Reorganization of Inhibitory Synapses and Increased PSD Length of Perforated Excitatory Synapses in Hippocampal Area CA1 of Dystrophin-Deficient mdx Mice

Dystrophin is a cytoskeletal membrane-bound protein expressed in both muscle and brain. Brain dystrophin is thought to be involved in the stabilization of γ-aminobutyric acid (GABA) receptor (GABA\(_{\AA}\)R) clusters in postsynaptic densities (PSDs) at inhibitory synapses onto pyramidal cells, and its loss has been linked to cognitive impairments in Duchenne muscular dystrophy. Dystrophin-deficient mdx mice have learning deficits and altered synaptic plasticity in cornu ammonis (CA1) hippocampus, but the possibility that altered synapse morphology or distribution may underlie these alterations has not been examined. Here we used in vivo magnetic resonance imaging and histological analyses to assess brain volumetric and cytoarchitectonic abnormalities and quantitative electron microscopy to evaluate the density and ultrastructure of CA1 hippocampal synapses in mdx mice. We found that mdx mice have increased density of axodendritic symmetric inhibitory synapses and larger PSDs in perforated asymmetric excitatory synapses in the proximal, but not distal, CA1 apical dendrites that normally express dystrophin, in the absence of gross brain malformations. Data are discussed in light of the known molecular and neurophysiological alterations in mdx mice. We suggest that increased inhibitory synapse density reflects tenuous compensation of altered clustering of \(\alpha2\) subunit-containing GABA\(_{\AA}\)-Rs in CA1 dendrites, whereas increased PSD length in perforated synapses suggests secondary alterations in excitatory synapse organization associated with enhanced synaptic excitation.

**Keywords:** DMD, MRI, quantitative electron microscopy, stereology, structural plasticity

**Introduction**

Dystrophin is a 427-kDa spectrin-like cytoskeletal protein that links a complex of intrinsic membrane proteins (dystrophin-associated glycoprotein complex) to the actin-based cytoskeleton in both muscle and brain cells and shows tissue-specific interactions with membrane receptors and/or ion channels (Blake and Kröger 2000). Dystrophin loss is responsible for Duchenne muscular dystrophy (DMD), an X-linked neuromuscular disease characterized by progressive muscle degeneration and nonprogressive cognitive deficits. DMD patients display variable degree of cognitive impairment ranging from mild deficits in verbal skills, selective attention, and memory performance to mental retardation with IQs below 70 (Billard et al. 1992; Hinton et al. 2001; Anderson et al. 2002). It has long been suggested that genetic loss of dystrophin is responsible for part of these deficits, as brain dystrophin is normally expressed in brain structures involved in diverse cognitive functions, such as the hippocampus, neocortex, and cerebellum (Gorecki et al. 1991; Lidov et al. 1993). Although macroscopic brain alterations such as ventricular enlargement and cortical atrophy have been reported in some DMD patients, brain autopsies and imaging studies have not led to consistent results, and a possible link between dystrophin loss and gross and/or ultrastructural brain abnormalities remains unclear (for reviews, see Septien et al. 1991; Anderson et al. 2002). However, the detection of glucose hypometabolism in hippocampus, cerebellum, and sensorimotor cortex, as well as dendritic abnormalities and of nonspecific electroencephalographic (EEG) disturbances, suggest altered synaptic function (Jagadha and Becker 1988; Anderson et al. 2002; Lee et al. 2002), an hypothesis consistent with the enrichment of dystrophin in postsynaptic densities (PSDs) in both human and mouse brains (Lidov et al. 1990; Kim et al. 1992, 1995).

Numerous studies in the dystrophin-deficient mdx mouse further support a role for dystrophin in brain and synapse function. Dystrophin-deficient mdx mice show specific learning and memory deficits (Muntoni et al. 1991; Vail lend et al. 1995, 1998, 2004), altered neuronal calcium homeostasis (Hopf and Steinhardt 1992), enhanced sensitivity of cornu ammonis (CA1) hippocampal neurons to hypoxia (Mehler et al. 1992), and morphological alterations of some cortical neurons (Sbriccoli et al. 1995). The absence of dystrophin in mdx mice leads to a marked reduction in the number and size of γ-aminobutyric acid (GABA) receptor (GABA\(_{\AA}\)R) clusters in hippocampal area CA1 and cerebellum (Knuesel et al. 1999). Recent studies underscored that dystrophin is a PSD component of central inhibitory synapses involved in long-term stabilization of membrane GABA\(_{\AA}\)R clusters and the organization of GABAergic networks (Knuesel et al. 2001; Brüning et al. 2002; Lévi et al. 2002), suggesting that altered GABAergic function might play a key role in the cognitive deficits associated with dystrophin deficiency. We previously showed that mdx mice have abnormal enhancement of CA1 hippocampal long-term potentiation (LTP), and pharmacological analyses suggested that altered GABAergic function may be responsible for the altered threshold of N-methyl-D-aspartic acid receptor (NMDAR) activation and abnormal activity-dependent synaptic plasticity (Vail lend and Billard 2002; Vail lend et al. 2004). Modifications in the number, proportion, and morphology of synapses are thought to underlie changes in synaptic efficacy associated with normal development, lesion repair, synaptic plasticity, learning, and memory processes (Geinisman 2000; Marrone and Petit 2002), with major contribution to a variety of synaptic functions.
of inherited forms of mental retardation and cognitive dysfunctions in mice (for review, see Vailland et al. 2008). Although congenital alterations in GABA\(_A\)-R clustering in dystrophin-deficient brains may have important consequences for synapse ultrastructure and integrity of hippocampal synaptic networks, this to date has not been studied in detail.

In the present study, we used quantitative serial electron microscopy (EM) and physical dissector stereological methods to evaluate the density and PSD length of distinct synapse subtypes in the CA1 hippocampal area. Asymmetric excitatory, symmetric inhibitory, axospinous, axodendritic, axosomatic synapses, and multiple spine boutons (MSBs) were analyzed at 2 distinct levels of CA1 apical dendritic arbors in proximal stratum radiatum enriched in dystrophin and GABA\(_A\)-R clusters and in distal radiatum, a region of wide interest to investigate the structural integrity of synaptic networks involved in synaptic plasticity. In the same tissue samples, we also estimated the relative density of CA1 pyramidal cells to identify putative neuronal loss and to determine the synapse-to-neuron ratio. Finally, volumetric in vivo magnetic resonance imaging (MRI) and histological volume estimation of distinct hippocampal regions by light microscopy was conducted to weight any ultrastructural abnormalities against possible macroscopic brain alterations.

