European Journal of Cell Biology 96 (2017) 402–406

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.com/locate/ejcb

Short communication

Loss of Huntingtin stimulates capture of retrograde dense-core vesicles to increase synaptic neuropeptide stores

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ARTICLE INFO

Article history:
Received 3 November 2016
Received in revised form
23 December 2016
Accepted 4 January 2017

Keywords:
Huntingtin
Axonal transport
Neuromuscular junction
Synapse

ABSTRACT

The Huntington's disease protein Huntingtin (Htt) regulates axonal transport of dense-core vesicles (DCVs) containing neurotrophins and neuropeptides. DCVs travel down axons to reach nerve terminals where they are either captured in synaptic boutons to support later release or reverse direction to reenter the axon as part of vesicle circulation. Currently, the impact of Htt on DCV dynamics in the terminal is unknown. Here we report that knockout of *Drosophila* Htt selectively reduces retrograde DCV flux at proximal boutons of motoneuron terminals. However, initiation of retrograde transport at the most distal bouton and transport velocity are unaffected suggesting that synaptic capture rate of these retrograde DCVs could be altered. In fact, tracking DCVs shows that retrograde synaptic capture efficiency is significantly elevated by Htt knockout or knockdown. Furthermore, synaptic boutons contain more neuropeptide in Htt knockout larvae even though bouton size, single DCV fluorescence intensity, neuropeptide release in response to electrical stimulation and subsequent activity-dependent capture are unaffected. Thus, loss of Htt increases synaptic capture as DCVs travel by retrograde transport through boutons resulting in reduced transport toward the axon and increased neuropeptide in the terminal. These results therefore identify native Htt as a regulator of synaptic capture and neuropeptide storage.

1. Introduction

Huntington's disease is a dominantly inherited neurodegenerative disorder caused by an abnormal polyglutamine expansion in the N-terminal part of the Htt protein. Htt is a large scaffold protein that tethers many partners into complexes to coordinate different cellular processes including autophagy, transcription, vesicle trafficking, and endocytosis (Saudou and Humbert, 2016). The progression of Huntington's disease is linked to toxic accumulation of mutant Htt, but loss of wild type Htt function might also contribute to neuronal cell death. Yet, although Htt is ubiquitously expressed and conserved from *Drosophila* to humans and its mutation is linked to Huntington's disease, its native role is not fully understood.

Native Htt is predominantly found in cytoplasm where it binds to vesicles and microtubules. Growing evidence links Htt to regulation of fast axonal transport of various organelles including BDNF (brain-derived neurotrophic factor) and APP (amyloid precursor protein) vesicles (Gunawardena et al., 2003; Gauthier et al., 2004; Caviston et al., 2011; White et al., 2015). Htt can regulate axonal transport through binding to its adaptor Huntingtin-associated protein 1 (HAP1), which interacts with kinesin (Mcguire et al., 2006) and dynein (Engelender et al., 1997; Li et al., 1995). A direct interaction between Htt and the dynein/dynactin complex has also been reported (Caviston et al., 2011). In addition, Htt scaffolds GAPDH (glyceraldhyde 3-phosphate dehydrogenase) on vesicles and Htt depletion induces detachment of GAPDH from vesicles leading to decreased axonal transport of BDNF and APP (Zala et al., 2013b). Finally, Htt may induce secondary effects on axonal transport by regulating dynein heavy chain expression (Weiss and Littleton, 2016).

