ORIGINAL ARTICLE

Neurotrophin-3 restores synaptic plasticity in the striatum of a mouse model of Huntington's disease

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Summary

Aims: Neurotrophin-3 (NT-3) is expressed in the mouse striatum; however, it is not clear the NT-3 role in striatal physiology. The expression levels of mRNAs and immune localization of the NT-3 protein and its receptor TrkC are altered in the striatum following damage induced by an *in vivo* treatment with 3-nitropropionic acid (3-NP), a mitochondrial toxin used to mimic the histopathological hallmarks of Huntington's disease (HD). The aim of this study was to evaluate the role of NT-3 on corticostriatal synaptic transmission and its plasticity in both the control and damaged striatum.

Methods: Corticostriatal population spikes were electrophysiologically recorded and striatal synaptic plasticity was induced by high-frequency stimulation. Further, the phosphorylation status of Trk receptors was tested under conditions that imitated electrophysiological experiments.

Results: NT-3 modulates both synaptic transmission and plasticity in the striatum; nonetheless, synaptic plasticity was modified by the 3-NP treatment, where instead of producing striatal long-term depression (LTD), long-term potentiation (LTP) was obtained. Moreover, the administration of NT-3 in the recording bath restored the plasticity observed under control conditions (LTD) in this model of striatal degeneration.

Conclusion: NT-3 modulates corticostriatal transmission through TrkB stimulation and restores striatal LTD by signaling through its TrkC receptor.

KEYWORDS

Huntington's disease, neurodegeneration, neurotrophins, NT-3, TrkC

1 | INTRODUCTION

Huntington's disease (HD) is a neurodegenerative ailment which results from a trinucleotide CAG repeat expansion in the chromosomes of affected individuals and is distinguished for movement, psychiatric, and cognitive disturbances.¹ As other neurodegenerative disorders, HD exhibits progressive alteration in neuronal function that leads to cell death of medium spiny neurons (MSNs) in the striatum. The causes of neuronal degeneration are not completely understood, but alterations in mitochondrial function, oxidative

stress, and changes in trophic support contribute to HD pathogenesis.² In particular, brain-derived neurotrophic factor (BDNF) action on MSNs is well known, and reduced level of BDNF and its TrkB receptor have been documented in HD,³ but it is not completely understood if other neurotrophins exert neuroprotective effect on MSNs and could be tested for therapeutic treatment in HD.

Neurotrophin-3 (NT-3), a trophic factor from the neurotrophin family, is widely expressed in the nervous system. Similar to other neurotrophins, the physiological actions of NT-3 are mediated by the activation of two membrane receptors: the low-affinity receptor p75

and the high-affinity receptor “tropomyosin receptor kinase” (Trk). Each neurotrophin binds to one specific subtype of Trk; however, NT-3 activates the TrkC receptor and binds with less affinity to TrkB and TrkA.⁴⁻⁶ Through the activation of its receptors, NT-3 reduces cellular damage, improves neuronal regeneration in different models of lesions or neurodegeneration, and participates in synaptic reorganization, synapse formation, and neuronal plasticity.⁷⁻¹³

Although NT-3 protects striatal cells from excitotoxicity,¹⁴ the trophic and modulatory effects of NT-3 on striatal physiology have been poorly explored. As shown in our previous study, NT-3 is expressed in the striatum¹⁵ and modulates corticostriatal synaptic activity; furthermore, this modulatory effect is reduced in the presence of striatal neurodegeneration induced by 3-nitropropionic (3-NP) *in vivo*. Modifications in the modulatory effects of NT-3 are accompanied by a reduction in NT-3 levels in the striatum.¹⁶ 3-NP is a mitochondrial complex II inhibitor,^{17,18} which has been widely used as a pharmacological model of HD. As a result of impairing the mitochondrial respiratory chain, 3-NP produces abnormal movements, cognitive deficits, and striatal degeneration that mimics the histopathology observed in the postmortem tissue from patients afflicted with this illness.¹⁹⁻²³

Our former observations showed that NT-3 plays an important role in striatal physiology; however, the mechanisms involved remain to be elucidated. Then, this study evaluates the modulatory effect of NT-3 on corticostriatal synaptic plasticity in the 3-NP-treated animal model of HD and describes the Trk receptors involved in its effects. Based on our data, we conclude that NT-3 modulates corticostriatal synaptic transmission and corticostriatal LTD. Furthermore, in the 3-NP model of HD, the exogenous addition of NT-3 modulates corticostriatal transmission by activating TrkB receptors while restoring corticostriatal LTD through the activation of TrkC receptors.

