Brain function in Duchenne muscular dystrophy

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Summary
Duchenne muscular dystrophy (DMD) is the second most commonly occurring genetically inherited disease in humans. It is an X-linked condition that affects approximately one in 3300 live male births. It is caused by the absence or disruption of the protein dystrophin, which is found in a variety of tissues, most notably skeletal muscle and neurones in particular regions of the CNS. Clinically DMD is characterized by a severe pathology of the skeletal musculature that results in the premature death of the individual. An important aspect of DMD that has received less attention is the role played by the absence or disruption of dystrophin on CNS function. In this review we concentrate on insights into this role gained from investigation of boys with DMD and the genetically most relevant animal model of DMD, the dystrophin-deficient mdx mouse. Behavioural studies have shown that DMD boys have a cognitive impairment and a lower IQ (average 85), whilst the mdx mice display an impairment in passive avoidance reflex and in short-term memory. In DMD boys, there is evidence of disordered CNS architecture, abnormalities in dendrites and loss of neurones, all associated with neurones that normally express dystrophin. In the mdx mouse, there have been reports of a 50% decrease in neurone number and neural shrinkage in regions of the cerebral cortex and brainstem. Histological evidence shows that the density of GABA_4 channel clusters is reduced in mdx Purkinje cells and hippocampal CA1 neurones. At the biochemical level, in DMD boys the bioenergetics of the CNS is abnormal and there is an increase in the levels of choline-containing compounds, indicative of CNS pathology. The mdx mice also display abnormal bioenergetics, with an increased level of inorganic phosphate and increased levels of choline-containing compounds. Functionally, DMD boys have EEG abnormalities and there is some preliminary evidence that synaptic function is affected adversely by the absence of dystrophin. Electrophysiological studies of mdx mice have shown that hippocampal neurones have an increased susceptibility to hypoxia. These recent findings on the role of dystrophin in the CNS have implications for the clinical management of boys with DMD.

Keywords: dystrophin; CNS; mdx

Abbreviation: DMD = Duchenne muscular dystrophy

Introduction
Duchenne muscular dystrophy (DMD) is an X-linked recessive disease and has the second highest incidence of all inherited diseases, approximately one in 3300 live male births (Emery, 1991). In boys the disease presents as muscle weakness that is first apparent at 3–4 years of age. The muscle weakness is due to an irreversible, ongoing loss of skeletal muscle, and results in the need for a wheelchair at ~10 years of age and death at ~20 years, normally due to cardiopulmonary complications. The disease is a consequence of a mutation of the dystrophin gene, which comprises nearly 0.1% of the entire human genome (Koenig et al., 1987). The dystrophin gene has a high mutation rate, with approximately one-third of all new cases of DMD resulting from a spontaneous mutation (Barbujani et al., 1990). The product of the dystrophin gene was identified in the first successful application of the reverse genetics technique by both Kunkle (Kunkle et al., 1985) and Worton (Ray et al., 1985). Since the identification of the dystrophin protein and its location close to the inner surface of the plasmalemma there has been a substantial research effort aimed at investigating its physio-
logical role in the cell membrane. These studies have mainly focused on skeletal muscle pathophysiology present in DMD and in animal models where dystrophin is known to be absent or present in a non-functional form. This work has given rise to two major hypotheses of the functional role of dystrophin in skeletal muscle. The first is a calcium hypothesis, where calcium homeostasis of the cell is disrupted (Wrogemann and Pena, 1976; Duncan, 1989; Head, 1993) resulting in an increase in intracellular calcium and cell necrosis. The second hypothesis suggests that dystrophin plays a structural role in maintaining the integrity of the lipid bilayer when it undergoes mechanical and osmotic stresses, and that in its absence the membrane ruptures allowing an influx of extracellular ions, in particular calcium (Roses et al., 1975; Roses and Appel, 1976; Head, 1993) resulting in an increase in intracellular calcium and cell necrosis. The second hypothesis suggests that dystrophin plays a structural role in maintaining the integrity of the lipid bilayer when it undergoes mechanical and osmotic stresses, and that in its absence the membrane ruptures allowing an influx of extracellular ions, in particular calcium (Roses et al., 1975; Roses and Appel, 1976; Head, 1993).

