



# Cortical Axonal Secretion of BDNF in the Striatum Is Disrupted in the Mutant-huntingtin Knock-in Mouse Model of Huntington's Disease

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Deficient BDNF signaling is known to be involved in neurodegenerative diseases such as Huntington's disease (HD). Mutant huntingtin (mhtt)-mediated disruption of either BDNF transcription or transport is thought to be a factor contributing to striatal atrophy in the HD brain. Whether and how activity-dependent BDNF secretion is affected by the mhtt remains unclear. In the present study, I provide evidence for differential effects of the mhtt on cortical BDNF secretion in the striatum during HD progression. By two-photon imaging of fluorescent BDNF sensor (BDNF-pHluorin and -EGFP) in acute striatal slices of HD knock-in model mice, I found deficient cortical BDNF secretion regardless of the HD onset, but antisense oligonucleotide (ASO)-mediated reduction of htt only rescues BDNF secretion in the early HD brain before the disease onset. Although secretion modes of individual BDNF-containing vesicle were not altered in the pre-symptomatic brain, the full-fusion and partial-fusion modes of BDNF-containing vesicles were significantly altered after the onset of HD symptoms. Thus, besides abnormal BDNF transcription and transport, our results suggest that mhtt-mediated alteration in activity-dependent BDNF secretion at corticostriatal synapses also contributes to the development of HD.

**Key words:** BDNF, Huntington's disease, antisense oligonucleotide, corticostriatal synapse

## INTRODUCTION

Huntington's Disease (HD) is a neurodegenerative disorder caused by the mutation of the huntingtin gene (*htt*), which is autosomal dominant [1]. The age of the HD onset varies inversely with the length of CAG repeat expansion in the *htt*, normally at midlife

[2-4]. Typical symptoms include motor dysfunction, cognitive decline, and psychiatric disorders, all of which are believed to be caused by mutant huntingtin (mhtt)-dependent degeneration of many brain areas including the cortex and striatum.

Neurotrophin signaling is known to have a protective function in HD by promoting striatal neuronal survival [5, 6]. Deficiency in brain derived-neurotrophic factor (BDNF) is linked to diverse brain dysfunctions, and the close relationship between HD pathology and BDNF loss has been extensively demonstrated [5-8]. In order to reverse the lowered striatal BDNF level and striatal atrophy in the HD brain, various manipulations for increasing BDNF signaling have been found to enhance cell survival and al-

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leviate HD symptoms in HD mouse models. One of approaches to recover the striatal BDNF level is overexpressing *bdnf* gene in the striatum of HD brain [9-13]. However, there is no direct evidence that overexpressed BDNF could be efficiently secreted in the HD brain. Under the normal condition, cortical BDNF is transported to the striatum anterogradely [9, 14, 15], and both cellular transport and secretion of BDNF are activity-dependent [16, 17]. Therefore, understanding how BDNF transport and activity-dependent secretion is regulated at corticostriatal synapses under normal and diseased states is important for designing BDNF-related strategies for HD therapy.

In this study, I have utilized a fluorescent BDNF sensor, that is expressed by a viral vector encoding *Bdnf* gene conjugated with pH-sensitive or enhanced green fluorescent protein (pHluorin and EGFP, respectively), to examine the spatiotemporal pattern of BDNF secretion in freshly isolated brain slices [17, 18]. Heterozygous knock-in mice model for HD were used because they are known to most closely reflect the genetic mutation that is found in human HD patients [19, 20]. The present study revealed a selective role of mHTTs in an impairment of BDNF secretion and suggests a novel therapeutic strategy for preventing deficiency of BDNF secretion in the HD brain by lowering the mHTT level before the onset of disease phenotypes.

## MATERIALS AND METHODS

### Animals

Animal protocols were approved by the Animal Care and Use Committee of University of California, Berkeley. All mice were purchased from Jackson Laboratory except the Q140 and Q175 mice, both of which were provided by the CHDI Foundation and shipped via Jackson Laboratory. *Emx1-Cre* heterozygotes (genetic background of C57BL/6; *Emx1*<sup>tm1(cre)Krf/J</sup>) were crossed with *Q140* homozygotes (B6J.129S1-Htt<sup>tm1Mfc</sup>/140Chd1J) or *Q175* homozygotes (B6J.129S1-Htt<sup>tm1Mfc</sup>/190Chd1J) to produce *Emx1-Cre/Q140* or *Emx1-Cre/Q175* heterozygotes. As a negative control, *Emx1-Cre* heterozygotes were used.

### Expression of BDNF sensor

AAV-DIO-BDNF-pHluorin was used as described previously [17]. AAV-DIO-BDNF-EGFP was constructed by replacing the pHluorin fragment into the PCR-amplified EGFP fragment. Sequencing and restriction enzyme reactions were performed to verify the plasmid. Custom packaging and purification of both BDNF sensors were performed through UNC Vector Core. 500 nl (per each hemisphere) of AAV-packaged BDNF sensors were bilaterally injected into the M1 of *Emx1-Cre* or *Emx1-Cre/Q140*

(*Q175*) heterozygote.