2 | METHODS

2.1 | Animals

C57/BL6 male mice (ENVIGO, México) that were ~35 days old at the beginning of the experiments were used in this study. Upon arrival, mice were housed in groups of 4 animals in plastic cages under a 12/12 hours dark/light cycle at room temperature (24°C), with free access to water and food. Animal care and manipulation procedures, which include efforts to reduce the number of mice used in this protocol, were approved by the Bioethics and Bio-security Committees of our institution and followed guidelines established by NIH regulations (8th edition) and the Mexican Official Norm (NOM-062-ZOO-1999).

2.2 | 3-NP treatment

Mice were randomly assigned to the control and 3-NP-treated groups. Mice in the 3-NP group received one daily intraperitoneal (i.p.) injection of 3-NP (15 mg/Kg, dissolved in 0.1 mol L⁻¹ phosphate buffer (PB), pH 7.4) for 5 days, to produce early stages of HD.^{22,23}

Mice in the control group received an equivalent volume of the vehicle for 3-NP (PB) according to mouse weight. Electrophysiological recordings and Western blot experiments were performed 2 days after the administration of the last 3-NP treatment.

2.3 | Slice preparation

Mice were anesthetized with halothane in a drop-in induction chamber and decapitated prior to brain extraction. Brains were submerged in a chilled low Ca²⁺ and high Mg²⁺ saline solution (in mmol L⁻¹: 130 NaCl, 3 KCl, 1 CaCl₂, 5 MgCl₂, 26 NaHCO₃, 1.25 NaH₂PO₄, and 10 glucose), saturated with a 95% O₂/5% CO₂ mixture. Sagittal brain slices (400 µm) were obtained using a vibratome (PELCO, 1000 Plus Sectioning System, Ted Pella Inc., Redding, CA, USA). The sections were maintained in the same low-calcium saline solution and were continuously oxygenated (95% O₂/5% CO₂) for 1 hour prior to the electrophysiological experiments.

2.4 | Recordings of extracellular synaptic population spikes

Slices were immersed in a recording chamber and continually perfused with an artificial cerebrospinal saline (in mmol L⁻¹: 125 NaCl, 3 KCl, 1 MgCl₂, 26 NaHCO₃, 2 CaCl₂, 0.2 thiourea, 0.2 ascorbic acid, and 10 glucose) for *in vitro* recordings. The saline was constantly bubbled with 95% O₂/5% CO₂, and the pH was adjusted to 7.4 with NaHCO₃ when necessary.

A bipolar, concentric (25 µm) tungsten electrode coupled to a constant voltage stimulator isolator (DS2A-MK.II, Digitimer Ltd., Hertfordshire, UK) was placed in the corpus callosum to stimulate glutamatergic corticostriatal fibers and obtain population synaptic responses. A stimulation of 5-30 mV with duration of 50-100 µs was administered to evoke a synaptic response.