Many case reports of DMD have indicated a significant CNS involvement, along with the skeletal muscle pathology that is characteristic of DMD. Indeed, Duchenne in his initial description noted that some boys displayed cognitive impairment (Duchenne, 1868). It is now well established that dystrophin is present in many cells throughout the body, including neurons of the CNS. In this review we will present recent evidence demonstrating that the absence or mutation of dystrophin in the CNS results in a significant disruption of neuronal and brain function. We will focus on work carried out in humans and in a dystrophin deficient murine model of DMD, the mdx mouse.

Evidence linking the absence of dystrophin with a cognitive impairment

In DMD boys

Since the original description of the disease by Duchenne (1868) in which he reported five patients with some degree of cognitive impairment, there has been debate as to whether there is in fact a cognitive deficit in the boys. This debate has been fuelled largely by conflicting reports about the prevalence of cognitive impairment in individual cases of boys with DMD. Gowers noted that ‘in most recorded cases the mind has been unaffected’ and concluded that intellectual impairment is not part of the disease (Gowers, 1879). Similar findings were reported by many investigators up until the 1970s (Morrow and Cohen, 1954; Walton and Natrass, 1954; Schoelly and Fraser, 1955; Truitt, 1955; Sherwin and McCully, 1961; Lincoln and Staples, 1977). However, there is now an overwhelming amount of evidence supporting Duchenne’s original observation that in many cases there is a significant cognitive impairment. The average IQ of a boy with DMD is 85. The distribution of IQ in boys with DMD is shifted 1 SD lower, and consequently 30% of boys with DMD have an IQ of <70 (Allen and Rogkin, 1960; Worden and Vinos et al., 1962; Dubowitz, 1965, 1979; Zellweger and Niedermeyer, 1965; Zellweger and Hanson, 1967; Cohen et al., 1968; Prosser et al., 1969; Black, 1973; Marsh and Munsat, 1974; Florek and Karolak, 1977; Karagan and Zellweger, 1978; Karagan, 1979; Leibowitz and Dubowitz, 1981; Bresolin et al., 1994).

Although some earlier reports found a decrease in IQ in DMD boys compared with age-matched controls (Scheinfield, 1950; Black, 1973; Florek and Karolak, 1977), the majority of studies, both longitudinal and correlational, found no such difference (Allen and Rodgin, 1960; Worden and Vignos, 1962; Zellweger and Niedermeyer, 1965; Zellweger and Hanson, 1967; Cohen et al., 1968; Prosser et al., 1969; Leibowitz and Dubowitz, 1981). Others have reported an increase in verbal IQ and a decrease in performance IQ with age, with full IQ remaining static (Prosser et al., 1969; Karagan and Zellweger, 1976; Sollee et al., 1985). Smith and colleagues reported that boys with DMD who were <6 years of age had a global developmental delay, particularly severe in language and locomotor areas, and showed a slight improvement with age (Smith et al., 1990). Deficits in verbal expression and in memory (Karagan et al., 1980), reading, mathematics and spelling (Dorman et al., 1988; Billard et al., 1998) and serial position memory (Anderson et al., 1988) have all been reported in DMD patients. Most recently Hinton and colleagues reported that regardless of IQ there was a specific cognitive profile, namely poor performance in digit span, story recall and comprehension, in a sample of 80 boys with DMD (Hinton et al., 2000). The authors note that, although there was a consistent profile of cognitive impairment, it was variable in degree, possibly due to the different dystrophin gene mutations in the boys sampled.

The search for a genotype/phenotype correlation for cognitive impairment in DMD has not led to any conclusive results. It has been considered that deletions more towards the 3′ end have a higher incidence of cognitive impairment (Bushby, 1992; Lenk et al., 1993). Rapaport found that 70% of patients with a deletion in exon 52 had cognitive impairment (Rapaport et al., 1991). Most recently, an association between the degree of cognitive impairment and the presence of mutation in the Dp71 isoform has been reported (Moizard et al., 2000). (For a complete account of the full-length dystrophin and the various isoforms, see Blake and Kröger, 2000; Mehler, 2000.)