### Antisense oligonucleotide (ASO) injection

Injection of 700 µg of ASO (in 200~500 nl of PBS) targeting htt [21, 22], which was provided by ISIS (now IONIS) pharmaceutical via the exclusive material transfer agreement (MTA) among the UC Berkeley, CHDI, and ISIS pharmaceutical, was performed in the right lateral ventricle of Q175 mice. As a control, the same volume of PBS was injected. One week later a single bolus ASO or PBS injection, the same mice were again injected with AAV-hSyn-BDNF-EGFP into the motor cortex (M1). After additional three more weeks to achieve full BDNF-EGFP expression in the M1 cortex, striatal slices were then prepared to examine activity-induced changes in BDNF-EGFP fluorescence using a two-photon microscope.

### Acute brain slice preparation

Standard artificial cerebral spinal fluid (ACSF) consisted of (in mM) 130 NaCl, 3.5 KCl, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 24 NaHCO<sub>3</sub>, 2 CaCl<sub>2</sub>, 2 MgCl<sub>2</sub>, and 10 glucose (pH 7.3). Mice were deeply anesthetized with isoflurane, and then transcardially perfused with ~20 ml of slicing ACSF (ACSF containing 10 mM Mg<sup>2+</sup> and 0.5 mM Ca<sup>2+</sup>) before the brain was dissected. Parasagittal striatal slices (400 µm thick) were prepared using a vibratome (Leica) using ice-cold slicing ACSF (below 4°C) and maintained at 30~32°C in normal ACSF for 1 hour before electrophysiological recording or two-photon imaging.

### Two-photon laser-scanning microscopy

Two-photon laser-scanning microscopy was performed using an LSM 510 META/NLO Axioimager system (Zeiss; Molecular Imaging Center at UC Berkeley) equipped with a Spectra-Physics MaiTai HP DeepSee laser (700 to 1,020 nm) and 403 water-immersion infrared objective (NA 0.8). BDNF-pH or BDNF-EGFP was excited by the 880 nm laser. The emission signals of BDNF-pH or BDNF-EGFP were acquired by using 500~550 nm band-pass filter. The field of view (512×512 pixels, 0.21 mm/pixel, 0.8 ms pixel time) was chosen in the striatal slice where cortical projections remained intact and BDNF-pH or BDNF-EGFP was significantly expressed at synaptic bouton-like structures (1~2 µm). Slices were placed in a recording chamber, submerged, and continuously perfused (2~3 ml/min) with oxygenated ACSF (containing 100 µM picrotoxin to isolate the glutamatergic synaptic transmission) at room temperature (20~25°C).

To record changes in BDNF-pH or BDNF-EGFP intensity in response to electrical stimulation, I acquired at least 100 consecutive images (at 1 Hz) as a baseline, then applied electrical stimula-



tion (HFS: 4 trains of stimuli spaced at 10 s intervals, with each train containing bursts of 100 spikes at 100 Hz) using a tungsten bipolar electrode (WPI) placed on the cortical layer 6 close to the white matter, and then at least additional 200 images at 1 Hz after stimulation were taken. In some experiments, iso-osmotic ACSF containing 50 mM  $\text{NH}_4\text{Cl}$  (pH 7.4) was applied to identify axonal BDNF-pH or BDNF-EGFP at the end of each experiment.

### Image analysis

Images were processed and analyzed with ImageJ software (NIH). I presented data as normalized fluorescence changes ( $\Delta F_i/F_0$ ), in which fluorescence changes ( $\Delta F_i$ ) at a given time were divided by the baseline fluorescence before stimulation ( $F_0$ ). Puncta with the amplitude of fluorescence reduction  $\geq 2$  standard deviations (SDs) from the baseline fluorescence were categorized as undergoing full fusion, and those showing a fluorescence increase  $\geq 2$  SDs from the baseline fluorescence were categorized as undergoing a transient event. The rest of the events were defined as no fusion.

### Statistical analysis

Statistical analyses were performed by using Prism 6.0 software (GraphPad). Unpaired Student's t-test and one-way ANOVA with post-test were used for testing significance between two groups and among three or more groups, respectively.

## RESULTS

### Activity-dependent BDNF secretion is selectively impaired at cortical axons in the HD brain

To test whether activity-dependent secretion of BDNF is altered in the brain of HD model mice, BDNF secretion was monitored with acute striatal slices containing corticostriatal projections as previously described [17], using a knock-in type of HD model mice (*HdhQ140*). *Cre/Q140* hybrid mice were obtained by mating *Emx1-Cre* transgenic mice with *HdhQ140* homozygotes, resulting in heterozygotes carrying a single copy of *HdhQ140* and *Cre* genes (*Emx1-Cre Q140/+*; Fig. 1A). At least 1-year old (48 weeks) *Emx1-Cre Q140/+* or *Dlx6-Cre Q140/+* mice were injected with AAV-DIO-BDNF-pHluorin (Fig. 1A, B) and used for experiments because Q140 mice are known to display prominent abnormal HD-like phenotypes at this age [19].

