Miranda *et al. Behavioral and Brain Functions* (2024) 20:21
https://doi.org/10.1186/s12993-024-00246-x

Behavioral and Brain Functions

RESEARCH

Open Access

Social and emotional alterations in mice lacking the short dystrophin-gene product, Dp71

Rubén Miranda[1,2*], Léa Ceschi[1], Delphine Le Verger[1], Flora Nagapin[1], Jean-Marc Edeline[1], Rémi Chaussenot[1] and Cyrille Vaillend[1*]

Abstract

Background The Duchenne and Becker muscular dystrophies (DMD, BMD) are neuromuscular disorders commonly associated with diverse cognitive and behavioral comorbidities. Genotype–phenotype studies suggest that severity and risk of central defects in DMD patients increase with cumulative loss of different dystrophins produced in CNS from independent promoters of the *DMD* gene. Mutations affecting all dystrophins are nevertheless rare and therefore the clinical evidence on the contribution of the shortest Dp71 isoform to cognitive and behavioral dysfunctions is limited. In this study, we evaluated social, emotional and locomotor functions, and fear-related learning in the Dp71-null mouse model specifically lacking this short dystrophin.

Results We demonstrate the presence of abnormal social behavior and ultrasonic vocalization in Dp71-null mice, accompanied by slight changes in exploratory activity and anxiety-related behaviors, in the absence of myopathy and alterations of learning and memory of aversive cue-outcome associations.

Conclusions These results support the hypothesis that distal *DMD* gene mutations affecting Dp71 may contribute to the emergence of social and emotional problems that may relate to the autistic traits and executive dysfunctions reported in DMD. The present alterations in Dp71-null mice may possibly add to the subtle social behavior problems previously associated with the loss of the Dp427 dystrophin, in line with the current hypothesis that risk and severity of behavioral problems in patients increase with cumulative loss of several brain dystrophin isoforms.

Keywords Dystrophin, Dystrophinopathies, Social behavior, Ultrasonic vocalizations, Anxiety, Fear memory, Dp71

Background

DMD is a common X-linked recessive 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 muscle and brain. This syndrome is associated with non-progressive cognitive and behavioral disabilities that are independent from skeletal muscle impairment and associated with the loss of brain dystrophin [52] for a review). Cognitive profile of DMD patients is however heterogeneous and includes deficits in language abilities, reading, visuospatial learning and in both short- and long-term memories [62] for a review). Boys with

*Correspondence:
Rubén Miranda
rubenmir@ucm.es
Cyrille Vaillend
cyrille.vaillend@universite-paris-saclay.fr
[1] Université Paris-Saclay, CNRS, Institut des Neurosciences Paris-Saclay, 91400 Saclay, France
[2] Department of Psychobiology and Methodology in Behavioral Sciences, Universidad Complutense de Madrid, Ciudad Universitaria, 28040 Madrid, Spain

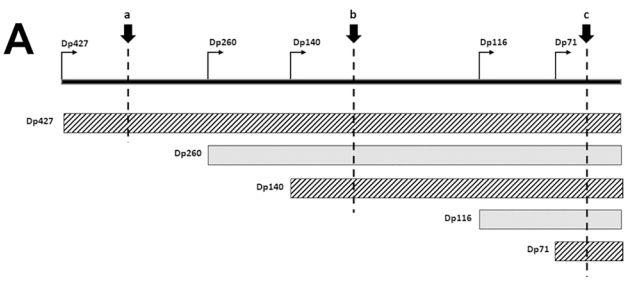
DMD also exhibit important neurobehavioral comorbidities, including attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorders (ASD), obsessive–compulsive disorder, anxiety, and intellectual disability (ID) [3, 62]. Individual differences in the mutation profiles within the *DMD* gene might explain this heterogeneity, as they may lead to the cumulative loss of shorter brain dystrophins derived from independent alternative promoters, such as Dp140 and Dp71 [44, 52, 55] (Fig. 1).

The cellular role of the distinct brain isoforms is not yet fully understood. Nevertheless lack of brain dystrophins has been proved to impact the structural and functional brain organization affecting critical aspects of synaptic transmission and plasticity in cognitive-relevant areas such as hippocampus, amygdala, cortex and cerebellum [11, 28, 42, 52]. In particular, mice with a selective loss of Dp71 (Dp71-null mice), which do not show muscle histopathology or a motor phenotype [20], display alterations of spatial learning, working memory and behavioral flexibility, reduced exploratory and novelty-seeking behaviors, which have been associated with altered glial-vascular functions, water homeostasis, abnormal glutamatergic synapse organization, enhanced central excitation and impaired synaptic plasticity [4, 11, 20, 29, 42].

Many lines of evidence from genotype–phenotype studies suggest that severity and risk of cognitive deficits in DMD patients increases with distal mutations leading to the cumulative loss of different dystrophins (e.g., [3, 18, 22, 55, 64]). For instance, ADHD and ID in DMD patients are closely related to distal mutations affecting expression of Dp140 and Dp71, with Dp71 loss being a major aggravating factor [19, 47, 50, 64]. However, no clear genotype–phenotype relationship was reported hitherto for other common neurobehavioral disturbances such as social disturbances, associated to or reminiscent of ASD [32–34, 46, 50]. The sole loss of Dp427 in one patient with DMD and autism suggested that the lack of Dp427 is sufficient to induce vulnerability to autism [23], which was also supported by our previous study in the Dp427-deficient *mdx* mouse model revealing presence of context-dependent changes in their social behavior and socially-induced vocalizations [43]. Two cohort studies of DMD patients reported that the patients diagnosed with ASD frequently (> 60%) had mutations disrupting Dp140 expression [3, 71]. However, in a cohort of 87 DMD patients the prevalence of ASD associated with disrupted Dp427 (4/26, 15%; upstream of exon 30) and Dp140 (11/54; 20%; exon 31–62) was comparable, while a higher percent of cases with ASD was observed when mutations disrupted Dp71. Although this was calculated from a rather small sample size (3/7; 43%; downstream of exon 63) [55], a putative increase in the risk for social behavior deficits with mutations affecting Dp71 was hypothesized. However, mutations downstream exon 63 affecting expression of all dystrophins including Dp71 are rarely observed ([44] for a review), and these results should therefore be interpreted cautiously and confirmed in future meta-analysis studies.

The etiology of social disturbances in DMD is still unknown, but it might be influenced in part by the enhanced emotional reactivity observed across mutation profiles in both patients [55] and DMD mouse models [56, 66]. In this regard, the Dp71-null mouse model selectively lacking Dp71 might be useful to isolate the specific contribution of this isoform to the emergence and/or aggravation of cognitive, emotional and social deficits.

In this study, we undertook a large-scale behavioral study of Dp71-null mice using a variety of tests to characterize, for the first time in this model, social behavior and associated ultrasonic vocalizations, emotional reactivity and aversive cue-outcome associative processes. We



B

	mdx	*mdx52*	*Dp71-null*	*mdx3cv*
Dp427	-	-	+	-
Dp260	+	-	+	-
Dp140	+	-	+	-
Dp116	+	+	+	-
Dp71	+	+	-	-

Fig. 1 *DMD* gene and dystrophin isoforms. **A** Diagram showing the *DMD* gene (79 exons) and its internal promoters (angled arrows) that give rise to several dystrophin proteins expressed in different tissues/regions: The full-length dystrophin Dp427 that exists as 3 isoforms is expressed in muscle, brain and cerebellar Purkinje cells, Dp260 in retina, Dp140 in brain and kidney, Dp116 in peripheral nerves and Dp71 in brain, retina and liver. The dystrophins expressed in brain are represented as hatched bars. The thick vertical arrows and dotted lines indicate the three main regions where disease-causing mutations lead to the unique loss of Dp427 (a), or a deficiency in Dp427, Dp260 and Dp140 (b), or a deficiency in all dystrophins (c). **B** Table showing the expression pattern of dystrophins in DMD mouse models. The mouse model analyzed in the present study is highlighted in grey. +: normal expression; – deficient

show that the absence of Dp71 is associated with selective alterations of social behavior and socially-induced vocalizations, as well as with a moderate increase in anxiety-related behaviors, supporting the hypothesis that distal mutations affecting expression of Dp71 may increase the risk for social and affective comorbidities in DMD.

Methods

Animals

Dp71-null mice were a kind gift from Prof. David Yaffe and Uri Nudel (Weizmann Institute of Science, Rehovot, Israel), who originally generated these transgenic mice by homologous recombination replacing most of the first and unique exon and a small part of the first intron of Dp71 by the promoter-less gene encoding a β-galneomycin resistance chimeric protein. This specifically abolished the expression of Dp71 without interfering with the expression of other *dmd*-gene products [57]. Breeders were initially used to establish colonies of Dp71-null and mdx3cv mice in the laboratory of Dr. Alvaro Rendon (Institut de la Vision, Paris, France), who then kindly transferred new breeders to our laboratory. Both lines were backcrossed for > 10 generations to C57BL/6JRj mice (Janvier Labs, France) in CDTA (Orléans, France). Production and maintenance of the distinct mutant lines was undertaken in our animal facility by crossing in each case heterozygous females with C57BL/6JRj mice to generate either Dp71-null or mdx3cv and their respective littermate controls (WT) for experiments. Genotypes were determined by PCR analyses of tail DNA.

Animals were kept under a 12-h light–dark cycle (light on 7.00 a.m.) with food and water ad libitum. Studies were conducted blind to the genotype and following guidelines of our local mouse facility in compliance with European Communities Council Directive (CEE 86/609) for animal care and experimentation, EU Directive 2010/63/EU and French National Committee (87/848).

Experimental groups

Independent cohorts of 10–16 weeks male siblings were used for ultrasonic recordings and behavioral tests. For social interactions, test mice were placed in individual caging at least 8 days before testing and protocols conducted as previously described [12, 43, 66]. Two cohorts were used for recordings of socially-elicited ultrasonic vocalizations during interaction with anesthetized and freely-moving females (12 WT and 15 Dp71-null mice, 12–14 weeks old) and for interactions with males (23 WT and 15 Dp71-null mice12-14 weeks old). Sociability and preference for social novelty were assessed in a third cohort of 10 WT and 13 Dp71-null mice submitted to the three-chamber test. Agonistic behaviors were studied using resident-intruder paradigm in a fourth cohort of mice (WT = 9, Dp71-null = 8, 14–16 weeks old). Anxiety-related behavior was evaluated in a fifth cohort of mice (WT = 12; Dp71-null = 12; 10 weeks old) successively submitted to the light–dark choice test, the elevated plus-maze test and to the exploration of an open field, with a 72 h delay between tests. A sixth cohort (WT = 7, Dp71-null = 10; 12 weeks old) was used to evaluate auditory brainstem responses (ABRs). Associative learning and fear memories were assessed using three additional cohorts of mice submitted to delayed cued fear conditioning under two different acquisition protocols: standard acquisition (WT = 20; Dp71-null = 18) and short acquisition (WT = 9; Dp71-null = 8); and trace fear conditioning (WT = 13; Dp71-null = 13; 12 weeks old). For unconditioned fear response a cohort of 9-months old Dp71-null (n = 9), mdx3cv (n = 5) and WT (B6) (n = 11) mice were used.

Ultrasonic vocalizations (USVs)

Mouse ultrasonic calls were recorded and analyzed as previously described [43]. Socially elicited ultrasonic vocalizations (USVs) were recorded in adult male mice confronted with anesthetized or freely-moving awake females, and with other unfamiliar males (see behavioral testing section. USVs were recorded with a condenser ultrasound microphone (Avisoft Bioacoustics, Germany; CMPA-P48/CM16, frequency response ± 3 dB within the range 10–180 kHz suspended 15 cm above the cage and connected to a TASCAM HD-P2 digital recorder. Vocalizations were sampled at 192 kHz with a 16-bit dynamic. Recordings were analyzed with the Avisoft SASLab Pro (v4.40) signal processing software. Spectrograms were generated (FFT-length of 1024 points, overlap of 75%, 100% Frame, Hamming window) to follow the frequency modulation. A high-pass FFT filter (cut-off frequency of 25 kHz) was applied to reduce the background noise. A call was defined as a unit of sound separated by silence from other sound units that may consist of one or more 'notes' or continuous markings on a sonogram [36]. A sequence was defined as a succession of at least 2 calls separated by silent intervals less than 200 ms. Number and mean duration of calls and sequences of calls were quantified and the total time spent calling was computed by summing durations of each call. The peak frequency (Pfreq) was defined as the frequency of maximum amplitude in the spectrum, while the peak amplitude (Pamp) corresponded to the amplitude at peak frequencies at the start- and endpoints of each call.