Materials and Methods

**Animals**

Mice of the C57BL/10ScSn-Dmd\(^{m2}J\) (mdx) mutant and C57BL/10J control strains were bred in our own colony as described (Vailland et al. 2004). Heterozygous mdx females were mated with control males to provide the mdx and wild-type (WT) littermate male mice used in these experiments. A total of 31 male mice aged at least 6 months were used in this study (WT, \(n = 17\); mdx, \(n = 14\)). Siblings were kept in the same cage (2–4 mice per cage) under a 12-h light–dark cycle (lights on: 7.00 AM) with food and water ad libitum. Mouse genotype was determined as previously described (Vailland et al. 2004). Plasma analyses were undertaken to reveal high levels of serum creatine kinase in the mutants, which is a characteristic of the early phase of the disease in both DMD patients and mouse models of DMD, and additional postmortem histological analysis of quadriceps muscle confirmed that mutants had characteristic changes within the muscle fibers (i.e., centralization of the nuclei). All experiments were conducted blind to the genotype and in accordance with the European Communities Council Directive of 24 November 1998.

**Magnetic Resonance Imaging**

Mice (>6 months old) were imaged under isoflurane anesthesia (induction 2%; flow rate 0.8% in 50% \(\text{O}_2\), 50% \(\text{N}_2\)) controlled on the basis of respiratory parameters. The body temperature was maintained at 37°C using heated mattress. MRI measurements were performed on a 7-T horizontal bore magnet (Oxford, UK) driven by Paravision (Bruker\(^\text{®}\), Wissembourg, France) and equipped with a 300 mT/m actively shielded gradient device (internal diameter [ID] = 90 mm, Bruker). For MRI examination, the animal's head was introduced in a "bird-cage" 1-H coil (ID = 36 mm). Images were acquired perpendicular (axial) and parallel (coronal) to the brain anteroposterior axis. The mouse brain was positioned using scouting gradient-echo images in the 3 orthogonal directions. After the shimming process, Bruker sequences were acquired with fat suppression as 2D axial-Rapid acquisition with relaxation enhancement (RARE) (TR/TE eff = 4000/40 ms [repetition time/effective echo time]; rare factor = 8; 2 averages; 31 contiguous 0.5-mm-thick sections; 30 × 30 mm field of view; 256 × 256 matrix; pixel size = 117 \(\mu\)m\(^2\)) and 3D coronal TurboRARE sequences (TR/TE eff = 2500/42.2 ms; rare factor = 16; 30 × 15 × 15 mm field of view; 256 × 128 × 128 matrix; voxel size = 117 \(\mu\)m\(^3\)). The total time spent by the mouse in the magnet was around 50 min. After each experiment, mice were recovered from anesthesia and returned to their home cages with free access to food and water.

**Tissue Preparation**

For light microscopy, mice were deeply anesthetized by intraperitoneal injection of sodium pentobarbital and then intracardially perfused with a 4% paraformaldehyde fixative solution in 0.1 M phosphate buffer, pH 7.3–7.4. Brains were cryoprotected in 30% sucrose and then quickly frozen in 2-methylbutane (Roth, Karlshruhe, Germany) cooled with dry ice to −40°C. Coronal serial sections (30-μm thick) were collected and stored at −80°C.

For EM, mice aged 6 months were perfused with a washing solution (0.1 M cacodylate buffer, pH 7.3–7.4, 1 mM) followed by a fixative solution (2% paraformaldehyde, 2% glutaraldehyde, 0.002% CaCl\(_2\), 0.15% picric acid in 0.1 M cacodylate buffer, pH 7.3–7.4). Brains were quickly removed from the skull, and −3-mm-thick slabs, containing the whole hippocampus, were cut with the aid of a brain matrix (Harvard Apparatus, Les Ulis, France). Both hippocampi were postfixed in the same fixative solution overnight at 4°C. An average of fifteen 250-μm-thick sections of the right and left hippocampi were made in the coronal plane with a vibrating microtome (VT 1000 S, Leica, Vienna, Austria). The anterodorsal hippocampus was dissected under stereo-microscope, and tissue samples were then osmicated in 1% buffered osmium tetroxide, soaked in 1% osmium tetroxide and potassium ferrocyanide (10 mg/mL) in 0.1 M cacodylate buffer, rinsed in buffer and water, contrasted in aqueous solution of uranyl acetate (1%) overnight at 4°C, and dehydrated in graded ethanol. Finally, the tissue was flat embedded in Epon 812 (embed-812, EMS Co. Ltd, Fort Washington, PA) and polymerized at 60°C for 48 h. Resin blocks were pseudorandomly selected within a given range of coordinates (bregma −1.70 to −2.04) (Paxinos and Franklin 2001). Serial semithin sections (2-μm thick) were then cut on a Leica UC7 ultramicrotome (Leica), collected on superfrost plus (Roth) glass slides, and stained with toluidine blue for neuron number quantification. For synapse number estimation, the same blocks were then trimmed to a trapezoidal-like region (~1 mm\(^2\)) containing both the CA1 pyramidal cell bodies and the entire apical dendritic field of CA1. Ribbons of at least 4 ultrathin serial sections (70 nm thick) of silver gray interference color were collected on 2 × 1-mm-slot grids coated with a formvar–carbon support film and counterstained with uranyl acetate and lead citrate.

**Brain Volumetric Analyses**

MRI areas and volumes were estimated on coronal and axial brain sections using the AMIRA software (TGS Inc., San Diego, CA) (WT, \(n = 4\); mdx, \(n = 4\)). Analyses were performed as previously described (Sebiri et al. 2008). Five main brain areas were delineated according to the cerebellum (including colliculus), olfactory bulbs, whole cortical area, ventricles, and hippocampus (Fig. 1). Brain volumes were extracted by multiplying areas by slice thickness.