In the mdx mouse

The mdx mouse is a murine model of DMD that lacks the full length dystrophin protein, but retains all the smaller dystrophin isoforms, including Dp71. Muntoni et al. (1991) were the first to report a cognitive deficit in the mdx mouse. Mice from 16–22 weeks old were found to have an impairment in passive avoidance learning. The cognitive impairment was further characterized by Vaillend and colleagues who found that mdx mice do not appear to have an impairment in task acquisition or in procedural memory, but rather they forget newly learned information more quickly than controls (Vaillend et al., 1995). Of interest is the finding that the mdx mouse, and the mdx<sup>3cv</sup> mouse (which
lacks the full-length dystrophin as well as the smallest dystrophin isof orm Dp71) are no different from controls in spatial discrimination tasks (Sesay et al., 1996; Vaillend et al., 1998). Although the evidence is not as strong as in DMD, it appears that in mdx mice the mutation of the full-length dystrophin protein is also correlated with a degree of cognitive impairment.

**Histological evidence for a CNS deformity**

**In DMD boys**

The results of brain autopsy, and more recently brain imaging, on DMD patients have not been consistent. Dubowitz and Crome investigated the brains on autopsy of 21 cases of classical DMD, and found only one case of abnormal brain weight and two cases where there were ‘striking histological abnormalities’ (Dubowitz and Crome, 1969). They concluded that DMD is not associated with any consistent gross or histological abnormality of the brain. Similarly, an MRI study by Bresolin and colleagues found no focal or generalized changes (Bresolin et al., 1994), but the sample was very small (n = 4). Rae and colleagues in a study of 15 DMD boys and 15 age-matched controls found no significant difference between these two groups in relative ventricular size, but stressed the young age (<13 years) of the boys in the sample (Rae et al., 1998b).

In contrast, other investigators have reported brain abnormalities in DMD patients ranging from slight to severe (Rosman and Kakulas, 1966; Rosman, 1970; Jagadha and Becker, 1988; Itoh et al., 1999). Among the abnormalities noted are neuronal loss, heterotopias, gliosis, neurofibrillary tangles, Purkinje cell loss, dendritic abnormalities (length, branching and intersections), disordered architecture, astrocytosis and perinuclear vacuolation. In 13 of 50 DMD patients studied, Bresolin and colleagues reported that the presence of macroglossia was significantly correlated with a low performance IQ (Bresolin et al., 1994). Schmidt et al. (1985), in agreement with Appleton et al. (1991), reported an increased head circumference in DMD patients, although these patients’ fathers also showed greater head circumference as a group compared with normal. This was not the case in the mothers of these patients. They scanned (CT) three of the 36 subjects who were found to have a large head circumference and reported findings of ‘probable megaencephaly’. The results of in situ brain measurements carried out on 30 DMD patients by Yoshioka et al. (1980) yielded more striking results. They found 67% of DMD patients studied had slight cortical atrophy, 60% had slight ventricular dilation and 30% had cortical atrophy, although clear signs of atrophy were only observed in older and more physically disabled patients (slight was defined as an enlargement of interhemispheric cisterns and sulci of 3–5 mm). Chen et al. (1999) reported a single DMD patient with mild brain atrophy, measured by CT scan.

There is some evidence to suggest that those DMD patients with identifiable brain abnormalities also show a cognitive impairment. Septien et al. (1991) studied 15 DMD patients, aged 4–16 years, in whom the average IQ was 84. In CT scans, 60% of the patients presented with slight cortical atrophy, with minimal dilation of the ventricles, interpreted as atrophy of the white matter. The nine cases presenting with cortical atrophy had an average IQ of 81, while the six presenting with normal CT scans had an average IQ of 90, although this difference was not statistically significant. In agreement with Yoshioka et al. (1980), they did find that cortical atrophy was more common in patients older than 10 years. In contrast, al-Qudah et al. (1990) found no correlation between MRI findings and verbal intelligence scores for four patients, two of whom were found to have mild atrophy consisting of dilation of lateral ventricles, CSF spaces and cerebral sulci. Overall these studies indicate that an abnormality of the brain, either gross or histological, is by no means common in DMD patients, and a correlation between abnormality and IQ impairment has yet to be clearly established. In those boys that do display abnormality in brain structure, the defects range from very mild to severe.

