Elsevier Editorial System(tm) for Neuroscience Research
Manuscript Draft

Manuscript Number: NSR-D-08-00172R1

Title: Restorative Effects of Uridine Plus Docosahexaenoic Acid in a Rat Model of Parkinson's Disease

Article Type: Rapid Communication

Section/Category: Neuroscience of Disease

Keywords: Parkinson's Disease; Uridine; Docosahexaenoic Acid; Dopamine; Tyrosine Hydroxylase Activity; Synapse

Corresponding Author: Professor Richard Wurtman,

Corresponding Author's Institution: Massachusetts Institute of Technology

First Author: Mehmet Cansev, PhD

Order of Authors: Mehmet Cansev, PhD; Ismail H Ulus, PhD; Lei Wang, PhD; Timothy J Maher, PhD; Richard Wurtman

Manuscript Region of Origin: UNITED STATES

Abstract: Administering uridine-5'-monophosphate (UMP) and docosahexaenoic acid (DHA) increases synaptic membranes and dendritic spines in rodents. We examined their effects on rotational behavior and dopaminergic markers in rats with partial unilateral 6-hydroxydopamine (6-OHDA)-induced striatal lesions. Rats receiving UMP, DHA, both, or neither, daily, and intrastriatal 6-OHDA 3 days after treatment onset, were tested for d-amphetamine-induced rotational behavior and dopaminergic markers after 24 and 28 days, respectively. UMP/DHA treatment reduced ipsilateral rotations by 57% and significantly elevated striatal dopamine, tyrosine hydroxylase (TH) activity, TH protein and Synapsin-1 on the lesioned side. Hence, giving uridine may increase nigrostriatal synapses and dopaminergic neurotransmission in this model of Parkinson's Disease.

*** Referee Suggestions**

l. Dr. Robert McCarley – Harvard University, Neuroscience Laboratory
Robert_mccarley@hms.harvard.edu
2. Dr. John H. Growdon – MIT, Brain and cognitive Sciences
growdon@HELIX.MGH.HARVARD.EDU
3. Dr. Steven Zeisel – University of North Carolina, Michael Hooker Research Center
steven_zeisel@unc.edu
4. Dr Robert Roth – Yale University, Dept. of Psychiatry
Robert.roth@yale.edu
5. Dr. Anders Bjorklund - Lund University, Section of Neurobiology
Anders.Bjorklund@med.lu.se

*** Response to Reviewers**

Dear Dr. Tsumoto:

Thank you for sending us your very helpful comments, and those of the two referees, concerning our manuscript submitted to Neuroscience Research (NSR-D-08-00172). The attached revision incorporates, we believe, responses to all of your and their suggestions, as described below:

1. We now comment on the fact that in animals receiving the DHA plus UMP, striatal phospholipid levels rose in both the lesioned and the control sides. (We state, page 5, that, since dopaminergic terminals constitute only a small proportion on striatal structures, 6-OHDA administration failed to affect striatal phospholipid levels, and administration of UMP plus DHA increased these levels in both the lesioned and control sides.) We previously showed that the UMP/DHA treatment elevates phospholipid levels more-or-less uniformly in all brain regions (Cansev and Wurtman, 2007), thus it seems probable that most or all cell constituents are affected: as far as is known, brain cells contain Kennedy Cycle enzymes that are probably unsaturated with substrate at normal brain substrate levels.

2. We acknowledge that it would probably have been better if our laboratories had been able also to show histochemically that DA synapses had increased in number or in size. (We state, page 6, that "unfortunately our laboratories were not able to perform histochemical studies that might have provided evidence of increases in the number or size of migrostriatal synapses.....so "this explanation remains an hypothesis".)

3. We have, as reviewer # 2 suggested, tested the correlation between the decrease in rotations and the biochemical changes in our treated animals. As now stated (page 4, "the numbers of rotations in all experimental groups were inversely correlated with the dopamine contents".)