The volume of the hippocampus was also estimated by histological analysis in formalin-fixed brain frozen sections (WT, \(n = 4\); mdx, \(n = 4\)). The first and last hippocampal sections (30 μm thick) were identified according to mouse brain atlas (Paxinos and Franklin 2001). For systematic sampling, the first serial section containing hippocampus was randomly chosen within the 6 first sections. Then, one in every 6 sections were stained with cresyl violet and photographed at 1.25× with a Sony DFW-X700 digital camera (Sony Co., Tokyo, Japan) coupled to Olympus BX60 light microscope (Olympus Optical Co., Hamburg, Germany). The volumes of the whole hippocampus (bregma −0.94 to −4.04) and of anterodorsal hippocampus (bregma −0.94 to −2.30) were determined by boundary tracing of cross-sectional areas. Similarly, serial sections photographed with a ×10 objective were used to estimate unilateral volumes of specific regions of interest for both the synapse and neuron counts in our study, that is, the anterodorsal CA1 subfield, stratum radiatum, and stratum pyramidale (Fig. 2a). Calibrated digital images were imported into the ImageJ software (http://rsb.info.nih.gov/ij/), and cross-sectional areas were outlined using a graphic tablet (Intuos3 Pen Tablet, Wacom Co. Ltd, Saitama, Japan). The volume was determined as the sum of the traced areas multiplied by the distance between sampled sections (180 μm) (Gundersen and Jensen 1987).
**Figure 1.** MRI evaluation of brain volumetric integrity in WT (top) and mdx mice (bottom). Sample MRI coronal images ([a], WT; [d], mdx), corresponding 2D cutting planes with brain regions delineated according to mouse brain atlas ([b], WT; [e], mdx) and 3D reconstructions of mouse brains ([c], WT; [f], mdx), show comparable brain gross anatomy in the 2 genotypes. Brain regions considered in the analysis are represented by distinct colors as follows: ventricles, red; cortex, yellow ([b], [e]) or translucent golden gray ([c], [f]); hippocampi, orange; other miscellaneous subcortical structures, white; olfactory bulbs, green; cerebellum/colliculus, purple.

**Figure 2.** Light and electron micrographs showing methodological aspects of the hippocampal volume estimation and quantification of neuron and synapse density. (a) Micrograph of violet cresyl-stained coronal frozen section (30 μm) of the anterodorsal hippocampus (mdx mouse) showing the boundaries of CA1 field (solid line), stratum pyramidale, and stratum radiatum (dotted lines at top and bottom of image, respectively) as traced for volume estimations. DG, dentate gyrus. Scale bar, 350 μm. (b) Pair of serial adjacent toluidine blue-stained semithin sections (2 μm) of CA1 pyramidal cell layer with a test grid randomly overlaid on each section used for cell density estimation with physical disector method. Arrows indicate neuron profiles, which appear in one section (reference) but not in the adjacent section (lookup). Scale bar, 20 μm. (c) Low-power micrograph (56×) of a serial ultrathin section (60 nm) showing the reference limits for control measurements of stratum radiatum length. Lighter areas correspond to the electron-beam traces resulting from picture shooting along the proximal (top) and distal radiatum (bottom). Solid versus dashed arrows indicate stratum radiatum length and distance between proximal and distal areas, respectively. p, stratum pyramidale; r, stratum radiatum; l–m, stratum lacunosum-moleculare. Scale bar, 100 μm. (d) Series of aligned ultrathin sections to show the principle of physical disector method for synapse quantification. Synapses are counted in all pairs of B and C sections (as in b). The rectangular frame is the counting frame. Arrows show synapses that are counted because they are present in one section but not in the adjacent one. Synapses touching solid lines of the counting frame were discarded. The first and last sections (A and D) were used as additional guard sections to improve identification of synapse subtypes in the z axis. Scale bar, 0.5 μm. (e) Sample series of 4 sections showing the benefits of using additional guard sections to improve identification and quantification of perforated synapses. Arrows show a synapse properly counted as perforated based on the perforation of the PSD (arrowhead) only evident in the first guard section (A). Scale bar, 0.5 μm.
To evaluate the integrity of hippocampal regions in the same conditions as for ultrastructural study of CA1 hippocampal synapses, the volume of hippocampus was also estimated from thick (250 μm) vibratome sections in 8 mouse brains processed as for EM (WT, n = 4; mdx, n = 4). Serial sections were floated on a bath filled with 0.1 M cacodylate buffer (pH 7.3–7.4) at room temperature and then placed onto clean slides to take pictures. Cross-sectional areas were outlined for every section containing the hippocampus and the values converted into volume estimation of the whole and anterodorsal hippocampus as above.

**Quantification of CA1 Hippocampal Neurons**

The numerical density (Nv) of pyramidal cells in anterodorsal CA1 was estimated by applying the disector method (Sterio 1984) to pairs of adjacent serial semithin sections. On average, 7 pairs of sections per animal with a 6-μm interpair separation were photographed with a 40× objective and aligned using the IGL Align software (http://synapses-web.org/tools/index.xtm). Quantification was performed with the aid of several macros adapted for stereology estimations in ImageJ software. Briefly, the CA1 pyramidal layer was delimited, and a test grid (rectangular unbiased counting frames of 350 μm2) was randomly overlaid on each pair of sections (Fig. 2b) to perform a uniform sampling of equally spaced counting frames. Frames with the upper left corner falling outside the pyramidal layer were discarded. On average, 102 neurons per animal were counted in 455 frames using the top of the counting frames (13,650–18,550 μm2) and the following parameters derived: 1) The number of neurons per counting, the boundaries of the stratum pyramidale were delimitated by the disector method when Rsb.info.nih.gov/ij/) (DeCoster 2007).

**Synapse Quantification**

The numerical density (Nv) of synapses was estimated from serial ultrathin sections. Following the procedure described by Eyre et al. (2003), each serial section of the ribbons mounted on slot grids was labeled with a letter (A, B, C, and D) and as shown in Figure 2d. Two distal series mounted on different grids were photographed taking at least 30 images from each section. For uniform sampling, an object or structure (e.g., blood vessel, dendritic segment, or cross-sectioned myelinated axon) present in all sections was taken as the first reference point. Images were converted to BMP16 bits gray scale files, aligned with the IGL software, and quantified with ImageJ. The physical disector method (described above) was applied to all pairs of images labeled B and C. The first and last images of a ribbon (i.e., A and D) were used as additional guard sections to facilitate the identification of synapses in the z axis (see example in Fig. 2e). For each mouse, stereological quantification of synapses was performed on an average of 33 disector pairs for the proximal radius and 26 disector pairs for distal radius. The reference and lookup sections were designated, and a counting frame (408 μm2) was randomly positioned at the top of the counting unit (Q) corresponding to the synaptic profiles with clearly identified PSD, synaptic cleft, and presynaptic component with synaptic vesicles (Mayhew 1996). The height of the disector b corresponded to the mean thickness of ultrathin sections calculated by the minimum fold method (Weibel 1979) and resulted in a mean value of 60 nm. The sampled area a corresponded to the total surface of the neuropil covered by the counting frames (1990 μm2 in proximal radius and 1503 μm2 in distal radius). Systematic sampling may introduce some bias when counts and reference area estimations are made in regions that contain large dendritic profiles and cell somata portions (DeFelipe et al. 1999). Because this was typically observed in the proximal radius, the dendritic and somata portions in this area were outlined and the resulting area (–408 μm2) subtracted from the total sampled area. In accordance with the disector method, the number of synaptic profiles that were present in the reference section but disappeared in the lookup section was divided by the disector volume (i.e., a × b, see above) to obtain the total number of synapses (i.e., asymmetric + symmetric) per cubic microns. For large, scarce, or complex sets of synaptic profiles (i.e., perforated, axodendritic, axosomatic synapses, and MSBs), the number of which is underestimated by the disector method when b ranges 60–70 nm, single-section quantification (i.e., 2D) was used to derive the number of synaptic types per square microns and optimize quantification and statistical comparisons (DeFelipe et al. 1999; Shi et al. 2005). To take into account a possible bias due to differential tissue shrinkage, the number of synapses was also normalized to the number of CA1 pyramidal neurons counted in the same resin blocks and was expressed as synapse-to-neuron ratio.
PSD Length
For all synapses identified in the proximal and distal radiatum, the PSD length was measured from single ultrathin sections. Measures were taken with ImageJ software in all frames of sections B because this second section of the ribbon was never distorted during digital aligning of serial images. As in Desmond and Levy (1986a) when the PSD was perforated, the sum of the individual trace lengths was considered (e.g., see Fig. 4).