Synaptic responses were recorded in the dorsal striatum using borosilicate glass micropipettes (1.0 mm OD × 0.5 mm ID 30-30-1, FHC, Inc., Bowdoinham ME, USA) filled with an isotonic solution (NaCl, 0.9%) as a conductor (2-4 MΩ), amplified (AC GRASS P15, Quincy, MA, USA), filtered (1 kHz), acquired, and digitalized with the aid of an ATM10 card coupled to a PC running an acquisition system written in the LabVIEW environment. In each recording, stimulation voltage was adjusted to 45%-50% with the aid of an intensity-response curve.¹⁶ In all recordings, the paired-pulse protocol (PPP) was used in each evoked response to address the pre- or postsynaptic origin of changes in synaptic efficacy. The PPP consisted of two stimulating pulses (S1, S2) of the same intensity and duration with an interval of 30-50 ms at 0.1 Hz, and paired-pulse ratio (PPR; S2/S1) was analyzed in each experimental condition. Bicuculline (10 µmol L⁻¹), an antagonist of GABA_A receptors, was always present in the solution to isolate corticostriatal responses. After 10-15 minutes of stable recordings, NT-3 (50 ng/mL, PeptoTech Inc., Rocky Hill, NJ, USA) was added to the bath containing slices from control or 3-NP-treated mice. As NT-3 activates TrkC and TrkB receptors, some experiments were conducted in the presence of the TrkB blocker

cyclotraxin B (CTX-B, 100 nmol L⁻¹, TOCRIS Biosciences, Bristol, UK), to identify the receptors that mediate the modulatory effects of NT-3 on corticostriatal synapses.

The neurotrophin was added to the recording bath, and a high-frequency stimulation protocol (HFS) was subsequently administered to evaluate the effects of NT-3 on corticostriatal plasticity. The HFS consisted of 3 trains of 100 Hz, with a 1 second duration and 20-ms interval. In the experiments designed to inquire about the activation of TrkC or TrkB receptors as the target of NT-3 to modulate striatal plasticity, CTX-B was present at the beginning of the experiment, and after 10 minutes, NT-3 was added to the recording bath and the HFS protocol was initiated in the presence of CTX-B and NT-3.

The amplitude of population spike was quantified and normalized, and the synaptic response of the last 5 minutes was averaged and related to the baseline value prior to drug administration or

plasticity induction. Data were analyzed off-line (Microcal Origin, 9.1, Microcal Origin Lab Corp., Northampton, MA), and Adobe Illustrator CS5.1 (Adobe Systems, Inc. San José, CA) was utilized to construct the images in the final figures.

2.5 | Western blot

Striatal slices from the control group and 3-NP-treated mice were treated with NT-3 (50 ng/mL), NT-3 (50 ng/mL) + high K⁺ (50 mmol L⁻¹), CTX-B (100 μmol L⁻¹), NT-3 (50 ng/mL) + CTX-B (100 μmol L⁻¹), or NT-3 (50 ng/mL) + CTX-B (100 μmol L⁻¹) + high K⁺ for 30 minutes and compared to a control group in the physiological saline used for electrophysiological experiments lacking any treatment to evaluate the phosphorylation status of TrkA, TrkB, and TrkC receptors. After incubation, striatal slices were manually homogenized in lysis buffer containing 26 mmol L⁻¹ Tris-HCl, 1% Triton x-100, 1.3 mol

FIGURE 1 NT-3 modulates corticostriatal transmission. A, Time course of the population spike amplitude in the presence of NT-3. The amplitude of the spike in response to the first stimulation (S1) increased $57\% \pm 6\%$ compared to the control condition ($t_{16} = -38.31$; $P < 0.001$) in the control tissue, whereas in slices from animals treated with 3-NP, the increase in the amplitude of S1 was $30\% \pm 5.7\%$ ($t_{17} = -22.6$; $P < 0.001$). The line in A indicates the time of administration and NT-3 concentration (50 ng/mL). B, Representative traces of population spikes in slices from control and (C) 3-NP-treated mice. D, PPR (S2/S1) in each experimental condition. Significant differences in the PPR were not observed. Dashed lines are the individual values for PPR in each experiment. The solid line illustrates average values ($n = 6$; Mean $\pm$ SE)

FIGURE 2 Corticostriatal LTD is modulated by NT-3. A, Time course of the population spike amplitude in control and NT-3-treated slices after HFS (arrow). Note that LTD is obtained under both conditions but to different extents. Control slices without NT-3 ($57.9\% \pm 3.7\%$, $t_{17} = 73.09$; $P < 0.001$) vs slices treated with NT-3 ($28.8\% \pm 2.4\%$, $t_{29} = 65.23$; $P < 0.001$). B, Representative traces of population spikes in control slices before and after HFS. C, Representative traces of population spikes in control slices in the presence of NT-3 before and after HFS. D, PPR (S_2/S_1) in untreated control slices and in the presence of NT-3. Note that presynaptic mechanisms are involved in the LTD generated in control slices; however, a postsynaptic mechanism mediates the effects of NT-3 on LTD ($n = 6$; Mean $\pm$ SE)