Ultrasonic calls (N = 38,412) were qualitatively classified using our previously described semi-automatic method [43]. Each call was assigned to one of the following categories [43]: *Upward*: continuous frequency increase of at least 1.5 Hz per 10-ms bins, with eventually

flat steps; *Downward*: continuous frequency decrease of at least 1.5 Hz per 10 ms bins, with eventually flat steps; *Peak*: frequency-modulated call showing continuous increase in frequency followed by a continuous decrease; *U-shape*: frequency-modulated call showing continuous decrease in frequency followed by a continuous increase; *Flat*: constant frequency with no modulation > 1.5 Hz per 10 ms bins; *Short*: Duration < 10 ms; *Sinusoidal*: two or more directional changes in frequency in distinct directions; *Frequency jump*: two or more components displaying discontinuous frequency steps on the sonographic representation but without gap on the time scale; *Unstructured*: without main sound component and frequency shape that could be assimilated to any of the other categories.

Behavioral testing

Social motivation in a three-chamber test

Apparatus and paradigm have been previously described [43]. The apparatus was a rectangular three-chambered box fabricated from clear polycarbonate. Dividing walls with manual retractable doorways (width: 10 cm, height: 20 cm) enabled access into each chamber (20 × 40 × 22 cm). The box was cleaned with alcohol and fresh bedding (1 cm high; Lignocel Prime-s, Genestil, France) was added between trials. The middle chamber was used to confine test mice at the beginning/end of test trials, while each of the two side chambers were used to place transparent polycarbonate tubes (8 cm in diameter) serving as confinement tubes for social stimuli (stranger mice) or remaining empty as nonsocial stimuli. These confinement tubes were drilled with holes (1 cm diameter; 2-cm spaced) to allow limited contact with the confined stimulus. Control male mice used as social stimuli (strangers, same age as test mice) were familiarized to the confinement tubes during four days (30 min per day) before the start of the experiment. Then, testing consisted of 3 trials: For trial 1 (habituation), the test mouse was placed in the middle chamber for 2 min, the doorways were then opened and the mice could freely explore the test box without confinement tubes for 10 min. For trial 2 (sociability) test mice were given the choice to explore an empty confinement tube versus an unknown mouse (stranger 1) enclosed in a second confinement tube located in an opposite side chamber. Location of stranger 1 in the left or right-side chamber was balanced across subjects. For trial 3 (preference for social novelty), a second novel unfamiliar mouse (stranger 2) was enclosed in the empty tube. The test mice were then given the possibility to interact with both stranger 1 and stranger 2 for 10 min. Behavior was videotracked during all trials (Any-maze software; Stoelting, USA) to analyze general activity (distance travelled, rearing and leanings, self-grooming, periods of activity and inactivity). Social behavior was reflected by the % time spent and number of entries in the side chambers containing the confined stimuli, as well as by the frequency and duration (% time) of sniffing episodes directed against the confinement tubes.

Agonistic behavior in resident-intruder test

The protocol was adapted from previously described methods [58]. At 8 weeks of age, test mice of both genotypes (Dp71-null and WT littermates) were changed to individual caging (20 × 30 cm cages) and thus maintained for 2 months without bedding change (1–2 cm high bedding; Lignocel Prime-s, Genestil, France). Resident test mice were confronted once with unknown C57BL/6JRj male mice aged 4 months acting as intruders. After 30-min acclimatization of residents to testing room, an intruder was placed in the resident's home cage and behavior was video recorded for 6 min. The following behaviors initiated by residents were coded manually using event-recorder keys (Any-maze software; Stoelting, USA): Sniffing, fights, pursuits (the mouse walking directly in the path of the other mouse that is also in motion, at a distance smaller than the mean body length), dominant behavior (putting a paw on the body of the other mouse and/or mounting).

Male–female and male-male dyadic social interactions

Mice were acclimatized to the test cage (transparent Plexiglas box, 20 × 30 × 14 cm) for 30 min; then an unknown control female or male (same age as test mice) was introduced to trigger social interaction for 3 min. Emission of USVs by resident males were recorded as described, and behavioral responses were video recorded. The sniffing or snout contacts elicited by resident males were quantified manually using event-recorder keys in Any-maze software (Stoelting, USA). We quantified the latency of first contact, mounting attempts, frequency and duration of contacts, number of dominant acts, number and duration of rearings (vertical activity), time spent rearing, grooming and making pursuit (mouse walking directly in the path of the other mouse that is also in motion, at a distance smaller than the mean body length) towards intruder. Contacts were classified as oro-facial (directed towards the head/neck/mouth area), oro-genital (towards the anogenital area) or directed towards other body parts (flank area).

Male exposure to anesthetized female

Following acclimatization to the test cage as above, the test mice were confronted for 3 min to an anesthetized control female (anesthesia: I.P. injections of 100 mg/kg ketamin and 12 mg/kg xylazin). The frequency and

duration of contacts performed by the test mice were manually quantified using event-recorder keys in Any-maze (Stoelting, USA).

Open field exploratory activity

The test box was a square open field (50×50×50 cm) with black walls and a floor covered with sawdust (1 cm high; Lignocel Prime-s, Genestil, France). Experiments were undertaken under constant room temperature (22–23 °C) and homogeneous dim illumination (50 lx). Each mouse was released near the wall and video-tracked for 30 min using the Any-maze software (Stoelting, USA). Recorded xy positions were used to generate tracking plot of the exploration paths, and to calculate the distance travelled, speed and time spent in the whole apparatus, as well as in distinct virtual zones of the box: The central area (30×30×30 cm) to estimate anxiety and a virtual corridor along walls (width: 10 cm) to estimate thigmotaxis. The percentage of time spent and distance traveled in the center zone were used as relative measures of anxiety. The number of rearings and leanings, referred to as vertical activity, were counted manually by the experimenter from saved videos. Thigmotaxis was further evaluated by counting the number of complete and unbroken revolutions along the virtual corridor around the open field.

Light-dark choice anxiety test

In the light–dark box test, mice had the choice to explore a brightly lit anxiogenic compartment or to stay in a more secure dark compartment. The apparatus had 20 cm-high Plexiglas walls and consisted of a brightly lit white compartment (40×15 cm; illumination: 600 lx) connected by a trap door (6×6 cm) to a dark compartment (15×15 cm; illumination < 10 lx), as previously described [56]. Each mouse was placed in the dark compartment for 10 s, the trap door was then opened and mice allowed to freely explore the whole apparatus for 5 min. Step through latency, number of entries and total time spent in the lit compartment were scored.

Elevated plus-maze anxiety test

The maze had two facing arms enclosed with high walls (20×8×25 cm), two open arms (20×8 cm) and a central area (8×8 cm) forming a plus sign 65 cm above the floor. Illumination was 150 lx in open and 30 lx in enclosed arms. Mice were individually placed at the center of the maze with the head facing an open arm. The number of entries and time spent in open or enclosed arms were recorded for 5 min. Head-dipping over sides of open arms (transiently putting the head outside open-arm boundaries to look down to the void) were counted and classified as protected head dips when the rest of the mouse's body remained in a closed arm, and as unprotected head dips when the whole mouse's body was located in the open arm.

Restraint-induced unconditioned fear

The mouse was restrained by grasping the scruff and back skin as previously described [66]. After 15 s, the mouse was released to a novel cage (24×19 cm, with 12-cm high walls) containing clean sawdust (1 cm high, Lignocel Prime-s, Genestil, France) and was then video-tracked for 5 min using the Any-maze software (Stoelting, USA). Unconditioned fear responses induced by this short acute stress were characterized by periods of tonic immobility (freezing) and quantified during a 5-min recording period. Complete immobilization of the mouse, except for respiration, was regarded as a freezing response [51]. The percent time spent freezing was calculated for group comparisons.

Auditory brainstem responses

ABRs were collected under deep anesthesia (95 mg/kg ketamine, 24 mg/kg xylazine, I.P.) by presenting acoustic stimuli presented monaurally using an insert earphone (Etymotic Research ER-1, USA). ABRs were recorded during 40–50 min by a Centor-USB interface (DeltaMed, France) connected to two subcutaneous electrodes (SC25, Neuro-Services, France) inserted just above the tympanic bulla and on the skull dorsal midline with one ground electrode placed in the thigh. Click presentations (100 µs; 2–32 kHz) were followed by pure tone bursts (10 ms; rise-fall time: 2 ms; frequencies: 8, 2, 16, 24, 4, & 32 kHz) and the session ended with click presentations to control the presence of any acoustic trauma. Stimuli were presented at 15 Hz across decreasing stimulus intensities (70, 50, 40, 30, 20, 10, 5 & 0 dB SPL; decibel in sound pressure level). Artifacts (potentially coming from myographic activity) with amplitudes > 40 µV were automatically discarded. The signal was filtered at 0.2–3.2 kHz with a 100-kHz sampling rate and 500–1000 waveforms were averaged per intensity.

Fear conditioning

Experiments were conducted using the StartFear System apparatus (Panlab, Spain) with continuous background white noise delivery (60 dB). Freezing behavior was detected by the piezoelectric accelerometer. For delay cued fear conditioning paradigm, a 2-min acclimatization period was followed by either 5 (strong acquisition) or 3 (weak acquisition) CS–US pairs delivered at variable random intervals of 60 to 180 s (acquisition). The US (0.4 mA, 2 s) was delivered immediately after the end of the CS (80 dB, 10 kHz, 30 s). During the next two days (retention sessions 1 and 2), mice were placed

in the new context and 16 CS were delivered alone (no electrical footshock) at intervals of 60 to 150 s. For the trace fear conditioning paradigm, mice were first allowed to freely explore the conditioning chamber for 5 min. On the next day acquisition started with a 3-min acclimatization period, followed by delivery of 6 CS-US pairs separated by pseudo-random intervals of 120, 180 or 240 s. The footshock (US, 0.4 mA, 2 s) was delivered 30 s (trace) after the end of the CS (80-dB tone, 10 kHz, 15 s). On the third day (retention), mice were placed in a new context (white metal walls and flat floor) and three CS were delivered alone with a 60-s interval between CS. At least three hours later, mice were placed back in the initial acquisition context for 5 min to evaluate contextual fear memory.

Statistics

Data are presented as mean ± S.E.M. Two-tailed parametric statistics were performed using GraphPad Prism7 (San Diego, California, USA), SPSS (IBM, USA) and Sigmastat (San Jose, California, USA). Repeated measures were analyzed using two-way ANOVAs with genotype as the between-group factor followed by Tukey post-hoc tests. Independent measures were analyzed using unpaired or paired t-tests. Correlations were evaluated using Pearson's coefficients and their significance using the r to z Fischer's test.

Results

Sociability and preference for social novelty

During habituation (trial 1), both genotypes spent more time exploring the side chambers than the central zone (Chamber effect, $F(2,42) = 13.160$, $p < 0.001$; side vs central chambers, $p < 0.05$, post-hoc tests), showing no place preference or bias for any side chamber. Sociability (trial 2) was optimal and comparable between genotypes, as both Dp71-null and WT littermates spent more time sniffing the social stimulus than the non-social object (WT, $t = 3.180$; Dp71-null, $t = 3.395$; $p < 0.05$; Fig. 2A). This preference for social vs non-social stimuli was observed in 80% of WT mice and in 84.62% of Dp71-null mice (criterion: individuals exploring social stimulus > 50% of their time).

In trial 3, WT mice spent more time (%) sniffing the novel than the familiar mouse ($t = 2.376$, $p < 0.05$) confirming typical preference for social novelty in WT controls. In contrast, Dp71-null spent comparable percent times sniffing the familiar and novel social stimuli ($t = 1.202$, $p > 0.05$, NS), with a trend for reversed preference for familiar over novel stimuli (NS). Dp71-null mice sniffed the familiar mouse significantly more time (%) ($t = 2.580$, $p < 0.05$) and the novel one significantly less time (%) as compared to controls ($t = 2.580$, $p < 0.05$) (Fig. 2B). Preference for social novelty was observed in 80% of WT mice but only in 53.85% of Dp71-null mice.

Agonistic interactions in the resident-intruder paradigm

Forced encounters with male intruders were performed in the resident's (test mouse) home cage to assess agonistic approaches and aggressiveness. Fights were absent in both genotypes except for one Dp71-null mouse showing a total of seven attacks. The absence of fights allowed reliable recording of social contacts. No differences between genotypes were observed in the number of sniffing, dominance and pursuit episodes ($F(1,30) = 0.0428$, $p > 0.05$, NS; Fig. 2C) or in the duration of sniffing episodes and pursuits ($F(1,15) = 0.0003$, $p > 0.05$, NS; Additional file 1). The scarce number of dominant acts and pursuits' episodes and duration initiated by WT and Dp71-null residents revealed a low aggressiveness, comparable between genotypes (all $p > 0.05$, NS; Additional File 1).