4. We now provide more detail in the Methods section, for example we provide general descriptions of the assays we used for DA, TH activity, phospholipids, and various proteins; we also provide more detail concerning how rotational behavior was measured. As suggested we have also decreased the size of the legend to Table 1.

Cecil H. Green Distinguished Professor
Massachusetts Institute of Technology
46-5023; 43 Vassar Street
Cambridge, MA 02139

Manuscript

Neuroscience of Disease: Dr. Tadaharu Tsumoto

Restorative Effects of Uridine Plus Docosahexaenoic Acid in a Rat Model of Parkinson's Disease

Mehmet Cansev[1,2], Ismail H. Ulus[1,2], Lei Wang[1], Timothy J. Maher[1,3], Richard J. Wurtman[1,*]

[1]Massachusetts Institute of Technology, Department of Brain and Cognitive Sciences, Cambridge MA, 02139, USA

[2]Uludag University School of Medicine, Department of Pharmacology and Clinical Pharmacology, Bursa 16059, Turkey

[3]Massachusetts College of Pharmacy and Health Sciences, Department of Pharmaceutical Sciences, Boston MA, 02115, USA

Numbers of pages, tables and figures: 12, 3, and 1

*Corresponding Author:

MIT, 43 Vassar St., Building 46-5023B

Cambridge MA, 02139

Phone: 617-253-6731, Fax: 617-253-6882

E-mail: dick@mit.edu

Summary

Administering uridine-5'-monophosphate (UMP) and docosahexaenoic acid (DHA) increases synaptic membranes (as characterized by pre-and post-synaptic proteins) and dendritic spines in rodents. We examined their effects on rotational behavior and dopaminergic markers in rats with partial unilateral 6-hydroxydopamine (6-OHDA)-induced striatal lesions. Rats receiving UMP, DHA, both, or neither, daily, and intrastriatal 6-OHDA 3 days after treatment onset, were tested for *d*-amphetamine-induced rotational behavior and dopaminergic markers after 24 and 28 days, respectively. UMP/DHA treatment reduced ipsilateral rotations by 57% and significantly elevated striatal dopamine, tyrosine hydroxylase (TH) activity, TH protein and Synapsin-1 on the lesioned side. Hence, giving uridine and DHA may partially restore dopaminergic neurotransmission in this model of Parkinson's Disease.

Keywords: Parkinson's Disease; Uridine; Docosahexaenoic Acid; Dopamine; Tyrosine Hydroxylase Activity; Synapse

Parkinson's disease (PD) is characterized by the progressive degeneration of dopaminergic nigrostriatal neurons, and reductions in striatal dopamine (DA) levels, dopaminergic synapses, and the density of dendritic spines on striatal medium spiny neurons. No treatment for PD currently available is thought to restore the numbers of dopaminergic nigrostriatal terminals or striatal dendritic spines.

We previously observed that chronic oral administration of two circulating phosphatide precursors, uridine (as UMP) and the omega-3 fatty acid DHA, along with dietary choline, can increase neuronal levels of the phosphatides and of specific proteins that characterize synaptic membranes (Wurtman et al., 2006), as well as the numbers of dendritic spines, in rodent brain (Sakamoto et al., 2007). The present report examines the effects of giving these precursors in a rat model of PD with unilateral neurotoxin-induced nigrostriatal damage and impaired DA neurotransmission. In this model 6-hydroxydopamine (6-OHDA), injected into one corpus striatum, causes ipsilateral decreases in DA synthesis and release, and characteristic turning behavior.

Male Sprague-Dawley rats (200-250 g) consumed the control diet (containing 16% protein and 0.1% choline) fortified with UMP (0.5%) and received by daily gavage (1 ml/kg) 300 mg/kg of DHA in 5% gum arabic solution; control rats were gavaged with DHA's vehicle. Three days after the start of UMP/DHA administration, rats were injected with 6-OHDA (8 μg in 2 μl of 0.3% ascorbic acid/0.9% saline) into two different sites within their right striata (Kirik et al., 1998). In preliminary experiments that used DA levels, TH levels, and TH activity as markers, we found that pretreatment with UMP and DHA did not diminish the initial toxic responses to the 6-OHDA. Hence, subsequent

studies used animals killed 28 days after starting UMP/DHA, a period previously shown to reliably increase membrane phosphatides, synaptic proteins (Wurtman et al., 2006) and dendritic spines (Sakamoto, et al., 2007).