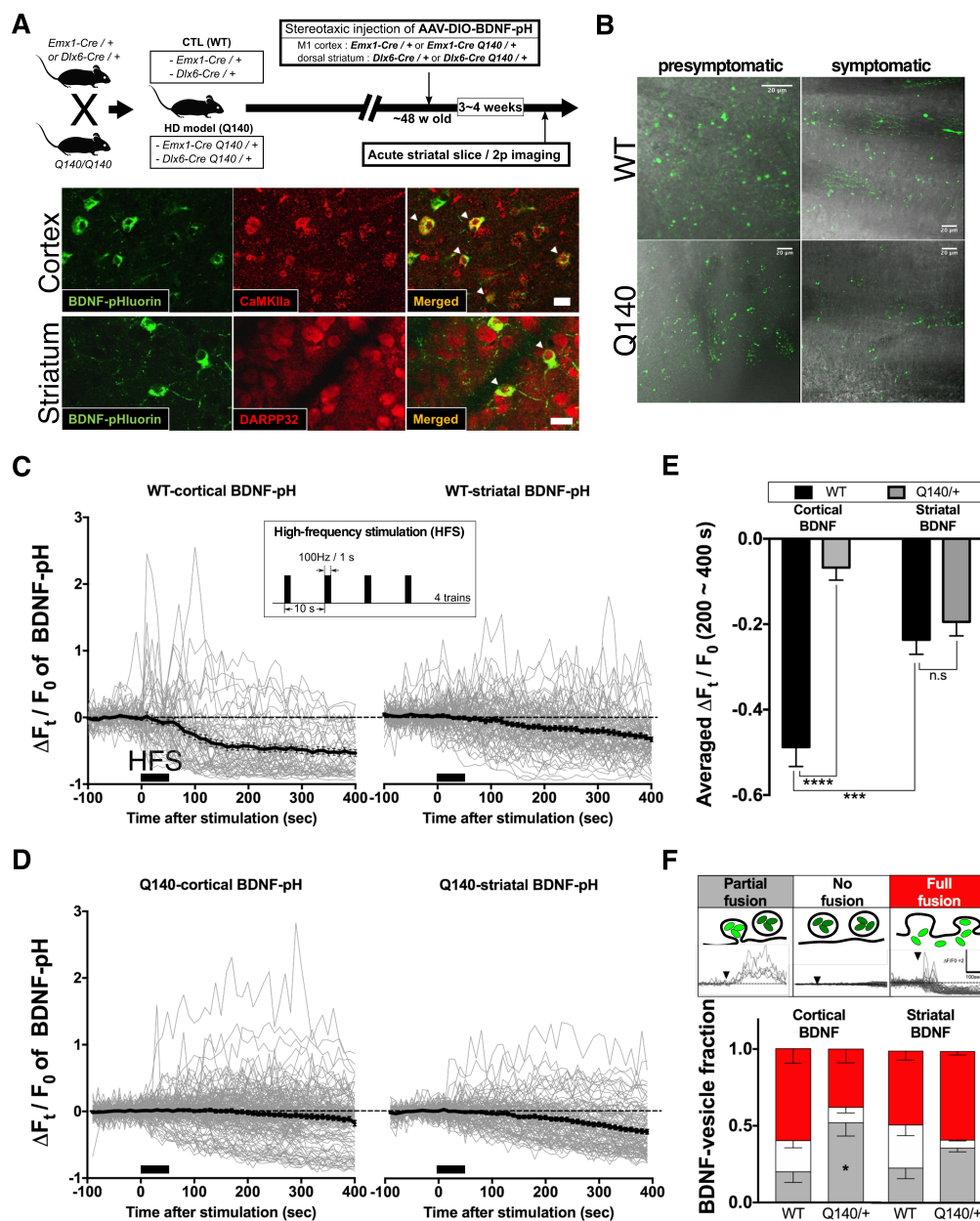
After applying electrical stimulation to cortical axons with the high-frequency stimulation (HFS) protocol (Fig. 1C, *inset*; see Materials and Methods for detailed information) to trigger activity-dependent BDNF secretion at corticostriatal synapses [17, 23], I found that presynaptic secretion of BDNF from cortical axons was greatly reduced in Q140 mice (Fig. 1C and E). Q140 mice

showed the reduced activity-induced change in  $\Delta F_i/F_0$  (averaged over 200~400 sec duration after stimulation) of BDNF-pHluorin (BDNF-pH) fluorescence in cortical axons (cortical BDNF-pH: wild-type (WT) =  $-0.49 \pm 0.04$  vs. Q140 =  $-0.07 \pm 0.03$ ;  $p < 0.0001$ , unpaired t-test). In contrast, there was no significant difference in BDNF secretion from medium spiny neuron (MSN) dendrites of Q140 mice, as compared to that found for WT mice (Fig. 1D and E). Activity-induced changes in  $\Delta F_i/F_0$  of BDNF-pHluorin from MSN dendrites of Q140 mice brain slices were not changed as observed in cortical axonal BDNF-pH (averaged  $\Delta F_i/F_0$  of striatal BDNF-pHluorin over 200~400 sec duration after stimulation: WT =  $-0.24 \pm 0.03$  vs. Q140 =  $-0.19 \pm 0.03$ ;  $p = 0.38$ , unpaired t-test). Collectively, these results indicate that cortical not striatal BDNF secretion is largely impaired in the striatum of mice with mhtts.