Dyadic social interactions with females and males

Here, social interactions were analyzed in a more neutral territory, as the test mice were only familiarized with the test cage for 30 min. During encounters with females and males, as well as during exposure to anesthetized females, no differences were observed in the number of episodes and time spent rearing and grooming, nor in the time spent making pursuits (with freely-moving males and females) (all, $p > 0.05$, NS; Additional file 2). This indicates unaffected vertical activity, agonistic behaviors and stress-induced grooming in Dp71-null mice. Moreover, there were no significant genotype differences in the number of social contacts and time spent sniffing anesthetized females or awake and freely-moving females and males (all $p > 0.05$, NS; Fig. 2D–F and Additional file 2). However, analyses of sniffing behavior as a function of time (Fig. 2G–I) revealed that Dp71-null mice spent significantly more time than WT sniffing anesthetized females during the first 120 s of the 3-min trial (Genotype x time interaction: $F(2,50) = 3.549$, $p < 0.05$; Fig. 2H). In contrast, this was not observed during interactions with the awake and freely-moving females and males, likely due to the active influence of intruders' behavior. Besides, no significant differences were observed between genotypes in the number and duration of contacts classified as a function of the body parts involved (i.e., oro-facial, oro-genital, other body parts) (all $p > 0.05$, NS; Additional file 3).

Ultrasonic vocalization during social interactions with females and males

Emission of ultrasonic vocalizations was abnormally enhanced in Dp71-null mice compared with WT mice, as shown by the increased percent time spent vocalizing

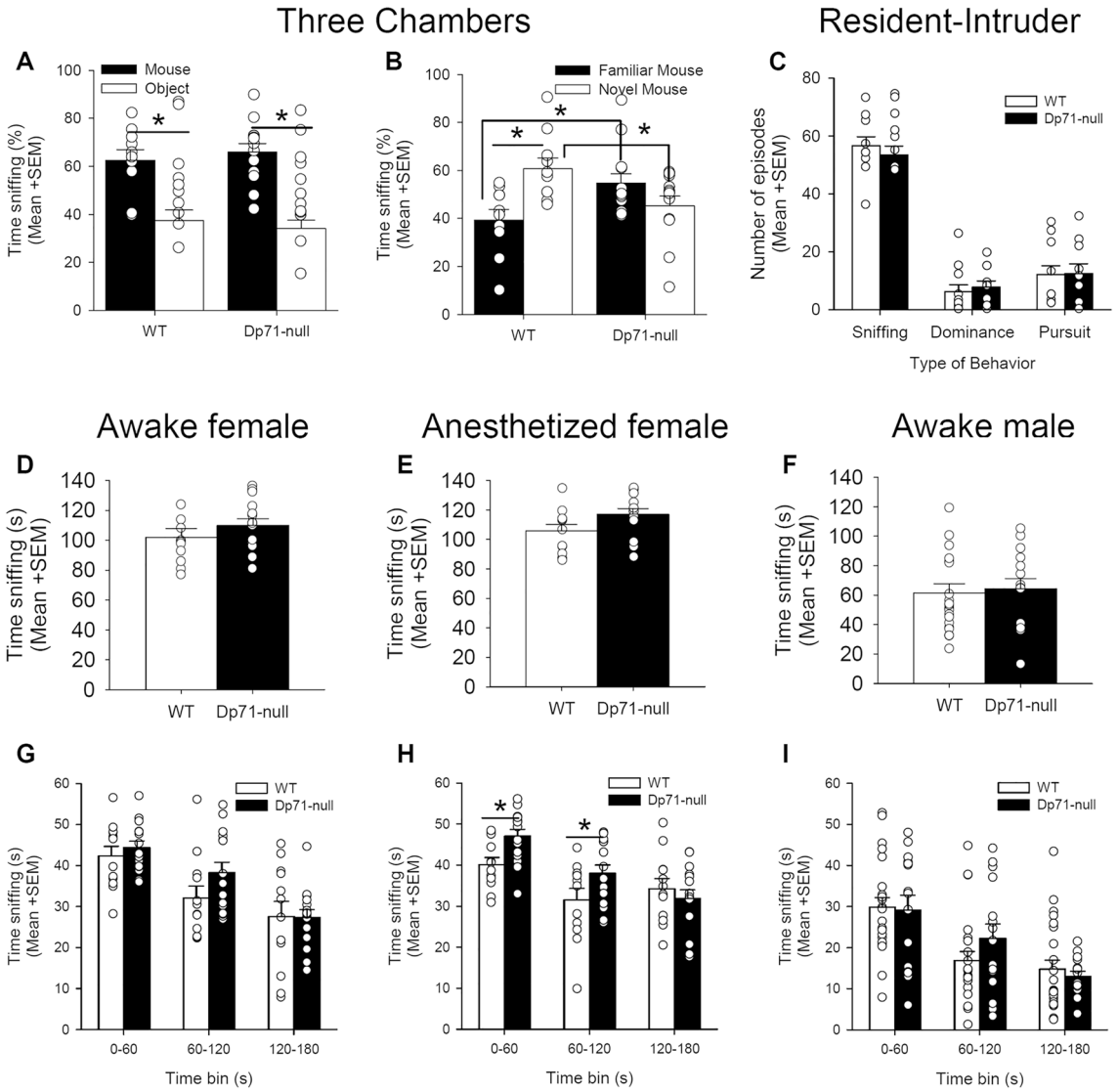


Fig. 2 Social motivation and social interactions. **A**, **B** Histograms represent exploratory behavior expressed as percent time spent sniffing the tubes containing either a mouse (black bars) or an object (white bars) during the sociability trial (**A**), and percent time spent sniffing towards the familiar (black bars) and novel mouse (white bars) during the trial assessing their preference for social novelty (**B**). **C** Number of sniffing episodes, dominant acts and pursuits initiated by WT and Dp71-null resident mice in the resident-intruder paradigm. **D**–**F** Total time spent by WT and Dp71-null male residents sniffing awake females (**D**), anesthetized females (**E**) and males (**F**). **G**–**I** Temporal evolution of social exploration initiated by residents exposed to awake females (**G**), anesthetized females (**H**) and males (**I**) across three 60-s time bins. The time spent sniffing the anesthetized females was significantly higher in Dp71-null than in WT mice during the first 120 s of the trial (*$p < 0.05$). White points represent individual data points for each mouse

in distinct contexts (Fig. 3A–C; Additional file 4). While this was only a tendency during interactions with awake females ($t = 1.974$; $p < 0.05$, NS; Fig. 3A), Dp71-null mice spent significantly more time vocalizing (%) in the presence of anesthetized females ($t = 2.260$, $p < 0.05$; Fig. 3B) and freely-moving males ($t = 2.081$, $p < 0.05$; Fig. 3C) compared to WT mice. USV production during interactions was further analyzed as a function of time (Fig. 3D–F). This revealed that the genotype difference in the % time vocalizing was homogenously expressed during the period of interaction, yet it was statistically significant during the first 60-s period during encounters with males (Genotype x time interaction: $F(2,28) = 4.288$, $p < 0.05$; post-hoc test: $p < 0.05$; Fig. 3F). The latency of the first call was significantly shorter in Dp71-null mice compared to WT during encounters with freely-moving females ($t = 2.260$, $p < 0.05$; Additional file 4). The mean call duration (ms) also showed slight increases in Dp71-null mice

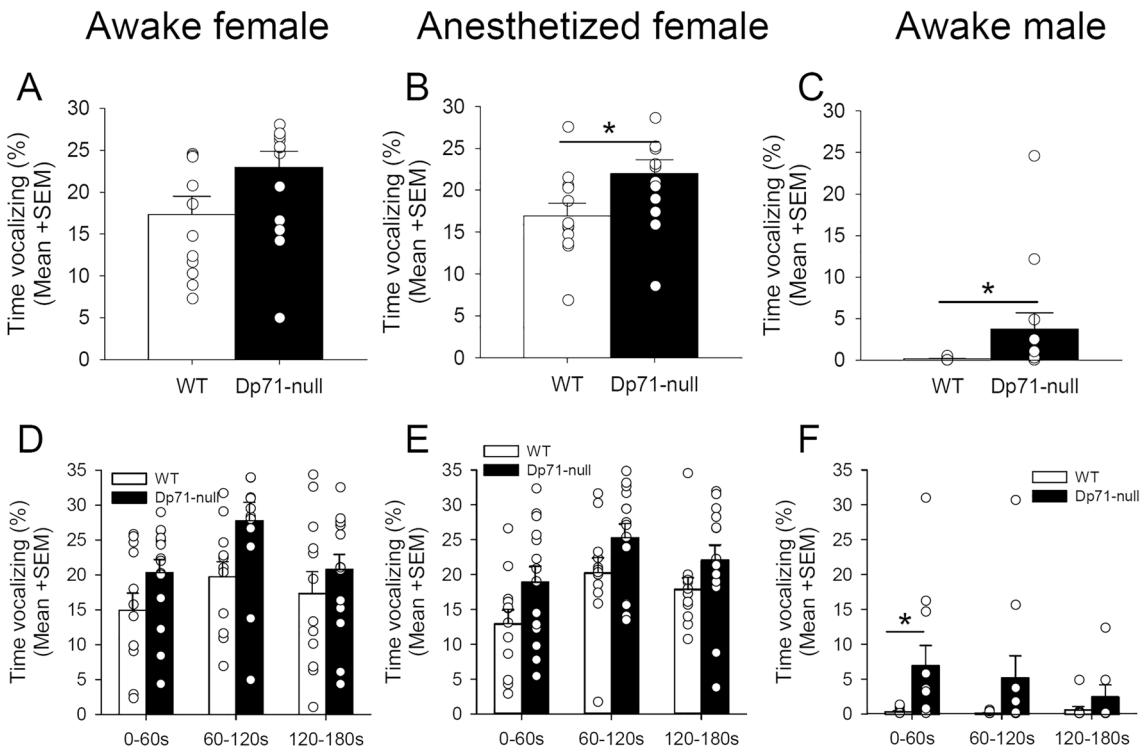


Fig. 3 Time spent vocalizing during social interactions. **A**–**C** Mean percent time vocalizing during interaction with awake females (**A**), anesthetized females (**B**) and awake males (**C**) (t-test, *p < 0.05). **D**–**F** Percent time spent vocalizing across 60-s time bins during interaction with awake females (**D**), anesthetized females (**E**) and awake males (**F**). Time spent sniffing (%) was significantly longer in Dp71-null than in WT mice across all time bins during interaction with anesthetized females (p < 0.05) and awake males (Significant during the first 60 s; Tukey posthoc test; *p < 0.05). White points represent individual data points for each mouse

compared to WT mice (Fig. 4A–C), but this was only significant during interactions with males (t = 2.934, p < 0.05; Fig. 4C). To better detail this parameter, we analyzed the distribution of call durations (Fig. 4D–F). This revealed that Dp71-null mice emitted a greater proportion of longer calls as compared to controls in all test conditions (KS-Tests, p < 0.001), a result consistent with the increased percent time spent vocalizing observed in this transgenic model. The rate (number per min) and duration of sequences as well as the number of calls emitted per sequence were selectively and significantly higher in Dp71-null mice as compared to controls in the presence of male encounters (all t > 2.2, p < 0.05; Additional file 4). Call peak frequencies and amplitudes were globally unaffected (p > 0.05, NS), indicating absence of genotype effects on the call bandwidth and intensity (Additional file 4).

Analysis of the correlations between behavioral and acoustic parameters first revealed in both genotypes a significant correlation between the call rate and the time spent in social contact with freely-moving females (p < 0.05; Additional file 5), suggesting that the ultrasonic vocalizations were related to social interaction regardless of the genotype. However, this was not observed for the contacts made with anesthetized females (Additional file 6) or the interactions with freely-moving males (Additional file 7) (p > 0.05, NS), suggesting context-specific differences in the stimuli triggering vocalizations.

Finally, we also detailed the ultrasonic vocal repertoire in response to social stimuli, which revealed significant differences between genotypes (Additional file 8). As shown in Fig. 5A–C, the proportion of peak calls emitted by Dp71-null mice was significantly higher as compared to WT controls in all experimental conditions (Male–Female (t = 2.779), Male-Anesthetized Female (t = 3.365), Male-Male (t = 2.390) (all, p < 0.05). Furthermore, Dp71-null mice also emitted a higher proportion of sinusoidal calls during investigation of anesthetized females (t = 2.392, p < 0.05; Fig. 5B) and of U-shape calls during the interaction with freely-moving males (t = 2.745, p < 0.05; Fig. 5C). Overall, this shows variation in Dp71-null mice of the frequency of use of specific vocalizations in distinct social contexts.

