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# Modulation of brain-derived neurotrophic factor as a potential neuroprotective mechanism of action of omega-3 fatty acids in a parkinsonian animal model

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ABSTRACT

While we recently reported the beneficial effects of omega-3 polyunsaturated fatty acids (n−3 PUFAs) in a mouse model of Parkinson's disease (PD), the mechanisms of action remain largely unknown. Here, we specifically investigated the contribution of the brain-derived neurotrophic factor (BDNF) to the neuroprotective effect of n−3 PUFA observed in a mouse model of PD generated by a subacute exposure to MPTP using a total of 7 doses of 20 mg/kg over 5 days. The ten-month high n−3 PUFA treatment which preceded the MPTP exposure induced an increase of BDNF mRNA expression in the striatum, but not in the motor cortex of animals fed the high n−3 PUFA diet. In contrast, n−3 PUFA treatment increased BDNF protein levels in the motor cortex of MPTP-treated mice, an effect not observed in vehicle-treated mice. The mRNA expression of the high-affinity BDNF receptor tropomyosin-related kinase B (TrkB) was increased in the striatum of MPTP-treated mice fed the high n−3 PUFA diet compared to vehicle and MPTP-treated mice on the control diet and to vehicle mice on the high n−3 PUFA diet. These data suggest that the modulation of BDNF expression contributes, in part, to n−3 PUFA-induced neuroprotection in an animal model of PD.


## 1. Introduction

Parkinson's disease (PD) is characterized by a massive and specific neuronal cell loss in the substantia nigra pars compacta (SNpc) leading to a drastic decrease in dopamine (DA) release to the striatum (Fearnley and Lees, 1991; Lang and Lozano, 1998). Clinically, PD patients suffer mainly from motor symptoms such as resting tremor, bradykinesia, muscular rigidity and postural instability, although non-motor impairments are also associated with the disease (see review Lim et al., 2009). Despite great efforts put forward to develop effective treatments for PD, only palliative strategies have been offered to patients thus far. The identification of modifiable environmental risk factors could translate into neuroprotective strategies to delay the onset of the disease.

Several studies now recognize the fundamental and central role of omega-3 polyunsaturated fatty acids (n−3 PUFAs) in brain health. Docosahexaenoic acid (DHA; 22:6n−3) is the most common n−3 PUFA within phospholipids of brain cell membranes, while eicosapentaenoic acid (EPA; 20:5n−3) is usually found in very limited amounts in the central nervous system (CNS) (Julien et al., 2006; Salem et al., 2001). The main dietary sources of long chain PUFA, namely EPA and DHA, remain fatty fish consumption. Besides their critical role in cell membrane fluidity, PUFAs can be released from the hydrophobic moiety of phospholipids via the enzyme phospholipase A2 (PLA2) from which they can be solicited in several intracellular events (Galli et al., 1993; Green et al., 2008). Although specific mechanisms of action through which n−3 PUFAs act remain unknown, some pathways have been suggested and studied *in vitro* as well as *in vivo*. These include anti-inflammatory (Bazan, 2005; Marcheselli et al., 2003), anti-oxidant (Bazan, 2005; Calon et al., 2004; Hashimoto et al., 2002) and anti-apoptotic properties (Calon et al., 2005; Kim, 2007).

Since n−3 PUFAs play such an important role in the CNS, their involvement in several prevalent diseases has been investigated. For example, preclinical (Calon et al., 2004; Green et al., 2007), clinical (Freund-Levi et al., 2006) and epidemiological (Morris et al., 2003; Schaefer et al., 2006) studies argue in favor of a preventive role of n−3

*Abbreviations:* AD, Alzheimer's disease; BBB, blood brain barrier; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; DA, dopamine; DAergic, dopaminergic; DHA, docosahexaenoic acid; DL, dorsolateral; DM, dorsomedial; GDNF, glial cell line-derived neurotrophic factor; HPLC, high performance liquid chromatography; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; n−3 PUFA, omega-3 polyunsaturated fatty acid; OD, optical density; PFA, paraformaldehyde; PBS, phosphate-buffered saline; PD, Parkinson's disease; SNpc, substantia nigra pars compacta; TH, tyrosine hydroxylase; TrkB, tropomyosin-related kinase B; VL, ventrolateral; VM, ventromedial.