**In the mdx mouse**

In the original description of the mdx dystrophin deficient mouse, Bulfield et al. (1984) noted that the older (12 month old) mice displayed muscular tremors, possibly due to the older mdx mice having gross structural deformities in skeletal muscle, ranging from simple fibre splitting to complex multiple branching (Head et al., 1992). Interestingly, no gross abnormalities in brain or spinal cord were detected by Bulfield et al. (1984), or in a later study by Torres et al. (1987). In contrast, Sbriccoli et al. (1995) employed retrograde axonal tracing to study the structure of the corticospinal system in mdx mice, and reported substantial differences between the brains of mdx mice and controls. They found that the absolute number of cells was decreased by 50% in mdx mice compared with controls. The cell packing density of cortical layer 5 neurones was higher in mdx than controls, while the cell packing density of corticospinal neurones was lower in mdx than controls. Corticospinal cells constituted 50% of neurones present in layer 5 of controls, but only 35% in mdx. The authors concluded that rather than a shrinkage of all layer 5 cell populations, the origin of the corticospinal tract was selectively damaged, and thus there were fewer labelled neurones in mdx than controls. It is important to note that they found the number of spinal motoneurones was no different between mdx and controls. The cross-sectional area of labelled neurones was ~20% lower in mdx than controls, with the labelled cells also differing in shape (the mdx cells were rounder than the pyramidal-shaped control layer 5 cells). A more recent study by the same group (Carretta et al., 2001) investigated the organization of spinal projecting brainstem neurones. Cell counts of neurones were made in the red nucleus, the vestibular nuclear complex, the
isms that give rise to the hypometabolism can be varied and occur in another subject of normal IQ with Wernicke-hippocampus, the number (but not size) of GABA<sub>A</sub> clusters soma of cerebellar Purkinje cells. In both the cerebellum and synaptic activity (Jueptner and Weiller, 1995). The mechanism of dystrophin (Knuesel et al., 1999) was found to be particularly striking around the soma of cerebellar Purkinje cells. In both the cerebellum and hippocampus, the number (but not size) of GABA<sub>A</sub> clusters was reduced by ~50%. In the cerebral cortex, dystrophin clusters and GABA<sub>A</sub> clusters were observed separately, as well as groups of dystrophin and GABA<sub>A</sub> clusters together, indicating a possible GABA<sub>A</sub>-subset-dependent co-localization of dystrophin (Knuesel et al., 1999).

Biochemical evidence for CNS involvement
In DMD boys
The search for the biochemical mechanisms underlying the mental deficit associated with lack of dystrophin has been necessarily limited. In 1994, Bresolin and colleagues published results from a PET study of fluorodeoxyglucose uptake showing decreased uptake in the cerebellum in DMD boys (Bresolin et al., 1994). This hypometabolism was shown not to occur in another subject of normal IQ with Wernicke-Hoffman disease, suggesting that the cerebellar hypometabolism was unrelated to the motor deficit. Glucose hypometabolism is a common feature of disorders with associated cognitive deficits, and is generally indicative of lowered synaptic activity (Jueptner and Weiller, 1995). The mechanisms that give rise to the hypometabolism can be varied and diverse.