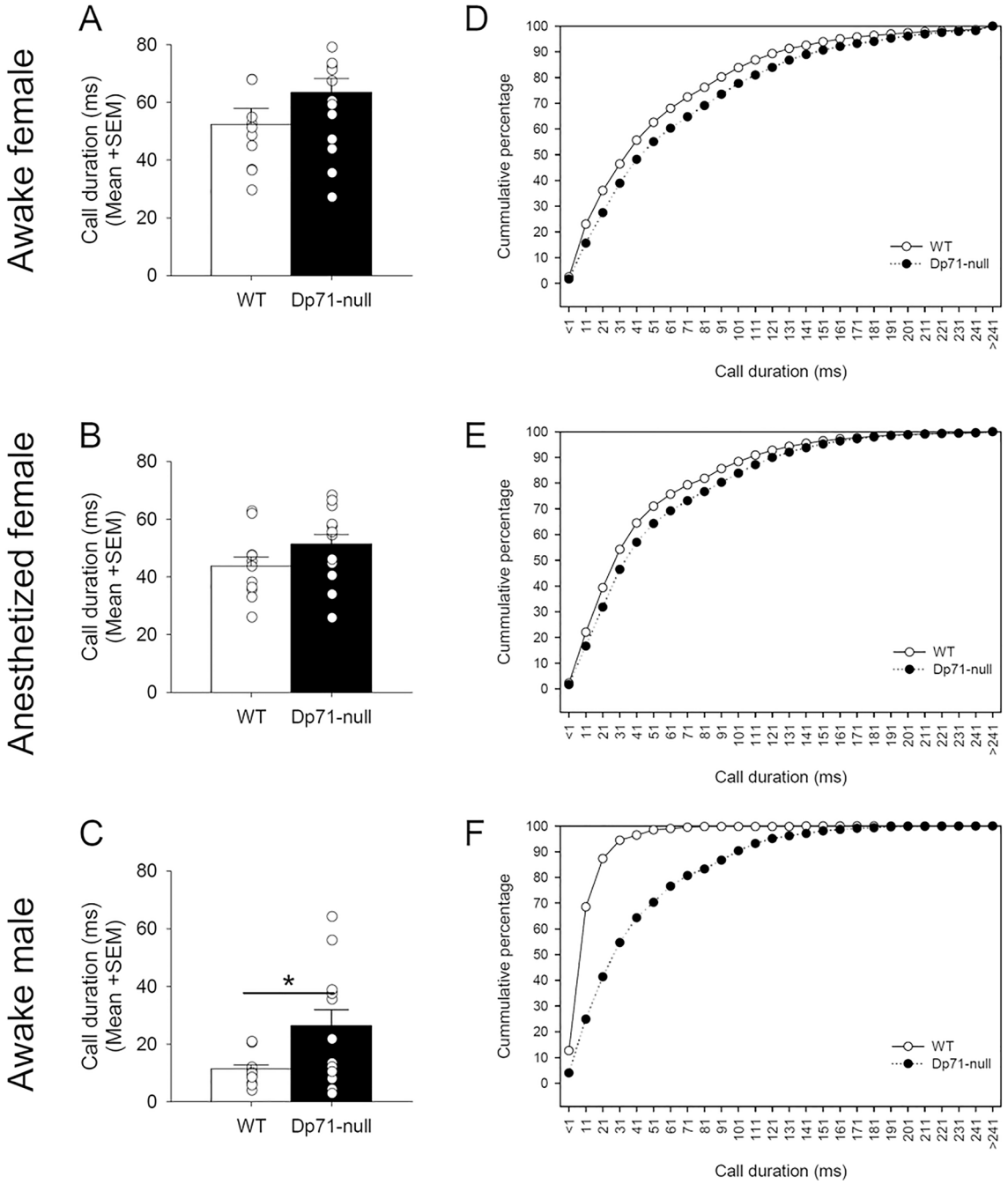


Fig. 4 Call duration and distribution of call durations. **A**–**C** Mean duration of calls emitted during interactions with awake females (**A**), anesthetized females (**B**) and awake males (**C**). The mean call duration of Dp71-null mice was particularly increased during interactions with males (*$p < 0.05$). White points represent individual data points for each mouse. **D**–**F** Frequency distribution (%) of ultrasonic vocalizations as a function of the call duration (ms) during exposure to freely-moving females (**D**), anesthetized females (**E**) and freely-moving males (**F**). Dp71-null mice emitted a greater proportion of longer calls as compared to controls in all test conditions (KS-Tests; all $p < 0.001$)

Anxiety and unconditioned fear behavior

Because it has been proposed that changes in the quantity of USVs might reflect emotional states in mice [61], we evaluated the presence of anxiety-related responses of Dp71-null and WT mice in three different standard anxiety tests. In the open field test, locomotion and exploration of a novel environment was not affected in Dp71-null mice. Indeed, total distance travelled

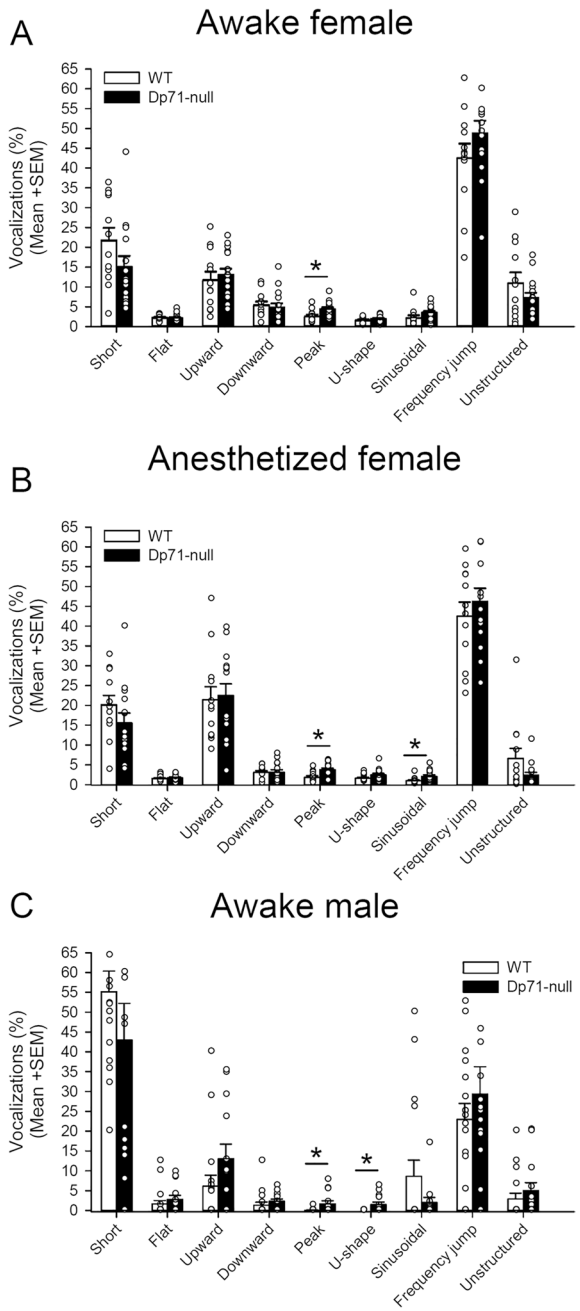


Fig. 5 Repertoire of socially-induced ultrasonic vocalizations. **A**–**C** Relative proportion of distinct call shapes emitted during interactions with awake females (**A**), anesthetized females (**B**) and awake males (**C**). Note that Dp71-null mice emitted a larger proportion of peak calls compared to WT mice in all experimental conditions (*t-tests, $p < 0.05$), while they produced a significantly higher proportion of other specific calls in distinct social contexts, i.e., more sinusoidal calls during the investigation of anesthetized females (**B**) (*t-test, $p < 0.05$) and more U-shape calls during the interaction with freely-moving males (*t-test, $p < 0.05$). White points represent individual data points for each mouse

was comparable between genotypes (Genotype effect: $F(1,22) = 0.855$, $p > 0.05$, NS, Fig. 6A), and its diminution across time (Time effect: $F(5,110) = 13.7$, $p < 0.001$) indicated a typical habituation to the apparatus in both genotypes (Genotype x Time interaction: $F(5,110) = 0.685$, $p > 0.05$, NS). Vertical activity and grooming behavior were also unaffected in the transgenic mice that showed comparable number of rearing and durations of grooming episodes compared with WT ($t = 0.78$ and $t = 0.71$, $p > 0.05$, NS). However, the percent distance travelled in the central area of the open field, typically considered as an anxiety-related behavioral parameter, was significantly reduced in Dp71-null mice as compared to WT (Genotype effect: $F(1,22) = 10.655$, $p < 0.01$). This was associated with a reduced number of entries into this zone ($t = 2.753$, $p < 0.05$) and an increased time spent running along the walls, where they completed a large number of unbroken revolutions along the virtual corridor (i.e., thigmotaxis) ($t = 2.236$, $p < 0.05$; Fig. 6A). This result was a first indication of a higher level of anxiety in Dp71-null mice.

In the light–dark box test (Fig. 6B), however, the number of entries and time spent in the anxiogenic lit compartment were comparable between genotypes ($t = -1.269$ and $t = -0.05$; all, $p > 0.05$, NS). In contrast the number of head dips from the dark compartment towards the lit one (hesitations) was significantly smaller in Dp71-null than in WT mice ($t = -3.433$, $p < 0.01$), suggesting a slight alteration of risk assessment behavior in the mutants.

In the elevated plus-maze test (Fig. 6C), in which anxiety results from the fear of heights in elevated open arms, the mean number of visits (entries in both open and closed arms) was lower in Dp71-null than in WT mice ($F(1,22) = 13.389$, $p < 0.01$), suggesting hypoactivity in Dp71-null mice. The percent time spent in the open areas (central area and open arms) tended to be shorter in Dp71-null mice as compared to controls but this was not significant ($t = 1.181$, $p > 0.05$, NS). Finally, the number of head dips towards the central area and open arms was smaller in Dp71-null mice $t = 2.293$, $p < 0.05$), which, again, suggested reduced risk assessment in this mouse model.

We then tested the unconditioned fear response induced by a brief manual scruff restraint, which is a robust phenotype reflecting enhanced stress reactivity in other DMD mouse models (*mdx*, *mdx52*) lacking Dp427 and Dp140 dystrophins [56, 66]. Here we compared the fear response in WT, Dp71-null mice and mdx3cv mice lacking all dystrophins. *Mdx*^{3cv} mice reproduced the strong phenotype consistently observed in other DMD mouse models, showing a large (>90%) increase in tonic immobility (freezing) following the short scruff restraint (Genotype effect: $F(2,24) = 207.557$, $p < 0.001$). In marked

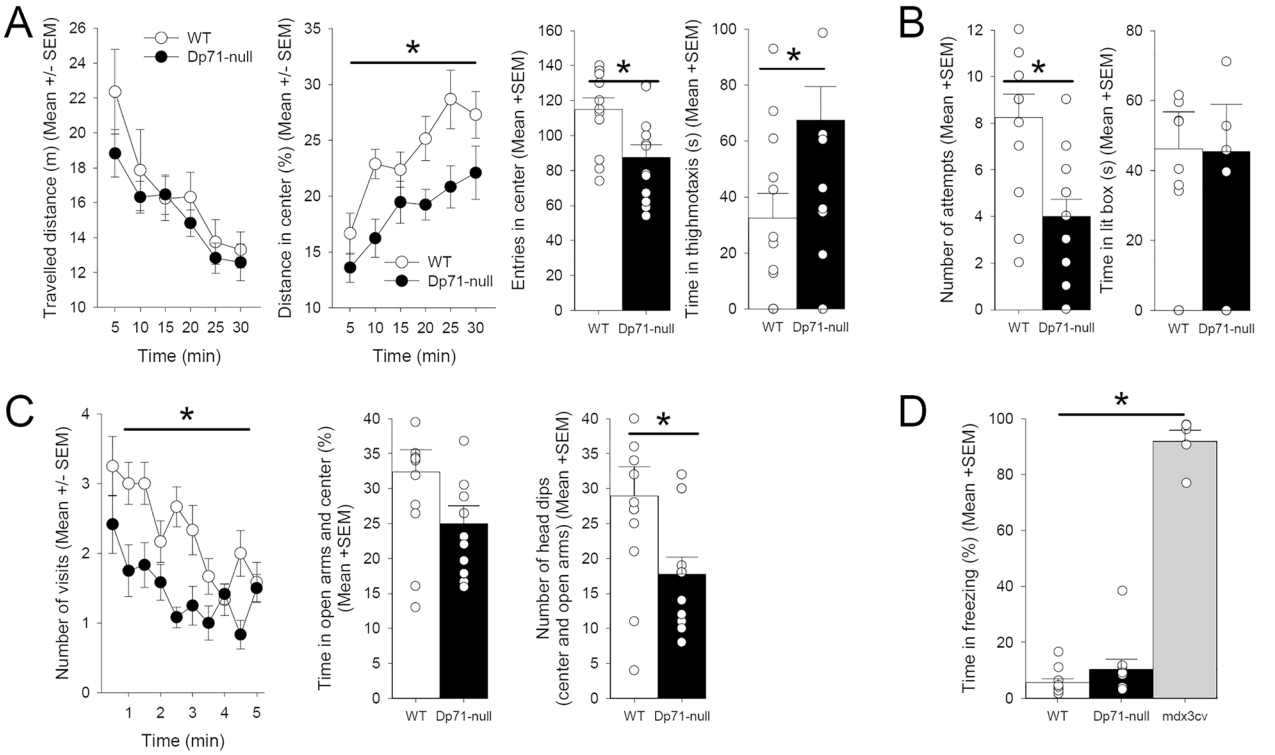


Fig. 6 Anxiety and unconditioned fear. **A** Open-field test. From left to right are shown the total distance travelled and percent distance travelled in central area as a function of time, then the mean number of entries in central area and time spent expressing thigmotaxis. **B** Light–dark test. Plots show the number of head dips towards the light compartment (left) and time spent in light compartment (right). **C** Plus-maze test. The plots show the number of visits to open and closed arms as a function of time (left), the percent time spent in open arms and central area (middle), and the number of head dips into the central area and open arms (right). **D** Unconditioned fear-related tonic immobility. Percent freezing in 5 min following brief scruff restraint. *$p < 0.05$. White points in bar plots represent individual data points for each mouse

contrast, however, the percent time spent freezing in Dp71-null mice was comparable to that of WT mice ($p > 0.05$, NS), indicating that the unique loss of Dp71 does not induce unconditioned fear responses (Fig. 6D).

