Huntingtin recruits KIF1A to transport synaptic vesicle precursors along the mouse axon to support synaptic transmission and motor skill learning

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Abstract

Neurotransmitters are released at synapses by synaptic vesicles (SVs), which originate from SV precursors (SVPs) that have traveled along the axon. Because each synapse maintains a pool of SVs, only a small fraction of which are released, it has been thought that axonal transport of SVPs does not affect synaptic function. Here, studying the corticostriatal network both in microfluidic devices and in mice, we find that phosphorylation of the Huntingtin protein (HTT) increases axonal transport of SVPs and synaptic glutamate release by recruiting the kinesin motor KIF1A. In mice, constitutive HTT phosphorylation causes SV over-accumulation at synapses, increases the probability of SV release, and impairs motor skill learning on the rotating rod. Silencing KIF1A in these mice restored SV transport and motor skill learning to wild-type levels. Axonal SVP transport within the corticostriatal network thus influences synaptic plasticity and motor skill learning.

Keywords:
Synaptic vesicle precursors, axonal transport, huntingtin phosphorylation, kinesin, molecular motors, motor skill learning, corticostriatal synapse, microfluidics, probability of release.
Introduction

Neurons communicate by transmitting chemical messages through their synapses. The number of synaptic vesicles that carry these chemical messengers (neurotransmitters), the probability of the vesicles releasing neurotransmitter, and the vesicle quantal size, all affect synaptic strength and thus the ability to learn and remember (Katz, 1969). Synaptic vesicles (SVs) actually begin life as SV precursors (SVPs), which are formed in the cell body and transported along the axon to the presynapse—a distance that can span meters (Guedes-Dias and Holzbaur, 2019; Rizalar et al., 2021; Rizzoli, 2014). One would expect that this long-distance axonal transport should influence SV homeostasis, because synapses must somehow be replenished with new vesicles as they release neurotransmitters. Thus far, however, evidence for this intuition has been lacking. It is thought that the synaptic SV pools, which contain hundreds of vesicles of which only a few percent participate in synaptic release (Reshetniak and Rizzoli, 2021), are sufficient to ensure a ready supply of SVs to be released, even with prolonged neuronal stimulation (Denker et al., 2011; Rizzoli, 2014). If necessary, neighboring synapses can draw on local SV pools that circulate between them (Wong et al., 2012).

Despite this substantial reserve of SVs, mutations that strongly affect SVP transport have been found to affect neuronal transmission and behavior in mice, flies, and worms. In Caenorhabditis elegans, null mutants for the kinesin-related gene unc-104 or the vesicle-associated protein SAM-4 lead to defects in SVP transport, with a consequent lack of SVs at synapses and locomotor deficits (Hall and Hedgecock, 1991; Zheng et al., 2014). In Drosophila, deletion of the imac gene, a kinesin-3 family member, impairs SVP axonal transport and the formation of synaptic boutons (Pack-Chung et al., 2007). In mice, loss of function of unc-104's mammalian homologue, KIF1A (Okada et al., 1995), leads to the accumulation of SVPs in the cell body and a dramatic reduction in the number of SVs at synapses, along with sensorimotor deficits and early postnatal death (Yonekawa et al., 1998). Completely blocking a molecular motor, however, does not tell us whether more modest enhancement or attenuation of axonal transport influences synapse homeostasis, synaptic transmission, or the function of specific brain circuits.

Mice bearing mutations in Huntingtin (HTT), a protein that plays a prominent role in axonal transport (Saudou and Humbert, 2016), provide one model system that could illuminate the role of SVP transport on synaptic homeostasis. HTT scaffolds various cargoes—endosomes,
autophagosomes, vesicles containing BDNF, APP, etc. (Bruyere et al., 2020; Cason et al., 2021; Caviston et al., 2011; Fu and Holzbaur, 2014; Gauthier et al., 2004; Her and Goldstein, 2008; Liot et al., 2013; Wong and Holzbaur, 2014)—along with the appropriate molecular motors for anterograde or retrograde transport and any adaptor proteins that may be needed. Because the direction of HTT-mediated transport is dictated by its phosphorylation at serine 421 (Bruyere et al., 2020; Colin et al., 2008; Ehinger et al., 2020; Vitet et al., 2020), we were able to investigate how mutations at this site affect axonal transport of SVPs. First we studied SVP transport in a reconstituted neuronal circuit on-a-chip, then in mice that bear either a constitutively phosphorylated HTT mutant (S421D) or an unphosphorylatable one (S421A). Our data reveal a functional link between anterograde transport of SVPs within corticostriatal projecting neurons, the synaptic SV pools, and the release probability of SVs at corticostriatal synapses, with consequences for motor skill learning.

Results

Constitutive HTT phosphorylation increases anterograde SVP transport and synaptic glutamate release

To investigate whether HTT and its phosphorylation affect the transport of SVP in a physiologically relevant system, we reconstituted mature corticostriatal circuits in microfluidic devices (Moutaux et al., 2018; Virlogeux et al., 2018). These devices have been used to establish HTT's role in the transport of organelles such as BDNF-containing vesicles and signaling endosomes (Gauthier et al., 2004; Liot et al., 2013; Scaramuzzino et al., 2022; Virlogeux et al., 2018). Microfluidics consist of a presynaptic and a postsynaptic compartment containing cortical and striatal neurons, respectively, and a middle synaptic compartment that receives axons from cortical neurons and dendrites originating from striatal neurons (Fig. 1A). The three compartments are connected by 3-μm-wide microchannels that are 500 μm long for axons and 75 μm long for dendrites (Lenoir et al., 2021). The number of striatal axons reaching the synaptic chamber at maturity is limited by the generation of a laminin gradient from the cortical chamber to the striatal chamber, whilst the poly-D-lysine concentration is kept constant (Scaramuzzino et al., 2022). In this configuration, isolated cortical axons unilaterally connect postsynaptic striatal dendrites in the middle compartment enriched in functional synaptic contacts (Ehinger et al., 2020; Moutaux et al., 2018; Scaramuzzino et al., 2022; Virlogeux et al., 2018).
We generated cortical and striatal neurons from embryos of wild-type (WT) mice and mice bearing either constitutively phosphorylated (HTT-SD mice) or unphosphorylatable HTT (HTT-SA mice) at embryonic day 15.5 (E15.5). HTT phosphorylation at serine 421 is mimicked by the replacement of the serine by an aspartic acid, which maintains the positive charge (S421D), whereas the unphosphorylatable form of HTT is obtained by mutating the serine into an alanine (S421A) (Thion et al., 2015). We transduced cortical presynaptic neurons at DIV 0 (day in vitro) with a lentivirus encoding the major SNARE protein of synaptic vesicles (v-SNARE), VAMP2 fused to mCherry protein (VAMP2-mCherry), a member of the vesicle-associated membrane protein (VAMP)/synaptobrevin family which labels SVPs (Pennuto et al., 2003). The circuit achieves functional maturity—as defined by the kinetics of neurite outgrowth, synapse formation, neuronal transport and synchronous activity—by DIV 10-12 and establishes functional excitatory connections transmitting information from cortical to striatal neurons (Moutaux et al., 2018). We therefore performed all experiments in the microfluidic devices at this time point. We used high-resolution spinning confocal videomicroscopy to record VAMP2-mCherry particles in the distal part of cortical axons (Fig. 1A) and generated kymographs to trace the movement of vesicles (Fig. 1A, right panels).

Constitutive HTT phosphorylation (S421D) increased the anterograde velocity of VAMP2-positive vesicles (Fig. 1B, left graph), the number of anterograde vesicles, and the linear flow rate (Figure 1-figure supplement 1A), leading to an increase in the net directional flux of VAMP2-mCherry vesicles traveling towards the presynapse (Fig. 1B, right graph). There was no significant effect on VAMP2-mCherry vesicle velocities or net directional flux of HTT-SA mutation in axons (Fig. 1B). HTT phosphorylation at S421 therefore influences transport of SVPs towards the presynapse.

We next investigated whether this increased presynaptic anterograde transport of SVPs affects the capacity of presynaptic neurons to release glutamate from SVs at the corticostriatal synapses. We transduced presynaptic cortical neurons with a lentivirus encoding the indicator of vesicle release and recycling VGLUT1-pHluorin, thanks to the fusion of a pH-sensitive GFP (pHluorin) to the vesicular glutamate transport VGLUT1 (Fernandez-Alfonso and Ryan, 2008). We then treated the presynaptic compartment with 4AP/bicuculline at DIV 10-12 to induce neuronal activity and measured the number of exocytic events per active synapse by recording fluorescence within the synaptic compartment (Fig. 1C, Figure 1-video 1). The amplitude of
VGLUT1 events was similar in WT, HTT-SA, and HTT-SD neurons (Figure 1-figure supplement 1B), but the frequency of release events at synapses was significantly greater only in HTT-SD neurons (Fig. 1C). HTT phosphorylation thus promotes axonal transport of SVPs and increases the capacity of synapses to release glutamate.

