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Influence of Dystrophin–Gene Mutation on *mdx* Mouse Behavior. I. Retention Deficits at Long Delays in Spontaneous Alternation and Bar-Pressing Tasks

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X-linked Duchenne muscular dystrophy (DMD) is frequently associated with a nonprogressive, cognitive defect attributed to the absence of dystrophin in the brain of DMD patients. The mutant *mdx* mouse, lacking in 427-kDa dystrophin in both muscle and brain tissues, is considered to be a valuable model of human DMD. In the present study, we compared *mdx* and C57BL/10 control mice and showed that *mdx* mice had impaired retention in a T-maze, delayed spontaneous alternation task 24 h, but not 6 h, after acquisition. *mdx* mice were not impaired in acquisition of a bar-pressing task on 4 consecutive days but showed poor retention 22 days after the last training session. Mutants and controls showed similar behavioral responses in free exploration and light/dark choice situations and did not differ in spontaneous locomotor activity or motor coordination. Retention impairments at long delays in *mdx* mice suggest a role of dystrophin in long-term consolidation processes.

KEY WORDS: Dystrophin gene mutation; *mdx* mouse; operant learning; delayed spontaneous alternation; novelty reaction; anxiety; motor activity.

INTRODUCTION

Duchenne muscular dystrophy (DMD) is a lethal, X-linked recessive neuromuscular disease affecting about 1 in 3500 live-born human males. It is characterized by severe muscle degeneration followed by highly damaging fibrosis, progressive muscular weakness, and elevated serum creatine kinase levels. Its molecular basis is now clearly known and involves the 14-kb DMD gene located at Xp21, which encodes dystrophin, a 427-kDa cytoskeletal protein. DMD is caused by alterations in the dystrophin gene, mainly deletions and point mutations disrupting the translational reading frame (Nicholson *et al.*, 1993). The major biological consequence is the absence of 427-kDa dystrophin. In muscles of normal individuals, dystrophin has been located on the intracellular side of the sarcolemma, where it is thought to preserve myofibre integrity during muscle contraction. However, its function is still largely unknown (Ahn and Kunkel, 1993). Dystrophin belongs to a family of related proteins; dystrophin and other alternative products of the DMD gene have been identified in nonmuscle tissues including brain and may serve different functions in particular neuronal cells or cerebral regions (Chelly *et al.*, 1990; Ahn and Kunkel, 1993). Full-length mRNAs encode the brain, cerebellar Purkinje cell, and muscle 427-kDa forms of dystrophin, which differ in their N-terminal amino acids (Nudel *et al.*, 1989). Apo-dystrophin-1 (71 kDa) and apo-dystrophin-2 (116 kDa) consist of only the C-terminal portion of 427-kDa dystrophin and have also been located in the CNS (Lederfein *et al.*, 1992; Jung *et*

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al., 1993; Mizuno *et al.*, 1993; Schofield *et al.*, 1994). In contrast to full-length dystrophin, which is expressed mainly in muscle and to a lesser extent in brain, apodystrophin-1 is found in many tissues (liver, testis, lung, skin, and kidney) and is relatively abundant in brain. Otherwise, utrophin is an autosomal homologue to the 427-kDa dystrophin also present in the brain (Jung *et al.*, 1991; Love *et al.*, 1991; ThiMan *et al.*, 1991; Uchino *et al.*, 1994a, b).

A nonprogressive cognitive impairment is observed in about one-third of DMD patients, compared to age-matched normal boys or patients suffering from other neuromuscular disorders, such as spinal muscular atrophy (Billard *et al.*, 1992). It has been reported that some cognitive functions are more affected than others (Billard *et al.*, 1992; Nicholson *et al.*, 1993; Bresolin *et al.*, 1994): verbal IQ, reading abilities, syntax comprehension, attention, story recall, and differed verbal recognition are particularly impaired in DMD patients. However, no overt morphological abnormalities were detected in the central nervous system (CNS) of 21 DMD patients (Dubowitz and Crome, 1969). Dystrophin is normally expressed in the brain of mammals in cerebral areas associated with cognitive functions, such as the limbic system and the cerebral neocortex, but also in areas associated with motor coordination, such as the cerebellum (Gorecki *et al.*, 1991; Jung *et al.*, 1991; Huard *et al.*, 1992; Lidov *et al.*, 1993). According to these data, it has been postulated that the absence or malfunction of dystrophin is causally related to the cognitive deficits described in DMD.