We next investigated the expression of conditioned fear responses, as this type of responses are also affected in other mouse models lacking distinct dystrophins. Because several fear-conditioning paradigms involve auditory tones as conditioned stimuli, we first compared audition of the two genotypes.

Auditory brainstem responses

The ABR thresholds (expressed in dB SPL) at presentation of clicks and pure tones are displayed in Fig. 7A. ABRs thresholds were comparable between genotypes in response to the click delivered at start ($p > 0.2$, NS) and end of the experimental session ($p > 0.1$, NS). The start and end threshold were comparable, indicating that the protocol did not induce any hearing loss in any genotype. ABRs threshold in response to pure tones exhibited a typical V−shape curve (Frequency effect: $p < 0.0001$) with an optimal threshold detected at 8 kHz [10, 12, 69]. No difference was found between genotypes (Genotype effect: $p > 0.7$, NS, genotype × frequency interaction: $p > 0.8$, NS). We further undertook a detailed analysis of the latencies of the first four waves composing the ABR triggered by the click at variable intensities (Fig. 7B), and of the amplitudes of the largest negative (I') and positive (III) waves that reflect the response of the auditory nerve fibers and the superior olivary complex, respectively. Both latencies and amplitudes were comparable between genotypes across stimulus intensities (all parameters, $p > 0.05$; Additional file 9).

Associative learning and fear memories

Cued-fear conditioning was first performed using five CS-US pairings for acquisition (Fig. 8A). Dp71-null and WT mice presented low and comparable freezing responses during acclimatization ($t = 1.729$, $p > 0.05$, NS). Both groups then showed a progressive acquisition of associative fear learning, as reflected by increases in the percent time spent expressing a fear response (% freezing; CS effect: $F(4,144) = 78.913$, $p < 0.001$) during delivery of the five 30 s conditioning auditory stimuli (CS). The

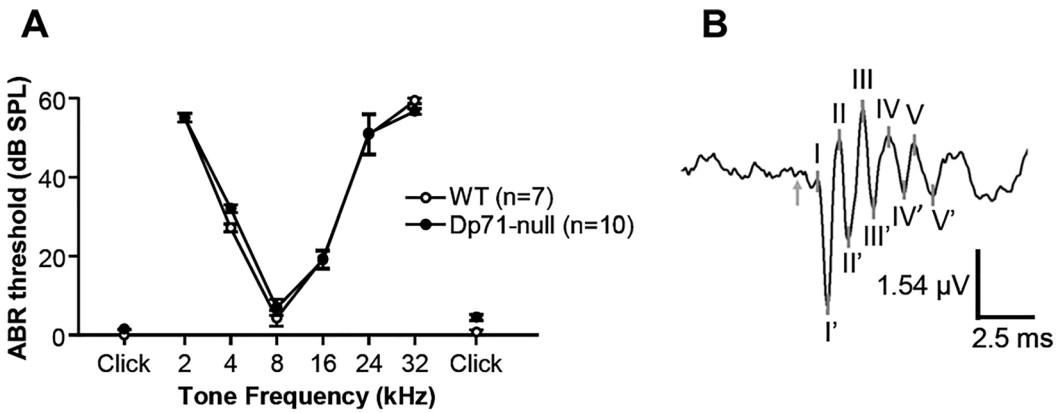


Fig. 7 Auditory brainstem responses (ABR). **A** ABR thresholds for clicks and pure tone frequencies demonstrate normal hearing in Dp71-null mice. **B** Sample ABR to a click at 70 dB, showing the successive waves labeled I to IV (positive peaks) and I' to IV' (negative peaks). Wave I arises from the auditory nerve and waves II to IV reflect evoked activity at different relays of the auditory brainstem. Gray arrow: click presentation

lack of differences between genotypes in the amount of freezing indicated a preserved acquisition of fear learning in Dp71-null mice (Genotype effect: $F(1,36)=0.0591$, $p>0.05$, NS; Genotype x CS interaction: $F(4,144)=0,361$, $p>0.05$, NS) (Fig. 8A). In contrast with the first session, the freezing response was abnormally enhanced in Dp71-null mice during the acclimatization period preceding the two retention sessions performed in a different context, a genotype effect that was significant in session 2 ($t=3,159$, $p<0.01$) (Fig. 8A). This might suggest some enhancement of a contextual memory of the experimental conditions. However, both genotypes showed similar freezing responses during CS presentation in both retention sessions (Retention 1: Genotype effect: $F(1,36)=2.684$, $p>0.05$, NS; Genotype x CS: $F(3,108)=0.974$, $p>0.05$ NS; Retention 2: Genotype effect: $F(1,36)=0.157$, $p>0.05$, NS; Genotype x CS: $F(3,108)=1.326$, $p>0.05$, NS), indicating unaffected memory retention of the CS-US association.

A distinct group of mice was submitted to an abbreviated protocol of cued-fear conditioning, by reducing the number of CS-US pairings during acquisition to diminish the strength of conditioning (Fig. 8B). Again, performance was comparable in both genotypes during the whole acquisition session (Acclimatization: $t=0.053$, $p>0.05$, NS; CS 1–3, Genotype effect: $F(1,15)=2.864$, $p>0.05$, NS; CS effect: $F(2,30)=57.019$, $p<0.001$; Genotype x CS interaction: $F(2,30)=0.968$, $p>0.05$, NS). Likewise, performance was comparable between genotypes during the two successive retention sessions: retention 1 (Acclimatization, $t=0.0172$, $p>0.05$, NS; CS 4–19, Genotype effect: $F(1,15)=1.212$, $p>0.05$, NS; Genotype x CS interaction: $F(3,45)=0.688$, $p>0.05$, NS) and retention 2 (Acclimatization: $t=0.159$, $p>0.05$, NS; CS 4–19, Genotype effect: $F(1,15)=0.949$, $p>0.05$, NS; Genotype x CS interaction: $F(3,45)=0.320$, $p>0.05$, NS), suggesting preserved cued fear learning and memory retention in Dp71-null even following weaker acquisition training.

In the trace fear conditioning paradigm (Fig. 8C), freezing was slightly higher in Dp71-null mice than in WT during habituation (H) (Freezing duration: 3.9% for Dp71-null, 1.4% for WT mice, $t=2.559$, $p<0.05$), as well as during acclimatization (A) on the next day (Freezing duration: 9.7% for Dp71-null, 4.7% for WT mice, $t=2.323$, $p<0.05$). Nevertheless the amount of freezing expressed in response to the six CS-US pairings was comparable between genotypes (Genotype effect: $F(1,24)=0.165$, $p>0.05$, NS; Genotype × CS-US interaction: $F(5,120)=0.678$, $p>0.05$, NS). During the cued trace-fear retention session performed 24 h later, Dp71-null mice exhibited no deficit in performance during acclimatization ($t=1.246$, $p>0.05$, NS) and presentation of CS (Genotype effect: $F(1,24)=0.024$, $p>0.05$, NS; Genotype × CS: $F(2,48)=2.436$, $p>0.05$, NS). Contextual fear memory was then evaluated by placing mice in the same context as during acquisition. Performance was comparable between genotypes reflecting normal retention of contextual fear memory in Dp71-null mice ($t=0.003$: $p>0.05$, NS).

Discussion

This study provides the first evidence of abnormal social behavior and socially-induced ultrasonic vocalizations in the Dp71-null transgenic mouse model that selectively lacks the shortest dystrophin-gene product, Dp71. These alterations of social behavior are accompanied by a moderate increase in anxiety-related behaviors, while learning and memory of emotional stimuli are preserved. Thus, the Dp71-null mouse displays a

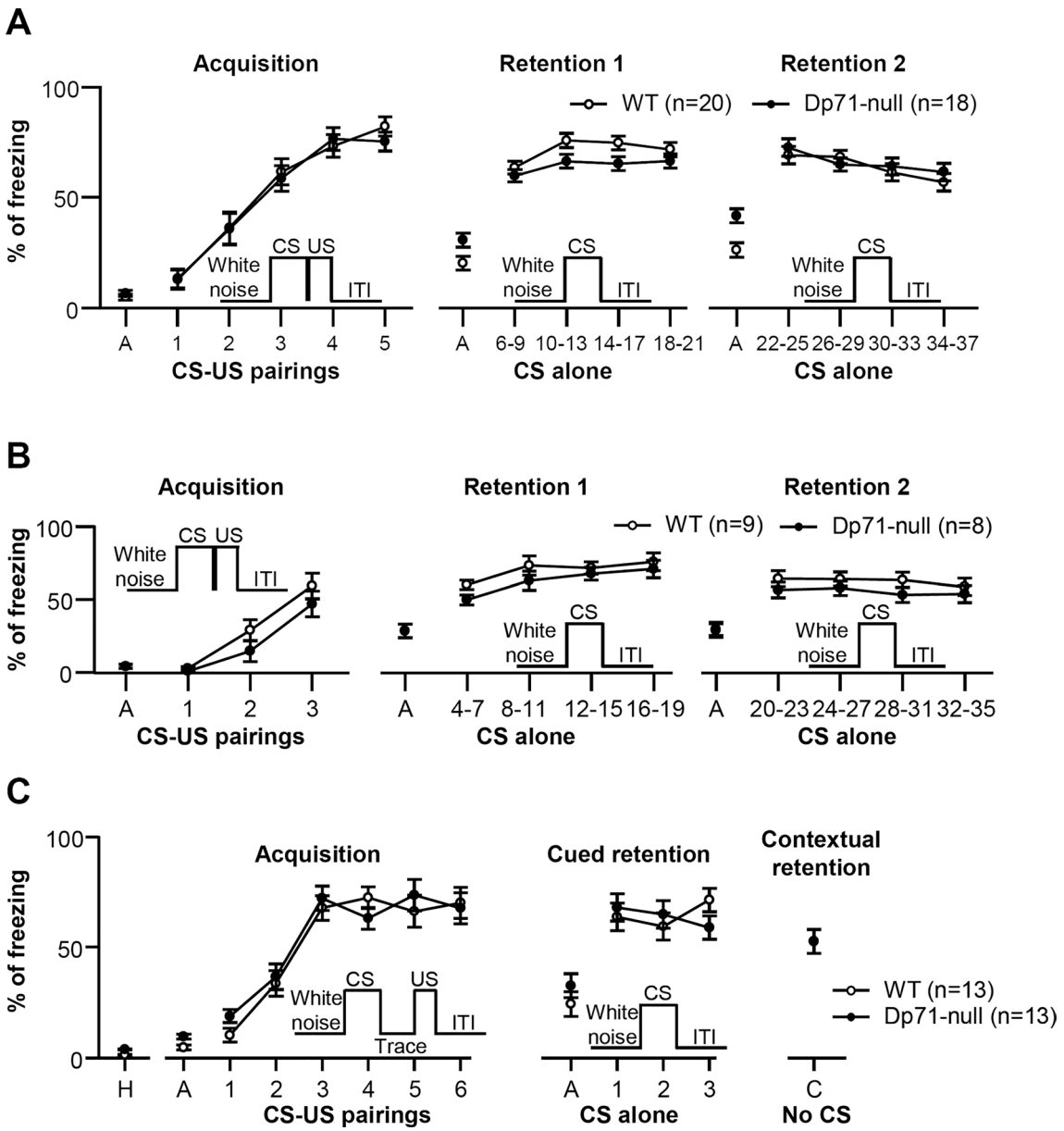


Fig. 8 Associative fear learning and memory. **A**, **B** Delayed cued fear conditioning. Performance is expressed as the percent time spent freezing during acclimatization periods (**A**), and in response to the CS during the acquisition session, consisting in five (**A**) or three (**B**) CS-US pairings, and in response to CS during the two retention sessions in a distinct context (blocks of four CS alone presentations) performed at 24 h intervals. **C** Trace Fear conditioning. Performance is expressed as the percent time spent freezing to the CS during the acquisition session, consisting in six CS-US pairings, and the two retention sessions (cued and contextual). Cued-retention session was performed 24 h post-acquisition by presenting CS alone in a different context. Contextual-retention session was performed by placing the animal in the same context without CS presentation 3 h after the cued-retention. H: habituation to apparatus before acquisition. A: Pre-session acclimatization. C: Contextual retention

specific profile of behavioral alterations that differs from DMD mouse models lacking other brain dystrophin isoforms (Table 1). The present results support the hypothesis that distal *DMD* gene mutations affecting the expression of Dp71 protein may contribute to the emergence of autistic and emotional traits in DMD.