Statistical Analysis
Data are presented as means ± standard errors of the mean (SEMs). All data successfully passed normality and equal variance tests (SigmaStat 2.0, SPSS Inc., Chicago, IL; P > 0.05) and were therefore analyzed with parametric tests. Volumes and neuron density were analyzed with 1-way analysis of variance (ANOVA) with genotype (WT and mdx) as the between-subject factor. Following the recommendations of DeFelipe et al. (1999) for the analyses of synapse density in different neuropil regions, the synapse-to-neuron ratio and the number of synapses per surface area recorded in proximal and distal radiatum were analyzed separately. For PSD length, a parameter that is not affected by differences between sampled areas, the region of interest (i.e., proximal vs. distal radiatum) was used in 2-way ANOVAs as a within-subject factor. P values <0.05 were considered statistically significant.

Results

In Vivo MRI and Histological Evaluation of Brain Integrity
We first explored possible changes in the volume of brain ventricles and of various brain structures in mdx mice to determine whether dystrophin loss results in macroscopic brain alterations as reported in a subset of DMD patients (Anderson et al. 2002). Table 1 shows volumes of different brain structures derived from in vivo MRI and “postmortem” histological analyses. MRI was performed on 4 mdx and 4 WT littermate mice of comparable age (>6 months). The volumes of the whole brain and ventricles were comparable between genotypes (P > 0.05). Similarly, there were no significant changes in the volumes of hippocampus and whole cortical areas. Finally, no significant differences were detected for the volume of the olfactory bulbs and cerebellum (P > 0.05). MRI analysis therefore revealed no major alteration of brain anatomy in mdx mice and no significant changes in brain structures that normally express dystrophin (cortex, hippocampus, and cerebellum) (Fig. 1).

Because preserved hippocampal volume is an important factor for reliable estimation of synaptic density in EM studies, we further analyzed the volumes of distinct hippocampal subregions in postmortem histological sections from 2 additional groups of mdx and WT mice. In the first group, brains were fixed with 4% paraformaldehyde (frozen-section group; mdx, n = 4; WT, n = 4), whereas in the second group, they were processed as for EM (vibratome-section group; mdx, n = 4; WT, n = 4). As shown in Table 1, hippocampal volumes estimated under such experimental conditions were smaller compared with the in vivo MRI analysis, which likely reflects tissue shrinkage due to the fixation and dehydration processes. However, hippocampal volumes estimated from both frozen and vibratome sections were comparable between genotypes.
The volume of lateral ventricles was not assessed in histological sections, as ventricular spaces are prone to mechanical distortion after sectioning, handling, and cover-slippping. The volume of the anterodorsal hippocampus was then determined in frozen sections, as it specifically corresponds to the portion of the hippocampus used for EM studies below. The spatial resolution of MRI measurements was not optimal for this purpose. As shown in Table 1, the volume of the anterodorsal hippocampus was comparable between genotypes in frozen sections ($P > 0.05$). The volume of the anterodorsal hippocampus was smaller when estimated from vibratome compared with frozen sections, which was likely due to the greater thickness and reduced number of sections containing this hippocampal portion in vibratome sections. However, the lack of difference between genotypes was confirmed in vibratome sections ($P > 0.05$), confirming with Table 1.

<table>
<thead>
<tr>
<th>Brain Structure</th>
<th>MRI (WT)</th>
<th>MRI (mdx)</th>
<th>Vibratome sections (WT)</th>
<th>Vibratome sections (mdx)</th>
<th>Frozen sections (WT)</th>
<th>Frozen sections (mdx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole brain</td>
<td>465.87 ± 6.82</td>
<td>473.19 ± 9.23</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Lateral ventricles</td>
<td>24.93 ± 4.17</td>
<td>33.08 ± 10.30</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Olfactory bulbs</td>
<td>22.60 ± 1.25</td>
<td>23.30 ± 1.06</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>63.99 ± 0.76</td>
<td>66.51 ± 1.74</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cortices</td>
<td>158.76 ± 2.58</td>
<td>158.79 ± 3.22</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>25.78 ± 1.26</td>
<td>26.04 ± 0.96</td>
<td>19.80 ± 0.49</td>
<td>19.99 ± 0.52</td>
<td>18.48 ± 0.46</td>
<td>19.56 ± 0.43</td>
</tr>
<tr>
<td>AD hippocampus</td>
<td>—</td>
<td>—</td>
<td>5.96 ± 0.47</td>
<td>5.69 ± 0.56</td>
<td>7.68 ± 0.25</td>
<td>7.57 ± 0.45</td>
</tr>
<tr>
<td>CA1 field</td>
<td>—</td>
<td>—</td>
<td>0.946 ± 0.060</td>
<td>0.912 ± 0.067</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CA1 pyramidal layer</td>
<td>—</td>
<td>—</td>
<td>0.086 ± 0.004</td>
<td>0.086 ± 0.006</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CA1 stratum radiatum</td>
<td>—</td>
<td>—</td>
<td>0.373 ± 0.028</td>
<td>0.365 ± 0.031</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Note: Estimated volumes in cubic millimeters are expressed as mean ± SEM (mdx, $n = 4$; WT, $n = 4$; for each condition). For anterodorsal (AD) CA1 hippocampal field, CA1 pyramidal cell and apical dendritic (stratum radiatum) layers, the volumes were estimated unilaterally.