### Fusion modes of BDNF-containing vesicles were differentially regulated in cortical and striatal neurites

Next, to explore the mechanism underlying differential effects of mhtts on BDNF secretion from cortical axons vs. striatal MSN dendrites, I analyzed behaviors of individual BDNF-containing vesicles by performing a population analysis of BDNF-pH puncta, which are indicative of BDNF-pH containing secretory vesicles [17, 24]. I was able to categorize fluorescence changes in BDNF-pH puncta into three differential secretion modes: partial-, full-, and no-fusion mode (Fig. 1F, above; see Materials and Methods for more details) [17].

I found that the major secretion mode of pools of BDNF-containing vesicles (BDNF-vesicles) in cortical axons and MSN neurites of WT mice was full-fusion (Fig. 1F; the fraction of the full-fusion mode, cortical,  $0.60 \pm 0.09$ ; striatal,  $0.49 \pm 0.06$ ). However, in Q140 mice the major secretion mode of cortical BDNF-vesicles was the partial fusion, and I found that an increase in the partial fusion mode of BDNF-vesicles in Q140 mice was statistically significant (Fig. 1F; the fraction of the partial-fusion mode, WT,  $0.20 \pm 0.07$ ; Q140,  $0.52 \pm 0.09$ ;  $p < 0.05$ , unpaired t-test). On the other hand, in Q140 mice striatal BDNF-vesicles rather showed the moderate increase in the fraction of either the full-fusion or partial-fusion mode compared to WT (the fraction of the partial-fusion mode: WT BDNF-vesicles =  $0.23 \pm 0.07$  vs. Q140 BDNF-vesicles =  $0.36 \pm 0.02$ ; the fraction of the full-fusion mode: WT BDNF-vesicles =  $0.49 \pm 0.06$  vs. Q140 BDNF-vesicles =  $0.59 \pm 0.02$ ), and this was likely due to the significant decrease in the no-fusion mode compared to WT ones (Fig. 1F; the fraction of the no-fusion mode, WT,  $0.29 \pm 0.07$ ; Q140,  $0.05 \pm 0.01$ ;  $p < 0.05$ , unpaired t-test). These results indicate a differential alteration of cortical and striatal mechanisms for activity-dependent BDNF in the HD brain and support the idea that cortical neurons express molecular ma-



**Fig. 1.** Differential activity-induced secretion of overexpressed BDNF from cortical axons or striatal neurites in the normal or Q140 heterozygote mice. (A) Above: Schematic diagram depicting experimental conditions including mouse generation, stereotaxic injection of AAV, and two-photon imaging of acute striatal slices. Below: Representative immunohistochemical analysis showing selective BDNF-pH expression in the cortex or dorsal striatum by an injection of AAV-DIO-BDNF-pH into the WT mice (*Emx1-Cre* / + or *Dlx5/6-Cre* / + mice). Note that CamKIIa and DARPP32 are markers for labeling cortical excitatory neuron and striatal medium spiny neurons, respectively. Scale bar = 20  $\mu$ m. (B) Representative images demonstrating BDNF-pH puncta signals in the striatum of WT (*Emx1-Cre* / +) or Q140 (*Emx1-Cre Q140* / +) mice at 10 weeks (presymptomatic) or 1 year old (symptomatic). AAV-DIO-BDNF-pH was injected into the primary motor cortex. (C, D) Two-photon imaging of striatal slices of *Emx1-Cre* / + (WT-cortical BDNF-pH) or *Dlx5/6-Cre* / + (WT-striatal BDNF-pH) mice or *Emx1-Cre Q140* / + (Q140-cortical BDNF-pH) or *Dlx5-Cre Q140* / + (Q140-striatal BDNF-pH) heterozygote mice (12~24 weeks old) injected with AAV-DIO-BDNF-pH in the primary motor cortex (cortical BDNF-pH) or dorsal striatum (striatal BDNF-pH). 4 times of 100 Hz (1 sec) electrical stimulation (black bar) was given to trigger activity-dependent BDNF secretion. Individual (gray) and averaged (black, mean  $\pm$  s.e.m.) traces of BDNF-pH fluorescence intensity measured by two-photon imaging were shown. Inset: Schematic diagram depicting the high-frequency stimulation (HFS) protocol. (E) Bar graphs depict average  $\Delta F_t / F_0$  ( $\pm$  s.e.m.) during 200~400 s after stimulation for all recorded BDNF-pH puncta. Numbers of puncta, at least 3 slices from 2 mice: WT-cortical BDNF-pH, 56; WT-striatal BDNF-pH, 86; Q140-cortical BDNF-pH, 155; Q140-striatal BDNF-pH, 98. \*\*\*\**p* < 0.0001, \*\*\**p* < 0.001, one-way ANOVA with Tukey's multiple comparisons test. (F) Above: Categorization of BDNF-containing vesicle fractions according to their changes in fluorescence intensities after electrical stimulation, as "Partial fusion (increased & recovered fluorescence intensity, gray)", "No fusion (no changes in fluorescence intensity, white)", and "Full fusion (decreased fluorescence intensity, red)". Below: Bar graphs depict average fractional changes of individual BDNF-pH puncta (putative BDNF-containing vesicles, mean  $\pm$  s.e.m.). \**p* < 0.05, WT vs. Q140, unpaired t-test.