In 1995 a paper showing altered bioenergetics in DMD was published (Tracey et al., 1995). This study used neuropsychological testing and <sup>31</sup>P-magnetic resonance spectroscopy to examine the brains of 19 boys with DMD and 19 age-matched control boys. Results showed significantly higher ratios of inorganic phosphate compared with ATP, phosphomonoesters (mainly phosphocholine and phosphoethanolamine) and phosphocreatine. There was no significant correlation between these ratios and any measure of intellectual ability employed. Interestingly, the skeletal muscle of dystrophic boys has been shown to have a reduced total creatine and/or phosphocreatine concentration (Tarnopolsky and Parise, 1999). Patients with neuromuscular disorders such as DMD have been shown to be chronically hypocapnic (Misuri et al., 2000) due to the pattern of rapid, shallow breathing and one might expect this to have an effect on brain energetics.

Boys with DMD have also been shown to have elevated levels of choline-containing compounds (glycero- and phosphocholine) in certain regions of their brains. An autopsy study showed increased (up to three times higher) choline-containing compounds in the frontal cortex in boys older than 17 years (Kato et al., 1997), while a study using magnetic resonance spectroscopy in vivo showed significantly increased choline-containing compounds in the cerebellum, but not the cortex (Rae et al., 1998b), of boys younger than 13 years. The ratio of choline-containing compounds to N-acetylaspartyl-containing compounds was shown to correlate significantly with the boys’ score on the Matrix Analogies Test, a measure of visuospatial cognitive ability that requires no verbal input and is tolerant of motor disability. As such, it is arguably a measure of DMD boys’ underlying ability (i.e. taking verbal and motor deficits into account). This suggested that the increase in choline-containing compounds was possibly a ‘compensatory’ effect, although the authors pointed out that there was also a relationship between elevated choline-containing compounds and age, with the level of the compounds increasing with age. Choline-containing compounds are seen to be elevated in a number of brain disorders and are traditionally interpreted as symptomatic of increased membrane turnover or decreased membrane stability.

The cerebellar and hippocampal focus of the biochemical lesions in DMD are of interest due to the normally high expression of dystrophin in neurones found in these regions. Both Dorman et al. (1988) and Billard et al. (1998) noted that the reading deficits seen in those with DMD are similar to those seen in phonological dyslexia (Castle and Coltheart, 1993). Persons with phonological dyslexia, either developmental (Rae et al., 1998c; Nicolson et al., 1999) or acquired (Levisohn et al., 2000), have been shown to have abnormalities in the right cerebellum. Similarly, deficits in verbal working memory, a large component of the DMD cognitive deficit (Hinton et al., 2000) are known to have a cerebellar focus (Desmond et al., 1997).

In the mdx mouse
The accessibility of the mdx mouse model has meant that more invasive biochemical measurements have been possible. The bioenergetic abnormalities reported in DMD brain have also been documented in the mdx mouse, with an increased inorganic phosphate to phosphocreatine ratio and increased intracellular brain pH reported using <sup>31</sup>P-magnetic resonance spectroscopy in vivo (Tracey et al., 1996a). The mice studied were aged in the range 180–240 days. These authors also reported a reduction in total creatine content in aged mdx mice compared with controls which was not found in younger (60–150 days) mice (Tracey et al., 1996b). A similar reduction of creatine compounds is also reflected in muscle tissue of mdx mice (Pullido et al., 1998).
Metabolism of glucose is altered in the brains of mice which lack dystrophin. A recent study (Rae et al., 2001), using intravenous injection of [1-13C]glucose and subsequent isotopomer analysis using NMR (nuclear magnetic resonance) spectroscopy, has shown significantly decreased free glucose in the brains of mdx mice compared with controls, but significantly increased fractional enrichment and increased flux of 13C into metabolites such as glutamate and GABA, suggesting a faster metabolic rate in the dystrophin-deficient brain. Furthermore, muscimol, a GABA_A (and partial GABA_C) agonist which normally induces sedation and decreases glucose use, has a decreased effect in mdx mice compared with controls. This suggests that the increased glucose use demonstrated in the mdx mouse brain may be due to decreased inhibitory input from the subset of abnormally clustered GABA_A receptors.