Rotational behavior was induced by intraperitoneal injection of *d*-amphetamine (5 mg/kg) 3 weeks after animals received the 6-OHDA treatment (day 25), and ipsilateral rotations by the rats, videotaped between 15 and 45 minutes following the *d*-amphetamine injection, were counted by two blind observers. Animals were sacrificed 3 days after testing for rotational behavior. Striatal DA was measured using an HPLC assay (Wang et al., 2005); and TH activity was determined by a radiometric method (Ulus and Wurtman, 1976). Striatal phospholipids were extracted and individual phospholipid classes were separated and quantified by measuring their phosphorus contents (Cansev and Wurtman, 2007). TH, Synapsin-1 and β-tubulin proteins were analyzed by Western blot (Cansev and Wurtman, 2007).

Data were analyzed using one-way analysis of variance (ANOVA) followed by *post hoc* Tukey tests. Comparisons between values obtained from intact and lesioned striata were made using Student's t-test. Data are presented as mean±SEM; P less than 0.05 was considered significant.

Animals that received UMP, DHA, or UMP plus DHA for 24 days exhibited significant reductions in the numbers of *d*-amphetamine-induced rotations, compared with those in control rats, by 48%, 47%, or 57% (all $P<0.05$), respectively (Table 1). The numbers of rotations exhibited by individual animals in all of the experimental groups were inversely correlated with the dopamine contents ($r=-0.447$;$P<0.05$) and TH activities ($r=-0.546$;$P<0.01$) in the lesioned striata.

Among control rats DA levels and TH activity (Table 2), and TH protein (Figure 1A) in the lesioned striata were 64%, 65%, and 35% lower (all $P<0.001$) than those in the intact striata, respectively, and levels of the two DA metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were reduced by about 50%. UMP administration, alone or with DHA, restored DA levels [by 41% ($P<0.01$) or by 37% ($P<0.05$)], as well as TH activity [by 53% ($P<0.05$) or 52% ($P<0.05$)] in lesioned striata (Table 2). Reductions in TH protein levels in lesioned striata were partially restored, increasing by 21% ($P<0.01$) or 22% ($P<0.01$), following DHA supplementation alone or after UMP plus DHA (Figure 1A). UMP plus DHA treatment also increased DOPAC levels from 0.18±0.01 to 0.22±0.01 nmol/mg protein ($P<0.05$) and HVA levels from 0.15±0.01 to 0.19±0.04 nmol/mg protein ($P<0.05$) in lesioned striata.

Levels of Synapsin-1, reduced in lesioned striata by 15% ($P<0.001$) (Figure 1B), were increased significantly by UMP, DHA, or UMP plus DHA (by 17%, 16%, or 25% respectively (Figure 1B). In contrast, levels of β-tubulin, our loading control, were unaffected by either 6-OHDA injection or dietary treatments (Figure 1). Since intrastriatal 6-OHDA is selectively neurotoxic to DA terminals, and since these terminals comprise only a small proportion of striatal structures, its administration failed to affect striatal phospholipid levels. Administration of UMP plus DHA did increase these levels (Table 3), as expected (Wurtman et al., 2006) in both the lesioned and the control sides, those of each of the individual phosphatides [i.e., phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) phosphatidylinositol (PI) and sphingomyelin (SM)] also increasing significantly (Table 3). This treatment is known to increase phosphatide levels significantly throughout the brain (Cansev and Wurtman,

2007), and probably affects most or all types of brain cells since all, apparently, utilize the same substrate-unsaturated Kennedy Cycle enzymes (Wurtman et al., In Press) for phosphatide synthesis.