The mutant C57BL/10-ScSn-*mdx* (*mdx*) mouse, discovered by Bulfield *et al.* (1984), lacks 427-kDa dystrophin in both muscle and brain due to a point mutation in the coding sequence (exon 23) introducing a STOP codon (Sicinski *et al.*, 1989). The mutant *mdx* mouse is considered to be a valuable animal model of human DMD. *mdx* mice exhibit many pathophysiological characteristics of the early phase of the human disease, such as elevated serum creatine kinase and focal necrosis of muscle fibers. However, limb muscles of *mdx* mice do not suffer long-term effects of necrosis as found in muscles of DMD patients but, instead, undergo rapid cycles of necrosis and regeneration. Muscle cell replication in *mdx* mice is maximal at 4–8 weeks of age and continues at lower levels until at least 44 weeks (DiMario *et al.*, 1991; McGeachie *et al.*, 1993; Pastoret and Sébille, 1993). Unlike humans, muscle fibers in *mdx* mice regenerate so successfully that no obvious motor disability is detected before 12 months of age (Bulfield *et al.*, 1984). Spontaneous motility and endurance of *mdx* mice, although slightly lower than those of C57BL/10 congenic mice, do not show significant differences from 5 weeks up to 6 months of age (Muntoni *et al.*, 1993).

In another way, behavioral studies of the mutant *mdx* mouse can greatly further our understanding of the mental disorders related to DMD, which are still poorly understood. The only behavioral study carried out in *mdx* mice (Muntoni *et al.*, 1991) reported a strong retention impairment in a passive avoidance learning task. To characterize more precisely the learning and memory capacities displayed by *mdx* mice, we investigated behavioral performance of both *mdx* and C57BL/10-ScSn (B10) control mice in a delayed spontaneous alternation task in a T-maze and in a positively reinforced operant learning task. The performance of animals in such tasks could be biased by emotional and/or motor disturbance. Therefore, we investigated behavioral reactivity of both *mdx* and control mice in a free exploration situation, in which animals were given the opportunity to move freely between a familiar and a novel environment. The animals of both strains were also tested in a light/dark choice apparatus to appreciate their reactivity in an anxiogenic paradigm (Belzung, 1992). Global motor activity and motor coordination were evaluated by a spontaneous locomotor activity test and traction reflex, respectively.

MATERIALS AND METHODS

Animals

The subjects were male mice of the C57BL/10-ScSn (B10) control strain and of the C57BL/10-ScSn-*mdx* (*mdx*) mutant strain. They originated from one pair of B10 mice (+/+, +/Y) obtained from CSEAL-CNRS (Orléans, France) and one pair of *mdx* mice (*mdx/mdx*, *mdx*/Y), which was a gift from Dr. Guénet (Institut Pasteur, Paris, France). Homozygote mutant mice are viable and fertile and were bred in the Centre de Neurochimie (Strasbourg, France). Histological analysis (M.-E. Stoeckel, URA CNRS 1446, ULP Strasbourg, France) showed characteristic changes in *mdx* mice

hindpaw skeletal muscle, such as centralization of the nuclei within the otherwise normal muscle fibers. An analysis of *mdx* mouse plasma (L. Metzinger, Faculté de Pharmacie, Département Immunologie, ULP Illkirch, France) detected high levels of serum creatine kinase, which is a characteristic of the early phase of the disease in both DMD patients and *mdx* mice. In addition, immunoblot analysis (A. Rendon and D. Filliol, INSERM U-338, Strasbourg, France) showed a lack of full-length dystrophin in both brain and muscle tissues, thus confirming *mdx* phenotype (data not shown).

Eight-week-old *mdx* (*mdx*/Y; $n = 25$) and control (+/+; $n = 23$) male mice were kept in group cages (2–5 mice/cage, same strain) under a reversed light cycle (light on: 7 PM to 7 AM) with food and water *ad libitum*. At 11 weeks old, animals were successively tested in a free-exploration test, a dark/light choice situation, and a spontaneous locomotor activity test, with a 48-h interval between each experiment. When they were 13 weeks old, mice were tested in a T-maze, delayed spontaneous alternation task. One-half of the animals was tested for retention 6 h after acquisition and the other half after a 24-h retention interval. Thereafter, mice were changed to individual caging. On the following day, they entered a 4-day food restriction period, followed by 4 consecutive days of training in a positively reinforced, operant learning task. One week later, mice were confronted with the delayed spontaneous alternation task for the second time: the animals previously tested at a 6-h retention interval were tested at a 24-h delay, and conversely those previously tested at the 24-h delay were tested 6 h after acquisition. Mice were submitted to a traction reflex test the following week. All experiments were carried out during the dark half of the reversed cycle.

Behavioral Testing

Delayed Spontaneous Alternation in a T-Maze

The apparatus was a gray, leukoplast T-maze composed of a central alley (40 × 10 × 25 cm) including a start box (15 × 10 × 25 cm) and two lateral alleys (30 × 10 × 25 cm) positioned at the end of the central alley. Sliding doors were placed at the entrance to each alley (central or lateral). The apparatus was placed in a room illuminated by two fluorescent tubes positioned each 1 m laterally to the central alley.