($P > 0.05$). The volume of lateral ventricles was not assessed in histological sections, as ventricular spaces are prone to mechanical distortion after sectioning, handling, and cover-slippping. The volume of the anterodorsal hippocampus was then determined in frozen sections, as it specifically corresponds to the portion of the hippocampus used for EM studies below. The spatial resolution of MRI measurements was not optimal for this purpose. As shown in Table 1, the volume of the anterodorsal hippocampus was comparable between genotypes in frozen sections ($P > 0.05$). The volume of the anterodorsal hippocampus was smaller when estimated from vibratome compared with frozen sections, which was likely due to the greater thickness and reduced number of sections containing this hippocampal portion in vibratome sections. However, the lack of difference between genotypes was confirmed in vibratome sections ($P > 0.05$), confirming with
different evaluation methods that hippocampal volume is not altered in mdx mice. Frozen sections were further used to specifically evaluate the volumes of the CA1 subfield, CA1 radiatum, and pyramidal layers where synapse and neuron densities were subsequently investigated. Consistent with the results above, no group differences were detected in any of these hippocampal subregions ($P > 0.05$; Table 1).

**CA1 Pyramidal Cell Density and Nuclear Morphology**

Previous studies in mdx mice suggested significant cell loss and altered shape of pyramidal cells in some brain structures (Sbriccoli et al. 1995; Carretta et al. 2001), but this had not been yet investigated in the hippocampus. Here we compared the numerical density of CA1 pyramidal cells using the disector method in semithin sections of brain tissue prepared for EM analysis (Fig. 2b) in 4 mdx and 4 WT littermate mice. As shown in Table 2, the number of neurons per cubic millimeters was comparable in the 2 groups of mice. In addition to neuron density, we analyzed the mean nuclear area and circularity of pyramidal cells in both genotypes because these parameters have been shown to be altered in apoptotic neurons that can be morphologically identified by neurite retraction, rounder nuclei, cell body, and nucleus shrinkage (Kasschke et al. 2001; Daniel and DeCoster 2004). However, as shown in Table 2, the nuclear area and circularity were not significantly different between genotypes ($P > 0.05$). The relative neuron packing was also calculated because this parameter, defined as the percentage of area covered by cell nuclei over the sampled area, could reveal alterations in cellular organization independently of neuronal loss (Carretta et al. 2004). No difference was found between mdx and control mice ($P > 0.05$; Table 2). These results, consistent with the unaltered volume of CA1 pyramidal cell layer, suggest that the lack of dystrophin does not induce neuron loss, changes in cellular organization, or apoptosis-related structural modifications in the anterodorsal hippocampal CA1 region.

**Synaptology**

**Tissue Ultrastructure and Qualitative EM Inspection**

Tissue was considered perfectly preserved when no signs of extended extracellular spaces, swelling of mitochondria, or selective swelling of dendrites were observed (Tao-Cheng et al. 2007). Qualitative inspection was also performed to evaluate typical signs of synapse pathology associated with neurocognitive disorders, such as distorted spine shape, electron-dense spines, varicosity formation, or hypertrophied spine apparatus (see Fiala et al. 2002). This analysis revealed no sign of synapse pathology in mdx mice. We also used low-power micrographs taken from ultrathin sections after synapse quantification to confirm that the CA1 stratum radiatum did not undergo differential shrinkage between genotypes. Figure 2c shows the electron-beam traces that were used as references to measure the distance from the CA1 pyramidal cell bodies to the limits of the proximal and distal regions. The radiatum length (microns) was comparable in WT (248.25 ± 19.04) and mdx mice (260.11 ± 13.84) ($P > 0.05$), thus confirming our above histological analysis. The distance (microns) from cell bodies to the limits of the proximal radiatum did not differ between WT (26.39 ± 1.39) and mdx mice (31.32 ± 4.66) ($P > 0.05$). As was the distance (microns) to the distal limit in WT (207.96 ± 16.53) and mdx mice (199.34 ± 16.73) ($P > 0.05$). This ensures that quantifications in the proximal and distal radiatum can be performed in comparable conditions among animals and genotypes.

At the ultrastructural level, a range of functionally different synapse subtypes were characterized according to standard morphological features (see "synapse nomenclature" in the Materials and methods). As shown in Table 3, asymmetric excitatory synapses mainly targeted dendritic spines (axospinous synapses) and to a much lesser extent dendritic shafts (axodendritic asymmetric). Symmetric inhibitory synapses were mainly located on dendritic shafts or somata portions and were in general larger (Table 4) and less numerous than asymmetric synapses (Table 3).

**Synapse Density**

Synapse density was estimated in both the proximal and distal radiatum of CA1 (mdx, $n = 4$; WT, $n = 4$). As shown in Table 3, in the proximal radiatum, the total number of synapses (i.e., asymmetric + symmetric) expressed as synapse-to-neuron ratio was similar in mdx and WT mice ($P > 0.05$). The density of each synaptic subtype expressed as synapses per 100 $\mu$m$^2$ was then compared between genotypes. The density of axosphomic synapses, only present in proximal radiatum, that of axospinous asymmetric excitatory synapses, perforated and nonperforated excitatory synapses, and MSBs were not statistically different between genotypes ($P > 0.05$). In contrast, mdx mice showed a large (42.85%) and statistically significant increase in the density of axodendritic symmetric inhibitory synapses as compared with the WT controls ($P < 0.02$) (Fig. 3). This selective change in the number of inhibitory synapses was the only significant change in synapse density detected in mdx mice in the proximal radiatum. The comparable density of perforated synapses and MSBs in the 2 groups of mice ($P > 0.05$) suggests that the number of excitatory synapses typically associated with activity-dependent plasticity (Geinisman 2000) was not altered by the lack of brain dystrophin in mdx mice.

In distal radiatum, the synapse-to-neuron ratio for total synapses was comparable in the 2 genotypes ($P > 0.05$), suggesting that dystrophin loss is not associated with a net change in synapse density in either distal or proximal radiatum areas (Table 3). As compared with proximal radiatum, both genotypes showed a reduced percentage of symmetric inhibitory synapses in distal radiatum and a consequent increase in the percentage of asymmetric excitatory synapses. This is in line with the common observation that asymmetric synapses are enriched in the distal stratum radiatum. In this specific region, the density of axodendritic symmetric synapses was comparable between genotypes ($P > 0.05$). This suggests that the increased density of inhibitory synapses in mdx mice is restricted to the proximal dendrites of CA1 pyramidal cells.