**HTT constitutive phosphorylation at S421 impairs motor skill learning in mice**

We had previously characterized HTT-SA, HTT-SD, and WT mice and found no differences between them in motor coordination (Figure 2-figure supplement 1A), forelimb strength (grip test), or anxious-depressive behavior (in the elevated plus maze test) (Ehinger et al., 2020). We therefore decided to reassess the three genotypes with a focus on the more subtle process of motor learning. To this purpose, we followed the mice daily as they developed skill on the rotarod (10 sessions per day for 8 consecutive days, for a total of 80 sessions) (Fig. 2A). Running is largely hard-wired in mice, but wild-type mice do become more adept at staying on the rod with training, so this presented a suitable test for the improvement of a basic motor skill. Over the eight days, the wild-type (WT) mice nearly doubled their latency to fall over the first three days/30 sessions of training (the learning phase) and then maintained that skill (the consolidation phase) (Fig. 2B). The HTT-SD mice did not improve as much during the initial learning phase and plateaued at a lower level of skill. The HTT-SA mice learned much more gradually than WT but by the eighth day were virtually equivalent.

To better characterize this motor learning deficit, we examined the first and last days of training more closely. HTT-SD had greater difficulty adjusting to the rod initially than WT mice but improved by the end of the day (Fig. 2C). On Day 8 the plateau noted above was in evidence throughout the sessions (Fig. 2D). The pattern with HTT-SA mice was less clear, at both 4 months (Fig. 2C and D) and 18 months (Figure 2-figure supplement 1B).

**HTT constitutive phosphorylation alters short-term plasticity**

Motor skill learning relies on communication between the dorsal striatum and layer V pyramidal neurons in the motor cortex via the release of glutamate by the cortical afferences (Graybiel and Grafton, 2015; Jin and Costa, 2015; Perrin and Venance, 2019; Yin and Knowlton, 2006). We therefore performed whole-cell recordings of medium-sized spiny neurons (MSNs) from the dorsolateral striatum in acute corticostriatal brain slices from WT, HTT-SD and HTT-
SA adult mice (Fig. 3A; see Methods) and analyzed the spontaneous excitatory postsynaptic currents (sEPSCs). The HTT-SD mice did not differ from WT or HTT-SA mice in sEPSC amplitude or frequency (Figure 3-figure supplement 1). We next recorded EPSCs evoked by paired-pulse stimulations of layer V cortical neurons from the somatosensory S2 cortex and the corresponding corticostriatal projection field in the dorsal striatum at various interstimulus intervals (ISIs: 25, 50, 100, 250 and 500 ms) to assess the probability of release at MSN corticostriatal synapses in WT, HTT-SD and HTT-SA mice (Fig. 3). Paired-pulse ratio (PPR) analysis revealed that in WT mice, corticostriatal short-term plasticity was facilitated for short ISIs (25 and 50 ms), with a lack of significant plasticity at 100 ms followed by a short-term depression for longer ISIs (250 and 500 ms), as previously described (Goubard et al., 2011). In HTT-SD mice, there was no short-term facilitation but only depression starting from 100 ms ISIs. HTT-SA mice showed a short-term plasticity similar to WT mice, except that the facilitation expression window widened up to 100 ms ISIs (Fig. 3). Thus, while all genotypes showed similar short-term depression, they exhibited marked differences in facilitation.

The lower facilitation in HTT-SD MSNs indicates that constitutive phosphorylation of HTT increases the probability of glutamate release in pyramidal cells. These findings are in agreement with the greater number of exocytic events in HTT-SD neurons within microfluidic devices (Fig. 1C).

**HTT phosphorylation increases the number and density of SVs at corticostriatal synapses**

Since decreased facilitation could indicate changes in the number of synaptic vesicles at the presynapse (Park et al., 2012; Pulido and Marty, 2017), we investigated the number of SVs at axon terminals within the corticostriatal network using electron microscopy. We focused on synapses formed between cortical neurons from the somatosensory cortex connecting with neurons from the dorsolateral striatum. According to the morphology of both the spines and the synapses, we counted the number of SVs in glutamatergic afferences within the dorsolateral striatum (Fig. 4A). With chronic HTT phosphorylation (HTT-SD mice), the number of synapses did not change (Fig. 4Bi) but there were a greater number of SVs at the axon terminals than in WT mice (Fig. 4Bii). The presynaptic size was smaller in the HTT-SD corticostriatal synapses (Fig. 4Biii), leading to an increase in SV density in HTT-SD presynaptic terminals (Fig. 4Biv). Although the total vesicle number may not be the major point of control for neurotransmission...
(Fernandez-Alfonso and Ryan, 2008; Fredj and Burrone, 2009; Sudhof, 2012) and vesicle pools are not anatomically segregated (Denker et al., 2009; Rizzoli, 2014), it is worth noting that the most proximal zone is likely to contain the readily releasable pool (RRP) while the most distal zone is likely to be enriched in vesicles from the reserve pool (RP) (Fig. 4C). We therefore divided the PSD into three 40 nm-wide zones so that we could measure vesicle density in each region. There was no difference between the genotypes in terms of the area of the zones (Figure 4-figure supplement 1A), but HTT-SD axon terminals exhibited a greater density of synaptic vesicles in the most distal zone (zone 3) (Fig. 4D). This indicates that constitutive HTT phosphorylation favors the anterograde transport of SVPs, leading to the accumulation of SVs in the distal presynapse in vivo. The fact that HTT-SA axon terminals had fewer vesicles lends further support to this explanation (Figure 4-figure supplement 1B).

Although we did not observe less anterograde transport or preferential retrograde transport of VAMP2-mCherry in our experimental conditions (Fig. 1B), previous reports have noted a preferential retrograde trafficking of vesicles in HTT-SA mice (Bruyere et al., 2020; Colin et al., 2008; Ehinger et al., 2020). This suggests that in our in vitro experiments, WT HTT is phosphorylated at low levels. Since, HTT-SD, but not HTT-SA, showed significant differences from WT in axonal transport, glutamate release, and motor skill learning, we focus on HTT-SD mice for the rest of this study.

**HTT recruits KIF1A to vesicles**

The anterograde transport of SVP is driven predominantly by the kinesin-3 motor KIF1A (Guedes-Dias and Holzbaur, 2019). HTT and KIF1A interactomes suggested a possible interaction between the two proteins (Shirasaki et al., 2012; Stucchi et al., 2018), but this has not been tested. We observed KIF1A in the proteome of HTT-associated vesicles (Fig. 5A)(Migazzi et al., 2021). We found that KIF1A colocalizes with HTT immunopositive puncta in free-cultured cortical neurons at DIV5 using a two-dimensional stimulated emission depletion (2D-STED) super-resolution microscope (Fig. 5B left panel). We confirmed this observation in HTT-SD neurons (Fig. 5B right panel). This suggests that phosphorylation of HTT could determine KIF1A recruitment on SVP.

We assessed this possibility by permeabilizing isolated axons within the distal part of microfluidic axonal compartments and, using Airyscan confocal high resolution microscopy, we...
observed greater colocalization of HTT with KIF1A and VAMP2 in HTT-SD neuronal circuits than in WT (Fig. 5C and Figure 5-figure supplement 1A). We then used proximity ligation assay (PLA) to confirm the in cellulo interaction between HTT and KIF1A. We observed a significantly greater PLA signal in HTT-SD neurons (Fig. 5D), despite no change in PLA levels of VAMP2 with HTT and KIF1A (Figure 5-figure supplement 1B and C). Finally, we prepared vesicular-enriched fractions from WT and HTT-SD mouse brains and immunoblotted them for KIF1A. HTT-SD showed a greater vesicular/cytosolic ratio for KIF1A (Fig. 5E), while p150 remained constant and the total brain levels of KIF1A did not differ between the genotypes (Figure 5-figure supplement 1D). KIF1A and HTT thus colocalize on VAMP2-positive vesicles, and S421 phosphorylation augments KIF1A interaction with HTT.

HTT-SD-mediated SVP transport depends on KIF1A

We next asked whether the phospho-HTT-mediated increase in SVP anterograde transport depends on KIF1A by using a validated sh-Kif1a (Kevenaar et al., 2016). Lentiviral expression of sh-Kif1a in cortical neurons reduced KIF1A expression by~83% (Figure 6-figure supplement 1A). We then treated corticostriatal projecting neurons plated in microfluidic devices with lentiviruses expressing either sh-scramble-GFP (sh-Scr) or sh-Kif1a-GFP. We recorded axonal transport of VAMP2-mCherry vesicles at DIV12 and generated kymographs as before (Fig. 6A). We found that silencing KIF1A in WT cortical neurons decreased VAMP2 anterograde vesicle velocity, the number of anterograde vesicles (Fig. 6B and Figure 6-figure supplement 1C), the linear flow (Figure 6-figure supplement 1C), and the net directional flux of VAMP2 vesicles toward the axon terminals (Fig 6B).