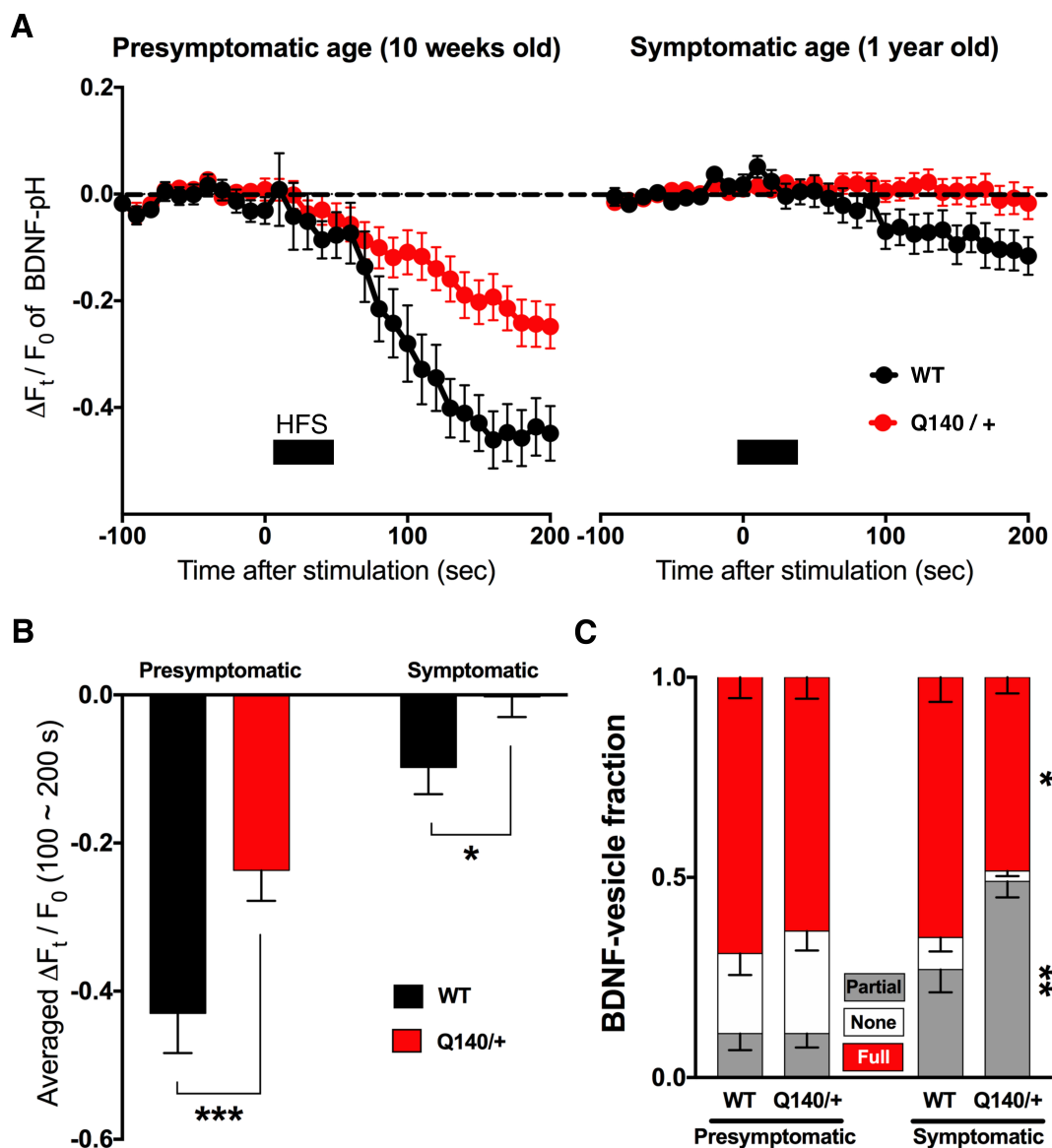
chinery that is either essential for BDNF secretion or sensitive to the mhtt.

**Differential effects of disease-state on cortical BDNF secretion**

There is evidence for early changes in the striatum in HD model mice and human patients before the onset of motor symptoms [19, 25-27]. It is thus possible that cellular components required for activity-dependent BDNF secretion are already altered before striatal

degeneration is observed. To test this idea, I next compared BDNF secretion from cortical axons of 10 week old (presymptomatic age) with that of 1 year old (symptomatic age) *Emx1-Cre Q140/+* mice (Fig. 1B). 10 weeks and 1 year old ages were chosen, because Q140 knock-in model mice showed significant htt nuclear staining beginning at 8 weeks, and this htt staining became widespread by 24 weeks of age [19].