---

### Table 2

Pyramidal neuron density and nuclear parameters in CA1 anterodorsal hippocampus

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>mdx</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurons/mm$^3$</td>
<td>165742.45 ± 27264.84</td>
<td>176361.01 ± 7669.50</td>
<td>0.721</td>
</tr>
<tr>
<td>Nuclear area ($\mu$m$^2$)</td>
<td>63.36 ± 1.86</td>
<td>67.62 ± 4.27</td>
<td>0.395</td>
</tr>
<tr>
<td>Area covered by nucle (%)</td>
<td>26.559 ± 1.269</td>
<td>24.689 ± 1.522</td>
<td>0.372</td>
</tr>
<tr>
<td>Nucleus circularity</td>
<td>0.903 ± 0.008</td>
<td>0.908 ± 0.005</td>
<td>0.611</td>
</tr>
</tbody>
</table>

Note: There was no difference between genotypes (WT, $n = 4$; mdx, $n = 4$) for these parameters ($P$ values from 1-way ANOVAs).

---

Downloaded from https://academic.oup.com/cercor/article-abstract/19/4/876/282778 by guest on 05 August 2018
Alterations in the size of synapses have been linked to different synaptic contacts and ultrastructural changes in the PSD of hippocampal CA1 proximal radiatum with dystrophin deficiency. Previous biochemical and electrophysiological studies in mdx mice have highlighted the functional importance of brain dystrophin for the stabilization of postsynaptic membrane GABA$_A$-R clusters containing the a2 subunit in hippocampus (Knuesel et al. 1999) and for the expression of CA1 hippocampal LTP (Vaillend et al. 2004), suggesting a possible role for GABAergic inhibitory processes in the genesis of cognitive deficits in DMD patients. Here we used a combination of in vivo MRI, light, and EM approaches to determine whether and how congenital loss of dystrophin may affect the cellular and synaptic organization of hippocampal networks in mdx mice. We demonstrate that dystrophin deficiency in hippocampus is associated with an increased number of inhibitory synaptic contacts and ultrastructural changes in the PSD of excitatory synapses.

**Discussion**

Previous biochemical and electrophysiological studies in mdx mice have highlighted the functional importance of brain dystrophin for the stabilization of postsynaptic membrane GABA$_A$-R clusters containing the a2 subunit in hippocampus (Knuesel et al. 1999) and for the expression of CA1 hippocampal LTP (Vaillend et al. 2004), suggesting a possible role for GABAergic inhibitory processes in the genesis of cognitive deficits in DMD patients. Here we used a combination of in vivo MRI, light, and EM approaches to determine whether and how congenital loss of dystrophin may affect the cellular and synaptic organization of hippocampal networks in mdx mice. We demonstrate that dystrophin deficiency in hippocampus is associated with an increased number of inhibitory synaptic contacts and ultrastructural changes in the PSD of excitatory synapses.

**Dystrophin Loss Does Not Alter Gross Brain Anatomy**

The presence of enlarged brain ventricles and slight cortical atrophy has been reported in a subset of DMD patients (Septien et al. 1991), suggesting white matter atrophy or neuronal cell...
death. Brain imaging and autopsy studies have shown inconsistent structural or volumetric differences in DMD patients compared with healthy controls, and no correlation between ventricle dilation and verbal intelligence could be demonstrated (Al-Qudah et al. 1990), which led to the general conclusion that DMD is not associated with gross anatomical brain abnormalities (for review, see Dubowitz and Crome 1969; Anderson et al. 2002). However, several methodological factors that could be sources of variability or misleading results have been pointed out, such as the small size of experimental groups, the patients’ young age, and the heterogeneity of the mutations that may differentially affect expression of other dystrophin gene products resulting from distinct internal promoters (Lidov 1996). A reduced number of neurons has been reported in the motor cortex of mdx mice (Sbriccoli et al. 1995), which is potentially compatible with cortical atrophy and/or ventricle dilation. However, no systematic study of mdx brain anatomy has been undertaken to confirm this hypothesis. Our present in vivo structural MRI investigation provides the first evidence that dystrophin loss is not associated with significant volumetric changes in the mdx mouse brain. Volumes of the whole brain, ventricles, and selected brain regions that normally express dystrophin, that is, neocortex, hippocampus, and cerebellum, were not significantly modified in mdx compared with WT littermate mice. Detection of subtle alterations in cortical cell number or distribution in specific cortices (Carretta et al. 2003) was beyond the resolution of this MRI analysis. Nevertheless, our results suggest that such alterations linked to the loss of brain dystrophin in mdx mice, although they might contribute to cognitive dysfunction, are not associated with gross brain volumetric abnormalities. Demonstrating the absence of volumetric changes in the hippocampus of this mutant was particularly important as it is a key structure in learning and memory processes (Squire 1992) and as abnormal synaptic plasticity has been reported in this brain area in mdx mice (Vaillend et al. 1998, 1999, 2004). Moreover, it is a prerequisite for reliable estimations of hippocampal neuron and synapse densities. Our histological analyses in both standard formalin-fixed frozen sections and in tissue sections processed as for EM confirmed the results of the MRI study. In addition, detailed volumetric analysis of the anterodorsal hippocampus, CA1 subfield, CA1 pyramidal cell, and dendritic layers did not reveal any difference between genotypes, thus confirming that the gross anatomy of the hippocampus is preserved in mdx mice.

Because previous studies in mdx mice revealed significant cell loss and altered pyramidal cell morphology in the corticospinal tract (Sbriccoli et al. 1995), the question arose as to whether the number of pyramidal cells could also be affected in the hippocampus of this mouse model. To address this question, we first estimated the relative density of CA1 pyramidal cells by histological examination on semithin sections and stereological methods to estimate the relative density of CA1 hippocampal synapses. The volume of CA1 stratum radiatum, the global synapse density, and the synapse-to-neuron ratio were unaffected in mdx mice, suggesting that dystrophin loss does not alter the basic processes of early synaptogenesis. Examination of the relative distribution of distinct synapse subtypes, however, revealed a selective and significant increase in the number of axodendritic symmetric inhibitory synapses in mdx mice, suggesting a major reorganization of inhibitory circuits as a consequence of impaired GABA<sub>α</sub>-R clustering.