Htt function in axonal transport is conserved between flies and mammals (Zala et al., 2013a). Therefore, *Drosophila melanogaster* is a powerful model system to study Htt function under physiological and pathological conditions. Recent observations revealed that knockout (KO) of *Drosophila* Htt leads to subtle changes in axonal transport of dense-core vesicles (DCVs) (i.e., modestly decreased anterograde and increased retrograde axonal flux) in specialized peptidergic neurons (Weiss and Littleton, 2016). DCVs travel by fast anterograde axonal transport to reach nerve terminals where they are either captured in synaptic boutons for many hours to support future neuropeptide release or reverse direction to travel

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http://dx.doi.org/10.1016/j.ejcb.2017.01.001
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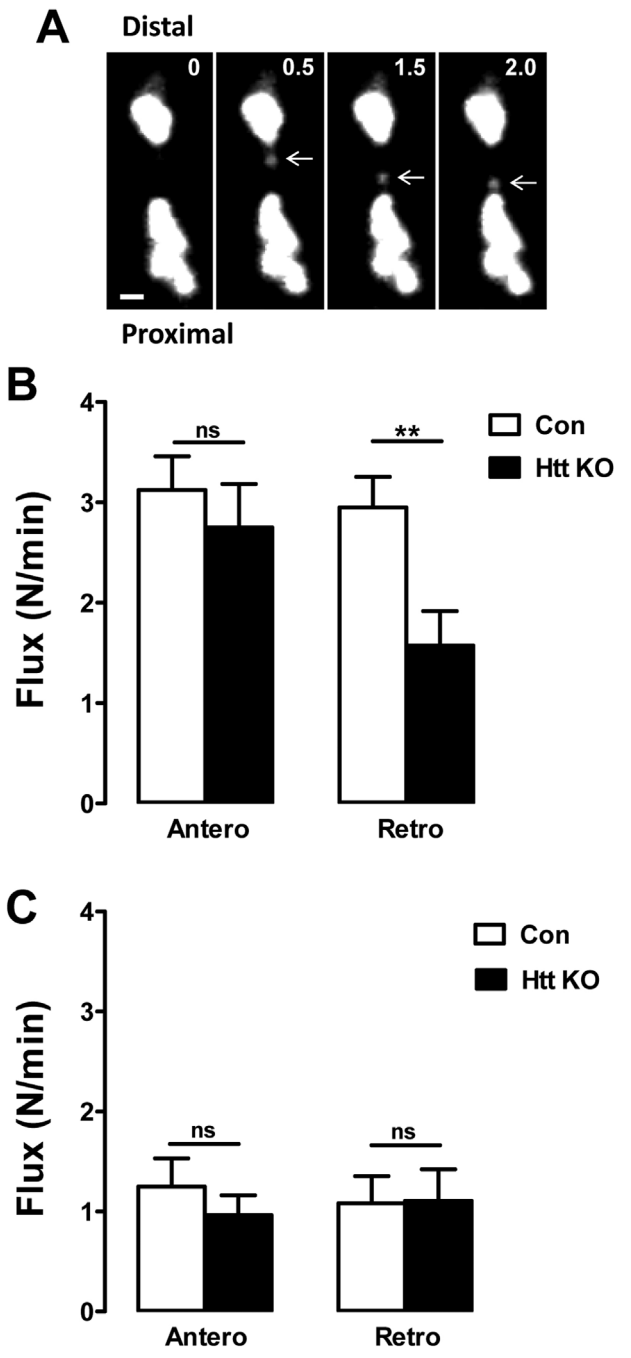

Fig. 1. Htt depletion in type Is synaptic boutons attenuates DCV retrograde flux. A. Contrast-enhanced time-lapse images of type Is boutons expressing AnfGFP show a DCV (arrows) traveling by retrograde transport between adjacent boutons. The time interval between frames is 0.5 s. Scale bar 1 μm. B. Htt knockout decreases retrograde DCV flux in proximal boutons expressing AnfGFP. Control (Con), n = 21; Htt KO, n = 20. $^{**}p < 0.01$, Unpaired *t*-test. C. Retrograde flux is not affected at the most distal boutons in Htt knockout larvae expressing AnfGFP. Con, n = 6; Htt KO, n = 14. Unpaired *t*-test shows that there is no significant (ns) change.