Consistent with the finding of impaired BDNF-pH secretion



**Fig. 2.** Disease state-specific alteration of activity-dependent BDNF secretion from cortical axons. (A) Average fluorescence changes with time ( $\Delta F_t / F_0$ ) for BDNF-pH puncta of cortical axons in wild-type (*Emx1-Cre*+, black) or Q140 (*Emx1-Cre Q140/+*, red) during the presymptomatic period (10 weeks old) or symptomatic period (1-year-old). Mean $\pm$ s.e.m. HFS (black bar) was given to trigger activity-dependent BDNF secretion. (B) Bar graphs depict average  $\Delta F_t / F_0$  ( $\pm$ s. e. m.) during 100–200 s after stimulation for all recorded BDNF-pH puncta. Numbers of puncta from at least 3 slices of 2 mice: presymptomatic wild-type (WT), 56; presymptomatic Q140 (Q140/+), 82; symptomatic wild-type (WT), 62; symptomatic Q140 (Q140/+), 155. \* $p < 0.05$ , \*\*\* $p < 0.001$ , unpaired t-test. (C) Bar graphs depict average fractional changes of individual BDNF-pH puncta (putative BDNF-containing vesicles, mean $\pm$ s.e.m.). WT vs. Q140: \* $p < 0.05$ , \*\* $p < 0.01$ , unpaired t-test.

from cortical axons in Q140 (Fig. 1), a replicated BDNF secretion assay still showed a significant reduction of HFS-induced BDNF secretion from cortical axons of symptomatic Q140 mice (Fig. 2A and B; averaged  $\Delta F_t/F_0$  over 100~200 sec duration after stimulation, symptomatic age: WT,  $-0.10 \pm 0.04$ ; Q140,  $-0.00 \pm 0.02$ ;  $p < 0.05$ , unpaired t-test). I also found that Q140 mice at the presymptomatic age showed a decrease in cortical BDNF secretion in response to HFS when compared to wild-type mice (Fig. 2A and B; averaged  $\Delta F_t/F_0$  over 100~200 sec duration after stimulation, presymptomatic age: WT,  $-0.43 \pm 0.05$ ; Q140,  $-0.23 \pm 0.04$ ;  $p < 0.001$ , unpaired t-test).

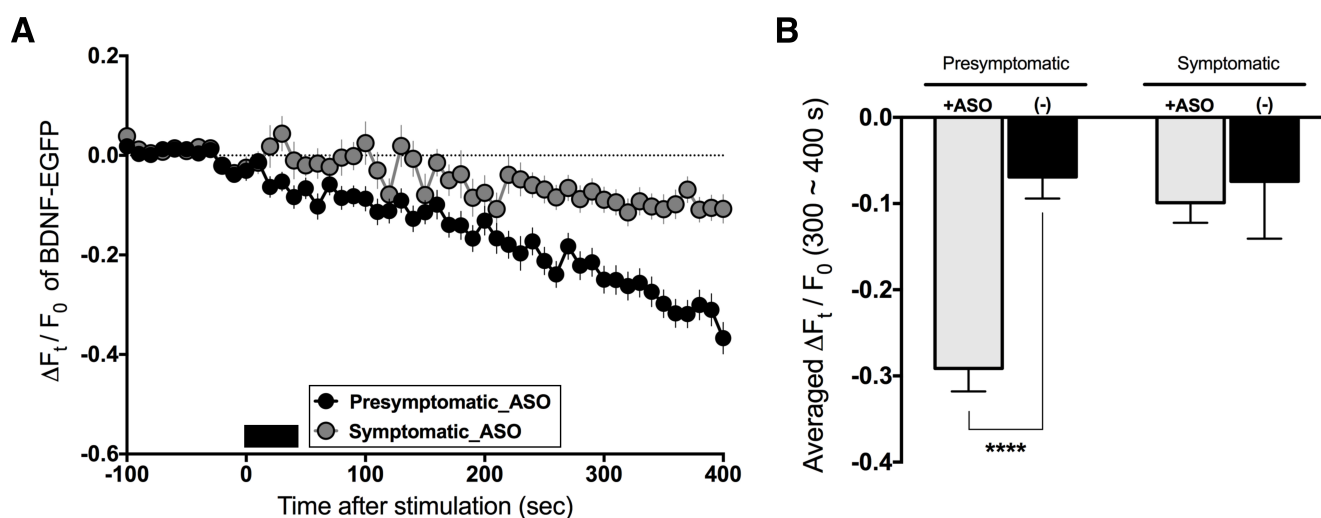
However, a population analysis of BDNF-vesicles in each condition revealed substantial changes in BDNF-vesicle fusion modes with the progress of the disease. At the presymptomatic age, reduced activity-dependent BDNF secretion was not correlated with any change in fusion modes of BDNF-vesicles (Fig. 2C), suggesting that molecular mechanisms involved in activity-dependent cortical BDNF secretion are relatively intact before the HD onset. By contrast, impaired BDNF secretion at the symptomatic age was accompanied by both a significant increase in the partial-fusion mode (fraction: WT,  $0.27 \pm 0.06$ ; Q140,  $0.49 \pm 0.04$ ;  $p < 0.01$ , unpaired t-test) and decrease in the full-fusion mode (fraction: WT =  $0.65 \pm 0.06$  vs. Q140 =  $0.48 \pm 0.04$ ;  $p < 0.05$ , unpaired t-test), indicating reduced activity-dependent full-fusion of BDNF-containing vesicles. These results suggest that mhtts not only affect BDNF transcription and transport but also inhibit BDNF secre-

tion by disrupting mechanisms for activity-dependent exocytosis of BDNF-containing secretory granules.