On the first day, the animal was allowed to explore the maze for 5 min. On the following day it was submitted, between 9 and 11:30 AM, to two consecutive trials during which it had access to only one lateral alley, the other being closed by the sliding door; half of the animals had access to the right alley, and the other half to the left alley. At the start of each trial, the animal was placed for 30 s in the start box and the sliding door was then opened; the latency to enter into the accessible alley was recorded; the animal remained confined in this alley for 30 s and was then submitted to a second trial in the same conditions. Six or twenty-four hours later, it underwent a third trial during which it could choose freely between the left and the right alley; half of the animals underwent this trial after a 6-h interval, and the other half after a 24-h interval. For each experimental group, retention of the two first trials was expressed by the percentage of mice alternating during the third trial.

Alternation rates were compared using a chi-square analysis of the total number of mice alternating. The latencies to enter the lateral alley during the two first trials (acquisition trials) and during the third trial (retention testing) were analyzed by means of a two-factor (strain, trial) ANOVA with repeated measures on one factor (trial) and by means of a two-tailed unpaired Student *t* test, respectively.

Positively Reinforced, Operant Learning Task

The apparatus was a translucent Plexiglas Skinner box (13 × 14 × 17 cm). The bar and the food cup were separated by a 5-cm-long partition so that, after a bar-press, the mouse had to go around the partition to reach the food cup. A continuous reinforcement schedule (CRF1) was applied; bar-presses and the presence of the animal in front of the food cup or in front of the bar were recorded on calibrated paper.

The animals were housed individually and food restricted 4 days before training, so that they reached 80–85% of their initial weight. They were then trained during 4 consecutive days. Each daily session ended after 20 bar-presses followed by food-pellet consumption (reinforced bar-presses) or after a maximum of 35 min. The number of reinforced bar-presses during the first 5 min and the last 5 min of each session was recorded to detect performance improvement within a session or be-

tween two consecutive sessions. In addition, the latency of the first bar-press and the time needed to make 20 reinforced bar-presses were recorded. Eight animals of each strain were also submitted to a retention session under the same conditions, 22 days after the last training day. Interstrain differences in the time needed to make 20 reinforced bar-presses and the latency of the first reinforced bar-presses were evaluated using a two-factor (strain, session) ANOVA with repeated measures on one factor (session). Within-session variations in the number of reinforced bar-presses were evaluated by a three-factor [strain (two levels: *mdx*, controls), session (four levels: D1, D2, D3, and D4), block of 5 min (two levels: first 5 min and last 5 min)] ANOVA with repeated measures on two factors (session, block of 5 min). Intersession variations in performance were evaluated by a three-factor [strain (two levels: *mdx*, controls), between-session (three levels: D1–D2, D2–D3, and D3–D4), block of 5 min (two levels: last 5 min, first 5 min)] ANOVA with repeated measures on two factors (between-session, block of 5 min). Subsequent *a posteriori* comparisons were performed using paired (between-strain comparisons) and unpaired (within-strain comparisons) two-tailed Student *t* tests.

Free-Exploration Test

The apparatus, previously described by Misslin and Ropartz (1981), consisted of an opaque PVC box (30 × 20 × 20 cm) covered with Plexiglas and subdivided into six (10 × 10-cm) exploration units which were all interconnected by small doors. It could be divided in half lengthwise in two compartments (three exploration units each) by closing three temporary partitions. Testing took place in the room where the mice were kept and the experimenter stood next to the testing box, always at the same place.

Each subject was placed for approximately 24 h in one compartment with the temporary partitions in place, to become familiarized with it. The floor of this compartment was covered by sawdust and the animal was given unlimited access to food and water. The following day, the temporary partitions were removed, giving the mouse the opportunity to choose between the familiar and the novel compartments. The subject was observed in red light ($I < 20$ lux) for 10 min. The time spent in the novel compartment, considered as an indication of novelty preference (Misslin and Ropartz, 1981), and the number of approach responses followed by avoidance reactions (attempts) toward the novel compartment were recorded each min using a keyboard connected to a computer system. Interstrain differences were evaluated statistically by a two-factor (strain, time) ANOVA with repeated measures on one factor (time). The sign test was used to reveal significant novelty preference.

Light/Dark Choice Situation

The apparatus consisted of a dark box and a transparent box made of PVC (20 × 20 × 15 cm each), covered with Plexiglas, and connected by an opaque plastic tunnel (5 × 7 × 10 cm). A 100-W desk lamp above the transparent box provided the only room illumination. Testing was performed between 2 and 4 PM. Mice were individually placed in the dark box to start the session. The time spent in the lit box and the number of transitions toward the lit box were recorded each minute for 5 min. Interstrain differences were evaluated statistically by a two-factor (strain, time) ANOVA with repeated measures on one factor (time).

Spontaneous Locomotor Activity Test

The apparatus consisted of two transparent PVC boxes (20 × 20 × 15 cm each) covered with translucent Plexiglas and connected by an opaque plastic tunnel (5 × 7 × 10 cm). Mice were individually placed in one of the two boxes and were allowed to explore the apparatus for 90 min. A computerized photocell detection system counted the number of transitions through the tunnel by blocks of 9 min. Interstrain comparisons of performance were evaluated statistically by a two-factor (strain, time) ANOVA with repeated measures on one factor (time).