Decreased bioenergetic buffering capacity would be expected to influence susceptibility to hypoxia. Mehler and coworkers have shown that hippocampal pyramidal neurones from mdx mice demonstrate increased sensitivity to hypoxia-induced loss of synaptic transmission (Mehler et al., 1992). A more recent study has shown more susceptibility of mdx hippocampal tissue slices to irreversible hypoxic failure compared with controls when kept in 10 mM glucose, but less susceptibility of mdx slices compared with controls when kept in 4 mM glucose (Godfraind et al., 2000). A substrate-dependent difference in oxygen consumption rates has also been demonstrated in mdx brain cortical tissue slices (Rae et al., 1998a, 2001), which consume oxygen at significantly higher rates than controls when metabolizing pyruvate at low oxygen partial pressures. In addition to decreased bioenergetic buffering capacity, the displayed altered response to hypoxia may also be influenced by the reported reduced GABA_A receptor clustering in dystrophin-deficient mice (Knuesel et al., 1999), as GABA_A receptor activation has been shown to exacerbate oxygen-glucose deprivation-induced neuronal injury (Muir et al., 1996).

A proton magnetic resonance study (Tracey et al., 1996b) reported normal levels of N-acetylaspartate, a neuronal viability marker (Bates et al., 1996), but increased whole-brain levels of choline-containing compounds (glycerol- and phosphocholine) and myo-inositol. We have subsequently confirmed the increase in choline-containing compounds and localized them to the cerebellum and hippocampus (Rae et al., 2001).

An analysis of tissue from various organs in the mdx mouse, including the cerebellum, has shown that dystrophic tissue is easily distinguishable from control tissue based on its metabolic profile. Griffin et al. (2001) identify discernible metabolic changes in glycolysis, β-oxidation, the TCA cycle, the phosphocreatine/ATP cycle, lipid metabolism and osmoregulation. These were all in the five dystrophic tissues tested: cardiac muscle, diaphragm, skeletal muscle, cerebral cortex and cerebellum.

There is also some recent evidence suggesting that the water transporter aquaporin-4 is deficient in the astrocytic end feet in mdx mice (Frigeri et al., 2001). Deficiency of the water transporter has also been reported in the muscle (Frigeri et al., 2001) and erythrocyte membrane in DMD (Serbu et al., 1986). The deficiency in erythrocyte water transport is probably due to altered membrane fluidity in the erythrocyte (Ferretti et al., 1990), which does not normally express dystrophin. Altered expression of the dystrophin homologue β-spectrin has been reported, which is symptomatic of findings of altered expression of a range of proteins homologous to dystrophin in DMD, with no consistent pattern emerging (Boivin, 1992), although it has been suggested that it may result from genetic heterogeneity (Ashley and Goldstein, 1983).

Tracey et al. (1996a) reported increased extracellular and decreased intracellular volumes in mdx brain suggesting that the alteration in aquaporin-4 levels might result in alterations in the ability of cells in the brain to regulate cell volume. Although there has been one report of altered response to hypo-osmotic shock by mdx mouse muscle (Menke and Jockusch, 1991), recent work (Rae et al., 2001) has shown no difference in efflux rates of [3H]taurine from mdx cortical brain tissue slices compared with controls when exposed to hypo-osmotic shock. The increase in total brain myo-inositol in mdx mice (Tracey et al., 1996b) might be interpreted as a response to chronic osmotic stress, as myo-inositol has been shown to act as an organic osmolyte in the brain (Strange et al., 1993).