These data show that chronic oral administration of uridine (as UMP) and/or DHA, which can stimulate brain phosphatide and synaptic protein synthesis (Wurtman et al., 2006), significantly reduces a behavioral consequence of unilateral damage to a nigrostriatal tract, i.e., amphetamine-induced ipsilateral rotational behavior, and partially restores biochemical markers of DA terminals. The observed effects of 6-OHDA on levels of DA and its metabolites, and on TH protein and activity, agree well with the previously-demonstrated 50-80% reduction in TH-immunoreactive-fiber density in rat striatum following two intrastriatal injections of 6-OHDA at doses (each 10 μg) (Kirik et al., 1998) in the range used here (8 μg). As expected (Ungerstedt, 1971; Hefti et al., 1980; Olds et al., 2006), the unilaterally-lesioned rats exhibited ipsilateral rotational behavior when given *d*-amphetamine 3 weeks after lesioning. If the animals also chronically received UMP alone, DHA alone, or a combination of UMP and DHA, d-amphetamine-induced ipsilateral rotations were reduced by 48%, 47%, or 57%, respectively, compatible with the partial restoration of striatal dopamine levels and, probably, release.

Giving UMP plus DHA does not protect nigrostriatal neurons from the initial toxic actions of 6-OHDA, but subsequently, it partially restores striatal DA, DOPAC and HVA levels, and TH levels and activity, possibly by increasing the number and/or size of nigrostriatal terminals and synapses. Unfortunately our laboratories were not able to perform histochemical studies that might have provided evidence of increases in the

number or size of nigrostriatal synapses, so this explanation remains an hypothesis. However in support of this explanation, the treatment also elevated striatal levels of Synapsin-1, a presynaptic vesicular protein (Ferreira and Rapoport, 2002) (Figure 1B), but not of β-tubulin (Figure 1), a ubiquitous structural protein, and in gerbil hippocampus, the treatment is known to increase the numbers of dendritic spines (Sakamoto et al., 2007) and the levels of pre- and postsynaptic proteins (e.g. PSD-95; GluR-1). That uridine can independently increase the storage and release of striatal DA in normal rats has been shown previously (Wang et al., 2005). Other investigators (Bousquet et al., 2008) have reported that supplementation with omega-3 fatty acids, including DHA, prior to administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine can be beneficial in a mouse model of PD.

Besides serving as a precursor for brain cytidyl-diphosphate, the rate-limiting constituent in membrane phosphatide synthesis (Ross et al., 1997; Cansev et al., 2005) by the Kennedy cycle (Kennedy and Weiss, 1956), uridine's phosphorylated product UTP also promotes neurite outgrowth by activating P2Y2 and other P2Y receptors, and then various intracellular signaling pathways (Pooler et al., 2005; Cansev, 2007). Conceivably, these receptors are involved in the increases in TH and Synapsin-1 proteins observed in our treated animals. Similarly, DHA, besides being a precursor preferentially incorporated into newly-synthesized brain phosphatides (DeGeorge et al., 1991), also interacts with brain receptors (e.g. syntaxin-3) to promote neuronal differentiation (Darios and Davletov, 2006). The ability of uridine and DHA to promote membrane phosphatide synthesis may also depend on the availability of a third circulating

compound, choline (Wurtman et al., 2006), inasmuch as choline kinase is highly unsaturated with choline (Millington and Wurtman, 1982).

In conclusion, these data show that chronic oral administration of the phosphatide precursors uridine and DHA can ameliorate the impairment in dopaminergic terminals and transmission in 6-OHDA-lesioned rat striata, probably by increasing the amount of synaptic membrane generated by surviving striatal neurons. Since clinical manifestations of PD apparently require the loss of as many as 70-80% of nigrostriatal neurons (Bernheimer et al., 1973), a treatment like UMP plus DHA which, hypothetically, could increase nigrostriatal synapses by 15-25% (as estimated from the increases in TH and Synapsin-1 levels in rat striatum) might confer clinical benefit in patients with Parkinson's Disease.