Reorganization of GABAergic synapses has been observed in several animal models characterized by a compromised balance between excitation and inhibition, such as in models of temporal lobe epilepsy (Knuesel et al. 2001) and in the mouse barrel cortex after increasing excitatory transmission by whisker stimulation (Knott et al. 2002). This indicates that structural plasticity at GABAergic synapses can occur to maintain excitatory/inhibitory balance (Liu 2004). The mechanisms underlying these compensatory responses are not fully understood. They may involve changes in the distribution of interneurons targeting principal cells and/or upregulation of different GABA<sub>α</sub>-R subunits and of the scaffolding protein gephyrin, as shown in the dentate gyrus after medial entorhinal cortex lesion (Simbürger et al. 2001) and in specific cerebellar and hippocampal interneurons of the α1 subunit–null mouse (Fritschy et al. 2006; Schneider Gasser et al. 2007). In the hippocampus of mdx mice, increased inhibitory synapse density was selectively found in proximal radiatum in which the CA1 hippocampal subfield of mdx mice. The results of our MRI study further support the idea that neuron loss in mdx mice cannot be generalized to all cortices. Moreover, such changes do not seem to occur in the anterodorsal part of the hippocampus investigated here, suggesting that the neuronal substrate of altered synaptic plasticity in mdx mice (Vaillend et al. 2004) is not at the cellular level but likely relies on altered synaptic or subcellular mechanisms.

**Increased Density of Axodendritic Inhibitory Synapses onto Dystrophin-Deficient Pyramidal Neurons: A Compensatory Mechanism?**

How a congenital loss of dystrophin and the resulting reduction in number and size of GABA<sub>α</sub>-R clusters in mdx mice (Knuesel et al. 1999) may impact on the organization of hippocampal synaptic networks and possibly mediate behavioral and cognitive dysfunctions? Cognitive function depends on the plastic nature of the nervous system expressed through its capacity to modify the number, proportion, shape, and size of synapses in an activity-dependent manner. Regulation of cytoskeleton dynamics and membrane receptor trafficking are largely involved in such processes (Matus 1999; Marrone and Petit 2002), and dysfunctions of these mechanisms underlie various forms of inherited mental retardation associated with changes in dendritic arbor complexity, synapse number, and ultrastructure (for reviews, see Fiala et al. 2002; Vaillend et al. 2008). We therefore investigated the ultrastructure and density of a variety of synapse subtypes in the hippocampus of mdx and WT littermate mice.

An initial qualitative inspection of hippocampal tissue did not reveal clear signs of synapse pathology in mdx mice, in contrast to other mouse models of neurodevelopmental disorders (Fiala et al. 2002). We thus performed a thorough quantitative EM study using serial ultrathin sections and stereological methods to estimate the relative density of CA1 hippocampal synapses. The volume of CA1 stratum radiatum, the global synapse density, and the synapse-to-neuron ratio were unaffected in mdx mice, suggesting that dystrophin loss does not alter the basic processes of early synaptogenesis. Examination of the relative distribution of distinct synapse subtypes, however, revealed a selective and significant increase in the number of axodendritic symmetric inhibitory synapses in mdx mice, suggesting a major reorganization of inhibitory circuits as a consequence of impaired GABA<sub>α</sub>-R clustering.
dystrophin is enriched and colocalizes with α2 GABAA-R subunits, but not in the distal radiatum where dystrophin and GABAA-R subunits are not detected (Knuesel et al. 1999, 2000). This high selectivity toward the proximal dendritic layer of CA1 suggests that it could rely on the reorganization of specific subpopulations of interneurons targeting proximal dendrites of pyramidal cells (Freund 2003), which would be reminiscent of the altered distribution of neurons expressing calcium-binding proteins in some cortical areas in the mdx mouse (Carretta et al. 2003). This also strengthens our conclusion of a rearrangement of inhibitory circuits that could correspond to a homeostatic response in an attempt to maintain inhibitory drive onto dystrophin-deficient principal neurons.

Whereas dystrophin seems dispensable for initiation of GABAA-R clustering, it is believed to be involved in the stabilization of postsynaptic GABAA-R complexes through interaction of its N-terminal domain with actin cytoskeleton, probably by limiting the lateral diffusion of GABAA-Rs outside the synapse (Brüning et al. 2002; Lévi et al. 2002). The functional role of such a mechanism, whether it is important for basal inhibitory transmission or for some form of activity-dependent plasticity at GABAergic synapses remains to be elucidated (Fritschy and Brüning 2003). In hippocampus of mdx mice, the reduced clustering of α2 subunits associated with dystrophin loss does not affect inhibitory postsynaptic currents (IPSCs) evoked in the pyramidal cell layer (Graciotti et al. 2008), perhaps due to a preserved perisomatic inhibition mediated by α1-containing receptors (Freund 2003). In contrast, we previously showed that field excitatory postsynaptic potentials (EPSPs) elicited and recorded in CA1 stratum radiatum are less sensitive to the GABA A-R antagonist bicuculline in mdx compared with control mice (Vaillend and Billard 2002), which suggests a reduction of GABAA-R-mediated transmission in dendrites. Moreover, we found facilitated NMDAR activation and abnormal enhancement of CA1 hippocampal LTP in mdx mice (Vaillend et al. 1999, 2004) and showed that these alterations are sensitive to bicuculline, thus suggesting a link with reduced dendritic inhibition. Strikingly, our present data demonstrate an increased density of axodendritic, not axosomatic, inhibitory synapses in CA1 hippocampal area of mdx mice. Such a specific rearrangement of inhibitory contacts may therefore reflect a compensatory mechanism against these alterations and also points to a major role of dystrophin and α2 subunit clusters at the dendritic rather than the somatic level in hippocampal pyramidal cells. In any event, if in the absence of dystrophin the increase in axodendritic inhibitory synapse density represents an attempt to compensate an altered inhibitory drive in dendrites, the electrophysiological data suggest that this process may not lead to full compensation.

**Altered GABA Function and Enhanced Hippocampal LTP in mdx Mice: What Is the Missing Link?**

How can GABAergic alterations reported in mdx mice lead to abnormally enhanced CA1 hippocampal synaptic plasticity? First, reports have shown that GABAA-R antagonists applied on hippocampal (Vaillend and Billard 2002) and cerebellar slices (Anderson et al. 2003) are less potent to modulate the strength of excitatory synaptic responses in mdx than in control mice. Second, mdx mice show impairments in paired-pulse facilitation of CA1 IPSCs (Graciotti et al. 2008), suggesting impaired adaptability and plasticity of inhibitory responses to repetitive stimulations. Moreover, the facilitated induction of CA1 hippocampal LTP in mdx mice can be prevented by a GABAA-R antagonist (Vaillend and Billard 2002). In all, these converging data suggest that neuronal disinhibition plays a key role in the regulation of synaptic plasticity in dystrophin-deficient neurons. This may involve reduced high-frequency stimulation (HFS)-induced feedforward inhibition, which can normally suppress LTP in the mature brain by reducing the time window for EPSPs summation and synaptic integration (Mittmann et al. 2004; Akerman and Cline 2007), although the contribution of nonsynaptic components of the GABA-mediated response evoked by HFS cannot be ruled out (Smirnov et al. 1999). The distribution of dystrophin along proximal dendrites, the dendritic reorganization of inhibitory synapses, and apparent reduction of dendritic GABAA-R-mediated transmission in mdx mice suggest a specific role of α2-containing GABAA-R clusters in signal integration in dendrites. Interestingly, spike backpropagation from axon to dendrites has been proposed to enhance dendritic depolarization and thereby facilitate synaptic plasticity (Mehta 2004). This may be normally modulated by various processes including local inhibition mediated by GABA (Tsubokawa and Ross 1996). Thus, it seems conceivable that reduced dendritic GABAergic inhibition in mdx mice may facilitate dendritic depolarization and thereby enhance synaptic plasticity.