Evidence from electrophysiological studies for an involvement of the CNS

In DMD boys

There have been very few electrophysiological studies carried out on DMD patients, due in large part to the practical and ethical problems associated with undertaking these types of measurements on sick boys. Overall the studies indicate a functional neural deficit in DMD. The majority of investigators, who have performed EEG studies on DMD patients, have found that a higher proportion (40–89%) of these patients display abnormalities compared with control groups or general age-matched population data (Wayne and Browne-Mayers, 1959; Perlstein et al., 1960; Zellweger and Niedermeyer, 1965; Nakao et al., 1968; Kozicka et al., 1971; Black, 1973; Florek and Karolak, 1977). The most consistent abnormality found was 14 and 6/s positive spikes, an abnormality also present in vegetative seizures, atypical seizures, migraines, autonomic nervous system dysfunctions, syncope and paroxysmal abdominal pain (Poser and Ziegler, 1958; Perlstein et al., 1960; Zellweger and Niedermeyer, 1965; Nakao et al., 1968). Unfortunately many of these studies lack appropriate control groups: Wayne and Browne-Mayers (1959), Perlstein et al. (1960), Zellweger and Niedermeyer (1965), Kozicka et al. (1971) and Florek and Karolak (1977) did not perform EEG studies on their own control groups. Barwick et al. (1965) did...
use age-matched control boys and found abnormal or borderline recordings in 28% of DMD patients (n = 20) compared with 45% (n = 18) in the age-matched controls. Interestingly they did not observe any 14 and 6/s positive spikes. Zellweger and Niedermeyer (1965) suggest that this relatively low incidence of EEG abnormalities in DMD patients is due to small patient numbers and the criteria set for definition of positive spike activity. Nakao et al. (1968) found 60% of a DMD patient group (n = 80) had abnormal EEG recordings compared with 19% of a healthy school children control group (n = 16). They concluded that abnormal EEG recordings occur in a higher percentage of DMD patients than normal. Wayne and Browne-Mayers (1959), Kozicka et al. (1971), Black (1973) and Florek and Karolak (1977) do not indicate the precise nature of the EEG abnormalities they observed, which makes their findings difficult to interpret.

It remains unclear if there is a correlation between IQ and EEG abnormalities in the DMD boys. Perlstein et al. (1960) did find a correlation between these two factors, while Zellweger and Niedermeyer (1965), Kozicka et al. (1971), Black (1973) and Florek and Karolak (1977) found no correlation between EEG abnormalities and IQ. Nakao et al. (1968) mentions that patients with EEG abnormalities were ‘more often inferior’ in intelligence to those with normal recordings. Perlstein et al. (1960) found a correlation between increasing age and increasing EEG abnormalities. In contrast Zellweger and Niedermeyer (1965) found no correlation between disease progression and EEG abnormalities. Similarly Wayne and Browne-Mayers (1959) and Nakao et al. (1968) found no correlation between severity of disease and EEG abnormalities.

More recently Di Lazzario et al. (1998), using transcranial magnetic stimulation, reported that the excitability of the motor cortex is reduced in DMD patients (n = 4) compared with controls (n = 4). Central motor conduction time and excitability to electrical stimulation were unchanged in DMD patients, leading the authors to conclude that the reduction in motor cortex excitability in the DMD boys arose as a result of aberrant synaptic functioning, which is in agreement with biochemical evidence (Bresolin et al., 1994; Jueptner and Weiller, 1995).

**In the mdx mouse**

The *mdx* mouse has enabled rigorous electrophysiological investigation of functional abnormalities in the dystrophin deficient CNS. Full-length dystrophin is absent in hippocampal cells in the *mdx* mouse (Lidov et al., 1996) and functional deficits in these cells have been the subject of investigation in recent times. Both Mehler et al. (1992) and Godfraind et al. (2000) have demonstrated that CA1 hippocampal neurones display a significantly increased susceptibility to hypoxia-induced reduction in synaptic transmission at Schaffer collateral/commissural CA1 synapses. Interestingly, there is no difference in long-term potentiation in CA1 and dentate gyrus cells in *mdx* mice (10–12 weeks) compared with controls (Sesay et al., 1996; Godfraind et al., 1997).