Acknowledgements: These studies were supported in part by grants from the National Institutes of Health (MH-28783), the Center for Brain Sciences and Metabolism Charitable Trust, and the Turkish Academy of Sciences (IH Ulus).

REFERENCES

Bernheimer, H., Birkmayer, W., Hornykiewicz, O., Jellinger, K., Seitelberger, F., 1973. Brain dopamine and the syndromes of Parkinson and Huntington. J. Neurol. Sci. 20, 415-455.

Bousquet, M., Saint-Pierre, M., Julien, C., Salem, N., Jr, Cicchetti, F., Calon, F., 2008. Beneficial effects of dietary omega-3 polyunsaturated fatty acid on toxin-induced neuronal degeneration in an animal model of Parkinson's Disease. FASEB J. 22, 1213-1225.

Cansev, M., Watkins, C.J., van der Beek, E.M., Wurtman, R.J., 2005. Oral uridine 5' monophosphate (UMP) increases brain CDP-choline levels in gerbils. Brain Res. 1058, 101-108.

Cansev, M., 2007. Involvement of Uridine-Nucleotide-Stimulated P2Y Receptors in Neuronal Growth and Function. Central Nervous System Agents in Medicinal Chemistry 7, 223-229.

Cansev, M., Wurtman, R.J., 2007. Chronic administration of docosahexaenoic acid or eicosapentaenoic acid, but not arachidonic acid, alone or in combination with uridine, increases brain phosphatide and synaptic protein levels in gerbils. Neuroscience 148, 421-431.

Darios, F., Davletov, B., 2006. Omega-3 and omega-6 fatty acids stimulate cell membrane expansion by acting on syntaxin 3. Nature 440, 813-817.

DeGeorge, J.J., Nariai, T., Yamazaki, S., Williams, W.M., Rapoport, S.I., 1991. Arecoline-stimulated brain incorporation of intravenously administered fatty acids in unanesthetized rats. J. Neurochem. 56, 352-355.

Ferreira, A., Rapoport, M., 2002. The synapsins: beyond the regulation of neurotransmitter release. Cell. Mol. Life. Sci. 59, 589-595.

Hefti, F., Melamed, E., Wurtman, R.J., 1980. Partial lesions of the dopaminergic nigrostriatal system in rat brain: biochemical characterization. Brain Res. 195, 123-137.

Kennedy, E.M., Weiss, S.B., 1956. The function of cytidine coenzymes in the biosynthesis of phospholipids. J. Biol. Chem. 222, 193-214.

Kirik, D., Rosenblad, C., Bjorklund, A., 1998. Characterization of behavioral and neurodegenerative changes following partial lesions of the nigrostriatal dopamine system induced by intrastriatal 6-Hydroxydopamine in the rat. Exp. Neurol. 152, 259-277.

Millington, W.R., Wurtman, R.J., 1982. Choline administration elevates brain phosphorylcholine levels. J. Neurochem. 38, 1748-1752.

Olds, M.E., Jacques, D.B., Kopyov, O., 2006. Relation between rotation in the 6-OHDA lesioned rat and dopamine loss in striatal and substantia nigra subregions. Synapse 59, 532-544.

Pooler, A.M., Guez, D.H., Benedictus, R., Wurtman, R.J., 2005. Uridine enhances neurite outgrowth in nerve growth factor-differentiated pheochromocytoma cells. Neuroscience 134, 207-214.

Ross, B.M., Moszczynska, A., Blusztajn, J.K., Sherwin, A., Lozano, A., Kish, S.J., 1997. Phospholipid biosynthetic enzymes in human brain. Lipids 32, 351-358.

Sakamoto, T., Cansev, M., Wurtman, R.J., 2007. Oral supplementation with docosahexaenoic acid and uridine-5'-monophosphate increases dendritic spine density in adult gerbil hippocampus. Brain Res. 1182, 50-59.

Ulus, I.H., Wurtman, R.J., 1976. Choline administration: activation of tyrosine hydroxylase in dopaminergic neurons of rat brain. Science 194, 1060-1061.