#### ASO-mediated Htt knock-down prevents disruption of axonal BDNF secretion in presymptomatic HD brains

My results demonstrated an association of the mhtt with disrupted activity-dependent BDNF secretion from cortical axons. I next examine whether the mhtt is directly responsible for impaired cortical BDNF secretion in the HD brain, by reducing the htt level with a htt-targeted antisense nucleotide (ASO), which was shown previously to down-regulate mhtt expression [21, 22]. Another knock-in HD line with ~180 CAG repeats in the htt gene (Q175 mice; *Emx1-Cre Q175/+* vs. *Emx1-Cre/+*), which was reported to show faster cortical and striatal atrophies (24~32 weeks old; [20]) than Q140 mice, was used in this experiment. For direct comparison of full-fusion modes of BDNF-vesicles, I utilized BDNF-EGFP instead of BDNF-pH, because a decrease in the fluorescent intensity of BDNF-EGFP mostly reflects the full-fusion of BDNF-vesicles [17, 18, 24].

I found that changes in fluorescence intensity of BDNF-EGFP in response to electrical stimulation in all five ASO-injected symptomatic Q175 mice were similar with those observed from PBS-injected Q175 mice (Fig. 3), indicating that ASO-mediated htt knock-down unable to prevent the impairment of BDNF secretion. However, ASO injection to the presymptomatic Q175 (8-week old) mice was effective in preventing defective activity-dependent



**Fig. 3.** Reduced Htt level is efficient for alleviating BDNF secretion deficit in the presymptomatic HD brain. (A) Average fluorescence changes with time (mean  $\pm$  s. e. m. of  $\Delta F_t/F_0$ ) for BDNF-EGFP puncta of cortical axons in Q175 (*Emx1-Cre Q175/+*, red) during the presymptomatic period (10 weeks old) or symptomatic period (1-year-old). 4 times of 100 Hz (1 sec) electrical stimulation (black bar) was given to trigger activity-dependent BDNF secretion. (B) Bar graphs depict average  $\Delta F_t/F_0$  ( $\pm$  s. e. m.) during 300~400 s after stimulation for all recorded BDNF-EGFP puncta. Numbers of puncta from at least 3 slices of 3 mice: presymptomatic Q175 with PBS injection (-), 32; presymptomatic Q175 with ASO injection (+ASO), 99; symptomatic Q175 with PBS injection, 29; symptomatic Q175 with ASO injection, 94. \* $p < 0.05$ , \*\*\* $p < 0.001$ , unpaired t-test.



BDNF secretion. I found that overall cortical BDNF secretion was significantly recovered in three of five presymptomatic Q175 mice, which were injected with the same amount of htt ASO with that injected in the symptomatic Q175 mice (Fig. 3; \*\*\* $p < 0.001$ ). These results indicate that a mhtt reduction during the early stage of HD could be beneficial for restoring BDNF level in the striatum through recovering activity-dependent BDNF secretion, although same application was not effective for reversing impaired BDNF secretion in the symptomatic HD brain.