We next measured VAMP2 transport in HTT-SD neurons and found greater anterograde velocity, number of anterograde vesicles, and positive net directional flux (and/or linear flow) than in WT neurons (Fig. 6B and Figure 6-figure supplement 1C), confirming our previous results (Fig. 1B). Silencing KIF1A in HTT-SD reduced the anterograde velocity of VAMP2 vesicles close to values observed in WT neurons (Fig. 6B). KIF1A silencing also reduced the number of anterograde vesicles (Figure 6-figure supplement 1B), linear flow, and net directional flux in HTT-SD to values found in WT (Fig 6B and Figure 6-figure supplement 1B). The velocity and number of retrograde-moving VAMP2 vesicles was also lower in HTT-SD neurons (Fig 6B). This attenuation of retrograde transport might be linked to KIF1A's reported
role as a dynein activator (Chen et al., 2019).

We considered the possibility that the observed increase in SV release might be due in part
to a synergistic action of BDNF at synapses, both because of the prominent role of HTT and its
phosphorylation in regulating BDNF transport (Colin et al., 2008; Ehinger et al., 2020; Gauthier
et al., 2004) and because synaptic BDNF levels regulate synaptic plasticity and SV release
(Gangarossa et al., 2020; Park and Poo, 2013; Park et al., 2014; Tyler et al., 2006; Walz et al.,
2006). Furthermore, dense-core vesicles (DCVs), including those containing BDNF, can be
transported by kinesin-3 (Hung and Coleman, 2016; Lim et al., 2017; Lo et al., 2011; Stucchi et
al., 2018). We therefore silenced KIF1A in cortical axons and measured BDNF-mCherry axonal
transport in the distal part of axons at DIV12 (Figure 6-figure supplement 2A). Chronic HTT
phosphorylation increased the anterograde transport, the linear flow, and the net directional flux
of BDNF-containing vesicles, in agreement with previous studies (Colin et al., 2008; Ehinger et
al., 2020) (Figure 6-figure supplement 2B). Silencing KIF1A did not affect BDNF dynamics
either in WT or in HTT-SD neurons. This indicates that the phospho-HTT-dependent increase in
SVP anterograde transport, in contrast to BDNF, is mediated by KIF1A.

HTT-KIF1A-mediated transport regulates the number of SVs at synapses

We next investigated whether SVP anterograde transport via the HTT-KIF1A complex
regulates the number of vesicles at synapses. We injected lentiviruses encoding either sh-
scramble-GFP or sh-Kif1a-GFP into layer V of the HTT-SD motor cortex (Fig. 7A), whose
neurons project mainly to the dorsolateral striatum (Hunnicutt et al., 2016). We then counted the
number of SVs at corticostriatal synapses from sections prepared from WT and HTT-SD brains
injected with lentiviral sh-Scr or sh-Kif1a. WT sh-Kif1a presynapses showed significantly fewer
SVs than WT sh-Scr presynapses (Fig. 7C). As previously shown (Fig. 4B), there were
significantly more SVs at presynapses in HTT-SD, but this number reverted to WT levels in
HTT-SD brains treated with sh-Kif1a (Fig. 7C). These data demonstrate that decreasing the
phospho-HTT-mediated anterograde transport of SVPs by reducing KIF1A levels in
corticostriatal projecting neurons reduces the number of synaptic vesicles at presynapses. This in
turn indicates a close relationship between axonal transport and synaptic SV content.

HTT-KIF1A-mediated axonal transport of SVPs in corticostriatal projecting neurons
regulates motor skill learning

To determine whether the modification in anterograde transport via the HTT-KIF1A complex within corticostriatal projecting neurons is responsible for the defect in motor skill learning we observed in HTT-SD mice, we injected lentiviral vectors encoding sh-Scr-GFP and sh-Kif1a-GFP into 3-month-old WT and HTT-SD mice. Three weeks later, we subjected the mice to the same rotarod protocol as before (Fig. 8A). HTT-SD mice did not show much improvement over 8 days (Fig. 8B), as with the non-injected mice (Figure 2B). Silencing KIF1A improved the performance of the HTT-SD mice over the first 6 days, but then the mice seemed to lose ground (Fig. 8B). Indeed, the improvement in motor learning of the HTT-SD mice via sh-Kif1a silencing was significant on the first day of training, but the effect did not last until the eighth day (Fig. 8C). This could be related to the duration of gene silencing in nondividing cells that is about 3 weeks while our experimental procedure extent up to 4 weeks (Bartlett and Davis, 2006). Nonetheless, these findings indicate that HTT-KIF1A-mediated axonal transport of SVPs in the corticostriatal projecting neurons, a process modulated by phosphorylation, influences the number of SVs at synapses, the probability of release, and the efficacy of motor skill learning.

Discussion

We show here that axonal transport of SVPs influences synaptic function. Specifically, HTT's phosphorylation status fine-tunes SVP transport efficiency through its recruitment of KIF1A. Genetically blocking dephosphorylation at S421 impaired motor learning, and abolishing KIF1A activity in the context of constitutive phosphorylation only partially restored motor performance on the rotarod. Such a genetic approach is rather blunt compared to the sensitivity of (de)phosphorylation in responding to cellular signals, yet it enabled us to answer the question that motivated the study and show that axonal transport does influence synaptic homeostasis, with consequences for circuit function and behavior.

Huntingtin and the regulation of SVP axonal transport

This work places HTT among the proteins that participate in SVP transport (Guedes-Dias and Holzbaur, 2019) and closes a loop opened by the discovery that DENN/MADD, a Rab3-GEP that binds to KIF1A (and KIF1Bβ), regulates SVP binding to microtubules according to Rab3's nucleotide state (Niwa et al., 2008). Rab3 is part of the HTT interactome (Shirasaki et al., 2012)
and enriched in SVs (Takamori et al., 2006); previous work in *Drosophila* larval axons showed
that reducing HTT levels decreases the transport of Rab3-positive vesicles (White et al., 2015).
In the context of Huntington disease, which is caused by polyglutamine expansions in HTT, both
Rab3 levels and the conversion from GTP to GDP state are dysregulated, which is consistent
with a role for HTT in the transport of SVPs.

Studies in Huntington disease (HD) models have revealed alterations in HTT
phosphorylation at S421 that could result from defects in Akt, the S421 kinase, or dysregulation
of the phosphatases Calcineurin (PP2B) and PP2A (Humbert et al., 2002; Metzler et al., 2010;
Pardo et al., 2006; Warby et al., 2005). Whether SVP trafficking is also altered and whether
restorating SVP transport through HTT phosphorylation could mitigate HD pathology are
questions that remain to be answered. That such a study would be worthwhile is suggested by the
fact that promoting HTT phosphorylation is neuroprotective, as it restores the transport and
release of BDNF (Humbert et al., 2002; Kratter et al., 2016; Pardo et al., 2006; Pineda et al.,
2009; Warby et al., 2009; Zala et al., 2008). It is interesting to note in this context that release of
glutamate at the corticostriatal synapse in altered in HD brains-on-chips (Virlogeux et al., 2018)
and in vivo (Fernandez-Garcia et al., 2020; Smith-Dijak et al., 2019) and that stimulating
glutamatergic corticostriatal connections in HD reverses motor symptoms in HD mice
(Fernandez-Garcia et al., 2020), suggesting that reestablishment of glutamate synaptic release
capacities could have therapeutic potential.

Fine-tuning SVP transport regulates synapse homeostasis and proper neurotransmission
Several studies have linked a reduction in axonal anterograde transport of SVPs to a
decline in synaptic function. Indeed, genetically impairing KIF1A reduces the number of SVs at
nerve terminals and causes postnatal lethality (Yonekawa et al., 1998). KIF1A loss-of-function
variants, most of them located within the conserved motor domain, reduce SVP transport and are
associated with four diseases: autosomal recessive hereditary sensory neuropathy IIC, autosomal
dominant mental retardation (ADMR) type 9, autosomal recessive spastic paraplegia (SPG) type
30, and autosomal dominant hereditary spastic paraplegia (HSP) (Pennings et al., 2020). That all
these disorders involve lower extremity spasticity and weakness reflects the challenge of
transporting vesicles down the extremely long axons of the peripheral nervous system, but the
cognitive deficits in ADMR type 9 show that disruptions in axonal transport clearly disturb
synaptic transmission and synaptic strength in the central nervous system as well, with obvious consequences for learning and memory (Guedes-Dias et al., 2019; Zhang et al., 2017). We found that increasing SVP axonal transport via phospho-HTT-mediated KIF1A activation is equally problematic: too many SVs in the synaptic pool are also detrimental to synaptic function and motor skill learning. This is consistent with the fact that the KIF1A gain-of-function mutation V8M, which causes another type of HSP, leads to greater anterograde transport of vesicles and abnormal accumulation of vesicles at synapses (Chiba et al., 2019; Gabrych et al., 2019). Further evidence for the sensitivity of synapses to both too little and too much SVPs comes from point mutations in the autoinhibitory domain of \textit{unc-104} that cause hyperactive axonal transport and abnormal accumulation of synaptic vesicles (Cong et al., 2021). Similarly, depletion of kinesin-binding protein, KBP, which inhibits KIF1A activity, leads to the abnormal accumulation of both KIF1A and vesicles at neurite terminals (Kevenaar et al., 2016), and nonsense mutations of KBP cause Shprintzen-Goldberg syndrome (also called marfanoid-craniosynostosis syndrome), which is characterized by intellectual disability, skeletal abnormalities, and axonal neuropathy (Dafsari et al., 2015; Valence et al., 2013). While we show here that S421 phosphorylation promotes anterograde transport and abnormal accumulation of SVs at the synapse, the lack of phosphorylation seemed to have only a limited effect on the transport and release of SVs \textit{in vitro}. This is consistent with previous studies analyzing the transport of other cargoes, which revealed more obvious differences for anterograde than for retrograde transport (Bruyere et al., 2020; Ehinger et al., 2020). This could be due to the culture conditions in microfluidic devices, in which the level of phosphorylation of endogenous WT HTT is rather low. We nonetheless observed slight alterations in HTT-SA mice, including fewer SVs at the presynapse and a more gradual learning curve. Although less marked, these changes are the opposite of the alterations observed when HTT is consistently phosphorylated. Together, these studies indicate that SVP transport is normally fine-tuned to ensure the proper quantity of SVs at the synapse and effective synaptic function.