Further studies have included another mouse model, the *mdx<sup>3cv</sup>*, which lacks the Dp140 and Dp71 dystrophin isoforms, both of which are present in control and *mdx* brain tissue but lacking in many boys with Duchenne’s. To this end, Vaillend et al. (1998) employed the brain slice technique to test synaptic plasticity in 21–24 week old *mdx* and *mdx<sup>3cv</sup>* mice. In agreement with Sesay et al. (1996), they found no deficiency in synaptic transmission in either strain of *mdx* mice; however, post-tetanic potentiation was increased in *mdx* mice. Vaillend et al. (1999) went on to further characterize the difference in post-tetanic potentiation seen previously in the *mdx* mouse using brain slices from 3–5 month-old control and *mdx* mice. In contrast to their previous study, post-tetanic potentiation was not significantly different in the *mdx* mouse compared with controls when the NMDA receptor antagonist D-APV (d-2-amino-5-phosphonovalerate) was present. Because of the indication that the NMDA receptor was affected by the lack of dystrophin, nNOS (nitric oxide synthase)-specific activity was also implicated due to NOS activation via NMDA-receptor mediated elevation of intracellular calcium levels. Accordingly they evoked pre-synaptic fibre volleys and field excitatory post-synaptic potentials mediated through both NMDA receptors and non-NMDA receptors. They found comparable synaptic responses in both NMDA and non-NMDA receptor subtypes of *mdx* and control mice. This indicates normal glutamatergic neuronal transmission, and hence preserved nNOS activity. The authors argue that the differences seen in short-term potentiation are due to a post-synaptic rather than a pre-synaptic mechanism. Using voltage-gated calcium channel antagonists nifedipine and NiCl₂ they found no difference in potentiation in either *mdx* or control mice, indicating that these channels are not responsible for their observations.

**Conclusion and clinical implications**

In recent years there has been a substantial increase in our understanding of the role of dystrophin in the CNS. These studies have been largely carried out on DMD boys and the dystrophin deficient *mdx* mouse and have demonstrated a range of abnormalities in CNS function, from behavioural and cognitive dysfunction to alterations in the clustering of ion channels in single identified neurones.

The major clinical issue in DMD is the skeletal muscle pathology, and this has understandably received the greatest attention. There has been very little clinical investigation of the role of dystrophin in the CNS in DMD. However, with the dramatic increase in our understanding of this role over the last decade or so, as detailed in this review, there is now a sufficient body of data on the functions of dystrophin in the CNS, and the implications of its disruption, for its clinical relevance to begin to be appreciated.
This potential clinical relevance may best be illustrated by consideration of the potential impact of a disruption in the distribution and function of the inhibitory receptor GABA_A. A disruption of GABA_A receptors may impact clinically in at least three areas: clinical efficacy of drugs, sleep disorders and motor control.

Many substances widely used in clinical medicine exert their influence exclusively or in part by binding to the GABA_A receptor. It is now reasonably well established that substances such as barbiturates, benzodiazepines, anaesthetics and corticosteroids interact with the GABA_A receptor in the CNS (for a review, see Johnston, 1996). If boys with DMD have an abnormality in distribution and function of these receptors then the efficacy of these substances could be altered. GABA disruption may also in part explain the sleep disorders suffered by many DMD boys. Sleep disorders in DMD are characterized by sleep fragmentation, increased arousal frequency and REM (rapid eye movement) sleep deprivation (Redding et al., 1985). Interestingly, a recent review on the role of GABA receptors in sleep regulation provides evidence suggesting that activation of GABA_A receptors is crucial in both the initiation and maintenance of non-REM sleep and in the generation of sleep spindles (Lancel, 1999). Finally, the motor problems experienced by boys with DMD are, of course, due largely to the skeletal muscle pathology. A disruption of normal motor control function may also have an impact on the ability of DMD boys to learn and carry out movement, and to compensate for the structural deformities in skeletal muscle. The cerebellum is a part of the motor control system, and has been shown to play a major role in such aspects of motor behaviour as motor task automation, motor learning and timing functions associated with motor tasks (Ivry and Keel, 1985; Jenkins et al., 1994). As detailed above, the cerebellar Purkinje cells in the mdx mouse have a disruption in GABA_A cluster size and, if a similar disruption occurs in DMD boys resulting in some abnormality in cerebellar function, it could influence their ability to learn and execute motor tasks. Although these three examples of possible clinical relevance of GABA_A receptor abnormalities are speculative, they indicate the potential importance of an understanding of the role of dystrophin in the CNS to the clinical management of boys with DMD.

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