If a deficit in dendritic inhibitory control can facilitate strengthening of excitatory synapses in mdx mice, could this find a morphological correlate at the synapse level? Changes in the efficacy of synaptic transmission are commonly associated with modifications of several synaptic-contact parameters including the size of the pre- and postsynaptic elements and the density or proportion of different synapse populations (Marrone and Petit 2002). In the present study, we quantified the incidence of perforated excitatory synapses and MSBs, which represent morphological states associated with adult synaptogenesis and enhanced synaptic efficacy (for reviews, see Geinisman 2000; Marrone and Petit 2002; Nikonenko et al. 2002). These 2 parameters were not affected in mdx mice. However, we found a significant and selective increase in PSD length of axospinous perforated excitatory synapses in mdx mice. Again, this difference was more pronounced in the proximal radiatum normally enriched in dystrophin than in the distal radiatum, thus supporting a causal role for dystrophin loss. Because perforated synapses are hallmarks of activity-dependent synaptic plasticity, this may represent a morphological correlate of the enhanced LTP and facilitated activation of NMDAR reported in mdx mice (Vaillend et al. 1999). Indeed, it is believed that modifications in the size of the PSD occur as a result of neurotransmitter receptors aggregation and changes in the density of various membrane proteins involved in signal transduction. For instance, NMDAR-dependent synaptic facilitation in CA1 pyramidal cells after ischemic episodes is associated with increased phosphorylation of NMDAR subunits 2A and 2B and increased PSD size due to recruitment of various signal transduction molecules to the PSD (Cheung et al. 2000; Martone et al. 2000). Moreover, proportional augmentation in PSD size and synaptic strength has been associated with LTP (Desmond and Levy 1986b; Toni et al. 2001), and conversely, a reduction in the size of perforated PSDs has been reported in the hippocampus of aged, memory-deficient rats (Nicholson et al. 2004), suggesting a positive correlation between PSD...
length and cognitive function. This assumption is further supported by the report of a decrease in PSD size along with impaired cognitive and synaptic functions in several animal models of mental retardation (Boda et al. 2004; Moretti et al. 2006). In mdx mice, the change in PSD length was specific to perforated synapses, suggesting a selective, and perhaps transitory, role for dystrophin and associated proteins in the formation of new excitatory synapses in adult brain. This is consistent with a model in which dystrophin, because of its late expression during development, may not be involved during the early stages of synaptogenesis, as suggested elsewhere (Fritschy and Brünig 2003). How the stabilization of GABAergic PSDs by dystrophin complexes may influence processes of activity-dependent remodeling of excitatory synapses, however, remains an open question for future studies.

Concluding Remarks

One main finding in the present study is the demonstration that dystrophin loss not only impairs clustering of specific GABA<sub>A</sub>-R subunits but also impacts on the general organization of inhibitory synapses and on the ultrastructure of a specific excitatory synapse subtype. Our results suggest that the lack of dystrophin in the CA1 hippocampal subfield is associated with a reorganization of dendritic inhibitory contacts reflecting tenuous compensation of altered GABA<sub>A</sub>-R clustering. Increase in the PSD length of excitatory perforated synapses suggests that dystrophin deficiency also affects the functional organization and/or remodeling of glutamatergic synapses. We believe that these morphological changes may explain the abnormal electrophysiological properties of hippocampal CA1 neurons found in mdx mice. Functional brain defects resulting from dystrophin loss therefore appear to be mainly expressed at the level of synaptic connectivity in the absence of gross anatomical abnormalities.

Many lines of electrophysiological data led to a functional model according to which dendritic inhibition may control the efficacy of excitatory synaptic inputs onto principal cells and their plasticity (Freund 2003). Recent work in the collybistin-deficient mouse, a genetic model displaying a specific loss of GABA<sub>A</sub>-R clusters in the hippocampus, has revealed reduced dendritic GABA<sub>A</sub>-ergic transmission and enhanced synaptic plasticity associated with impaired spatial learning (Papadopoulos et al. 2007). Although mdx mice only display a partial loss (about 50%) of α2 subunit clusters (Knuesel et al. 1999) and mild cognitive impairments (Vailld et al. 1995), both models support a role for dendritic GABA<sub>A</sub>-R clustering in regulating activity-dependent synaptic plasticity and cognitive functions. Our current knowledge of DMD physiopathology (for reviews, see Lidov 1996; Blake and Krüger 2000; Anderson et al. 2002) suggests that dystrophin loss may not account for the most severe cases of mental retardation, which can be due to further brain anomalies resulting from the additional loss of other short dystrophin gene products in patients carrying C-terminal mutations (Moizard et al. 1998). However, because all patients lack the full-length dystrophin as is the case of the mdx mice, defective GABA<sub>A</sub>-ergic function secondarily affecting synaptic plasticity at pyramidal cell glutamatergic synapses may be considered a common phenotypic feature associated with the DMD syndrome. This may open the search for effective treatments with therapeutic agents modulating dendritic GABA<sub>A</sub>-R function.

Funding

AFM (Association Francaise contre les Myopathies, France, Grant # DiT1 2006) to C.V., from OSR (Oficina de Suport a la Recerca, Universitat de les Illes Balears, Spain) to R.M. and by CNRS (Centre National de la Recherche Scientifique, France).

Notes

The authors are grateful to S. Vandergeest, N. Samson, and P. Veyrac for animal care. We also thank C. Rampon for fruitful advice, M. Guegan for her collaboration in the histology, and L. Damas for her participation to preliminary EM experiments. Conflict of Interest: None declared.

Address correspondence to Dr Cyrille Vaillend, Neurobiologie de l’Apprentissage, de la Mémoire et de la Communication, CNRS, Université Paris-Sud XI, Bât 416, UMR 8620, Orsay cedex, France. Email: cyrille.vaillend@u-psud.fr.

References


DeCoster M. 2007. The nuclear area factor (NAF): a measure for cell apoptosis using microscopy and image analysis. In: Méndez-Vilas A,