Traction Reflex Test

A thin horizontal wire (1.15 mm in diameter) was stretched 35 cm above the table surface. Each animal was submitted to three consecutive trials separated by 5-min intervals. At the start of each trial, the forepaws of the mouse were placed on the wire. The performance was evaluated in terms of latency to bring the hindpaws up to the wire. Each trial lasted for a maximum of 25 s. As the distri-

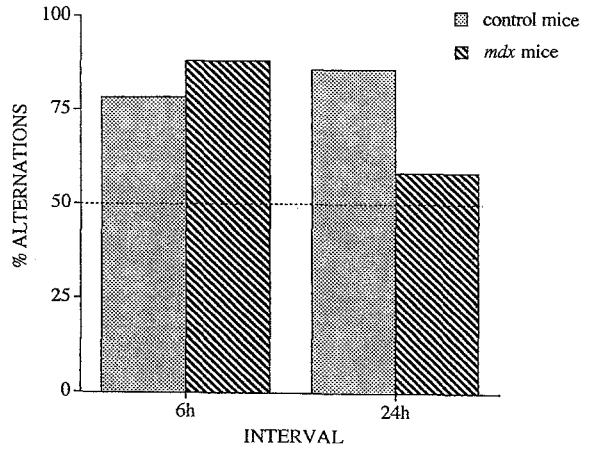

Fig. 1. Performance of *mdx* and control mice in a T-maze delayed spontaneous alternation task. Results are expressed as percentages of alternations on the third trial, at the 6-h [*mdx*, $n = 25$; controls, $n = 23$] and 24-h [*mdx*, $n = 24$; controls, $n = 21$] retention delays. The dotted line indicates the random level, defined as 50% alternations.

bution of traction reflex performance was not normal, a Mann–Whitney U test was used for between-strain comparisons.

RESULTS

Delayed Spontaneous Alternation Task in a T-Maze

For each strain, no difference was found between mice tested before and mice tested after the operant learning task, on alternation rates or latency to enter the lateral alley at the 6-h or the 24-h retention delay. Thus, we pooled the data of these two testing sessions for a given retention delay.

The percentages of mice of both strains alternating during the retention session are presented in Fig. 1. The χ^2 analysis revealed no difference between *mdx* and control mice at the 6-h retention interval [$\chi^2(1) = .82$, $p = .3657$], whereas a significant difference was detected after a 24-h retention interval [$\chi^2(1) = 4.09$, $p = .043$]. After a 6-h retention interval, the animals of both strains alternated at a rate which differed significantly from random [*mdx* mice, $\chi^2(1) = 14.44$, $p < .001$; control mice, $\chi^2(1) = 7.34$, $p < .01$], indicating that they remembered the alley visited during the training session. After a 24-h retention interval, *mdx* mice alternated at a rate close to random [$\chi^2(1) = .66$, NS], whereas the alternation rate of control mice differed significantly from random [$\chi^2(1) = 10.71$, $p < .01$].

On the other hand, no difference was observed between strains for latency to reach the goal alley, during either acquisition trials [strain effect, $F(1,91) = .22$, $p = .6405$; strain × trial interaction, $F(1,91) = .073$, $p = .7878$] or retention testing [6-h retention interval, $t(46) = .72$, $p = .4726$; 24-h retention interval, $t(43) = .79$, $p = .4359$].

Positively Reinforced Operant Learning Task

The time needed to make 20 reinforced bar-presses decreased significantly for both strains during training [$F(3,44) = 315.401$, $p < .001$], indicating a progressive acquisition of the task across the sessions. There was no significant difference between strains on this parameter [strain effect, $F(1,44) = 2.89$, $p = .0962$; strain × session interaction, $F(3,44) = 1.561$, $p = .2018$]. Moreover, there was no overall difference between the latency of the two strains of mice to make the first reinforced bar-press [strain effect, $F(1,43) = 2.358$, $p = .1319$; strain × session interaction, $F(3,43) = .127$, $p = .9439$].

The numbers of reinforced bar-presses recorded during the first 5 min and the last 5 min of each daily session of training (D1 to D4) are presented in Fig. 2. The number of reinforced bar-presses increased for both strains, across sessions [$F(3,44) = 172.109$, $p < .001$] and within sessions [block of 5-min effect, $F(1,44) = 13.446$, $p < .001$]. There was no significant strain effect [$F(1,44) = .875$, $p = .3548$] and no significant interaction. A significant improvement in performance was detected for both strains between the last 5 min of a session and the first 5 min of the following session [block of 5-min interaction, $F(1,44) = 63.163$, $p < .001$]. No overall difference was detected between *mdx* and control mice.