L^{-1} glycerol, $130 \text{ mmol } L^{-1}$ NaCl, and the protein phosphatase inhibitor cocktail complete mini tab (Roche Diagnosis, Mannheim, Germany). Then, homogenate samples were collected and centrifuged for 5 minutes at 4500 rpm; the supernatant was collected and stored at -70°C . Protein concentrations were quantified using the Bradford method, and 25–30 μg of protein were loaded on 8%–10% polyacrylamide gels and electrophoresed (90–100 V, 150 mA). Proteins were transferred to nitrocellulose or polyvinylidene fluoride (PVDF) membranes that were then blocked with PBS or TBS and 5%–10% nonfat milk overnight at 4°C . Membranes were incubated with primary antibodies against phospho-Tyr654/655-TrkA (1:1000, Cell Signaling Technology, Inc. Danvers, MA, USA), total TrkA, (1:1000, Santa Cruz, Biotechnology, Inc. Dallas, TX, USA), phospho-Tyr816-TrkB (1:2000, EDM Millipore, Temecula, CA, USA), total TrkB (1:2000, Cell Signaling Technology, Inc. Danvers, MA, USA), phospho-Tyr820-TrkC (1:6000, Novus Biologicals,

Littleton, CO, USA), total TrkC (1:3000, Cell Signaling Technology, Inc. Danvers, MA, USA), and α -Actin (1:500, kindly donated by Dr. Hernández²⁴), for 12 hours at 4°C all antibodies except; phospho-TrkA, total TrkA, phospho-TrkC, total TrkC, and α -actin (1–4 hour at 4°C) and with HRP-conjugated secondary antibodies (1:10 000) for 1–2 hours at room temperature. Proteins were detected using the chemiluminescence method (Immobilon™ Western, Chemiluminescent HRP Substrate, Millipore, Billerica, MA, USA). Images were acquired (Gel Documentation System, BioSens SC 645, Shanghai Bio-Tech Co., Ltd.) and densitometrically analyzed using ImageJ software (NIH).

2.6 | Drugs

All reagents were purchased from Sigma-Aldrich (St. Louis, MO), unless stated otherwise.

FIGURE 3 NT-3 reestablished corticostriatal LTD in an animal model of HD. A, Time course of the population spike amplitude in slices from 3-NP-treated mice after HFS. 3-NP-treated slices exhibited an increase in the spike amplitude after HFS ($38.8\% \pm 3.5\%$, $t_{19} = -48.7$, $P < 0.001$); however, in the presence of NT-3, 3-NP-treated slices exhibited a decrease in amplitude after HFS, ($36.3\% \pm 4.1\%$, $t_{19} = 38.81$; $P < 0.001$). B, Representative traces of population spikes in slices from 3-NP-treated mice before and after HFS (arrow). HFS triggers LTP in 3-NP-treated slices. C, Representative traces of population spikes in slices from 3-NP-treated mice before and after HFS and in the presence of NT-3. Note that NT-3 reestablished LTD to the levels observed in control tissues (Fig. 2). D, PPR (S_2/S_1) in 3-NP slices and 3-NP slices + NT-3. There were no significant differences in both experimental conditions. Note that postsynaptic mechanism mediates the effects of NT-3 on the reestablishment of LTD in slices from 3-NP-treated mice ($n = 6$; Mean $\pm$ SE)

2.7 | Statistical analysis

All data were analyzed with Sigma Plot 12 software (Softek, SPSS Inc., San José, CA) and expressed as Means $\pm$ SEM. One-way ANOVA with a post hoc test or Student's t test (paired or unpaired, depending on the experiment) was used for comparisons between groups. Experiments without normal distribution data were analyzed with nonparametrical test. Statistical significance was set to $P < 0.05$.