\textbf{Axonal SVP transport, the synaptic vesicle pool, and SV release probability}

Within the presynapse, SVs are organized into different pools—the readily releasable pool (RRP), the recycling pool, and the reserve pool—according to their composition, age, and distance from the active zone (Crawford and Kavalali, 2015; Kaeser and Regehr, 2017; Rizzoli,
The RRP contains the SVs ready to be released upon neural activity and is thought to be refilled by SVs from the reserve pool by a slow process (hundreds of milliseconds) (Pulido and Marty, 2017; Rizzoli, 2014). We observed an increased number of vesicles in the pool most distal from the membrane. This is consistent with the fact that vesicles are most likely to accumulate where the SVPs detach from the KIF1A motor, away from the membrane and the active zone (Bodaleo and Gonzalez-Billault, 2016). In addition, newly produced vesicles arriving at the presynaptic zone are preferentially released (Truckenbrodt et al., 2018).

Although the locations of SV populations relative to the membrane do not reliably define vesicular pool identities (Denker et al., 2009; Fernandez-Alfonso and Ryan, 2008; Fredj and Burrone, 2009), we propose that this population distal from the membrane influences the replenishment of the pools nearer the membrane, i.e., the RRP (Kidokoro et al., 2004) and/or the recycling pool (Ratnayaka et al., 2012). All the vesicle pools are dynamic and interdependent (Chamberland and Toth, 2016; Chanaday et al., 2019), and previous work in Drosophila has shown that impaired recruitment of SVs from the reserve pool to the RRP causes memory deficits and limited short-term synaptic plasticity (Kidokoro et al., 2004). Modulation of the reserve pool itself, in size or dynamicity, has also been shown to affect plasticity and memory (Corradi et al., 2008; De Rossi et al., 2020; Gitler et al., 2008; Skorobogatko et al., 2014).

**The dorsolateral striatum and motor skill learning**

We found that lowering KIF1A levels within M1 neurons that project mainly to the dorsolateral striatum (DLS) rescued motor skill learning, a process that is usually attributed to the dorsomedial striatum (DMS) (Costa et al., 2004; Yin et al., 2009). Recent studies have suggested that both DMS and DLS are engaged in learning (Bergstrom et al., 2018; Gremel and Costa, 2013; Kimchi et al., 2009; Kupferschmidt et al., 2017; Perez et al., 2022; Perrin and Venance, 2019; Stalnaker et al., 2010; Thorn et al., 2010), so targeting the DLS might be sufficient to rescue the development of motor skill during the first days of rotarod training. This would not, however, explain the lack of rescue upon KIF1A silencing in HTT-SD mice during the consolidation phase, when WT mice show that they maintain the skill level that peaked at day 3. Rather, this could be related to the observation that during consolidation, the number of neurons that are activated upon stimulation of the DLS falls as the circuit streamlines its
connections (Badreddine et al., 2022; Cao et al., 2015; Picard et al., 2013). In other words, as the motor skill is mastered, fewer neurons are needed to code the activity. Thus, it is possible that this small number of DLS connections responsible for consolidation of a learned skill would not have been targeted by the sh-Kif1a virus since it is unlikely that its expression would cover the entire DLS.

Altogether, our results highlight the importance of axonal SVP transport for synaptic transmission and identify a role for the HTT-KIF1A pathway as a regulator of SVP transport, synapse function, and motor skill learning within corticostriatal projecting neurons.
Material and Methods

Contact for Reagent and Resource Sharing

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Frédéric Saudou (frederic.saudou@inserm.fr)

Experimental Model and Subject Details

Mice

$Htt^{S421A/S421A}$ and $Htt^{S421D/S421D}$ mice (referred to as HTT-SA and HTT-SD mice, respectively) have been previously described (Thion et al., 2015). They were generated by the Mouse Clinical Institute (Strasbourg, France). Briefly, these C57BL/6J mice were knocked-in with a point mutation replacing the serine 421 by an alanine or an aspartic acid, respectively. All mice were maintained with access to food and water *ad libitum* and kept at a constant temperature (19-22 °C) and humidity (40–50%) on a 12:12 hour light/dark cycle. All experimental procedures were performed in an authorized establishment (Grenoble Institut Neurosciences, INSERM U1216, license #B3851610008) in strict accordance with the directive of the European Community (63/2010/EU). The project was approved by the French Ethical Committee (Authorization number: APAFIS#18126-2018103018299125 v2) for care and use of laboratory animals and performed under the supervision of authorized investigators. For behavior studies, only males were used at 3-4 months of age. Behavioral studies compared littermates, homozygous ($Htt^{S421DorA/S421DorA}$) or wild type ($Htt^{+/+}$) mice. Those mice were then used for electrophysiological and biochemical studies or processed to be imaged by electron microscopy. The number of animals was limited to the minimum number necessary per group in order to have at least an 80% chance of detecting a significant difference (power $1-\beta$) and a risk of error $\alpha$ of 5%. This number was determined using a statistical test for estimating the optimal sample size using the variances determined in a preliminary study.

For biochemistry and neuronal culture (E15.5), the sex distinction of homozygous or WT mice was not made. Specific ages used for each experiment are indicated in the figure legends. C57BL/6J mice, purchased from Charles River Laboratory, were used for backcrosses to maintain the colony and to obtain WT E15.5 pups.

Primary neuron culture and transduction
Primary cortical and striatal neurons were dissected from E15.5 wild type (C57Bl/6J) or HTT-SA or HTT-SD mouse embryos as previously described (Liot et al., 2013). They underwent a chemical dissociation with papain cysteine solution, DNase (1/100), and FBS (1/10) and were finally mechanically dissociated. They were re-suspended in a growing medium containing a Neurobasal medium, 2% B27, 1% Penicillin/streptomycin, and 2 mM glutamax (5 x 10^6 cells in 120 µl). Cortical neurons were plated in the presynaptic chamber coated with poly-D-lysine (0.1 mg/ml) and striatal neurons were plated in the postsynaptic chamber coated with poly-D-lysin and laminin (10 µg/ml) with a final density of ~7000 cells/mm^2. A growing medium was added to the synaptic chamber to equilibrate the flux. Neurons were left in the incubator for 2 hours and then all compartments were gently filled with growing medium. Neurons were cultured at 37°C in a 5% CO2 incubator for 10-12 days.

Between DIV0 and DIV4, cortical neurons within the presynaptic compartment were transduced with lentiviruses expressing VAMP2-mCherry, VGLUT1-pHluorin, or BDNF-mCherry. Neurons were washed out the next day. At DIV 8, cortical neurons were transduced with sh-Kif1a or sh-Scr lentiviruses.

Accelerating rotarod

Motor skill learning was assessed using an accelerating rotarod (LE8305, BIOSEB). The tests were performed during the beginning of the light phase on male littermates housed in the same cages. Cages were transported to the experimental room at least 30 minutes before the tests to allow habituation of the mice to the room kept at a constant temperature (19-22 °C) and humidity (40–50%). The day preceding the test, the mice were acclimated to the rod by one session (1 minute at 4 rpm). Then, the accelerating rotarod assay was performed over 8 consecutive days with 10 sessions per day per mouse, increasing the speed from 4 rpm to 40 rpm over 5 minutes. Each trial was separated by at least a 15-minute resting period. The latency and the speed to fall off from the rotarod were recorded.

Stereotaxic injections

3-month-old HTT-SD and WT male mice were anesthetized by inhalation of isoflurane associated with a mix of oxygen and room air (3-5% of isoflurane for induction and 1-2% in the mask). The mouse head was then shaved and placed within the stereotaxic frame. The skin was
incised, and the skull was bilaterally drilled. The capillary was inserted slowly. We injected bilaterally (position AP:1,54 ML: + or -1,6 DV:-0,8) 500 nl of the diluted lentivirus (1/3 dilution in saline solution of the KIF1A shRNA or the Scr shRNA) at 0.5 µl/min speed using a nanoinjector. The capillary was slowly removed one minute after the end of the injection to prevent the leak of the injected solution. The skull was then washed with saline solution, the skin was sutured and 1 ml of NaCl 0.9% was injected subcutaneously. After surgery, mice were put alone in a warmed cage and monitored daily throughout recovery.

