Miniature inhibitory postsynaptic current in cerebellar Purkinje cells of old dystrophic *mdx* mice

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Duchenne muscular dystrophy (DMD) is caused by the mutations in the X-linked dystrophin gene resulting in a deficiency in the protein dystrophin. About 1/3 of boys with DMD display some degree of cognitive impairments (Cotton *et al.*, 2001). In the cerebellum, dystrophin is normally localized at the postsynaptic membrane of GABAergic synapses of Purkinje cells. Previously, we showed a significant reduction in both the frequency and amplitude of miniature inhibitory postsynaptic current (mIPSCs) in cerebellar Purkinje cells of adult (3-4 months old) *mdx* compared with littermate control (Kueh *et al.*, 2011; Kueh *et al.*, 2008). Here, we investigated the mIPSCs of young (3-4 months old) and old *mdx* mice (23-26 months old). These aging mice were chosen because earlier reports showed both muscle and brain degenerative progression in old *mdx* mice resembles those found in DMD patients (Pastoret & Sebille, 1995; Rae *et al.*, 1998).

All experiments were conducted in accordance with the international guidelines on the care and use of experimental animals and approved by the Animal Care and Ethics Committee of Western Sydney University. Mice (*mdx*, n=6; littermate control, n=7) were anesthetized with isoflurane then decapitated for cerebellum collection. Cerebellum section (250 μm) was cut parasagittally using a vibroslicer (Leica VT1200s, Leica Microsystems) and maintained in artificial cerebrospinal fluid at 16°C in the Braincubator (Buskila *et al.*, 2014) until use. Whole-cell patch clamp recording of mIPSCs in Purkinje cells was recorded in the presence of TTX (0.4 μM) and confirmed with bicuculine (5 μM) at room temperature. Fire polished patch electrodes used in this study had resistance range from 2.9 MΩ to 5 MΩ when filled with internal solution. All data were sampled at 10 kHz and low pass filtered at 3 kHz. Recording of mIPSCs were analysed using Clampfit 10.6 and Graph Pad Prism 7, and all values are reported as mean ± SE. Statistical analysis was performed using two-tailed student unpaired t-test or Kolmogorov-Smirnov test and were considered significant at the $P<0.05$ level.

Our results showed that the mean frequency of mIPSCs was significantly reduced in old *mdx* (0.69 ± 0.17 Hz, n=9 cells) compared to littermate control (1.61 ± 0.26 Hz, n=10 cells), $P=0.009$ (unpaired student t-test). The peak amplitude was also significantly smaller in *mdx* (45.75 ± 0.82 pA) than littermate control (53.73 ± 0.74 pA), $P<0.0001$ (Kolmogorov-Smirnov test). These results are consistent with the findings in the younger group of *mdx* mice (3-4 months old).

We concluded that dystrophin deficiency reduces both frequency and amplitude of mIPSCs in Purkinje cells of young and aging mice. These results imply that lack of dystrophin disrupts the synaptic transmissions at GABAergic synapses. This perturbed synaptic transmission may be similar to human disease progression and contribute to the cognitive dysfunction in boys with DMD. Cognitive impairment in DMD boys is non-progressive, and it is of interest that the frequency and amplitude of mIPSCs were similar between young and old *mdx* mice.

Buskila Y, Breen PP, Tapson J, van Schaik A, Barton M & Morley JW. (2014) Extending the viability of acute brain slices. *Scientific reports*, **4**, 5309. doi: 10.1038/srep05309

Cotton S, Voudouris NJ & Greenwood KM. (2001) Intelligence and Duchenne muscular dystrophy: full-scale, verbal, and performance intelligence quotients. *Dev Med Child Neurol*, **43(7)**, 497-501.

Kueh SLL, Dempster J, Head SI & Morley JW. (2011) Reduced postsynaptic $GABA_A$ receptor number and enhance gabaxadol induced change in holding currents in Purkinje cells of the dystrophin-deficient *mdx* mouse. *Neurobiol Dis*, **43(3)**, 558-564.

Kueh SLL, Head SI, Morley JW. (2008) $GABA_A$ receptor expression and inhibitory post-synaptic currents in cerebellar Purkinje cells in dystrophin-deficient *mdx* mice. *Clin Exp Pharmacol Physiol*, **35(2)**, 207-210. doi: 10.1111/j.1440-1681.2007.04816.x

Pastoret C, Sebille A. (1995) *mdx* mice show progressive weakness and muscle deterioration with age. *J Neurol Sci*, **129(2)**, 97-105.

Rae C, Scoot RB, Thompson CH, Dixon RM, Dumughn I, Kemp GJ, Male A, Pike M, Styles P & Radda GK. (1998) Brain biochemistry in Duchenne muscular dystrophy: a 1H magnetic resonance and neuropsychological study. *J Neurol Sci*, **160(2)**, 148-157.