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PUFA in the development of Alzheimer's disease (AD) (see also reviews: Boudrault et al., 2009; Calon and Cole, 2007). However, much less data are available for PD. One recent prospective study conducted in 2005 by de Lau et al. has positively associated a high intake of PUFA, specifically n−3 PUFA, with a lower risk of developing PD (de Lau et al., 2005). Our group has recently demonstrated a beneficial effect of a chronic n−3 PUFA dietary intake in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) animal model of PD (Bousquet et al., 2008). Mice fed a high n−3 PUFA diet for ten months depicted a modified brain fatty acid profile while a number of nigral and striatal dopaminergic (DAergic) components, such as tyrosine hydroxylase (TH)-positive neurons, *Nurr1*, a nuclear receptor important for the regulation and the survival of dopaminergic neurons (Saucedo-Cardenas et al., 1998), and dopamine transporter mRNA levels in the SNpc as well as striatal dopamine content measured by high performance liquid chromatography (HPLC) were protected from MPTP-induced neurotoxicity (Bousquet et al., 2008).

Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor ubiquitously and most abundantly expressed in the mammalian brain, promoting growth and participating in the development of neurons. It has an essential role in the establishment of the proper number of DAergic neurons in the SN and reduced BDNF biosynthesis has been speculated to lead to degeneration of DAergic neurons (Baquet et al., 2005). Interestingly, BDNF protein levels are reduced in the caudate nucleus, putamen and in the SN of PD patients (Mogi et al., 1999; Parain et al., 1999). The neurotrophic action of BDNF is mediated by tropomyosin-related kinase B (TrkB), a tyrosine kinase protein receptor (Middlemas et al., 1991; Soppet et al., 1991). Partial deletion of this receptor leads to a reduced number of DAergic cells in the SN as well as the formation of α-synuclein aggregates in older mice (21–23 months of age), supporting a role of BDNF in PD (von Bohlen und Halbach et al., 2005). In contrast to BDNF, TrkB mRNA levels remain normal in PD patients in several structural components associated to the pathology (Benisty et al., 1998). BDNF has been recognized for years as a potential neuroprotective agent for PD (Peterson and Nutt, 2008). However, the blood brain barrier (BBB) completely blocks the entry of BDNF and other neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF) into the brain (Kastin et al., 2003), which severely compromises clinical application. Thus, a neuroactive compound that could stimulate brain secretion of these neurotrophic factors could be useful in the development of therapeutic strategies for PD. Notably, the upregulation of BDNF has been suggested as a potential mechanism of action of n−3 PUFA in a rat model of traumatic injury as well in the healthy rat (Wu et al., 2004; Wu et al., 2008). Here, we investigated the contribution of BDNF and its receptor to the beneficial action of n−3 PUFA in the structures relevant to PD using a subacute MPTP mouse model of parkinsonism.

## 2. Materials and methods

### 2.1. Animals, dietary treatment and MPTP lesion

Thirty-eight C57BL/6 male mice (Charles River, Wilmington, MA, USA) of two months of age were assigned to either a control low n−3 PUFA diet or a diet enriched in n−3 PUFA for a ten-month dietary treatment, as recently described (Bousquet et al., 2008). Briefly, long chain n–3 PUFA were obtained in a microencapsulated formulation (MEG-3) from Ocean Nutrition Inc. (Halifax, NS, Canada) to protect PUFA from oxidation and were incorporated in an isocaloric diet to obtain daily doses of approximately 425 mg/kg of DHA (5.3 g/kg of the diet) and 90 mg/kg of EPA (1.1 g/kg of diet), based on the verification of previous protocols using equivalent daily consumption per animal. Mice were housed five per cage under standard conditions throughout the experiments with free access to food and water and handled under the same conditions by one investigator. All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Policy of the Centre Hospitalier de l'Université Laval (CHUL). Twenty mice received seven intraperitoneal (i.p.) injections of MPTP–HCl (20 mg/kg free base; Sigma, St. Louis, MO, USA) dissolved in saline 0.9%, prepared fresh. MPTP was administered twice on the first two days of the experimental protocol at an interval of 12 hours (h) and once a day on the three subsequent days. Vehicle animals received saline 0.9% i.p. following identical MPTP administration schedule. The MPTP regimen utilized in this study was selected to produce a mild DAergic denervation suitable for neuroprotection study (Bousquet et al., 2008; Gibrat et al., 2009; Tremblay et al., 2006).

