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Striatum brain-derived neurotrophic factor levels are decreased in dystrophin-deficient mice

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ABSTRACT

Brain dystrophin is enriched in the postsynaptic densities of pyramidal neurons specialized regions of the subsynaptic cytoskeletal network, which are critical for synaptic transmission and plasticity. Lack of dystrophin in brain structures have been involved with impaired cognitive functions. The brain-derived neurotrophic factor (BDNF) is a regulator of neuronal survival, fast synaptic transmission, and activity-dependent synaptic plasticity. The present study investigated BDNF protein levels by Elisa analysis in prefrontal cortex, cerebellum, hippocampus, striatum and cortex tissues from male dystrophic *mdx* ($n = 5$) and normal C57BL10 mouse ($n = 5$). We observed that the *mdx* mouse display diminution in BDNF levels in striatum ($t = 6.073$; df = 6; $p = 0.001$), while a tendency of decrease in BDNF levels was observed in the prefrontal cortex region ($t = 1.962$; df = 6; $p = 0.096$). The cerebellum ($t = 1.258$; df = 7; $p = 0.249$), hippocampus ($t = 0.631$; df = 7; $p = 0.548$) and cortex ($t = 0.572$; df = 7; $p = 0.586$) showed no significant alterations as compared to wt mouse. In conclusion, we demonstrate that only striatum decreased BDNF levels compared with wild-type (wt) mouse, differently to the other areas of the brain. This dystrophin deficiency may be affecting BDNF levels in striatum and contributing, in part, in memory storage and restoring.

Deficiency of dystrophin, a 427-kDa protein coded by a gene in Xp21, is responsible for the early onset of genetic Duchenne muscular dystrophy (DMD). The protein is located at the inner-side of cell membrane in muscles and brain cells, in association with a membrane-bound cytoskeletal protein complex known as the dystrophin-associated proteins [3]. Brain dystrophin is enriched in the postsynaptic densities of pyramidal neurons specialized regions of the subsynaptic cytoskeletal network, which are critical for synaptic transmission and plasticity [11].

The lack of dystrophin in brain structures such as in hippocampus and neocortex has been involved with impaired cognitive functions in these regions [2]. However, the nature, magnitude and biological support of the cognitive deficits involving dystrophin deficiency still remain unclear, although they have been partly addressed by studies in the dystrophin-deficient *mdx* mouse, a genetic model of DMD [2,16].

The brain dystrophin is abundant in the hippocampus and absent in other subcortical areas [12]. Selective behavioural deficits involving hippocampal function were predicted to occur in the *mdx* mouse mutant. Pioneer studies showed that dystrophin deficiency in *mdx* mouse is associated with impaired memory retention at long delays in certain procedural learning tasks and spatial alternation tasks [14,16–18]. At cellular level, the absence of dystrophin in *mdx* mouse causes altered calcium homeostasis and hippocampal neuronal function [13], particularly specific alterations of hippocampal long-term potentiation, a form of plasticity widely believed to be critical for memory formation [16].

The neurotrophin brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family and the most widespread growth factor in the brain. BDNF has many functions in the adult brain as a regulator of neuronal survival, fast synaptic transmission, and activity-dependent synaptic plasticity [10,4]. In this context, the present study investigated BDNF protein levels in prefrontal cortex, cerebellum, hippocampus, striatum and cortex of dystrophin-deficient *mdx* mouse, as compared do normal mouse controls.

To this aim, we used male dystrophin-deficient *mdx* mouse ($n = 5$) and normal C57BL10 mouse ($n = 5$), all aged 3 months old, ceded by Human Genome Research Center, Biosciences Institute, University of São Paulo. The animals were housed 5 to a cage with food and water available *ad libitum* and were maintained on a 12-h light/dark cycle. All experimental procedures involving animals were performed in accordance with the NIH Guide for the

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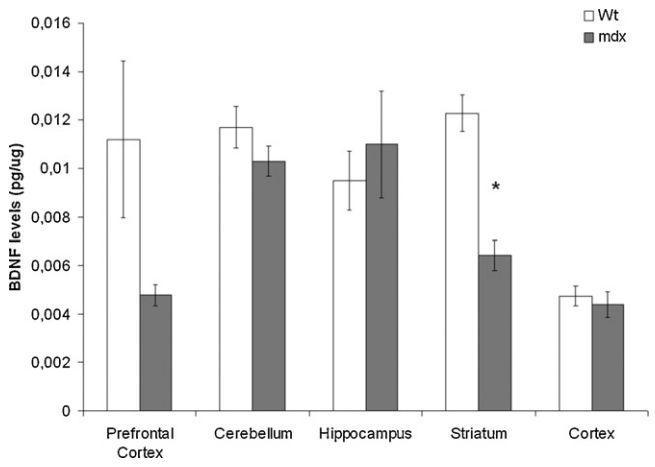

Fig. 1. BDNF protein levels in mdx mice and Wt controls. Bars represent means ± S.E.M. of 5 rats. $^{*}p < 0.05$ vs. wild-type according to Student's *t*-test.

Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care.