Ungerstedt, U., 1971. Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of nigro-striatal dopamine system. Acta Physiol. Scand. Suppl. 367, 69-93.

Wang, L., Pooler, A.M., Albrecht, M.A., Wurtman, R.J., 2005. Dietary uridine-5'-monophosphate supplementation increases potassium-evoked dopamine release and promotes neurite outgrowth in aged rats. J. Mol. Neurosci. 27, 137-145.

Wurtman, R.J., Ulus, I.H., Cansev, M., Watkins, C.J., Wang, L., Marzloff, G., 2006. Synaptic proteins and phospholipids are increased in gerbil brain by administering uridine plus docosahexaenoic acid orally. Brain Res. 1088, 83-92.

Wurtman, R.J., Cansev, M, Ulus, I.H. In Press. Choline and its products acetylcholine and phosphatidylcholine. In: Lajtha A. (Ed.), Neural Lipids, Handbook of Neurochemistry and Molecular Neurobiology, vol. 8, Springer-Verlag, Berlin-Heidelberg.

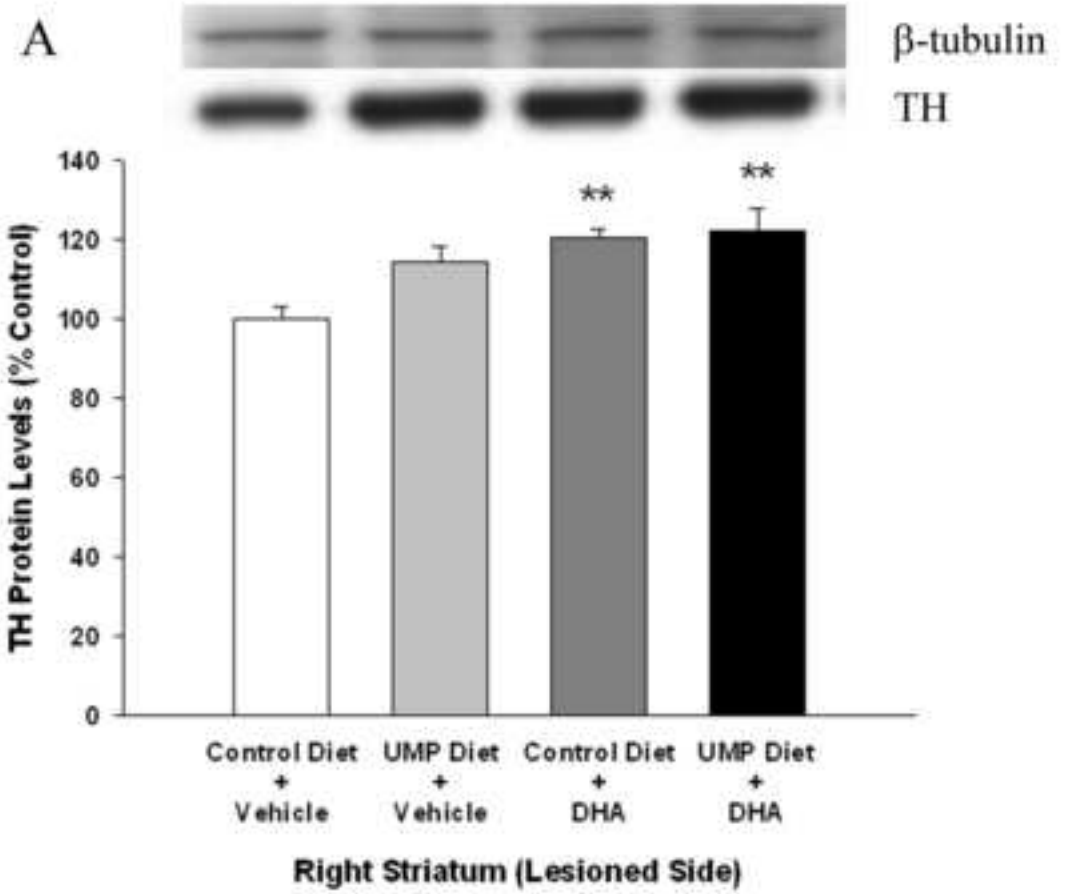
Figure Legend

Figure 1. TH Protein and Synapsin-1 Levels

Homogenates of the lesioned striata were analyzed for TH protein (A) and Synapsin-1 (B). Values obtained from rats treated with UMP, DHA, and UMP+DHA were expressed by reference to those (normalized to 100) obtained from rats that received the control diet+vehicle (control group). β -tubulin was used as the loading control. N=6 per group. *P<0.05 and **P<0.01 compared with control group using One Way ANOVA.