3 | RESULTS

3.1 | Modulatory effects of NT-3 on corticostriatal transmission

In the first set of experiments, the effects of NT-3 on corticostriatal synaptic responses were evaluated. NT-3 (50 ng/mL) significantly

increased the synaptic responses of both experimental groups. A greater increase in the mean amplitude was observed in the control group than in the 3-NP group, but this difference was not significant. However, the distribution of the data increased when NT-3 was administered to the control group, but not the slices from the 3-NP-treated group (Figure 1A). Figure 1B, C shows representative recording traces. Modulatory effects of NT-3 on the control and 3-NP groups seemed to rely on postsynaptic mechanisms, as the analysis of PPR did not show differences (Figure 1D).

3.2 | Modulatory effects of NT-3 on long-term corticostriatal synaptic plasticity

We first evaluated plasticity in control tissues to evaluate the effect on NT-3 on corticostriatal plasticity. The HFS protocol in the corticostriatal pathway produced LTD in the control group; the HFS also

produced stable LTD in the presence of NT-3 (Figure 2A). Although NT-3 produced a lower decrease in the amplitude of LTD, differences between groups were not observed (Figure 2B, C). In control slices, LTD expression was mediated by a presynaptic mechanism; however, NT-3 modulation of LTD induction was mediated by a postsynaptic mechanism (Figure 2D).

3.3 | NT-3 restores corticostriatal synaptic plasticity in the 3-NP-treated animal model of HD

We next evaluated synaptic plasticity in slices from 3-NP-treated mice. HFS produced LTP in slices from the 3-NP-treated mice. However, if NT-3 was present in slices from 3-NP-treated mice, HFS induced LTD. In other words, the presence of NT-3 reestablished LTD (Figure 3A, B, C). Both, the LTP and LTD generated in the corticostriatal synapses of 3-NP-treated tissues was mediated by postsynaptic mechanisms (Figure 3D).

3.4 | The modulatory effects of NT-3 on corticostriatal synapses are mediated by TrkB receptors, and effects on LTD are not mediated by TrkB receptors activation

NT-3 is known to bind TrkC and with less affinity to TrkB. Therefore, the TrkB antagonist CTX-B was used to discern which receptor was involved in the NT-3-mediated modulation of corticostriatal synapses. First, the effect of CTX-B on healthy slices was evaluated; modulatory effect of NT-3 was prevented by the CTX-B; however, CTX-B did not affect LTD produced by HFS + NT-3 (Figure 4A, B).

PPR analysis of NT-3 effect in the presence of CTX-B on modulation and striatal plasticity did not show statistical differences (Figure 4C, D, respectively).

Next, we evaluated whether the inhibition of TrkB in slices from 3-NP-treated mice changed NT-3 effects on synaptic modulation and on striatal LTD. In the presence of CTX-B, NT-3 did not produce any modulatory effect on corticostriatal synapses (Figure 5A, B) and changes were not observed in the PPR analysis (Figure 5C).

The next series of experiments were conducted in the presence of CTX-B and NT-3 and after 10-20 minutes of stable recording, the HFS protocol was administered. Under this condition, a strong LTD was obtained (Figure 5D, E). According to the PPR analysis, a postsynaptic mechanism mediated this effect of NT-3 on striatal LTD (Figure 5F). Based on the results from these experiments, we concluded that TrkB was not involved in NT-3-mediated modulation of striatal LTD. Therefore, the modulatory effects of NT-3 on corticostriatal plasticity were mediated mainly by the activation of postsynaptic TrkC receptors.