### 2.2. Tissue preparation for post-mortem analyses

Two weeks following the last MPTP injection, animals were sacrificed under deep anesthesia with ketamine/xylazine and perfused via intracardiac infusion with 0.1 M phosphate-buffered saline (PBS). After intracardiac perfusion, brains were collected and hemispheres separated. The left hemisphere was post-fixed in 4% paraformaldehyde (PFA) for 48 h and transferred to 20% sucrose in 0.1 M PBS for cryoprotection. Coronal brain sections of 25 µm thickness were cut onto a freezing microtome (Leica Microsystems, Montreal, QC, Canada). The right hemisphere was snap-frozen in 2-methyl-butane and then stored at −80 °C.

### 2.3. BDNF and TrkB mRNA expression

A specific [$^{35}$S]UTP-labeled complementary RNA (cRNA) probe was used to assess tissue mRNA levels of BDNF and TrkB, a high-affinity receptor for BDNF. The cDNA of BDNF was subcloned into pCR 2.1 and linearized with the restriction enzyme Xho and corresponded to a 350 base pairs (bp) (99–448 NM_007540). The cRNA probe for TrkB stems from a 284 bp (2597–2880 NM_008745) EcoRI–BamHI fragment of a full-length mouse TrkB cDNA subcloned into pBluescript SK+ and linearized with XbaI. The cRNA probes were synthesized and labeled by using Promega riboprobe kit (Promega, Madison, WI), [$^{35}$S]UTP (Perkin Elmer Inc., Boston, MA) and the RNA polymerase SP6 and T7, respectively (Roche Diagnostics, Indianapolis, IN). Brain sections were hybridized following the procedures described below and previously published protocols (Lapointe et al., 2004; Tremblay et al., 2006). Sense probes were also generated for all of these markers and no specific signal was obtained (data not shown).

Both *in situ* protocols ultimately underwent the same preparation. Slices were mounted onto Snowcoat X-tra™ slides (Surgipath, Winnipeg, Canada) and stored under vacuum overnight before use. Brain sections were fixed in 4% PFA pH 9.5 for 5 h for BDNF analyses and in 4% PFA pH 7.4 for 20 minutes (min) for TrkB. Pre-treatment was made with various consecutive baths PBS 0.1 M twice 5 min, proteinase K 0.1 µg/mL 10 min at 37 °C, acetylation bath (0.25% acetic anhydride, triethanolamine 0.1 M) 10 min, and twice for 5 min in standard saline citrate (SSC) (0.3 M NaCl, 30 mM sodium citrate). Repetitive baths of ethanol solutions (30%, 60%, 100%, 100%; 3 min each) were performed for dehydration. *In situ* hybridization of the riboprobes on tissue sections was performed at 58 °C overnight in a standard hybridization buffer (deionised formamide 50%, sodium chloride 5 M, tris 1 M, EDTA 0.5 M, Denhart's solution 50×, dextran sulfate 50%, tRNA 10 mg/mL, DTT 1 M, and $^{35}$S coupled $2\times10^6$ cpm/µL probe). Post-treatment was conducted using different successive baths: SSC 4× (30 min), removing coverslips, SSC 2× twice (5 min), RNase A 20 µg/mL (1 h) at 37 °C, milliQ water twice (15 seconds (s)), SSC 2× (15 min), SSC 0.5× (30 min) at 60 °C, SSC 0.1× (30 min) at 60 °C, and SSC 0.1× (5 min) at room temperature (RT). Consecutive baths of ethanol solutions (30%, 60%, 100%, 100%; 3 min each) were used for further dehydration. Tissue sections were then placed against BiomaxMR (Kodak, New Haven, CT) radioactive sensitive films for 3 days for either probe.