testing, or exploratory computational analyses like clustering and annotation enrichment.*

**USAGE NOTES\***

*This section should contain brief introductions to assist others with reuse of the presented data. This can include suggestions of software packages that are suitable for analyzing the presented data, or tips for further processing steps and for integrating or compare the presented data with other data.*

*Under a subsection called “SPATIAL ANCHORING”, information should be provided that facilitates the spatial anchoring of the presented data into the HBP interactive atlas viewers. If possible, brain image data and coordinates should be provided not only in native space, but in one of the following brain reference spaces: BigBrain template [v1], MNI Colin27 [v1], MNI ICBM 152 [2009c, nonlinear, asymmetric], Infant brain template [v4.0], Allen Mouse CCF [v2 or v3], WHS SD atlas template [v2.0]. Semantic links preferred to brain regions of one of the following atlases: BigBrain parcellation [v1], JuBrain probabilistic cytoarchitectonic atlas [v18], Infant brain atlas [v4.0], Allen Adult Mouse Brain Reference Atlas [v2 or v3], WHS SD atlas [v2.0].*

Data are provided in excel files.

**SPATIAL ANCHORING:**

**DATA RECORDS\***

*This section should be used to explain the data presented in this descriptor and the repository where they are stored. This should include an overview of data files and formats, and potentially a short content description and file-internal data structure for each file type. Example of a mock-up repository overview:*

In the repository the data are stored in the following structure (incl. info on file content):

**repository-root/**

**data-descriptor.pdf** *[contains a short description of the dataset*

**sub\_info.tsv** *[contains information on the subjects*

**experimental-methods\_info.json** *[contains information on the applied experimental method]*

**sub-XXX/**

**sub-XXX\_slice-XXXX.tif** *[brain slice scan of subject XXX; index XXXX equals physical slice position in µm, anterior to posterior]*

**derived-data/**

**analysis-methods\_info.json** *[contains information on the applied analysis methods]*

**analysis-X/**

**analysis-X\_set-X.tsv** *[contains result data of analysis X]*

**code/**

**analysis-X.py** *[script that produces result data of analysis X]*

Information on used file formats and file-internal data structures:

**Tab-Separated Value format (tsv):** *labels in first row; data of same type in columns*

**JavaScript Object Notation (json):** *nested key-value pairs [cf. templates in xx]*

**Tagged Image Format File (tif):** *100µm thickness; 1x1 pixel dimension; unit in µm*

**Python Script (py):** *Python 3.0 script*

In the repository, the data are stored under an excel format.

The datasheet ‘Emotional reactivity of *mdx52* mice’ contains 5 sheets:

1- Summary of metadata

2- Anxiety

3- Unconditioned fear response

4- Fear conditioning

5- Anxiogenic OF

In each sheet, the subjects are organized in lines.

**CODE AVAILABILITY\***

*This section should list all software / code (ready-made or custom-made) and their version used in the generation or processing of the presented data. This should include a statement indicating whether and how the software or code can be accessed, including any access restrictions. If relevant, provide also any specific variables or parameters used to run the software / code on the presented data.*

Data are provided in excel files.

**Acknowledgements**

*This section should contain brief acknowledgements of non-author contributors. Anonymous referees and editors or effusive comments are not accepted. Grant or contribution numbers can be acknowledged.*

The authors are grateful to Dr Jun Tanihata and Dr Shin’ichi Takeda from the National Center of Neurology and Psychiatry (Tokyo, Japan) for kindly providing the mdx52 breeder mice and to the Zootechnic platform of our institute for mouse breeding, care, and genotyping.

**Author contributions**

*This section should state briefly on a separate line each author’s contribution to producing or maintaining the presented data as well as their role in publishing them.*

Conceptualization: C.V.; Methodology: C.V.; Validation: C.V.; Formal analysis: A.S., F.Z., C.I.; Investigation: A.S., F.Z., C.I.; Resources: C.V.; Data curation: A.S., F.Z., C.V.; Writing - original draft: A.S.; Writing - review & editing: A.G., C.V.; Visualization: A.S., C.I., C.V.; Supervision: C.V.; Project administration: C.V.; Funding acquisition: A.G., C.V.

**REFERENCES\***

*This section should list all bibliographic information for all literature cited in the above sections using the standard Nature referencing style.*

1. Hinton, V. J. *et al.* Association of autistic spectrum disorders with dystrophinopathies. *Pediatr Neurol* **41**, 339–346 (2009).

2. Ricotti, V. *et al.* Neurodevelopmental, emotional, and behavioural problems in Duchenne muscular dystrophy in relation to underlying dystrophin gene mutations. *Dev Med Child Neurol* **58**, 77–84 (2016).