3.5 | Phosphorylation of Tyr816-TrkB and Tyr820-TrkC induced by NT-3

Finally, changes in the phosphorylation level of TrkB and TrkC were evaluated in slices from 3-NP-treated mice, using the Western blot technique to verify the participation of the TrkB receptor in the modulatory effect of NT-3 and exclude its participation in NT-3-induced plasticity of damaged striatum. Even though there was an increase in TrkB phosphorylation in the presence of NT-3, we did not detect statistical differences in the p-Tyr816-TrkB levels among groups that

FIGURE 4 CTX-B blocks synaptic modulation but not NT-3 effects on corticostriatal synapse plasticity of healthy slices. A, Time course of population spikes of slices from healthy mice recorded in the presence of the TrkB blocker CTX-B. In the presence of CTX-B, NT-3 did not modulate the synaptic transmission of the corticostriatal pathway (100 ± 0.82 vs 97.97 ± 0.52). However, CTX-B did not prevent striatal LTD after HFS and in the presence of NT-3 (100 ± 0.82 vs 31.5 ± 1.91 ; $t_{28} = 51.5$, $P < 0.001$). B, Representative traces in the presence of CTX-B (black), in the presence of CTX-B + NT-3 (gray) and after HFS (red). The block of TrkB impedes NT-3 modulation but not the plasticity of corticostriatal synapses. C, PPR analysis of NT-3 effect on corticostriatal transmission or (D) striatal LTD did not exhibit significant changes

FIGURE 5 CTX-B blocks synaptic modulation but not NT-3 effects on corticostriatal synapse plasticity of 3-NP-treated mice. A, Time course of population spikes of slices from 3-NP-treated mice recorded in the presence of the TrkB blocker CTX-B. In the presence of CTX-B, NT-3 did not modulate the synaptic transmission of the corticostriatal pathway. B, Representative traces in the presence of CTX-B (black), in the presence of CTX-B + NT-3 (red), and the overlap. Note that in the presence of the TrkB blocker, NT-3 did not modulate corticostriatal transmission. C, Changes in PPR were not observed between the CTX-B and CTX-B + NT-3 groups of slices from 3-NP-treated mice. D, Time course of the population spike amplitude of slices from 3-NP-treated mice incubated with the TrkB blocker and subjected to the HFS protocol. Note that the TrkB blocker did not impede LTD triggered by HFS in the presence of NT-3. The spike amplitude was significantly decreased by 41.31% after HFS (100.16 ± 0.73 vs 58.78 ± 0.62 , $t_{40} = 43.094$, $P < 0.001$). E, Representative traces of population spikes in slices from 3-NP-treated mice in the presence of CTX-B (black), CTX-B + NT-3 (gray), after HFS in the CTX-B + NT-3 group (red) and the overlap. In 3-NP-treated slices, the TrkB blocker did not prevent LTD induced by HFS in the presence of NT-3. F, PPR (S2/S1) in 3-NP slices in the presence of CTX-B + NT-3 before and after HFS. No significant differences were observed indicating that a postsynaptic mechanism mediates the effect of NT-3 on reestablishing striatal plasticity ($n = 5$; Mean $\pm$ SE)

received NT-3 or NT-3 + high K^+ treatment compared to control slices. The lack of increase in the levels of p-Tyr816-TrkB in CTX-B-treated slices in the presence of NT-3, even in the presence of high K^+ to emulate HFS in the slice, demonstrates that NT-3 effects on plasticity are mediated by the activation of a different receptor from TrkB. Levels of TrkB phosphorylation at Tyr816 were significantly different in slices of control, or receiving NT-3, and NT-3 + high K^+ treatments

compared to those receiving treatments in the presence of CTX-B (Figure 6A, B left).

The levels of p-TrkC were evaluated under the same experimental conditions described above. As expected, CTX-B did not decrease p-Tyr820-TrkC levels under any experimental conditions. In particular, p-Tyr820-TrkC levels increased when NT-3 was applied in the presence of high K^+ (Figure 6B right). These results