Figure(s)

Click here to download high resolution image



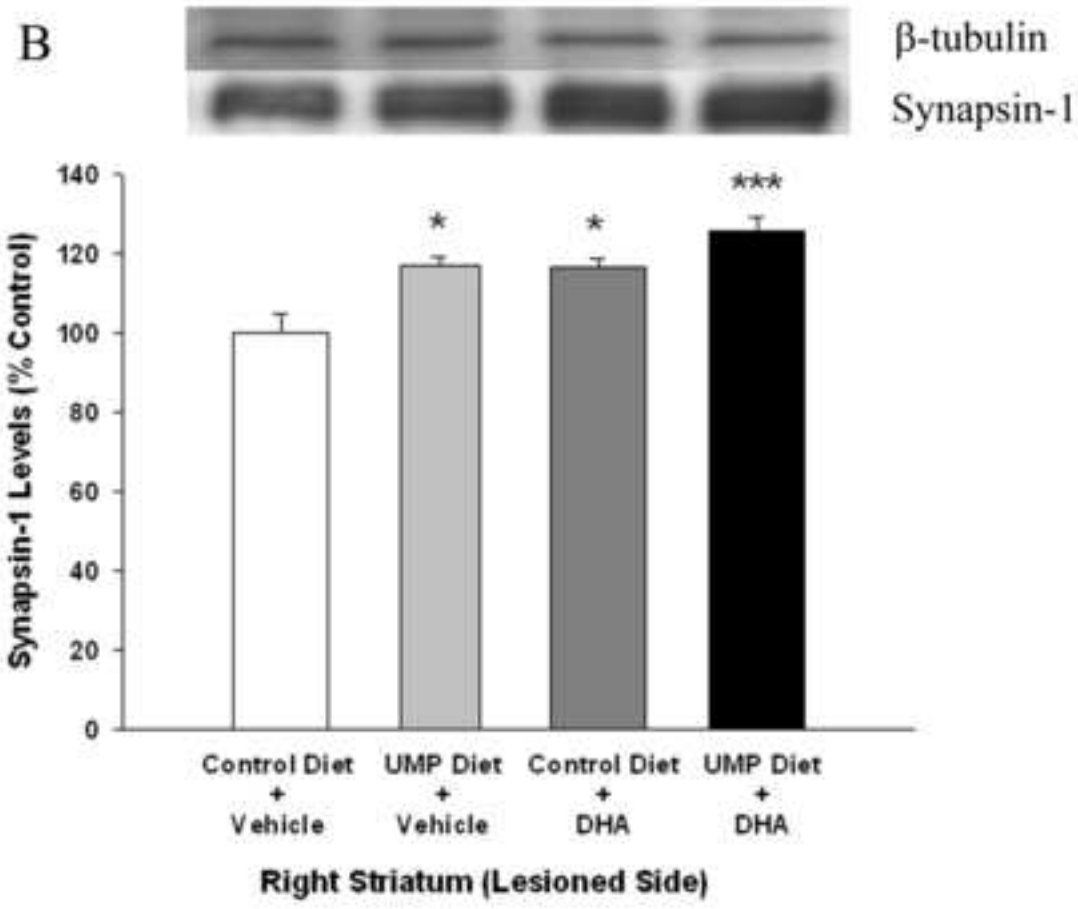


Table 1. Rotational Behavior

Table 1	
Treatment	Ipsilateral rotations/30 min
Control diet + Vehicle	151 ± 21
UMP diet + Vehicle	79 ± 22*
Control diet + DHA	81 ± 12*
UMP diet + DHA	65 ± 18**