Altered social behavior and socially-induced ultrasonic vocalizations

Deficits in social interaction are cardinal markers for autism risk, reproduced in diverse mouse models carrying mutations in candidate genes for autism (e.g., [16, 17, 24, 37, 39, 41, 65, 70], for a review). Context-specific changes in social behavior have been previously observed

Table 1 Emotional and social phenotypes in DMD mouse models

DMD mouse models →	***mdx***	***Mdx52***	***Dp71-null***	***Mdx3cv***
Brain dystrophin(s) deficiency →	Dp427	Dp427, Dp140	Dp71	Dp427, Dp140, Dp71
Anxiety	Mild increase (1,2,3)	Increased (2)	Mild increase (*)	Increased (4)
Unconditioned fear	Abnormally strong (1,2,3)	Abnormally strong (2,6)	Unaffected (*)	Abnormally strong (*)
Auditory cued fear conditioning	Impaired (1)	Impaired (2)	Unaffected (*)	*Not tested*
Sociability/social motivation	Unaffected (5)	Increased (6)	Unaffected (*)	*Not tested*
Social novelty preference	Unaffected (5)	*Not tested*	Impaired (*)	*Not tested*
Social interactions with WT male	Slightly increased (5)	*Not tested*	Unaffected (*)	*Not tested*
Social interactions with WT female	Slightly reduced (5)	*Not tested*	Unaffected (*)	*Not tested*
Social exploration of anesthetized WT female	Unaffected (5)	*Not tested*	Increased (*)	*Not tested*
Socially-induced vocalizations	Reduced rate in presence of anesthetized female (5)	*Not tested*	Increased rate in presence of male, female, anesthetized female (*)	*Not tested*

The table focuses on the phenotypes addressed in the present study. The mouse model analyzed in the present study is highlighted in grey. (1) [66], (2) [56], (3) [60], (4) [67], (5) [43], (6) [28], *The present study

in the *mdx* mouse model, suggesting a link between the loss of the full-length dystrophin (Dp427) and the emergence of social behavior disturbances or autistic traits in DMD [43]. However, some genotype–phenotype studies in DMD patients suggested that the prevalence of ASD increases with distal mutations [55, 71], suggesting that the lack of Dp71 dystrophin might increase the risk for social behavior deficits. In the present study, we found alterations in Dp71-null mice social behavior that differ in nature to those previously reported in *mdx* mice lacking Dp427. This is in agreement with the hypothesis that the loss of Dp71, and its cumulative loss with other dystrophins due to distal mutations in the *DMD* gene, might play a role in the emergence and/or aggravation of social problems in DMD patients.

We first addressed sociability and preference for social novelty using the three-chamber test, in which social motivation is evaluated from indirect social approaches. The original Dp427-deficient *mdx* mouse does not show any deficit in this test [43]. Here, Dp71-null mice showed unaltered sociability, initially suggesting unaffected social motivation. Nevertheless, Dp71-null mice showed impaired preference for social novelty that, as previously suggested by other authors, might reflect the presence of alterations in social recognition [6]. This social phenotype is considered less straightforward than a lack of sociability (i.e., preference for a new social stimulus over an inanimate object) to interpret a relationship with ASD-related behavior. However, it fits with models considering that qualitative changes in social interactions are central components in ASD individuals, who tend to avoid unfamiliar social partners and show diminished interest in novelty [1]. Moreover, a similar phenotype characterized by normal sociability and impaired preference for social novelty has been observed in diverse mouse models lacking ASD-related genes [8, 13, 15, 35, 63].

A putative deficit in novelty-seeking behavior that could relate to present results has been previously suggested in Dp71-null mice after observing a decrease in object investigation when mice were exposed to new sets of objects on successive sessions in an object recognition task [20]. Here, since Dp71-null mice showed normal preference for social versus inanimate stimuli and no alteration in the level of interaction when confronted with unfamiliar freely-moving females and males, it is unlikely that the impairment in social novelty could be only attributed to a deficiency in general interest for novelty. Besides, the total number and duration of sniffing episodes directed to novel and unfamiliar mice were comparable between genotypes suggesting no obvious deficiency in social-approach behavior and no overall restricted interest in specific stimuli. Alternatively, we cannot rule out that this deficit in preference for social novelty might be associated with altered executive functions and decision-making processes, in line with the

impaired function of the prefrontal cortex reflected through the cortical E/I unbalance previously observed in this mouse model [11].

Qualitative alterations in social behavior of Dp71-null mice are further evidenced by their unusually high interest in unresponsive (anesthetized) females. This behavior may be related to their tendency to persistently investigate familiar mice over new ones in the social novelty test. Despite the different nature of these situations, the behaviors displayed could reflect a perseverative trait, a difficulty in disengaging, and/or a lack of flexibility. A similar phenotype has been observed in other ASD- and/or ID-relevant mouse models, such as in the euchromatin histone methyltransferase 1 knockout mice, that demonstrate a prolonged sociability response and delayed or absent preference for social novelty in a variation of the three-chamber test. This was interpreted as social behavior disturbances reminiscent of autistic features [2]. Interestingly, in this latter model the social phenotype was also associated with increased anxiety, suggesting possible relationships between processing of emotional stimuli and qualitative changes in social behavior. Here, the prolonged social approaches in Dp71-null mice might reflect some difficulty in adaptive disengaging behavior and/or some problem to integrate feedback information emitted by social counterparts. Conversely, and in line with the unaffected sociability of Dp71-null mice, their unaltered aggressiveness and/or agonistic reactivity during direct forced encounters with conspecific male intruders, comparable to that of WT mice, suggests that other remaining domains of social cognition such as hierarchy, seem unaffected in Dp71-null mice.

Analyses of USV emissions by Dp71-null mice revealed both quantitative and qualitative alterations, which further supports the presence of alterations in social behavior in this model. First, Dp71-null mice showed an abnormal increase in the time spent vocalizing during forced social interactions, accompanied by the emission of a larger proportion of calls of longer duration with males as well as freely-moving and anesthetized females, suggesting that these alterations in USV production are not likely to be driven by specific sexual factors. Rate of vocalizations and call duration have been previously associated with social behaviors reflecting the level of social motivation [9, 49]. Accordingly, the significant increase in the time spent vocalizing by Dp71-null mice might be consistent with their longer social interactions and apparent difficulty to disengage and adapt their behavior, as also interpreted regarding their behavior towards unresponsive females.

Interestingly, this increased production of socially-induced USVs in Dp71-null mice, was not observed in Dp427-deficient *mdx* mice, which, in marked contrast, rather showed reduced interaction with females accompanied by decreased rate of vocalizations [43]. Secondly, Dp71-null mice showed an abnormal vocal repertoire with an increased emission of peak calls in all tested situations, of sinusoidal calls in presence anesthetized females and of U-shape calls in the presence of males. Again, this represents an opposite phenotype to *mdx* mice where we previously documented a significant reduction in the production of peak and sinusoidal calls upon interaction with anesthetized females and other *mdx* males [43]. The composition of an adult mouse repertoire normally depends on the context and previous social experience [59]. Short and composite calls (i.e., two or more components emitted simultaneously) are considered as 'basic' calls found in many behavioral contexts, whereas upward, frequency jump, U-shape, flat and peak calls are predominately found in social situations and therefore considered as more 'informative' of the behavioral, emotional or motivational content of these specific situations [9]. We therefore interpret the abnormal vocal repertoire observed in Dp71-null mice as reflecting an altered neural control of call production and/or an abnormal processing of relevant stimuli involved in the modulation of social responses/behavior.

In conclusion, the lack of Dp71 is not perturbing all domains of social cognition but rather inducing subtle qualitative alterations of specific social behaviors under particular situations that could in part be reminiscent of autistic traits and considered in line with the specific social problems described in DMD boys (e.g., clinging to adults, preference to play with younger children and poor facial affect recognition), regardless of meeting all criteria for an ASD diagnosis [33, 34]. We further suggest that the observed social phenotype in Dp71-null mice may relates to the executive deficits demonstrated in this model [11] and/or to the moderate emotional disturbances also identified in the present study, which might as well influence social motivation and/or behavior.

Disturbances in emotional behavior

Emotional disturbances, encompassing internalizing and externalizing problems, is a common phenotype in DMD that could contribute to social behavior disturbances in patients. A role for brain dystrophin in controlling emotional processes has been previously suggested since abnormal stress-induced unconditioned fear responses can be significantly reduced in *mdx* mice by rescuing brain dystrophin expression with intracerebral administration of exon-skipping oligomers [26, 54, 60, 66]. Since then, enhanced anxiety and stress reactivity were also reported in *mdx52* mice lacking Dp427 and Dp140 brain dystrophins [56]. Interestingly, such emotional disturbances were also associated with increases in social

approaches in adult *mdx52* mice and increased USV production in *mdx52* pups [28]. Overall, this suggest that in various models lacking brain dystrophins, the enhancement of specific social behaviors and socially-induced vocalizations may coexist with emotional problems. Moreover, anxiety is a common comorbidity in ASD [53] and a frequent trait in DMD patients [55].

We previously suggested that Dp71 may have a role in anxiety-related behaviors, since Dp71-null mice displayed a reduced number of entries and shorter distance travelled in the central area upon exploration of a hole board, a behavior reflecting increased levels of anxiety [29]. Anxiety is a complex phenomenon that cannot be described in a single test, and several studies demonstrated that the effects of anxiolytic compounds may vary depending on the targeted neurotransmission system, mouse genetic background and experimental conditions [14, 27]. Here we therefore extended behavioral testing of this trait submitting Dp71-null mice to a larger battery of anxiety tests. Consistent with our previous observations, Dp71-null mice showed comparable locomotor activity to that of WT mice, but increased thigmotaxis and reduced exploration of the central zone of an open field. Similarly, in the elevated-plus maze test Dp71-null mice spent proportionally less time in the center and open arms, which correspond to the most anxiogenic zones of the apparatus. Dp71-null mice also displayed a general hypoactivity, but this cannot be attributed to a locomotor deficit, since this was not observed in the open field test that more accurately evaluates locomotion. The reduced number of arm entries in elevated plus maze might rather be considered as a consequence of the enhanced emotional anxiety of Dp71-null mice. In contrast, however, in the light–dark choice test the time spent in the anxiogenic lit compartment was comparable in both genotypes, suggesting a normal emotional response of Dp71-null in this specific situation. The cause of these discrepancies observed among distinct anxiety tests remains unclear. At variance with previous results in *mdx* mice [66], here enhanced anxiety-related responses were observed in higher-motor demand tests (open-field exploration, elevated-plus maze test), but not in the light–dark choice test involving a low-motor demand. Since Dp71-null mice do not have myopathy and do not present a motor phenotype, it is unlikely that this variability among tests relates on the task motor demand. Alternatively, as suggested for *mdx* mice [66], the nature or strength of the anxiogenic stimulus could be a possible factor modulating emotional reactivity in Dp71-null mice. Different novelty-seeking motivation and attention states associated with distinct experimental situations, factors that together with the targeted neurotransmission system and mouse genetic background have been shown to influence the effects of anxiolytic compounds, could also be involved [14]. Interestingly, enhanced anxiety in Dp71-null mice was associated with reduced risk assessment behaviors, as detected here in elevated-plus maze and light–dark choice tests. Risk assessment represent a defensive behavior that is thought to facilitate information gathering concerning potential threat, and is particularly sensitive to anxiolytic and anxiogenic drugs [7, 68].