FIGURE 6 Phosphorylation levels of TrkB and TrkC in the presence of NT-3. A, Representative images showing Western blot analyses of protein extracts from slices of 3-NP-treated mice maintained under conditions that imitated electrophysiological recordings. In the absence of CTX-B (lines 1-3), increased levels of p-Tyr816-TrkB were observed in response to NT-3 and NT+ HFS (high K^+) treatments. In the presence of CTX-B (lines 4-6), p-Tyr816-TrkB levels decreased in all experimental conditions. In contrast, p-Tyr820-TrkC expression level evaluated under the same conditions was not affected by CTX-B and increased in the presence of NT-3 and high K^+ . Independent slices from 3-NP-treated mice were used for each experimental condition. B, Illustrates in box plot the percentage of p-TrkB induction (p-TrkB/total TrkB) relative to control condition. The effect of NT-3 treatment in the band intensities was normalized to that for the untreated condition for each experimental group. The statistical analysis was performed using a Kruskal-Wallis one-way analysis of variance on ranks test ($H_5 = 19.274$; $P = 0.002$); multiple comparisons were assessed using the Student-Newman-Keuls test. * $P < 0.05$. P-Tyr820-TrkC activation (p-Tyr820-TrkC/TrkC) increased in high K^+ in the absence of CTX-B (lines 1-3); the CTX-B did not affect its expression (line 4-6). There was a nonsignificant increase in p-Tyr820-TrkC after the addition of CTX-B and in the presence of NT-3+ high K^+

illustrated that CTX-B did not block the phosphorylation of TrkC receptors by NT-3, and this activation was increased in the condition that emulates HFS.

The phosphorylation level of TrkA receptors was also evaluated to discard its role in NT-3 effects on corticostriatal plasticity. TrkA receptors did not change its phosphorylation status in any of the experimental conditions (Figure 7), showing that TrkA receptors are not involved in NT-3 effects on striatal LTD.

4 | CONCLUSION

Based on our results, NT-3 modulates the glutamatergic synaptic responses of the corticostriatal pathway in the healthy and damaged striatum, consistent with our previous report.¹⁶ NT-3 also modulates the long-term plasticity of corticostriatal transmission through postsynaptic mechanisms. HFS produced LTP in response to 3-NP-induced striatal degeneration. Nevertheless, the presence

of NT-3 in the slices of 3-NP-treated mice restored long-term depression in corticostriatal synapses. The activated receptor involved in modulating corticostriatal transmission was TrkB, whereas TrkC seemed to be involved in restoring corticostriatal LTD.

A 3-NP treatment has been shown to trigger LTP after HFS,^{25,26} and mitochondrial inhibition affects the membrane potential and increases the sensitivity of NMDA and AMPA receptors to glutamate, favoring the activity of PKA, which has been linked to the generation of striatal LTP.^{27,28} Nevertheless, this LTP is considered pathological; meanwhile, LTD is the physiological form of plasticity most frequently observed in the striatum. Moreover, corticostriatal LTD involves endocannabinoid synthesis, which act as retrograde messengers at the corticostriatal synapses, activating CB1 receptors at the presynaptic neuron which, in turn, reduces neurotransmitter release and maintains LTD expression.²⁹ However, for LTD induction, different postsynaptic mechanisms are involved.³⁰ This study demonstrates that the mechanism by which NT-3 modulates corticostriatal LTD in healthy tissue and restores LTD of damaged

FIGURE 7 Phosphorylation levels of TrkA in the presence of NT-3. A, Western blot analyses of protein extracts from slices of 3-NP-treated mice maintained under conditions that imitated electrophysiological recordings. Lines 1-3 in the first row show the expression of p-Tyr674/675-TrkA in control and in response to NT-3 and NT+ HFS (high K^+) treatments. Lines 4-6 exhibit phospho-TrkA in the presence of CTX-B. There were no changes in the phosphorylation level in any of the experimental conditions. B, Illustrates in box plot the percentage of p-TrkA expression (p-TrkA/Total TrkB) relative to control condition

striatum involves postsynaptic TrkC receptor activation, but the signaling pathway remains to be elucidated. The possible function of NT-3 in the interplay between postsynaptic induction and pre-synaptic maintenance in corticostriatal LTD is interesting because it has been described that TrkC receptors in conjunction with an axonal protein tyrosine phosphatase receptor (PTP σ) form an organizing complex of excitatory synapses which depends on synaptic activity.^{13,31} So, it may be possible that NT-3 links postsynaptic induction and presynaptic expression of LTD stabilizing corticostriatal synapses through PTP σ -TrkC complex and regulates striatal LTD too. Future experiments will test this hypothesis.