However, the number of reinforced bar-presses was slightly lower in *mdx* than in control mice during the first 5 min of the third (D3) session [unpaired Student t test, $t(44) = 1.96619$, $p = .0556$]. As shown in Fig. 2, *mdx* mice performance did not improve significantly between D2 and D3 [paired Student t test, $t(24) = 1.82232$, $p = .0809$], contrary to control mice [paired Student t test, $t(20) = 3.28634$, $p < .01$]. This slight deficit in performance in *mdx* mice was correlated with a longer latency of the first reinforced bar-press [$t(44) =$

2.74923, $p < .01$] and a longer time to make 20 reinforced bar-presses [$t(44) = 2.05254$, $p = .0461$]. Nevertheless, the animals of the two strains reached similar performances on the fourth (D4) training session [last 5 min, $t(44) = .36852$, $p = .7142$].

The numbers of reinforced bar-presses made during the last 5 min of the fourth training session (D4-L) and the first 5 min of the retention session (R) are presented in Fig. 3. Although *mdx* ($n = 8$) and control mice ($n = 8$) displayed similar performances during D4-L [$t(14) = .58043$, $p = .5709$], mutants showed a significant impairment in performance during the first 5 min of the retention session (R) compared to controls [$t(14) = 2.65935$, $p = .0187$]. Moreover, control mice improved their performance between D4-L and R [$t(7) = 4.07527$, $p < .01$], whereas *mdx* mice showed no improvement between the training and the retention sessions [$t(7) = 0$, NS]. The time needed to make 20 reinforced bar-presses was significantly greater in *mdx* than in control mice during the retention session [$t(14) = 2.29376$, $p = .0378$]. There was no difference between strains on the latency of the first reinforced bar-press.

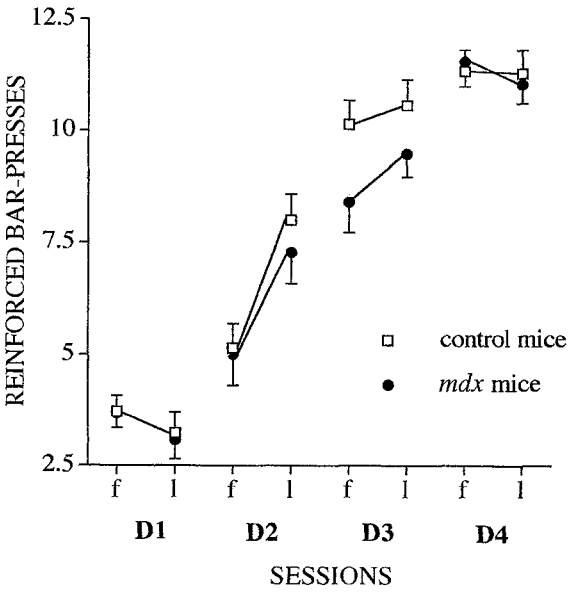

Fig. 2. Number of reinforced bar-presses recorded in *mdx* ($n = 25$) and control ($n = 21$) mice during the first 5 min (f) and the last 5 min (l) of each daily session of training (D1, D2, D3, and D4).

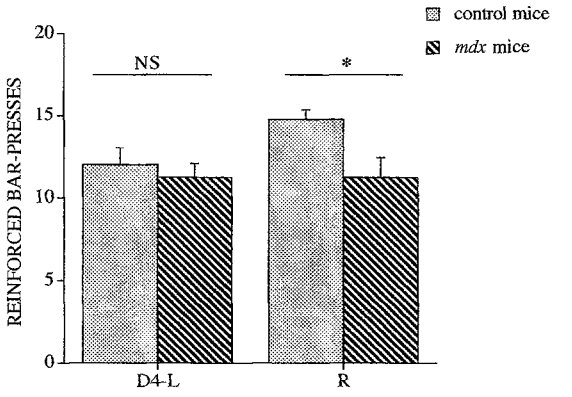

Fig. 3. Number of reinforced bar-presses recorded in *mdx* ($n = 8$) and control ($n = 8$) mice during the last 5 min of the fourth training session (D4-L) and the first 5 min of a retention session (R) performed 22 days after training. Means ± SE. *$p < .02$.

Free-Exploration Test

The time spent in the novel compartment is presented in Fig. 4A, for both *mdx* and control mice. For each group, the time spent in the novel compartment increased up to a maximal amount of time (>40 s) during the first 3 min and stabilized until the end of the 10-min testing session. A two-factor ANOVA showed no significant strain effect [$F(1,45) = .025$, $p = .8753$] and no significant strain × time interaction [$F(9,45) = .654$, $p = .7505$] on this parameter. The sign test indicated that both mutants and controls exhibited a significant preference for the novel compartment from the second min ($p < .01$) to the end of the testing session ($p < .001$).