Plasmids

The VAMP2-mCherry construct was a kind gift from T. Ryan’s laboratory. Vglut1 cDNA sequence was amplified from an adult mouse brain. Its sequence from the 104th amino acid to the end was cloned after the pHluorin sequence in a Smal site in a superecliptic pHluorin containing vector used in (Fernandez-Alfonso and Ryan, 2008). KIF1A shRNA construct (JL-35, target sequence: GACCGGACCTTCTACCAGT) has already been published (Kevenaar et al. , 2016). It has been inserted in a EGFP-pSuper vector between NdeI and PstI sites. The control Scramble shRNA is a mouse universal scramble obtained from the scrambled order of HIF-1α nucleotides (Target Sequence: GGGTGAACTCACGTCAGAA)(Yu et al., 2004). It has been inserted in a EGFP-pSuper vector between NdeI and PstI sites. BDNF-mCherry construct and lentivirus, previously published (Hinckelmann et al., 2016) have been used for axonal transport experiments.

For lentivirus production, all the plasmids were cloned into a pSin vector (Drouet et al., 2009) by Gateway system (Life Technology) at the GIN virus production facility as described before (Bruyere et al. , 2020). VAMP2-mCherry, sh-Kif1a, and sh-Scr lentiviruses were produced by the ENS Lyon Vectorology Facility.

Microfluidic fabrication

Microfluidic devices were generated as previously described (Lenoir et al. , 2021; Virlogeux et al. , 2018). Briefly, we modified the size of the microchannels (3 µm width, 3 µm height, and 500 µm length) of polydimethylsiloxane microfluidic device (Taylor et al., 2005). After amplification and production, microfluidic devices were sealed on Iwaki boxes using plasma cleaner. The upper chamber was then coated with poly-D-lysine (0.1 mg/ml) and the lower
chamber was coated with poly-D-lysine (0.1 mg/ml) and laminin (10 μg/ml). After overnight incubation at 4°C, microfluidic devices were washed 2 times with the growing medium. Microchambers were then placed in the incubator until neurons were plated.

**Videomicroscopy**

Videorecording of neurons plated in microfluidic devices was performed at DIV 12. Before recordings, DIV 12 neurons in the microchamber were carefully inspected and selected based on the absence of cell contamination. For double transductions (with sh-RNA lentiviruses), the transport of mCherry-tagged cargo was analyzed within GFP-positive axons. Images were acquired every 200 ms for 1 minute on an inverted microscope (Axio Observer, Zeiss) with X63 oil-immersion objective (1.46NA) coupled to a spinning-disk confocal system (CSU-W1-T3; Yokogawa) connected to an electron-multiplying CCD (charge-coupled device) camera (ProEM+1024, Princeton Instrument) at 37 °C and 5% CO2. For the study of the exocytosis events, images were acquired every 200 ms for 1 min on an inverted microscope (Axio Observer, Zeiss) with X63 oil-immersion objective (1.46NA) coupled to a spinning-disk confocal system (CSU-W1-T3; Yokogawa) with TIRF microscopy (Nikon/Roper, Eclipse Ti) equipped with a camera Prime 95B sCMOS (Telelyne Photometrics) at 37 °C and 5% CO2. The same three fields per microchambers were acquired before and after a 4AP-bicuculline (respectively 2.5mM and 50µM) stimulation of the presynaptic chamber, four times in total (1 before and three after stimulation).

**Immunostaining**

Neurons from the reconstituted corticostriatal network were fixed with a PFA/Sucrose solution (4%/4% in PBS) for 20 minutes at room temperature (RT). After three washes of PBS, neurons were incubated first with a blocking solution (BSA 1%, NGS 2%, Triton X-100 0.1%) and then with primary antibodies for KIF1A (Abcam, #ab180153, 1:100, rabbit), HTT (Millipore, #MAB2166, 1:500, mouse), and mCherry (Fisher Scientific, #16D7, 1:200, rat) overnight at 4°C. The next day, neurons were washed three times with PBS followed by one-hour incubation at RT of appropriate secondary antibodies (1:1000) and finally washed again three times with PBS. Images were acquired with a X63 oil-immersion objective (1.4 NA) using an inverted confocal microscope (LSM 710, Zeiss) coupled to an Airyscan detector. For 2D-
STED microscopy, we used the Abberior kit containing the secondary antibodies (STAR RED anti mouse or rabbit, STAR ORANGE anti mouse or rabbit) and coverslips were mounted with the Abberior mount solid. Images were taken with a 100X oil-immersion objective (1.46 NA) using the Abberior 2D-STEDYCON upright confocal microscope.

For brain slices, brains were incubated in PFA 4% overnight and washed with PBS three times the next day. Then, brains were cut into 100 µm-thick slices using a vibratome. The slices were incubated with a blocking solution (0.3% triton, 10%NGS in PBS) for 2 hours at RT and then with antibody against GFP (Institut Curie, A-P-R#06) overnight at 4°C. The day after, the primary antibody was removed by 3 washes of PBS before incubating the slices with the associated secondary antibody and finally with 3 washes of PBS. Finally, slices were incubated with DAPI (1/4000) for 15 minutes, washed three times with PBS, mounted on Superfrost slides by using Dako Faramount Aqueous Mounting Medium solution and coverslips. The slices were acquired with a x10 objective (0.45 NA) using a slide scanner (AxioScan Z1, Zeiss) and with a x10 objective (0.3 NA) using an inverted confocal microscope (LSM 710, Zeiss) coupled to an Airyscan detector to improve signal-to-noise ratio and to increase the resolution.

In situ proximity ligation assay (PLA)

The NaveniFlex MR kit (Navinci #NF.MR.100) was used to study the interaction of endogenous HTT with endogenous KIF1A/VAMP2 in cortical neurons. The assay was performed following manufacturer’s instructions. Primary antibodies were incubated with the same dilutions used for immunocytochemistry experiments. Images were acquired with a X63 oil-immersion objective (1.4 NA) using an inverted confocal microscope (LSM 710, Zeiss).

Western blotting

Cortical neurons were plated in free culture, transduced at DIV1 with sh-Kif1a or Sh-scr, and lysed at DIV5 in NetN buffer (20 mM Tris-HCl pH8, 120 mM NaCl, 1mM EDTA, 0.5% NP40) complemented with protease inhibitor cocktail (Roche).

A vesicular fraction from brains was prepared as described in (Hinckelmann et al., 2016). Briefly, brains were homogenized in lysis buffer (10mM HEPES-KOH, 175 mM L-aspartic acid, 65 mM taurine, 85 mM betaine, 25 mM glycine, 6.5 mM MgCl2, 5mM EGTA, 0.5 mM D-glucose, 1.5 mM CaCl2, 20 mM DTT pH 7.2, protease inhibitor from Roche) on ice with a glass
potter and then with a 25G needle. Lysates were then centrifuged (12000 RPM) and the supernatant, considered as the total fraction, is then centrifuged (3000 RPM for 10 minutes). The resulting supernatant was centrifuged (12 000 RCF for 40 minutes). The supernatant was then ultracentrifuged (100 000g) to obtain the vesicular fraction (the pellet) and the cytosolic fraction (the supernatant).

All types of lysed samples were dosed by a Bradford reagent to quantify the protein concentration and then analyzed by SDS-PAGE transferred to PVDF membranes. Then, membranes were incubated for 45 minutes in a 5% BSA TBST (10mM Tris pH 8, 150 mM NaCl, 0.5% Tween 20) solution and incubated with primary antibodies against KIF1A (Abcam # ab180153, 1:5000), VAMP2 (Synaptic Systems #104211, 1:1000), pS421 (Chemicon #AB9562, 1:500), Vinculin (Sigma #V9131, 1:10000), p150 (BD laboratories, # 612708, 1:1000), Tubulin (Sigma #T9026, 1:1000) at 4°C, overnight. The next day, membranes were washed at least three times with TBST and incubated with secondary antibodies conjugated with Horseradish Peroxidase (HRP) against mouse or rabbit (1:1000) for two hours at RT. Membranes were finally revealed with ECL (Thermo Scientific) after three washes of TBST.

Electron microscopy

We anesthetized 3- to 4-month-old animals with 1ml/kg of Doléthal® and perfused them transcardially with cold PBS followed by 2% paraformaldehyde 2% glutaraldehyde and 0,1M cacodylate cold solution. We removed brains from the skull and fixed them in a 0.1M phosphate buffer pH7.2 with 2% of glutaraldehyde and 2% of paraformaldehyde for 48 hours at 4°C before obtaining 2 mm-thick or 100µm-thick slices from a mold and a vibratome, respectively. A 1mm square piece of tissue was removed from the dorsolateral striatum; samples were then fixed again with the same solution for 72 hours, washed with phosphate buffer, and then post-fixed in a 0.1M phosphate buffer pH 7.2 with 1% Osmium tetroxide for 1 hour at 4°C. After extensive washes with water, samples were then stained with a solution of 1% uranyl acetate pH 4 in water for 1 hour at 4°C. They were further dehydrated through a gradient of ethanol (30%-60%-90% and three at 100%) and infiltrated with a solution of 1/1 epon/alcohol 100% for 1 hour and followed by several baths of fresh epon (Fukka) for 3 hours. The resin was then poured into capsules containing the samples, heated at 60°C for 72 hours for polymerization, and finally cut into ultrathin sections with an ultramicrotome (Leica). Sample sections were then post-stained
with fresh solutions of 5% uranyl acetate and 0.4% of lead citrate, observed with a transmission electron microscope at 80 kV (JEOL 1200EX) and images were acquired with a digital camera (Veleta, SIS, Olympus). Analysis was performed with ImageJ and quantification of the number of synapses was performed on axon-free neuropil regions (Zhang et al., 2015).