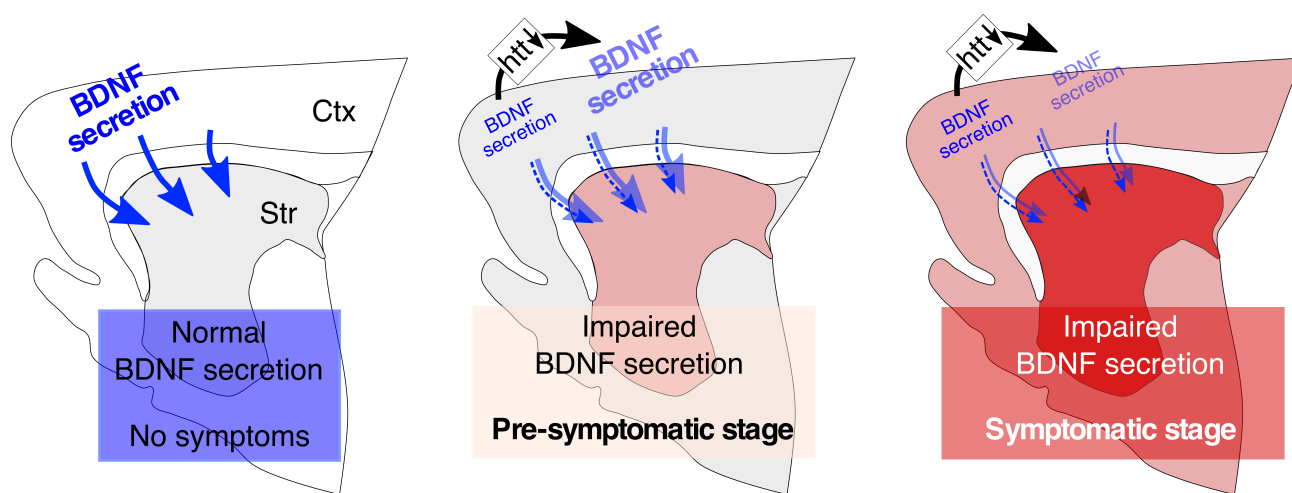
## DISCUSSION

In this study, I show that cortical activity-dependent BDNF secretion is abnormal, but no alteration of striatal BDNF secretion was observed in the brain with HD (Fig. 1E). These results suggest that cortical activity-dependent BDNF secretion is more vulnerable to the mhtt than striatal BDNF secretion from MSNs. Since striatal MSN do not express a significant level of BDNF [14, 15], insensitivity of activity-dependent BDNF secretion to the mhtt might result from the lack of molecular machinery essential for BDNF secretion. Overall activity-induced cortical BDNF-pH secretion in WT striatal slices was significantly higher than striatal one by same stimulation (Fig. 1E;  $p < 0.001$ , unpaired t-test), but this is probably because cortical axons directly received electrical stimulation, whereas activity in striatal neurites was evoked by electrical stimulation-induced synaptic transmission. Since pre-

synaptic glutamate release at corticostriatal synapses was shown to be already reduced at this HD stage [26, 27], the reduced no-fusion mode of striatal BDNF-vesicles in the HD brain might be reminiscent of the increased postsynaptic receptor sensitivity as a compensatory mechanism caused by decreased presynaptic inputs, and this alteration may affect activity-dependent BDNF secretion from striatal neurons of HD brains.

The exact molecular mechanisms of BDNF secretion affected by mhtts are still unclear. Several studies provide molecular evidence for mhtt-dependent alteration of vesicular exocytosis. Overexpression of mutant huntingtin in PC12 cells depleted vesicular machinery such as complexin II, resulting in impaired  $Ca^{2+}$ -triggered vesicular exocytosis [28]. Moreover, impaired astrocytic BDNF release in Q140 knock-in mice is reported to be caused by the abnormal function of Rab3a, a small GTPase localized on membranes of dense-core vesicles [29]. Since an ATP release from astrocytic dense-core vesicles was also significantly reduced in Q140 mice [29], it is possible that mhtts disrupt general  $Ca^{2+}$ -dependent exocytosis of dense-core vesicles. Despite normal fusion modes of BDNF-vesicles in the presymptomatic HD brain (Fig. 2C), I cannot exclude a possibility that a mhtt reduction reverses a deficient BDNF transport in the presymptomatic HD brain that was found to be present during early developmental stages [6, 7], still capable of increasing the chance of BDNF secretion in the striatum.

My study did not directly show whether the ASO treatment restored impaired secretion of a pro-form (proBDNF) or mature



**Fig. 4.** Proposed model for a disease progress-dependent alteration of activity-dependent BDNF secretion from cortical axons to the striatum. A schematic illustration of cortical presynaptic BDNF secretion in the normal brain or HD brains at the presymptomatic or symptomatic stage. In addition to impaired synthesis and transport of BDNF, I propose that BDNF secretion is also downregulated by mutant huntingtin (htt), probably through altered actions of molecular mechanisms regulating activity-dependent docking or exocytosis of BDNF-containing vesicles. Since mhtt-mediated disruption of BDNF secretion is prominent during the presymptomatic stage of HD, and this could be reversed by selective reduction of mhtt, a combined treatment targeting both the presymptomatic stage of HD and restoration of BDNF expression could be a promising strategy for reversing striatal degeneration in the HD brain.



form of BDNF (mature BDNF). A line of evidence suggests that the mature BDNF is a major form of BDNF secreted from cortical axons in the striatum. A previous study demonstrated that forebrain overexpression of BDNF in the HD model mice increases striatal TrkB signaling and cell survivals [9], both of which are mediated by mature BDNFs. Moreover, induction of corticostriatal long-term potentiation (LTP) was dependent on mature BDNF secretion from cortical presynaptic axons [30, 31] as shown at hippocampal synapses, where LTP and long-term depression (LTD) were selectively regulated by mature and proBDNF signaling, respectively [16, 30-33]. Thus, I suggest that restoration of BDNF signaling by the ASO treatment is mostly mediated by mature BDNFs.

Together, not only providing additional evidence for a mhtt-mediated disruption of BDNF secretion, the present study also points to a combination of the mhtt reduction and BDNF overexpression for prevention of HD after early detection of the disease (Fig. 4). In addition to a neuronal delivery of BDNF, I expect that glial BDNF overexpression or implantation of genetically modified cells with BDNF expression would also be useful [10, 11, 13] in promoting striatal BDNF level and reducing HD symptoms, with the efficacy greatly enhanced if accompanied with the mhtt reduction before HD symptoms occur.

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