For each strain, the time course of attempts to enter the novel compartment followed by avoidance responses (attempts) are presented in Fig. 4B. A two-factor ANOVA was performed only for the first 3 min of the testing session, as almost no attempt was observed after 3 min of testing. The number of attempts decreased significantly during the first 3 min for both strains [$F(2,45) = 40.217$, $p < .001$]. There was a significant strain × time interaction [$F(2,45) = 3.469$, $p = .035$] but no strain main effect [$F(1,45) = .161$, $p = .6905$]. To specify whether mutants and controls differed in the number of attempts during the first 2 min of testing, the difference (A1–A2) between the number of attempts recorded during the first minute (A1) and that during the second minute (A2) was calculated for both *mdx* and control mice. As

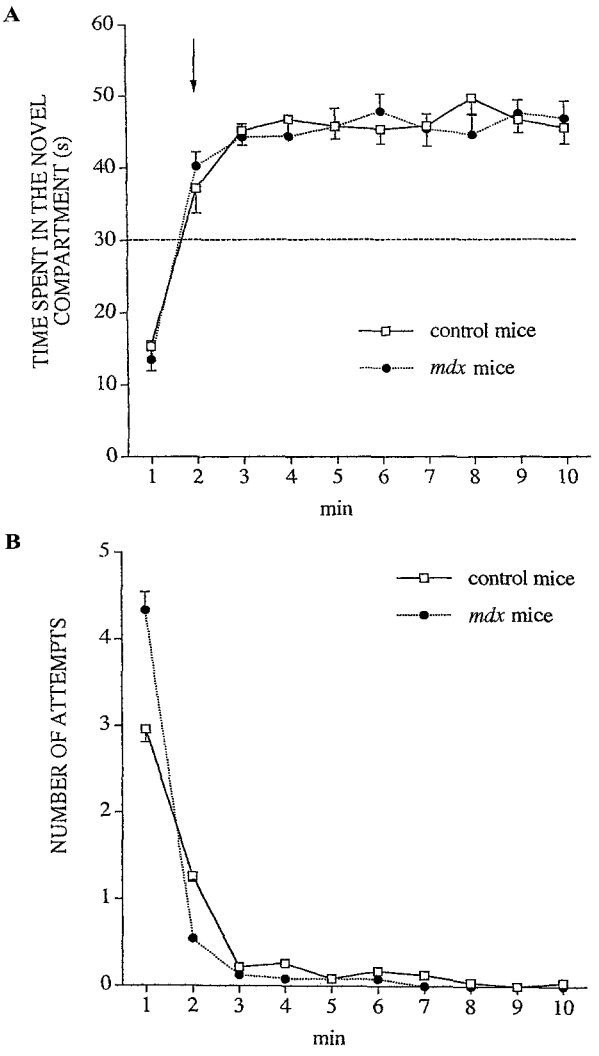

Fig. 4. Time course of behavioral variables in *mdx* (n = 24) and control (n = 23) mice in a free-exploration test, during each min of a 10-min test: (A) time spent in the novel compartment, with the arrow indicating a significant preference for the novel compartment; (B) number of attempts to enter the novel compartment followed by avoidance responses (attempts).

shown in Fig. 5, the A1–A2 difference was statistically greater in *mdx* than in control mice [two-tailed unpaired Student t test, $t(45) = 2.3$, $p = .0261$], indicating that the decrease in the number of attempts was more pronounced in mutants.

Light/Dark Choice Situation

There was no significant difference between strains in the time spent in the lit box [strain effect, $F(1,46) = .028$, $p = .8677$; strain × time interaction, $F(4,46) = 2.166$, $p = .0745$]; the mean time spent in the lit box by *mdx* mice (73.78 ± 4.74 s) was similar to that of controls (74.88 ± 4.63 s). In addition, no difference was detected between *mdx* (8.28 ± 0.49) and control (8.39 ± 0.49) mice on the number of transitions [strain effect, $F(1,46) = .025$, $p = .8754$; strain × time interaction, $F(4,46) = .892$, $p = .4698$].

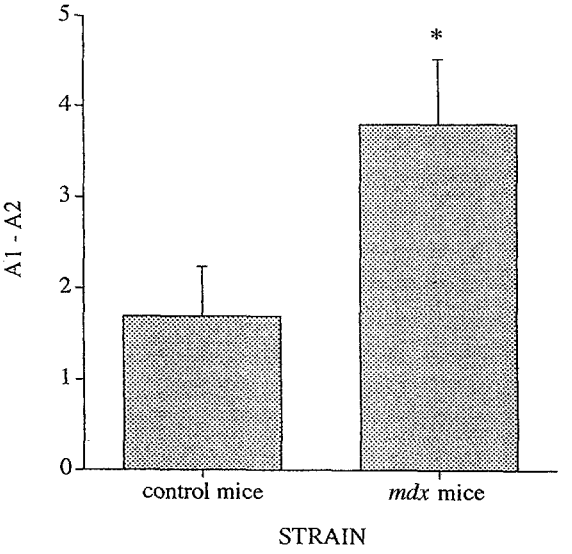

Fig. 5. Difference (A1–A2) between the number of attempts recorded during the first minute (A1) and that during the second minute (A2) of a 10-min free-exploration test in *mdx* (left; n = 24) and control (right; n = 23) mice. Means ± SE. *$p < .05$.