**Brain slice preparation and whole-cell patch-clamp recordings**

All experiments were performed in accordance with the guidelines of the local animal welfare committee (Center for Interdisciplinary Research in Biology Ethics Committee) and the EU (directive 2010/63/EU). We prepared horizontal brain slices containing the somatosensory S2 cortex and the corresponding corticostriatal projection field in the dorsal striatum from mice (2-3-months old) using a vibrating blade microtome (VT1200S, Leica Micosystems, Nussloch, Germany). Brains were sliced in a 5% CO2/95% O2-bubbled, ice-cold cutting solution containing (in mM): 125 NaCl, 2.5 KCl, 25 glucose, 25 NaHCO3, 1.25 NaH2PO4, 2 CaCl2, 1 MgCl2, and 1 pyruvic acid, and then transferred into the same solution at 34°C for 60 minutes and then moved to room temperature.

For whole-cell patch-clamp recordings, borosilicate glass pipettes of 4-6 MΩ resistance contained (in mM): 105 K-gluconate, 30 KCl, 10 HEPES, 10 phosphocreatine, 4 ATP-Mg, 0.3 GTP-Na, 0.3 EGTA (adjusted to pH 7.35 with KOH). The composition of the extracellular solution was (in mM): 125 NaCl, 2.5 KCl, 25 glucose, 25 NaHCO3, 1.25 NaH2PO4, 2 CaCl2, 1 MgCl2, and 10μM pyruvic acid, bubbled with 95% O2 and 5% CO2. Signals were amplified using EPC10-2 amplifiers (HEKA Elektronik, Lambrecht, Germany). All recordings were performed at 34°C using a temperature control system (Bath-controller V, Luigs & Neumann, Ratingen, Germany) and slices were continuously superfused at 2-3 ml/min with the extracellular solution. Slices were visualized on an Olympus BX51WI microscope (Olympus, Rungis, France) using a 4x/0.13 objective for the placement of the stimulating electrode and a 40x/0.80 water-immersion objective for localizing cells for whole-cell recordings. The series resistance was not compensated. Recordings were sampled at 10 kHz, using the Patchmaster v2x32 program (HEKA Elektronik).

For paired-pulse protocols, electrical stimulations were performed with a bipolar electrode (Phymep, Paris, France) placed in the layer V of the somatosensory S2 cortex. Electrical stimulations were monophasic at constant current (ISO-Flex stimulator, AMPI, Jerusalem,
Israel). Currents were adjusted to evoke 50-200pA EPSCs. Repetitive control stimuli were applied at 0.1Hz. For each ISI, 20 successive EPSCs were individually measured and then averaged. Variation of input and series resistances above 20% led to the rejection of the experiment. Off-line analysis was performed with Fitmaster (Heka Elektronik). Statistical analysis was performed with Prism 5.02 software (San Diego, CA, USA). All results are expressed as mean ± SEM. Statistical significance was assessed in non-parametric Mann Whitney, one-sample t-tests using the indicated significance threshold (p).

**Mass spectrometry**

This analysis follows that of (Migazzi et al., 2021). Briefly, vesicular fraction from brains obtained as described earlier was first pre-cleared for an hour at 4°C with protein A Sepharose beads (Sigma Aldrich-P9424) and then immunoprecipitated for 3 hours at 4°C by agarose beads preincubated with rabbit anti-HTT D7F7 antibody (Cell Signaling, Cat#5656). To remove the non-specific binding, the beads were washed three times with the lysis buffer and bound proteins are finally eluted with Laemmli buffer. The HTT corresponding band on the western blot was cut and analyzed. MS was performed with a LTQ Orbitrap XL mass spectrometer (Thermo Scientific), equipped with a nanoESI source (Proxeon). The top eight peaks in the mass spectra (Orbitrap; resolution, 60,000) were selected for fragmentation (CID; normalized collision energy, 35%; activation time, 30 ms, q-value, 0.25). Proteins were identified using the MaxQuant software package version 1.2.2.5 (MPI for Biochemistry, Germany) and UniProt database version 04/2013.

**Quantification and Statistical Analyses**

**Transport analysis**

Vesicle velocity, directional flow, and vesicle number were measured on 100 µm of neurite using KymoTool Box ImageJ plugin, as previously described (Virlogeux et al., 2018). Anterograde or retrograde speeds describe, respectively, the mean speed of anterograde or retrograde segmental movement of a vesicle. Static vesicles are those without any movement during the recording. Linear flow and directionality were calculated as in (Virlogeux et al., 2018).
Electrophysiology analysis

For each ISI, 20 successive EPSCs were individually measured and then averaged. Variation of input and series resistances above 20% led to the rejection of the experiment. Offline analysis was performed with Fitmaster (Heka Elektronik). Statistical analysis was performed with Prism 5.02 software (San Diego, CA, USA). All results are expressed as mean ± SEM. Statistical significance was assessed in non-parametric Mann Whitney, one-sample t-tests using the indicated significance threshold (p).

Immunostaining

Immunostained vesicles in distal axons were quantified as previously shown (Scaramuzzino et al., 2022). Briefly, we used a customized macro for imageJ where the images are enhanced using a DoG filter adapted to the vesicle size. Masks are created on each channel using manual thresholding that is kept constant for each individual channel and replicates. Finally, the number of particles is automatically counted for the single and dual channels and expressed as the percentage of co-localization. For PLA analysis, the number of fluorescent dots in 100µm² was normalized on the total number of nuclei (DAPI⁺).

Exocytosis events

The same three fields per microchamber were acquired before and after a 4AP-bicuculline (respectively 2.5mM and 50µM) stimulation of the presynaptic chamber, four times in total (1 before and three after stimulation). The movies were analysed using a customized macro for imageJ, thus the recording of the amplitude and the number of exocytosis events were automatized. The number of events was expressed as follows:

\[
\gamma_i = \frac{(y_{i,\text{post}} - y_{i,\text{pre}}) \cdot \frac{\sum x_i}{n} + 100}{100}
\]

Where

- \( y_i \) expresses the difference of the number of events after \((y_{i,\text{post}})\) minus before \((y_{i,\text{pre}})\) stimulation in HTT-SD neurons of the field \( i \),
- \( x_j \) expresses the difference of the number of events after minus before stimulation in WT neurons of the field \( j \) and \( n \) of them have been averaged.
• the final value was normalized to 1, i.e. a given HTT-SD neuron field, whose activity after stimulation increased as much as that of the average of the WT neuron fields, will display a value of 1.

• The amplitude of the signal from stimulated neurons was normalized by that of the same neuron before stimulation.

Electron microscopy analysis

We used ImageJ to analyze synapse morphology. We counted the number of synapses in axon-free neuropil regions (Zhang et al., 2015). Synaptic vesicles were numbered according to their physical features (size, grey scale and shape). The presynaptic zone, which contains the active zone, was defined as the zone facing the PSD. The 40-nm-wide zones in the presynapse were defined according to their location relative to the active zone.

Statistical analysis

Statistical calculations were performed using GraphPad Prism 6.0. Statistical parameters (Replication, sample size, SEM, etc) are reported in the figure legends. For each dataset, we identified outliers using the ROUT test (Q=1%) and removed them from analysis. We performed a Shapiro-Wilk normality test with the threshold set at $\alpha = 0.05$; if the data followed a normal distribution, we used parametric tests, and if not, we used non-parametric tests. If we were analyzing two conditions we used a t-test (or a Mann-Whitney test if nonparametric). If comparing more than two conditions we used a one-way ANOVA followed by Tukey's post-hoc analysis (or a Kruskal-Wallis test followed by Dunn’s post hoc analysis if nonparametric). If the datasets were interdependent, we used a two-way ANOVA followed by Tukey's post-hoc analysis if more than two groups are compared, or a Sidak’s post-hoc analysis if only two groups are analyzed. For a nonlinear fit, we did a run test to determine whether the curve deviates systematically from the data. Low P value (ns) indicates that the curve poorly describes the data.

*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; ns, not significant.