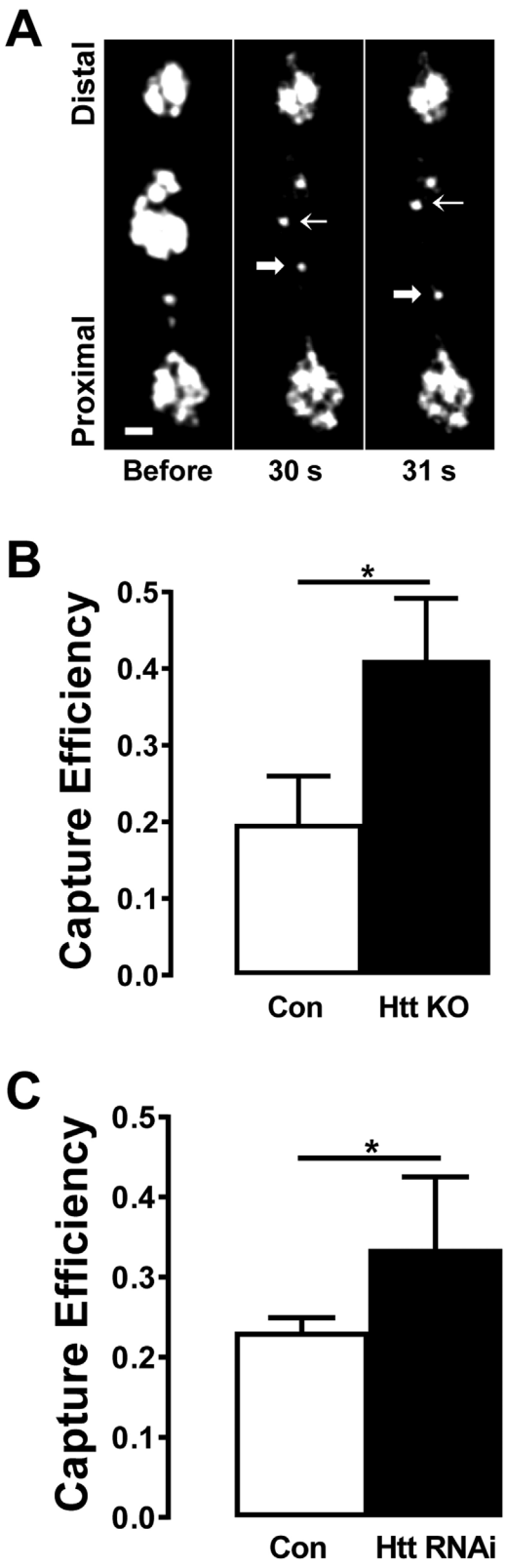

Fig. 2. Htt loss increases DCV retrograde synaptic capture efficiency. A. Detection of anterograde and retrograde DCV movement through a photobleached bouton. Contrast-enhanced images of type Is boutons expressing AnfGFP before, 30 s and 31 s after photobleaching of bouton. Note the DCVs moving through photobleached area in anterograde (thin arrow) and retrograde (bold arrow) direction. Scale bar 1 μm. B. Retrograde capture efficiency is increased in Htt knockouts expressing AnfGFP. Control (Con), n = 10; Htt KO, n = 12. C. Htt RNAi-mediated knockdown increases retrograde capture efficiency in boutons expressing Dilp2GFP. Con, n = 12; Htt RNAi, n = 7. $^{*}p < 0.05$, Unpaired *t*-test.

by dynein/dynactin-dependent retrograde transport as part of DCV circulation (Shakiryanova et al., 2006; Wong et al., 2012; Cavolo et al., 2015). Notably, due to circulation of excess DCVs in strings of synaptic boutons, the main parameter that determines neuropeptide accumulation in nerve terminals is DCV capture efficiency, not the rates of DCV delivery or exit of resident DCVs (Wong et al., 2012; Bulgari et al., 2014). Yet, the impact of Htt on synaptic capture is not known.