To further explore if the altered emotional reactivity of Dp71-null could be associated with altered defensive behaviors, we also submitted the mice to a different threatening situation consisting in a mild and brief scruff restraint stress. This is typically associated with a pronounced enhancement of defensive freezing responses in other DMD mouse models lacking Dp427 (*mdx*) or Dp427 and Dp140 (*mdx52*), and an enhancement of unconditioned fear responses have also been recently reported in DMD patients [40]. In the case of Dp71-null mice, however, the freezing response after restraint was very low and comparable to that of WT mice, while it was markedly expressed in the mdx3cv model lacking all dystrophins, similarly to what was reported in *mdx* and *mdx52* models. This strongly support that Dp71 is not involved in this phenotype that can be mainly attributed to the loss of Dp427.

Despite alterations in risk assessment and anxiety in Dp71-null mice, they did not display aggressive behaviors in the resident-intruder test. Aggression and anxiety are believed to be co-regulated, presumably based on the involvement of overlapping neurochemical and neuroanatomical pathways including prefronto-amygdaloid circuits. However, anxiolytic drugs can either reduce or potentiate aggressive behavior, specific strains of mice bred based on their aggressive behavior may present high or low levels of anxiety, and there are even transgenic mouse models in which aggression and anxiety can be altered independently [45]. Therefore altered anxiety and risk assessment in Dp71-null mice do not seem to be modulatory factors of their aggressive behavior.

In all, our results indicate that a selective loss of Dp71 is associated with anxiety-related behaviors that may show differential expression depending on the experimental context. It is therefore likely that a cumulative loss of several brain dystrophins may aggravate emotional disturbances in DMD.

Unaffected emotional learning in Dp71-null mice

Brain activation studies related to risk assessment behaviors have shown involvement of a brain network including different hypothalamic nuclei and their connections with amygdala nuclei, bed nucleus of stria terminalis, lateral septum, hippocampus, gray periaqueductal area and prefrontal cortex (reviewed in [7]). Some of these

centers, such as amygdalar nuclei, have also been implicated in contextual and cued- fear conditioning [38]. Because enhanced anxiety in Dp71-null mice suggests putative alterations in the processing of emotional stimuli, we further assessed such mechanisms involved in associative emotional learning. However, no significant deficits in learning acquisition and memory retention were observed in Dp71-null mice in distinct paradigms involving the amygdala (cued fear conditioning) or functional interactions between amygdala and hippocampus (contextual and trace fear conditioning) [5]. Interestingly, Dp71-null mice only showed enhanced emotional responses during acclimatization periods that could reflect some potentiation of a contextual memory of the experimental conditions. However, depending on the experiment, this was observed before or after learning, suggesting a rather unspecific expression of their spontaneous enhanced anxiety. Accordingly, we can conclude that the lack of Dp71 enhances anxiety-related behaviors that cannot be attributed to major alterations of the cognitive processes involved during emotional learning. This is at variance with the phenotype of *mdx* and *mdx52* mice showing significant impairments in both acquisition and retention of fear conditioning [56, 66]. Such variability in behavioral outcomes between mouse models reflects that Dp71 is involved in distinct brain mechanisms, which might explain the distinct phenotypes and variable severity of deficits that emerge after the cumulative loss of several brain dystrophins.

Conclusions

Clinical heterogeneity in the cognitive, behavioral and neuropsychiatric profile of DMD patients is one of the main intriguing questions associated with this neuromuscular syndrome, which stimulates research on the different functions of the multiple brain dystrophins. The severity of the cognitive impairment and the risk for comorbid diagnosis of neuropsychiatric disorders increase when the site of mutation is distally located within the DMD gene and affects expression of shorter dystrophins such as Dp140 and Dp71. The behavioral profile of the mouse model lacking Dp71 seems in line with an increased risk of observing deficits in social behavior and ASD when mutations alter Dp71 expression [21, 55]. Although the presence of ASD in DMD patients could sometimes be associated with the sole loss of Dp427 [23, 43], our results indicate that the lack of Dp71 may also induce alterations in social behavior. This suggests that a cumulative loss of Dp427 and Dp71 could lead to more severe alterations of social behavior compared to the unique loss of Dp427, which would be in line with the hypothesis of an increased risk and severity of the phenotype in patients with distal mutations. Such deficits in social behavior could be moreover associated with executive dysfunction and decision-making deficits that could be induced by the previously documented alterations in prefrontal function of Dp71-null mice [11]. Similarly, our present data are consistent with recent clinical studies suggesting that emotional disturbances are common in DMD patients [3, 21, 55] and support the existence of a central component in the genesis of emotional and neuropsychiatric disturbances in this syndrome [3, 25, 48]. Again, the evidence of an abnormally enhanced emotional reactivity resulting from a selective loss of Dp71 suggest that mutations affecting Dp71 could aggravate emotional disturbances in DMD, which was not so far supported by available clinical evidence [50]. Our present results also further highlight that the different dystrophin-gene products have distinct functions in brain [52, 55]. Processing of emotional stimuli is a crucial aspect in mammalian cognition determining adaptation and problem solving in specific situation by adjusting behaviors relying on different neural systems. In this regard, the social behavior alterations described in Dp71-null mice could be influenced by their abnormal emotional reactivity, suggesting that the phenotypes associated with the loss of brain dystrophins may depend on a complex interplay of multiple systems rather than to specific and independent neural networks.

Abbreviations

ABR	Auditory brainstem response
ADHD	Attention-deficit/hyperactivity disorder
ANOVA	Analysis of variance
ASD	Autism spectrum disorders
BMD	Becker muscular dystrophy
CNS	Central nervous system
CS	Conditioned stimulus
DMD	Duchenne muscular dystrophy
ID	Intellectual disability
IP	Intraperitoneal injection
NS	Not significant
PCR	Polymerase chain reaction
SEM	Standard error of mean
US	Unconditioned stimulus
USV	Ultrasonic vocalization
WT	Wild type

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12993-024-00246-x.

Additional file 1.

Additional file 2.

Additional file 3.

Additional file 4.

Additional file 5.

Additional file 6.

Additional file 7.

Additional file 8.

Additional file 9.

Acknowledgements
We are grateful to the Zootechnic and genotyping platforms of the Paris-Saclay Institute of Neuroscience for mouse breeding, care, and genotyping.

Author contributions
R.M. and C.V. contributed to the conception and design of the study; L.C., D.L.V., F.N., J.M.E., R.C., R.M. and C.V. contributed to the acquisition and/or analysis of data; R.M., L.C., R.C. and C.V. contributed to drafting the text and figures; C.V. provided resources and funding. All authors reviewed the manuscript.

Funding
This work was supported by Centre National de la Recherche Scientifique (CNRS, France), Université Paris-Sud and Paris-Saclay (France), and by grants from AFM (Association Française contre les Myopathies, France; Grants numbers #15299 and #17117) and Agence Nationale de la Recherche (ANR, France; grant number ANR-14-CE13-0037-01, DYSther) to C.V, and European Union's Horizon 2020 research and innovation program "Brain Involvement iN Dystrophinopathies" under grant agreement No. 847826 to C.V. and R.M. A PhD fellowship from Ministère de l'Enseignement Supérieur et de la Recherche (France) was attributed to R.C.

Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Animal care and all experimental procedures complied with the European Communities Council Directive (CEE 86/609/EEC), EU Directive 2010/63/EU, French National Committee (87/848) and Ethic Committee (Paris Centre et Sud #59; APAFIS authorization #2635).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 13 March 2024 Accepted: 5 August 2024
Published online: 24 August 2024

References

1. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th ed. Arlington (VA): American Psychiatric Publishing; 2013.
2. Balemans MC, Huibers MM, Eikelenboom NW, Kuipers AJ, van Summeren RC, Pijpers MM, Tachibana M, Shinkai Y, van Bokhoven H, Van der Zee CE. Reduced exploration, increased anxiety, and altered social behavior: autistic-like features of euchromatin histone methyltransferase 1 heterozygous knockout mice. Behav Brain Res. 2010;208:47–55.
3. Banihani R, Smile S, Yoon G, Dupuis A, Mosleh M, Snider A, McAdam L. Cognitive and neurobehavioral profile in boys with Duchenne muscular dystrophy. J Child Neurol. 2015;30:1472–82.
4. Belmaati Cherkaoui M, Vacca O, Izabelle C, Boulay AC, Boulogne C, Gillet C, Barnier JV, Rendon A, Cohen-Salmon M, Vaillend C. Dp71 contribution to the molecular scaffold anchoring aquaporine-4 channels in brain macroglial cells. Glia. 2021;69:954–70.
5. Bergstrom HC. The neurocircuitry of remote cued fear memory. Neurosci Biobehav Rev. 2016;71:409–17.
6. Bicks LK, Koike H, Akbarian S, Morishita H. Prefrontal Cortex and Social Cognition in Mouse and Man. Front Psychol. 2015;6:1805.
7. Blanchard DC, Griebel G, Pobbe R, Blanchard RJ. Risk assessment as an evolved threat detection and analysis process. Neurosci Biobehav Rev. 2011;35:991–8.
8. Carter MD, Shah CR, Muller CL, Crawley JN, Carneiro AM, Veenstra-VanderWeele J. Absence of preference for social novelty and increased grooming in integrin β3 knockout mice: initial studies and future directions. Autism Res. 2011;4:57–67.
9. Chabout J, Serreau P, Ey E, Bellier L, Aubin T, Bourgeron T, Granon S. Adult male mice emit context-specific ultrasonic vocalizations that are modulated by prior isolation or group rearing environment. PLoS ONE. 2012;7: e29401.
10. Chabout J, Cressant A, Hu X, Edeline JM, Granon S. Making choice between competing rewards in uncertain vs. safe social environment: role of neuronal nicotinic receptors of acetylcholine. Front Hum Neurosci. 2013;7:468.
11. Chaussenot R, Amar M, Fossier P, Vaillend C. Dp71-dystrophin deficiency alters prefrontal cortex excitation-inhibition balance and executive functions. Mol Neurobiol. 2019;56:2670–84.
12. Chaussenot R, Edeline JM, Le Bec B, El Massioui N, Laroche S, Vaillend C. Cognitive dysfunction in the dystrophin-deficient mouse model of Duchenne muscular dystrophy: a reappraisal from sensory to executive processes. Neurobiol Learn Mem. 2015;124:111–22.
13. Choleris E, Gustafsson JA, Korach KS, Muglia LJ, Pfaff DW, Ogawa S. An estrogen-dependent four-gene micronet regulating social recognition: a study with oxytocin and estrogen receptor-alpha and -beta knockout mice. Proc Natl Acad Sci U S A. 2003;100:6192–7.
14. Clément Y, Le Guisquet AM, Venault P, Chapouthier G, Belzung C. Pharmacological alterations of anxious behaviour in mice depending on both strain and the behavioural situation. PLoS ONE. 2009;4: e7745.
15. Clipperton-Allen AE, Page DT. Pten haploinsufficient mice show broad brain overgrowth but selective impairments in autism-relevant behavioral tests. Hum Mol Genet. 2014;23:3490–505.
16. Coba MP, Ramaker MJ, Ho EV, Thompson SL, Komiyama NH, Grant SGN, Knowles JA, Dulawa SC. Dlgap1 knockout mice exhibit alterations of the postsynaptic density and selective reductions in sociability. Sci Rep. 2018;8:2281.
17. Courchet V, Roberts AJ, Meyer-Dilhet G, Del Carmine P, Lewis TL Jr, Polleux F, Courchet J. Haploinsufficiency of autism spectrum disorder candidate gene NUAK1 impairs cortical development and behavior in mice. Nat Commun. 2018;9:4289.
18. D'Angelo MG, Lorusso ML, Civati F, Comi GP, Magri F, Del Bo R, Guglieri M, Molteni M, Turconi AC, Bresolin N. Neurocognitive profiles in Duchenne muscular dystrophy and gene mutation site. Pediatr Neurol. 2011;45:292–9.
19. Daoud F, Angeard N, Demerre B, Martie I, Benyaou R, Leturcq F, Cossée M, Deburgrave N, Saillour Y, Tuffery S, Urtizberea A, Toutain A, Echenne B, Frischman M, Mayer M, Desguerre I, Estournet B, Réveillère C, Penisson-Besnier, Cuisset JM, Kaplan JC, Héron D, Rivier F, Chelly J. 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. 2009;18:3779–94.
20. Daoud F, Candelario-Martínez A, Billard JM, Avital A, Khelfaoui M, Rozenvald Y, Guegan M, Mornet D, Jaillard D, Nudel U, Chelly J, Martínez-Rojas D, Laroche S, Yaffe D, Vaillend C. Role of mental retardation-associated dystrophin-gene product Dp71 in excitatory synapse organization, synaptic plasticity and behavioral functions. PLoS ONE. 2009;4: e6574.
21. Darmahkasih AJ, Rybalsky I, Tian C, Shellenbarger KC, Horn PS, Lambert JT, Wong BL. Neurodevelopmental, behavioral, and emotional symptoms common in Duchenne muscular dystrophy. Muscle Nerve. 2020;61:466–74.
22. Desguerre I, Christov C, Mayer M, Zeller R, Becane HM, Bastuji-Garin S, Leturcq F, Chiron C, Chelly J, Gherardi RK. Clinical heterogeneity of duchenne muscular dystrophy (DMD): definition of sub-phenotypes and predictive criteria by long-term follow-up. PLoS ONE. 2009;4: e4347.
23. Erturk O, Bilguvar K, Korkmaz B, Bayri Y, Bayrakli F, Arlier Z, Ozturk AK, Yalcinkaya C, Tuysuz B, State MW, Gunel M. A patient with Duchenne muscular dystrophy and autism demonstrates a hemizygous deletion affecting Dystrophin. Am J Med Genet A. 2010;152A:1039–42.
24. Ferhat AT, Halbedl S, Schmeisser MJ, Kas MJ, Bourgeron T, Ey E. Behavioural phenotypes and neural circuit dysfunctions in mouse models of autism spectrum disorder. Adv Anat Embryol Cell Biol. 2017;224:85–101.