3. Colombo, P. *et al.* Assessing mental health in boys with Duchenne muscular dystrophy: Emotional, behavioural and neurodevelopmental profile in an Italian clinical sample. *Eur J Paediatr Neurol* **21**, 639–647 (2017).

4. Hoffman, E. P., Brown, R. H., Jr. & Kunkel, L. M. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. *Cell* **51**, 919–28 (1987).

5. Felisari, G. *et al.* Loss of Dp140 dystrophin isoform and intellectual impairment in Duchenne dystrophy. *Neurology* **55**, 559–564 (2000).

6. Daoud, F. *et al.* Analysis of Dp71 contribution in the severity of mental retardation through comparison of Duchenne and Becker patients differing by mutation consequences on Dp71 expression. *Hum Mol Genet* **18**, 3779–3794 (2009).

7. Taylor, P. J. *et al.* Dystrophin gene mutation location and the risk of cognitive impairment in Duchenne muscular dystrophy. *PLoS One* **5**, e8803 (2010).

8. Lorusso, M. L. *et al.* Specific profiles of neurocognitive and reading functions in a sample of 42 Italian boys with Duchenne Muscular Dystrophy. *Child Neuropsychol* **19**, 350–369 (2013).

9. Knuesel, I. *et al.* Short communication: altered synaptic clustering of GABAA receptors in mice lacking dystrophin (mdx mice). *Eur J Neurosci* **11**, 4457–4462 (1999).

10. Vaillend, C., Rendon, A., Misslin, R. & Ungerer, A. Influence of dystrophin-gene mutation on mdx mouse behavior. I. Retention deficits at long delays in spontaneous alternation and bar-pressing tasks. *Behav Genet* **25**, 569–579 (1995).

11. Vaillend, C., Billard, J.-M. & Laroche, S. Impaired long-term spatial and recognition memory and enhanced CA1 hippocampal LTP in the dystrophin-deficient Dmd(mdx) mouse. *Neurobiol Dis* **17**, 10–20 (2004).

12. Vaillend, C. & Chaussenot, R. Relationships linking emotional, motor, cognitive and GABAergic dysfunctions in dystrophin-deficient mdx mice. *Hum Mol Genet* **26**, 1041–1055 (2017).

13. Goyenvalle, A. *et al.* Functional correction in mouse models of muscular dystrophy using exon-skipping tricyclo-DNA oligomers. *Nat Med* (2015) doi:10.1038/nm.3765.

14. Razzoli, M. *et al.* Social stress is lethal in the mdx model of Duchenne muscular dystrophy. *EBioMedicine* **55**, 102700 (2020).

15. Sekiguchi, M. *et al.* A deficit of brain dystrophin impairs specific amygdala GABAergic transmission and enhances defensive behaviour in mice. *Brain* **132**, 124–35 (2009).

16. Vaillend, C. *et al.* Spatial discrimination learning and CA1 hippocampal synaptic plasticity in mdx and mdx3cv mice lacking dystrophin gene products. *Neuroscience* **86**, 53–66 (1998).

17. Vaillend, C. & Ungerer, A. Behavioral characterization of mdx3cv mice deficient in C-terminal dystrophins. *Neuromuscul Disord* **9**, 296–304 (1999).

18. Beroud, C. *et al.* Multiexon skipping leading to an artificial DMD protein lacking amino acids from exons 45 through 55 could rescue up to 63% of patients with Duchenne muscular dystrophy. *Hum Mutat* **28**, 196–202 (2007).

19. Araki, E. *et al.* Targeted disruption of exon 52 in the mouse dystrophin gene induced muscle degeneration similar to that observed in Duchenne muscular dystrophy. *Biochem Biophys Res Commun* **238**, 492–497 (1997).

20. Vaillend, C. *et al.* Rescue of a dystrophin-like protein by exon skipping in vivo restores GABAA-receptor clustering in the hippocampus of the mdx mouse. *Mol Ther* **18**, 1683–8 (2010).

21. Dallérac, G. *et al.* Rescue of a dystrophin-like protein by exon skipping normalizes synaptic plasticity in the hippocampus of the mdx mouse. *Neurobiol Dis* **43**, 635–641 (2011).

22. Aoki, Y. *et al.* In-frame dystrophin following exon 51-skipping improves muscle pathology and function in the exon 52-deficient mdx mouse. *Molecular therapy : the journal of the American Society of Gene Therapy* **18**, 1995–2005 (2010).

23. Aupy, P. *et al.* Long-Term Efficacy of AAV9-U7snRNA-Mediated Exon 51 Skipping in mdx52 Mice. *Mol Ther Methods Clin Dev* **17**, 1037–1047 (2020).