Data and Software Availability

All datasets generated and analyzed during the study are included in the manuscript and in the supporting files. Source Data files have been provided for Figure 1, Figure 1-
Supplement 1, Figure 2, Figure 2- Figure Supplement 1, Figure 3, Figure 3- Figure Supplement 1, Figure 4, Figure 4- Figure Supplement 1, Figure 5, Figure 5- Figure Supplement 1, Figure 6, Figure 6- Figure Supplement 1, Figure 6- Figure Supplement 2, Figure 7, and Figure 8.
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Additional information

Supplementary files include 7 Supplementary Figures and Video 1

Competing financial interests:
The authors declare that they have no competing interests
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Figure 1. HTT phosphorylation at S421 increases SVP anterograde axonal transport and SV exocytosis. (A) Diagram of the microfluidic device for reconstituting a corticostriatal network compatible with live-cell imaging of axons. Cortical axons grow in the cortical chamber (yellow) and connect with the striatal dendrites in the striatal chamber (green) through synapses in the synaptic compartment (purple). On the right, representative kymographs of VAMP2-mCherry vesicle transport in axons for each genotype. Scale bar = 25 µm. (B) Segmental anterograde (**** p < 0.0001, N = 1078 WT vesicles, 1886 HTT-SD vesicles and 1384 HTT-SA vesicles), retrograde velocities (ns: non significant; N = 1029 WT vesicles, 1564 HTT-SD vesicles, 2019 HTT-SA vesicles) and directional net flux (**** p<0.0001; N = 118 WT axons, 157 HTT-SD axons, 132 HTT-SA axons) of VAMP2-mCherry vesicles. Histograms represent means ± SEM of 3 independent experiments. Significance was determined using one-way ANOVA followed by Dunn’s multiple comparison test. (C) Schematic of the 3-compartment microfluidic device. Cortical neurons were infected with a lentivirus expressing VGLUT1 linked to a pH-sensitive variant of GFP (pHluorin); they were stimulated with 4 AP bicuculline at DIV11. The number of VGLUT-1 pHluorin exocytosis events within the synaptic chamber of the corticostriatal network, as compared to that of WT and to that of non-stimulated condition is shown here (*p<0.05; N = 6712 events in WT, 4640 events in HTT-SD and 5176 events in HTT-SA neurons). The box-whisker plots show the median, the 25th and the 75th percentiles, the smallest and the largest values of 3 independent experiments using a total of N = WT 11, 10 HTT-SD and 10 HTT-SA neurons seeded within microfluidic devices with at least three fields per device. Significance was determined using one-way ANOVA followed by Dunn’s multiple comparison test.

Figure 2. Constitutive phosphorylation of HTT at S421 impairs motor skill learning in mice. (A) D of the accelerating rotarod protocol assessing motor skill learning over 8 days with 10 sessions per day. (B) Mean latency to fall each day for 8 days, for HTT-SD mice (*p<0.05: two-way ANOVA followed by Sidak's multiple comparisons test where p<0.01 at day 7) and HTT-SA mice (ns: non significant: two-way ANOVA). (C) Mean time to fall off the rotarod per session, over 10 sessions, during the first or (D) the last day for HTT-SD (ns: non significant: two-way ANOVA at day 1 and *p<0.05: two-way ANOVA at day 8 followed by Sidak's multiple comparison test where p<0.01 at trial 3 and 10) and HTT-SA (ns: non significant: two-way ANOVA at day 1 and 8). We compared 3-month-old male mice: 20 WT with 20 HTT-SD littermates, and 13 WT with 18 HTT-SA littermates.

Figure 3. HTT phosphorylation at S421 increases short-term plasticity in the corticostriatal network ex vivo. (A) Schematic of medium-sized spiny neurons (MSNs) recording in the dorsolateral striatum (DLS) after paired-pulse stimulations in S2 cortex of mice at 2 to 3 months of age. (B) Representative traces of the paired-pulse ratio per interstimulus interval of electrophysiological response of MSNs in the DLS after stimulation in S2 in 2- to 3-month-old WT (gray), HTT-SD (pink) and HTT-SA (orange) mice (C). Quantification of (B). In contrast to WT and HTT-SA MSNs, HTT-SD MSN responses from 25 to 50 ms showed no facilitation (paired-pulse ratio~1) but only depression from 100 ms. (*p<0.05, **p<0.001, and ***p<0.0001; ns means non-significant). Paired pulse ratios were recorded from 13 WT, 12 HTT-SD and 12 HTT-SA MSNs from at least N = 3 mice.

Figure 4. HTT phosphorylation increases the number of synaptic vesicles distally to the presynaptic active zone. (A) Representative images of SVs at the corticostriatal synapse, obtained by electronic microscopy, in DLS slices from three WT and HTT-SD mice 3-month-old male. Scale = 200 nm. (B) quantification of (i) the number of synapses at the corticostriatal synapse per 100 µm² in DLS on n = 74 WT and 74 HTT-SD striatal areas (ns: non significant), (ii) the number of SVs per corticostriatal synapse
from five WT and three HTT-SD mouse brains (N = 279 WT and 171 HTT-SD axon terminals; ****p<0.0001) (iii) Size of the cortical axon terminal in 158 WT and 156 HTT-SD corticostriatal synapses (** p<0.05) and (iv) the density of synaptic vesicles within these axon terminals (number of vesicles per μm²) in N=157 WT and 162 HTT-SD corticostriatal synapses (***p<0.001). (C) Representative images showing the 40nm-wide zones in the axon terminal. Zone 1 is the closest to the synaptic cleft and contains the active zone. Zone 2 40-80 nm is adjacent to Zone 1, and zone 3 (80-120 nm) is farthest from the active zone. Dark orange denotes the PSD within the striatal postsynaptic element. Scale= 100 nm. (D) The number of SVs per zone within the distal 120 nm of the axon terminal in at least 149 ±2 axon terminals (ns: non significant, *p<0.05). The box-whisker plots show the median, the 25th and the 75th percentiles, the smallest and the largest value from at least three brains for each condition. Significances were determined using the Mann-Whitney test.

Figure 5. HTT phosphorylation recruits KIF1A on VAMP2-mCherry vesicles. (A) Mass spectrometry analysis of vesicles purified from mouse brains identifies KIF1A (red) among HTT-associated vesicular proteins. (B) Confocal and 2D-STED images of free-cultured neurons at DIV5 showing the colocalization of KIF1A and HTT. Scale bar: 1μm. (C) Representative immunofluorescence labeling revealing HTT (cyan), KIF1A (green), and VAMP2-mCherry (magenta) within WT and HTT-SD cortical axons in the long channels of the microfluidic devices. The images were acquired in a specific region of interest and processed by an Airyscan detector (Scale bar: 1 µm). Distribution analysis shows that HTT and KIF1A were more likely to colocalize on KIF1A+ vesicles in the HTT-SD condition. The graph represents means ±SEM of 3 independent experiments reproducing a corticostriatal network of WT or HTT-SD neurons in at least 3 microfluidic devices per experiment. Significance determined by the Mann-Whitney test (*p<0.05; N = 61). (D) Proximity Ligation Assay (PLA) in WT or HTT-SD neurons, nuclei stained with DAPI. Representative images are from 3 independent experiments. Scale bar: 10 µm. Significance was determined by the Mann-Whitney test (*** p<0.0001; N = 32-34). (E) Western blot analysis for HTT, KIF1A (both bands), p150Glued, and tubulin from vesicular fractions from 6 WT and 6 HTT-SD brains. Significance was determined using the Mann-Whitney test (*p < 0.05).

Figure 6. HTT-dependent axonal transport of SVPs is mediated by KIF1A. (A) Diagram indicating lentiviral transduction of VAMP2-mCherry and sh-scramble (sh-Scr-GFP) or sh-Kif1a (sh-Kif1a-GFP) lentiviruses at DIV8 in the microfluidic device. On the right, representative kymographs of VAMP2-mCherry vesicle transport in axons for each condition. Scale bar = 25 μm. (B) Segmental anterograde and retrograde velocities (anterograde: * p<0.05, ****p<0.0001, ****p < 0.0001; N = 548 vesicles WT sh-Scr, 318 vesicles WT sh-Kif1a, 1129 vesicles HTT-SD sh-Scr, 628 vesicles HTT-SD sh-Kif1a) (Retrograde: *p<0.05, **p<0.01, ****p<0.0001; N = 583 vesicles WT sh-Scr, 396 vesicles WT sh-Kif1a, 1282 vesicles HTT-SD sh-Scr, 620 vesicles HTT-SD sh-Kif1a) and directional net flux (* p<0.01; N = 79 axons WT sh-Scr , 59 axons WT sh-KIFA,112 axons HTT-SD sh-Scr , 89 axons HTT-SD sh-Kif1a; one-way ANOVA test) of VAMP2-mCherry vesicles in WT and HTT-SD neurons transduced with sh-Scr or sh-Kif1a lentiviruses. Histograms represent means ± SEM of 3 independent experiments. Significance was determined using a one-way ANOVA followed by Dunn’s multiple comparison test.