Spontaneous Locomotor Activity Test

No difference was found between *mdx* and control mice in this test [$F(1,46) = .166$, $p = .6859$]. As shown in Fig. 6, spontaneous locomotor activity decreased significantly [$F(9,46) = 33.525$, $p < .001$] for both strains. No significant strain × time interaction was detected [$F(9,46) = .533$, $p = .8509$], suggesting that habituation was similar in mutants and controls. The animals of the two strains displayed similar baseline locomotor activity from approximately 36 min until the end of testing (Fig. 6).

Traction Reflex Test

The mean latency to bring the hindpaws up to the wire was similar in *mdx* (10.567 ± 1.078 s)

and control (10.175 ± 1.143 s) mice across the three consecutive trials, and a two-tailed Mann–Whitney U test showed no significant difference between strains over trials [$U \geq 168.5$, $p \geq .53$].

DISCUSSION

The results obtained in the six behavioral tests are summarized in Table I. We report that *mdx* mutant mice had retention impairments compared to C57BL/10 control mice in a delayed spontaneous alternation task in a T-maze and in a positively reinforced operant learning task. Our results did not detect global learning and memory deficits but, rather, suggest moderate and specific deficits at long retention delays. In the operant learning task, which involves procedural memory, *mdx* and control mice reached similar scores during training. This suggests that *mdx* mice were not impaired in acquisition of the task and had no deficit in procedural memory. A slight impairment in performance was observed in mutants during the third training session, which was correlated with a longer latency of the first reinforced bar-press and a longer time to make 20 reinforced bar-presses, but this could probably not account for learning disturbance.

On the other hand, *mdx* mice exhibited significantly lower performance than control mice during the retention session. In fact, control mice improved their performance after a 22-day retention interval, contrary to *mdx* mice. It has been shown that long-term spontaneous improvement in performance can occur in rodents over periods of several days to several weeks posttraining (Kamin, 1957; Huppert and Deutsch, 1969; Gisquet-Verrier and Alexinsky, 1988). Furthermore, Meziane *et al.* observed improvement in performance in BALB/c mice performing a positively reinforced operant learning task 16 days after partial acquisition of the task (personal communication). *mdx* mice did not show any improvement in performance in the operant learning task at long delays, suggesting that they were impaired in long-term consolidation processes (Squire and Spanis, 1984). Interestingly, *mdx* mice also showed impaired memory retention after a 24-h, but not after a 6-h, interval in a T-maze, delayed spontaneous alternation task. The theory of alternation postulates the existence of a memory trace relative to the information (or events) received on the first trials (acquisition). Delayed spontaneous alternation tasks could account for short-term memory processes or declarative memory in mice, and any increase in delay between acquisition and retention trials leads to a rapid decay in alternation behavior (Durantou *et al.*, 1989). In our experiment, short-term memory or retrieval deficits seem unlikely since mutants were comparable to controls at the 6-h retention delay. The impaired retention observed at the 24-h delay rather suggests that *mdx* mice tend to forget newly acquired information faster than C57BL/10 control mice. Other investigations are needed to specify the nature of the deficits underlying the long-term forgetting displayed by *mdx* mice in the two tasks used.

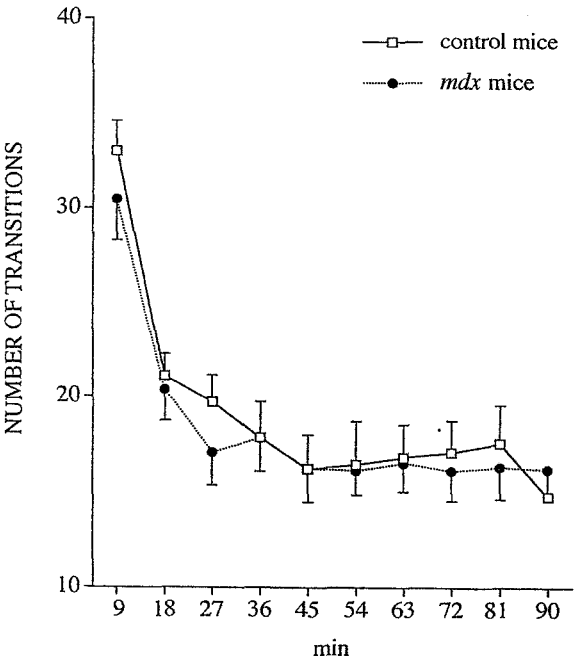

Fig. 6. Spontaneous locomotor activity in *mdx* (n = 25) and control (n = 23) mice. Number of transitions made by mice through the tunnel during a 90-min test, in blocks of 9 min.

Otherwise, mutants and controls showed similar behavioral responses in a free exploration situation and in the light/dark choice situation. However, in the free-exploration test, a rapid and pronounced decrease in avoidance responses toward the novel environment was noted in *mdx* mice. This suggests that mutants had reduced defensive reactions to novel environmental stimuli, compared to controls (Misslin and Ropartz, 1981).