Rats receiving UMP, DHA, both, or neither, daily, and intrastriatal 6-OHDA 3 days after treatment onset, were tested for *d*-amphetamine-induced rotational behavior after 24 days. Ipsilateral rotations were recorded for 30 minutes, between 15 and 45 min, after i.p. injection of 5 mg/kg of d-amphetamine. N = 6-9 in each group. *P<0.05; and **P<0.025 compared with Control diet + Vehicle group using One Way ANOVA followed by *post hoc* Tukey test.

Table 2. Dopamine (DA) Levels and Tyrosine Hydroxylase (TH) Activity

Table 2A	**Dopamine Levels (nmol/mg protein)**	
Treatments	Left (Intact) Striatum	Right (Lesioned) Striatum
Control diet + Vehicle	0.704 ± 0.024	0.252 ± 0.018[a]
UMP diet + Vehicle	0.749 ± 0.022	0.355 ± 0.025**
Control diet + DHA	0.745 ± 0.021	0.311 ± 0.016
UMP diet + DHA	0.830 ± 0.022**	0.345 ± 0.017*
Table 2B	**TH Activity (nmol DOPA formed/h/mg protein)**	
Treatments	Left (Intact) Striatum	Right (Lesioned) Striatum
Control diet + Vehicle	3.983 ± 0.26	1.405 ± 0.06[a]
UMP diet + Vehicle	3.591 ± 0.20	2.144 ± 0.19*
Control diet + DHA	4.014 ± 0.12	1.906 ± 0.17
UMP diet + DHA	4.189 ± 0.24	2.131 ± 0.17*

Rats receiving the treatments for 28 days were sacrificed on day 29. Striata were assayed for DA levels (A) and TH activity (B). N = 6-9 in each group. *P<0.05; and **P<0.01 compared with Control diet + Vehicle group within the same localization using One Way ANOVA followed by *post hoc* Tukey test. [a]P<0.001 compared with values from left striata of animals that received the Control diet + Vehicle using Student's t test.

Table 3. Phospholipid Levels

Left (Intact) Striatum	**Phospholipid Levels (nmol/mg protein)**					
Treatments	Total PL	PC	PE	PS	SM	PI
Control diet + Vehicle	377 ± 16	128 ± 5	125 ± 8	22 ± 1	16 ± 1	10 ± 1
UMP diet + Vehicle	394 ± 15	126 ± 4	139 ± 5	25 ± 1	16 ± 1	11 ± 1
Control diet + DHA	402 ± 9	142 ± 3	149 ± 4*	28 ± 1**	16 ± 1	20 ± 1**
UMP diet + DHA	455 ± 9***	152 ± 4**	158 ± 7**	32 ± 1***	21 ± 2*	27 ± 3***
Right (Lesioned) Striatum	**Phospholipid Levels (nmol/mg protein)**					
Treatments	Total PL	PC	PE	PS	SM	PI
Control diet + Vehicle	376 ± 11	130 ± 4	127 ± 6	22 ± 1	15 ± 1	10 ± 1
UMP diet + Vehicle	424 ± 14	142 ± 5	138 ± 3	25 ± 1	19 ± 1	14 ± 1
Control diet + DHA	419 ± 13	145 ± 5	144 ± 7	25 ± 1	16 ± 1	15 ± 2*
UMP diet + DHA	433 ± 14*	153 ± 4**	150 ± 3*	29 ± 1***	24 ± 4*	15 ± 1*

Rats receiving the treatments for 28 days were sacrificed on day 29. N = 6-9 in each group. *P<0.05; **P<0.01; and ***P<0.001 compared with Control Diet + Vehicle group within the same localization using One Way ANOVA followed by *post hoc* Tukey test. Total PL, Total Phospholipids; PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; PS, Phosphatidylserine; PI, Phosphatidylinositol; SM, Sphingomyelin.