The modulatory effect of NT-3 on corticostriatal synapses was mediated by the activation of TrkB receptors; the use of the TrkB blocker prevented the NT-3-mediated modulation. However, the effect of NT-3 on striatal plasticity in both healthy and slices from 3-NP-treated mice was not inhibited by CTX-B. NT-3 has been reported to use different mechanism to modulate acute and long-term plasticity.^{8,32} Western blots showed that TrkC phosphorylation was increased by NT-3 in the presence of high K^+ which was used to mimic HFS, indicating that NT-3 restored LTD in the 3-NP model by activating preferentially TrkC receptors. Although NT-3 also activates TrkA, it is unlikely that NT-3 effects on LTD are mediated by TrkA stimulation, as we have shown that NGF did not affect corticostriatal

transmission,¹⁶ and we did not find changes in the phosphorylation state of TrkA receptors in our experimental conditions that could suggest its participation in the NT-3 effects in the present study.

The effects of NT-3 on neuronal plasticity in other cerebral areas^{11,33-35} or at the neuromuscular junction^{36,37} have been described. In the striatum, the research on neurotrophins has focused on describing the trophic effects of brain-derived neurotrophic factor (BDNF) on striatal MSNs, because defective huntingtin generated by the HD mutation impedes BDNF transcription.³ However, as stated above, NT-3 also activates TrkB receptors,³⁸ and some of the actions ascribed to BDNF may be mediated by NT-3. As shown in the present study, the modulatory effects of NT-3 on corticostriatal synapses are mediated by TrkB activation; however, LTD was modulated and reestablished by the activation of TrkC. In previous studies, we documented a decrease in the NT-3 levels in response to 3-NP-induced striatal degeneration.³⁹ If the 3-NP treatment reduced NT-3 levels, HFS produced LTP; however, the exogenous addition of NT-3 to the recording bath reestablished the LTD by activating TrkC receptors, the levels of which are known to be increased in this model of striatal degeneration.³⁹

Our animal model of neurodegeneration mimics the early histopathological and neurophysiological events of HD, such as dysregulation of glutamatergic cortical inputs to the striatum along with an increase in the postsynaptic excitatory currents.^{40,41} These changes partially explain how HFS induces LTP in our model of HD. Neuronal damage, as evidenced by changes in synaptic activity and the number of dendritic spines in the MSNs, also produces a synaptic disconnection.¹⁶

The progressive reduction in corticostriatal synaptic activity affects BDNF release and subsequently enhances cellular dysfunction and degeneration of the MSNs.⁴¹ Similar to patients with HD and the transgenic mouse model, BDNF levels are also reduced in the 3-NP-induced neurodegeneration model.^{16,39} The decreased BDNF levels will fail to trigger neuronal survival and neuronal protection of MSNs. However, if NT-3 is present in the striatum, it would stimulate TrkB and TrkC receptors and trigger signaling pathways related to neuroprotective mechanisms as well modulatory actions.⁴² Therefore, experimental strategies that increase NT-3 levels in the degenerated striatum would be a good candidate to overcome with neuronal death.

To our knowledge, the present study is the first one to report that NT-3 restores striatal LTD in a degeneration model of HD. Also, our results show that NT-3 actively participates in striatal physiology, and as synaptic plasticity is important to maintain striatal function, we are currently investigating which signaling pathway(s) is (are) involved in these modulatory effects.

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blot experiments, acquired, and analyzed data; CI Vivar-Cortés and FM Torres Cruz carried out Western blot experiments and analyzed data; and E Hernández-Echeagaray conceived and designed the experimental work, interpreted data, and wrote the article. Víctor G Gómez-Pineda is a Ph.D. student from Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México (UNAM), and receives a PhD fellowship 816819 from CONACyT. FM Torres Cruz is a postdoctoral trainee and receives a fellowship from the Programa de Becas Posdoctorales de la DGAPA-UNAM. This work was supported by CONACyT 180660 and DGAPA-PAPIIT IN216515, IN211718 Grants to EHE*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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