25. Filippo TD, Parisi L, Roccella M. Psychological aspects in children affected by Duchenne de Boulogne muscular dystrophy. Ment Illn. 2014;4: e5.
26. Goyenvalle A, Griffith G, Avril A, Amthor H, Garcia L. Functional correction and cognitive improvement in dystrophic mice using splice-switching tricyclo-DNA oligomers. Med Sci (Paris). 2015;31:253–6.
27. Harro J. Animals, anxiety, and anxiety disorders: How to measure anxiety in rodents and why. Behav Brain Res. 2018;352:81–93.
28. Hashimoto Y, Kuniishi H, Sakai K, Fukushima Y, Du X, Yamashiro K, Hori K, Imamura M, Hoshino M, Yamada M, Araki T, Sakagami H, Takeda S, Itaka K, Ichinohe N, Muntoni F, Sekiguchi M, Aoki Y. Brain Dp140 alters glutamatergic transmission and social behaviour in the mdx52 mouse model of Duchenne muscular dystrophy. Prog Neurobiol. 2022;216: 102288.
29. Helleringer R, Le Verger D, Li X, Izabelle C, Chaussenot R, Belmaati-Cherkaoui M, Dammak R, Decottignies P, Daniel H, Galante M, Vaillend C. Cerebellar synapse properties and cerebellum-dependent motor and non-motor performance in Dp71-null mice. Dis Model Mech. 2018;11:dmm033258.
30. Hendriksen JG, Vles JS. Neuropsychiatric disorders in males with duchenne muscular dystrophy: frequency rate of attention-deficit hyperactivity disorder (ADHD), autism spectrum disorder, and obsessive–compulsive disorder. J Child Neurol. 2008;23:477–81.
31. Hendriksen JGM, Thangarajh M, Kan HE, Muntoni F, ENMC 249th workshop study group. 249th ENMC International Workshop: The role of brain dystrophin in muscular dystrophy: Implications for clinical care and translational research, Hoofddorp, The Netherlands, November 29th-December 1st 2019. Neuromuscul Disord. 2020;30:782–94.
32. Hinton VJ, Cyrulnik SE, Fee RJ, Batchelder A, Kiefel JM, Goldstein EM, Kaufmann P, De Vivo DC. Association of autistic spectrum disorders with dystrophinopathies. Pediatr Neurol. 2009;41:339–46.
33. Hinton VJ, Fee RJ, De Vivo DC, Goldstein E. Poor facial affect recognition among boys with duchenne muscular dystrophy. J Autism Dev Disord. 2007;37:1925–33.
34. Hinton VJ, Nereo NE, Fee RJ, Cyrulnik SE. Social behavior problems in boys with Duchenne muscular dystrophy. J Dev Behav Pediatr. 2006;27:470–6.
35. Hisaoka T, Komori T, Kitamura T, Morikawa Y. Abnormal behaviours relevant to neurodevelopmental disorders in Kirrel3-knockout mice. Sci Rep. 2018;8:1408.
36. Holy TE, Guo Z. Ultrasonic songs of male mice. PLoS Biol. 2005;3: e386.
37. Krishnan V, Stoppel DC, Nong Y, Johnson MA, Nadler MJ, Ozkaynak E, Teng BL, Nagakura I, Mohammad F, Silva MA, Peterson S, Cruz TJ, Kasper EM, Arnaout R, Anderson MP. Autism gene Ube3a and seizures impair sociability by repressing VTA Cbln1. Nature. 2017;543:507–12.
38. LeDoux J. The amygdala. Curr Biol. 2007;17:R868–74.
39. Lu DH, Liao HM, Chen CH, Tu HJ, Liou HC, Gau SS, Fu WM. Impairment of social behaviors in Arhgef10 knockout mice. Mol Autism. 2018;9:11.
40. Maresh K, Papageorgiou A, Ridout D, Harrison NA, Mandy W, Skuse D, Muntoni F. Startle responses in Duchenne muscular dystrophy: a novel biomarker of brain dystrophin deficiency. Brain. 2023;146:252–65.
41. Michetti C, Caruso A, Pagani M, Sabbioni M, Medrihan L, David G, Galbusera A, Morini M, Gozzi A, Benfenati F, Scattoni ML. The knockout of synapsin II in mice impairs social behavior and functional connectivity generating an ASD-like phenotype. Cereb Cortex. 2017;27:5014–23.
42. Miranda R, Nudel U, Laroche S, Vaillend C. Altered presynaptic ultrastructure in excitatory hippocampal synapses of mice lacking dystrophins Dp427 or Dp71. Neurobiol Dis. 2011;43:134–41.
43. Miranda R, Nagapin F, Bozon B, Laroche S, Aubin T, Vaillend C. Altered social behavior and ultrasonic communication in the dystrophin-deficient mdx mouse model of Duchenne muscular dystrophy. Mol Autism. 2015;6:60.
44. Muntoni F, Torelli S, Ferlini A. Dystrophin and mutations: one gene, several proteins, multiple phenotypes. Lancet Neurol. 2003;2:731–40.
45. Neumann ID, Veenema AH, Beiderbeck DI. Aggression and anxiety: social context and neurobiological links. Front Behav Neurosci. 2010;4:12.
46. Pagnamenta AT, Holt R, Yusuf M, Pinto D, Wing K, Betancur C, Scherer SW, Volpi EV, Monaco AP. A family with autism and rare copy number variants disrupting the Duchenne/Becker muscular dystrophy gene DMD and TRPM3. J Neurodev Disord. 2011;3:124–31.
47. Pane M, Lombardo ME, Alfieri P, D'Amico A, Bianco F, Vasco G, Piccini G, Mallardi M, Romeo DM, Ricotti V, Ferlini A, Gualandi F, Vicari S, Bertini E, Berardinelli A, Mercuri E. Attention deficit hyperactivity disorder and cognitive function in Duchenne muscular dystrophy: phenotype-genotype correlation. J Pediatr. 2012;161:705–9.
48. Pangalila RF, van den Bos GA, Bartels B, Bergen M, Stam HJ, Roebroeck ME. Prevalence of fatigue, pain, and affective disorders in adults with Duchenne muscular dystrophy and their associations with quality of life. Arch Phys Med Rehabil. 2015;96:1242–7.
49. Panksepp JB, Jochman KA, Kim JU, Koy JJ, Wilson ED, Chen Q, Wilson CR, Lahvis GP. Affiliative behavior, ultrasonic communication and social reward are influenced by genetic variation in adolescent mice. PLoS ONE. 2007;2: e351.
50. Pascual-Morena C, Cavero-Redondo I, Reina-Gutiérrez S, Saz-Lara A, López-Gil JF, Martínez-Vizcaíno V. Prevalence of neuropsychiatric disorders in Duchenne and Becker muscular dystrophies: a systematic review and meta-analysis. Arch Phys Med Rehabil. 2022;103:2444–53.
51. Paylor R, Zhao Y, Libbey M, Westphal H, Crawley JN. Learning impairments and motor dysfunctions in adult Lhx5-deficient mice displaying hippocampal disorganization. Physiol Behav. 2001;73:781–92.
52. Perronnet C, Vaillend C. Dystrophins, utrophins, and associated scaffolding complexes: role in mammalian brain and implications for therapeutic strategies. J Biomed Biotechnol. 2010;2010: 849426.
53. Postorino V, Kerns CM, Vivanti G, Bradshaw J, Siracusano M, Mazzone L. Anxiety disorders and obsessive-compulsive disorder in individuals with autism spectrum disorder. Curr Psychiatry Rep. 2017;19:92.
54. Relizani K, Griffith G, Echevarría L, Zarrouki F, Facchinetti P, Vaillend C, Leumann C, Garcia L, Goyenvalle A. Efficacy and safety profile of Tricyclo-DNA antisense oligonucleotides in duchenne muscular dystrophy mouse model. Mol Ther Nucleic Acids. 2017;8:144–57.
55. Ricotti V, Mandy WP, Scoto M, Pane M, Deconinck N, Messina S, Mercuri E, Skuse DH, Muntoni F. Neurodevelopmental, emotional, and behavioural problems in Duchenne muscular dystrophy in relation to underlying dystrophin gene mutations. Dev Med Child Neurol. 2016;58:77–84.
56. Saoudi A, Zarrouki F, Sebrié C, Izabelle C, Goyenvalle A, Vaillend C. Emotional behavior and brain anatomy of the mdx52 mouse model of Duchenne muscular dystrophy. Dis Model Mech. 2021;14:dmm049028.
57. Sarig R, Mezger-Lallemand V, Gitelman I, Davis C, Fuchs O, Yaffe D, Nudel U. Targeted inactivation of Dp71, the major non-muscle product of the DMD gene: differential activity of the Dp71 promoter during development. Hum Mol Genet. 1999;8:1–10.
58. Saudou F, Amara DA, Dierich A, LeMeur M, Ramboz S, Segu L, Buhot MC, Hen R. Enhanced aggressive behavior in mice lacking 5-HT1B receptor. Science. 1994;265:1875–8.
59. Scattoni ML, Gandhy SU, Ricceri L, Crawley JN. Unusual repertoire of vocalizations in the BTBR T+tf/J mouse model of autism. PLoS ONE. 2008;3: e3067.
60. Sekiguchi M, Zushida K, Yoshida M, Maekawa M, Kamichi S, Yoshida M, Sahara Y, Yuasa S, Takeda S, Wada K. A deficit of brain dystrophin impairs specific amygdala GABAergic transmission and enhances defensive behaviour in mice. Brain. 2009;132:124–35.
61. Simola N, Granon S. Ultrasonic vocalizations as a tool in studying emotional states in rodent models of social behavior and brain disease. Neuropharmacology. 2019;159: 107420.
62. Snow WM, Anderson JE, Jakobson LS. Neuropsychological and neurobehavioral functioning in Duchenne muscular dystrophy: a review. Neurosci Biobehav Rev. 2013;37:743–52.
63. Takayanagi Y, Yoshida M, Bielsky IF, Ross HE, Kawamata M, Onaka T, Yanagisawa T, Kimura T, Matzuk MM, Young LJ, Nishimori K. Pervasive social deficits, but normal parturition, in oxytocin receptor-deficient mice. Proc Natl Acad Sci U S A. 2005;102:16096–101.
64. Taylor PJ, Betts GA, Maroulis S, Gilissen C, Pedersen RL, Mowat DR, Johnston HM, Buckley MF. Dystrophin gene mutation location and the risk of cognitive impairment in Duchenne muscular dystrophy. PLoS ONE. 2010;5: e8803.
65. Um SM, Ha S, Lee H, Kim J, Kim K, Shin W, Cho YS, Roh JD, Kang J, Yoo T, Noh YW, Choi Y, Bae YC, Kim E. NGL-2 deletion leads to autistic-like behaviors responsive to NMDAR modulation. Cell Rep. 2018;23:3839–51.
66. Vaillend C, Chaussenot R. Relationships linking emotional, motor, cognitive and GABAergic dysfunctions in dystrophin-deficient mdx mice. Hum Mol Genet. 2017;26:1041–55.
67. Vaillend C, Ungerer A. Behavioral characterization of mdx3cv mice deficient in C-terminal dystrophins. Neuromuscul Disord. 1999;9:296–304.

68. Wall PM, Messier C. Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement instrument of animal anxiety-like behavior. Neurosci Biobehav Rev. 2001;25:275–86.
69. Willott JF. Measurement of the auditory brainstem response (ABR) to study auditory sensitivity in mice. Curr Protoc Neurosci. 2006; Chapter 8:Unit8.21B.
70. Wong CT, Bestard-Lorigados I, Crawford DA. Autism-related behaviors in the cyclooxygenase-2-deficient mouse model. Genes Brain Behav. 2019;18: e12506.
71. Wu JY, Kuban KC, Allred E, Shapiro F, Darras BT. Association of Duchenne muscular dystrophy with autism spectrum disorder. J Child Neurol. 2005;20(10):790–5.

Publisher's Note