After mouse euthanasia by decapitation, prefrontal cortex, cerebellum, hippocampus, striatum and cortex were immediately dissected, isolated and stored at −80 °C for analyses of BDNF protein levels. BDNF levels were measured by anti-BDNF sandwich-ELISA, according to the manufacturer instructions (Chemicon, USA). Briefly, the structures were homogenized in phosphate buffer solution (PBS) with 1 mM phenylmethylsulfonyl fluoride and 1 mM EGTA. Microtiter plates (96-well flat-bottom) were coated for 24 h with the samples diluted 1:2 in sample diluent. The plates were then washed four times with sample diluent, and a monoclonal anti-BNDF rabbit antibody diluted to 1:1000 in sample diluent was added to each well and incubated for 3 h at room temperature. After washing, a peroxidase conjugated anti-rabbit antibody (diluted 1:1000) was added to each well and incubated at room temperature for 1 h. After addition of streptavidin-enzyme, substrate and stop solution, the amount of BDNF was determined by absorbance in 450 nm. A standard curve was produced and it ranged from 7.8 to 500 pg/ml of BNDF. This curve was obtained from a direct relationship between Optical Density and BDNF concentration. Total protein was measured by Lowry's method using bovine serum albumin as a standard.

The Statistical Package for the Social Sciences (SPSS) 15.0 was utilized for statistical analyses. All data are expressed as mean ± standard error of the mean of *n* animals, and have been statistically analyzed with the Student's *t*-test for unpaired data. *P* values less than 0.05 were considered statistically significant.

As depicted in Fig. 1, *mdx* mouse displays alteration in the availability of BDNF levels in striatum compared with wild-type (wt) mouse ($t = 6.073$; df = 6; $p = 0.001$). Prefrontal cortex showed a tendency of decrease in BDNF levels as compared with wt mouse ($t = 1.962$; df = 6; $p = 0.096$). In cerebellum ($t = 1.258$; df = 7; $p = 0{,}249$), hippocampus ($t = 0.631$; df = 7; $p = 0.548$) and cortex ($t = 0.572$; df = 7; $p = 0.586$) no significant alterations were observed as compared with wt mouse.

Normally, the protein dystrophin is present in all regions of the brain, mostly in the cerebellum. Dystrophin, is present in neurons, but not in glia or myelin, and forms punctate foci associated with the plasma membrane of perikarya and dendrites, but not axons. While dystrophin is abundant in cerebral cortical neurons and cerebellar Purkinje cells, it is absent in most subcortical neurons, granule cells of fascia dentata, and cerebellar neurons other than Purkinje cells. The distribution and localization of dystrophin suggest a role in organizing the plasma membrane, probably as an anchor of the postsynaptic apparatus [12].

There are several ways in which the lack of dystrophin can affect the brain. First, dystrophin is expressed in the developing brain [15], and mutations that affect the dystrophin complex can affect neuronal migration and differentiation [13]. Second, lack of dystrophin affects neuronal excitability, since this protein is found in the postsynaptic apparatus, serving to anchor receptors, including the $GABA_A$ receptor [9]. Lack of dystrophin also affects long-term synaptic plasticity [5]. Third, loss of dystrophin may lead to neuronal death [11]. It leaves the neuron more susceptible to metabolic and physiological insults [5]. Motor neurons may die when their dystrophin-deficient targets degenerate [15]. The *mdx* mouse has a mutation that prevents the production of the full-length dystrophin molecule, although the production of shorter isoforms is intact. This mutant has relatively mild but widespread changes in the brain, including a reduced number of projections to the spinal cord from the cerebral cortex and red nucleus, and changes in the number of certain types of interneurons in the cerebral cortex [6].

In this study, only striatum presented low levels of BDNF compared with mouse control. The striatum is highly innervated by BDNF-releasing synapses. BDNF is delivered to the striatum via activity-dependent anterograde release from excitatory corticostriatal axons. Diminished striatal volumes and behavioural abnormalities are characteristic in striatal dysfunction caused for BDNF levels alteration [8]. The striatum did not present high levels of distrophin, but it participates of the distribution of cortical memory together with the cerebellum, through two major cortical afferent pathways. These pathways relay information from peripheral receptors and higher cerebral cortical centers, receive afferent inputs from the cerebral cortex, striatum, red nucleus and brainstem reticular formation and send axonal afferents via climbing fiber pathways to form sparse synaptic contacts at proximal sites on Purkinje cell dendrites. These two pathways process and relay informational signals from the external environment and distributed cortical memory retention and from internal autonomicrhomeostatic and proprioceptive sources, respectively (for a review see [12]). This reduced dystrophin expression in striatum has been associated with alterations on these pathways. However, the striatum also has relation to the start, stop and direction of motor movement [8]. Thus the decrease BDNF protein levels in striatum also may be related with locomotor activity beyond memory performance.

In this regard, the BDNF function is associated with synaptic function and plasticity [1]. Also, normal BDNF levels in the brain are essential to the maintenance of normal learning and memory function by a process referred to as synaptic consolidation. Recent studies showed that reductions in BDNF levels have been reported in a number of neurodegenerative diseases or associated models [7,19]. However, there are no evidences of how the dystrophin may be affecting the BDNF levels in striatum. In conclusion, we demonstrated that only striatum decreased BDNF levels compared with wild-type mouse, differently to the other areas of the brain. This study showed the first evidence that dystrophin deficiency may be affecting BDNF levels in striatum and may be contributing, in part, in memory storage and restoring. Future studies should be realized to clarify how dystrophin alters the BDNF levels in striatum of the *mdx* mice.

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