Spontaneous locomotor activity and motor coordination were not impaired in *mdx* mice, confirm-

Table I. Summary of the Results Obtained in the Six Behavioral Tests (Means ± SE)

Test and behavioral variable	C57BL/10 (n = 23)	*mdx* (n = 25)	p value		
			Strain	Strain × session[a]	Strain × time[a]
Delayed spontaneous alternation					
6-h delay (%)	78.26	88	.365[b]		
24-h delay (%)	85.71	58.33	.043[b]		
Bar-pressing task					
Training on the 4 sessions, reinforced bar-presses	7.92 ± .30	7.44 ± .29	.354	.228	
Retention 22 days posttraining, reinforced bar-presses during the first 5 min	14.75 ± .59	11.25 ± 1.17	.018		
Free exploration					
Novelty preference (time in s)	42.24 ± .98	41.96 ± .95	.875		.750
Attempts	1.47 ± .28	1.66 ± .35	.690		.035
Light/dark choice					
Time spent in the lit box (s)	74.88 ± 4.63	73.78 ± 4.74	.867		.074
Number of transitions	8.39 ± .49	8.28 ± .49	.875		.469
Locomotor activity (transitions)	19.06 ± .62	18.32 ± .57	.685		.850
Traction reflex (latency in s)	10.17 ± 1.14	10.56 ± 1.07	.530[c]		

[a] ANOVA with repeated measures.
[b] Chi-square (χ^2) analysis.
[c] Mann–Whitney U test.

ing the data reported by others (Bulfield *et al.*, 1984; Muntoni *et al.*, 1993; Pastoret and Sébille, 1993). Therefore, the memory deficits observed in *mdx* mice cannot be explained by a high level of emotional reactivity and/or motor disturbance.

Correlations between variables of all of the six tests (33 variables) were analyzed in a triangular correlation matrix (528 squares), using Fisher's r-to-z test. For each strain, significant correlations ($p < .01$) were mainly found within the memory tests or between emotional and motor tests but not between memory tests and the other tests. This suggests that memory performance was not correlated with emotional or motor performance in our experiments.

The lack of dystrophin protein (427 kDa) in both muscles and brain is the primary biochemical defect in DMD patients as well as in *mdx* mice. A relationship between the cognitive defects related to DMD and the lack of dystrophin in brain regions associated with cognitive function has been suggested. Dystrophin has been specifically located in the pyramidal cells of the hippocampus (CA1) and of the deep layers of the cerebral neocortex (Ahn and Kunkel, 1993; Lidov *et al.*, 1993) of normal mice. Minciacchi *et al.* (1994) observed that corticospinal neurons from *mdx* mice are markedly reduced in number and appear smaller and round-shaped, compared to control mice neurons. This suggests that the lack of dystrophin in the brain could result in complex alterations of CNS structural and functional properties. Hippocampal pyramidal cells from *mdx* mice are more sensitive to hypoxia-induced loss of synaptic transmission (Mehler *et al.*, 1992). The lack of 427-kDa dystrophin in these neurons was related to an impaired activity of L-type calcium channels. However, Lidov *et al.* (1993) emphasized that dystrophin is expressed predominantly in cerebellar Purkinje cells and suggested that cognitive deficits in DMD could be due to an impairment in cerebellar nonmotor functions. Moreover, Bresolin *et al.* (1994) observed cerebellar hypometabolism in four DMD patients and variable metabolic reduction in associative cortical areas. However, the performance displayed by *mdx* mice in the operant learning task and the absence of ataxia in this mutant indicate no apparent cerebellar dysfunction.

In the present study, specific learning and memory deficits observed in *mdx* mice parallel some defects reported in DMD patients and are consistent with the hypothesis of a relationship between memory disorders and genetic deficiency in 427-kDa dystrophin. DMD patients appear to be

impaired in some specific memory abilities, such as remembering a short story or recognizing a short sentence, which could involve either a left hemisphere or a frontal lobe dysfunction (Billard *et al.*, 1992). Defects of short-term memory have also been reported (Bresolin *et al.*, 1994). However, the variability in location and size of the mutations in DMD patients can lead to a lack of several products of the dystrophin gene in addition to the absence of the full-length dystrophins. This may explain why the cognitive deficits reported in different populations of DMD patients are heterogeneous. Some authors have proposed that mutations within the carboxy terminus of dystrophin correlate with mental retardation in DMD patients, although this is not yet clearly demonstrated (DenDunnen *et al.*, 1991; Lenk *et al.*, 1993; Nagai *et al.*, 1993; Nicholson *et al.*, 1993). Furthermore, the possibility that alternative products of the DMD gene could compensate for the absence of dystrophin should be taken into consideration. *mdx* mice lack the full-length forms of the 427-kDa dystrophin but still express other DMD gene products. Hence, behavioral studies in mutant mice with different mutations in the DMD gene (Cox *et al.*, 1993) will be useful to understand the biochemical abnormalities underlying the cognitive deficits related to DMD and the functional properties of cytoskeletal proteins in the brain (Fallon and Hall, 1994).

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