Figure 7. In vivo KIF1A silencing in mice restores the SV synaptic pool. (A) Immunolabeling of GFP within the injection site on a slice located at 1.5 mm before the bregma (left). Scale = 1 cm (insets, 100 μm). Immunolabeling of GFP within the projection site on a slice located at -0.3 mm after the bregma (right). Scale = 1 cm (inset, 250 μm). Nuclei are labeled with DAPI. (B) Representative images from electron microscopy of corticostriatal synapses and (C) quantification of the number of SVs at the corticostriatal synapse of 3 WT male mice injected with either sh-Scr or sh-Kif1a and 3 HTT-SD mice injected with sh-Scr or sh-Kif1a (** p < 0.01, *** p < 0.001, **** p < 0.0001; N = 360 WT sh-Scr
synapses, 324 WT sh-Kif1a synapses, 417 HTT-SD sh-Scr synapses, 337 HTT-SD sh-Kif1a synapses). Scale = 200 nm. Histograms represent means ±SEM. Significance was determined using one-way ANOVA followed by Dunn’s multiple comparison test.

Figure 8. Motor skill learning defects of S421D mice are rescued by KIF1A silencing in vivo. (A) Schematic of the experimental procedure consisting in bilateral stereotaxic injections in the mouse brain followed three weeks later by the accelerating rotarod protocol over 8 days. (B) Mean time to fall off the rotarod per day over 8 days. Two-way ANOVA comparing the four conditions showed significant differences between genotypes and silencing conditions (**p<0.0001). Holm-Sidak’s post-hoc analysis revealed significant differences between WT sh-Scr and HTT-SD sh-Scr mice at day 4 and day 8 (*p<0.05). (C) Mean time to fall off the rotarod the first day (*p<0.01) (left) and the last day (ns: non significant, right), per sessions. Holm-Sidak’s post-hoc analysis revealed significant differences between HTT-SD sh-Scr and HTT-SD sh-Kif1a mice during the first day (* p<0.05). At least 3 cohorts containing 12 WT sh-Scr, 11 WT sh-Kif1a, 10 HTT-SD sh-Scr, and 12 HTT-SD sh-Kif1a 3-month-old littermate male mice were analyzed.

Figure supplement legends

Figure 1-figure supplement 1. HTT phosphorylation at S421 increases SVP anterograde axonal transport without affecting the quantity of SV released. (A) Left: Number of anterograde (*p<0.05; N = 117 WT axons, 156 HTT-SD axons and 132 HTT-SA axons) and retrograde (*p<0.05; 118 WT axons, 159 HTT-SD axons and 134 HTT-SA axons) VAMP2-mCherry vesicles in 100 µm of axon. Right: their linear flow rate (*p<0.05; 118 WT axons, 158 HTT-SD axons and 133 HTT-SA axons). Histograms represent means ±SEM of 3 independent experiments. Significance was determined using a one-way ANOVA followed by Dunn’s multiple comparison test. (B) Diagram of the three-compartment microfluidic device with an indication of lentiviral transduction and stimulation with 4AP bicuculline. The amplitude of VGLUT-1 pHluorin exocytosis events within the synaptic chamber of the corticostriatal network was compared to that of non-stimulated condition (see Methods). Histograms represent means ± SEM of 3 independent experiments. Significance was determined using one-way ANOVA followed by Dunn’s multiple comparison test (ns: non significant).

Figure 2-figure supplement 1. HTT phosphorylation at S421 impairs motor skill learning without affecting motor performance. (A) Results of the first rotarod trial of the first day for HTT-SD and HTT-SA mice (ns: non significant, Mann-Whitney test). The box-whisker plots show the median, the 25th and the 75th percentiles, the smallest and the largest values for N = 20 WT vs. 20 HTT-SD and 13 WT vs. 18 HTT-SA 3-month-old littermate male mice. (B) Performance during the 10 sessions of the first day for 8 WT, 10 HTT-SD and 12 HTT-SA 18-month-old mice. Significance was determined using two-way ANOVA followed by Holm-Sidack’s multiple comparison test (*p<0.05 between WT and HTT-SD).

Figure 3-figure supplement 1. HTT phosphorylation does not regulate the sEPSCs in the corticostriatal synapse. (A) Schematic of the procedure for sEPSC recording in MSNs within the DLS. (B) Representative traces, cumulative probability of the mean amplitude, and mean interevent intervals (p<0.05) of sEPSCs in MSNs within the DLS in 2-3-month-old WT and HTT-SD mice. sEPSCs were recorded from WT (10 MSN) and HTT-SD mice (10 MSN). There were no significant differences in cumulative probability between WT, HTT-SD and HTT-SA mice (ns: non significant).
Figure 4-figure supplement 1. Characterization of the three presynaptic zones of HTT-SD corticostriatal axon terminals and analysis of HTT-SA corticostriatal synapses. (A) Area of the three zones in WT and HTT-SD axon terminals studied in 145±9 axon terminals from three WT and HTT-SD 3-month-old mice (ns: not significant, two-way ANOVA followed by Dunn’s multiple comparisons test). (B) Left (i). The number of corticostriatal synapses in 72 WT and 77 HTT-SA striatal areas from three WT and HTT-SA 4-month-old mice (ns: non significant, Mann and Whitney). Right (ii). The number of vesicles at 4 months in 148 WT and 130 HTT-SA corticostriatal axon terminals (****p<0.0001, Mann and Whitney). The box-whisker plots show the median, the 25th and the 75th percentiles, using at least three brains of each genotype.

Figure 5-figure supplement 1. HTT phosphorylation and subcellular localization and interaction of HTT and KIF1A with VAMP2. (A) Representative immunostainings revealing HTT (cyan), KIF1A (green), and VAMP2-mCherry (magenta) within cortical axons localized in the long channels of the microfluidic devices. The images were acquired in a specific region of interest and processed by an Airyscan detector (Scale bar: 1 µm). The distribution analysis shows that there is greater colocalization of HTT and KIF1A on KIF1A+ vesicles in the HTT-SD condition compared to WT neurons. The graph represents means ±SEM of 3 independent experiments reconstituting corticostriatal networks of WT or HTT-SD neurons in at least 3 microfluidic devices per experiment. Significance was determined using the Mann-Whitney test (ns: non-significant; n = 61). (B) Western blot analysis for HTT, HTT S421 phosphorylation, KIF1A (both bands), p150Glued, and tubulin from vesicular fractions of N = 3 WT and 3 HTT-SD brains. Significance was determined using the Mann-Whitney test (ns: non-significant). As previously reported, HTT-SD is not recognized by the anti pS421-HTT antibody. (C-D) Proximity Ligation Assay (PLA) in WT and HTT-SD neurons. Nuclei were stained with DAPI. Shown are representative images from three independent experiments. Scale bar: 10 µm. Significance was determined using the t-test (ns: non-significant; N = 29-31).

Figure 6-figure supplement 1. KIF1A levels in HTT-SD neurons regulate VAMP-2 axonal transport. (A) Analysis of KIF1A levels by western blot in cortical neurons either not-transduced or transduced with sh-Kif1a or sh-Scr lentiviruses (*p<0.05, **p<0.01, ***p<0.001; one way ANOVA followed by Dunn’s multiple comparisons test). Histograms represent the means ±SEM. (B) Number of anterograde (*p<0.05, **p<0.01; n = 76 axons WT sh-Scr, 59 axons WT sh-Kif1a, 110 axons HTT-SD sh-Scr and 86 axons HTT-SD sh-Kif1a) and retrograde (*p<0.05; n = 60 WT sh-Scr axons, 114 WT sh-Kif1a axons, 79 HTT-SD sh-Scr axons and 95 HTT-SD sh-Kif1a axons) VAMP2-mCherry axonal vesicles along 100 µm of axon in WT and HTT-SD and their linear flow rate (*p<0.05; 75 WT sh-Scr axons, 59 WT sh-Kif1a axons, 107 HTT-SD sh-Scr axons and 85 HTT-SD sh-Kif1a axons. One way ANOVA followed by Dunn’s test). Histograms represent the means ±SEM of at least 3 independent experiments.

Figure 6-figure supplement 2. KIF1A silencing doesn’t affect BDNF-mCherry transport. (A) Diagram indicating transduction of BDNF-mCherry and sh-scramble (sh-scr-GFP) or sh-Kif1a (sh-Kif1a-GFP) lentiviruses (left). Representative kymographs of BDNF-mCherry vesicle transport within WT or HTT-SD axons transduced with sh-Scr or sh-Kif1a at DIV8. Scale bar = 25 µm. (B) Segmental anterograde (****p<0.0001; n = 618 WT sh-Scr vesicles, 901 WT sh-Kif1a vesicles, 1735 HTT-SD sh-Scr vesicles, and 2830 HTT-SD sh-Kif1a vesicles) and retrograde velocities. There were no significant differences between genotypes in the retrograde segmental velocities and KIF1A silencing conditions (ns=non significant), linear flow (**** p<0.0001; 75 WT sh-Scr axons, 102 WT sh-Kif1a axons, 114 HTT-SD sh-Scr axons and 191 HTT-SD sh-Kif1a axons), or net flux (**** p<0.0001; n = 75 WT sh-Scr axons, 103 WT sh-Kif1a axons, 123 HTT-SD sh-Scr axons and 186 HTT-SD sh-Kif1a axons) of BDNF-
mCherry vesicles. Histograms represent means ±SEM of 3 independent experiments. Significance was
determined using one-way ANOVA followed by Dunn’s multiple comparisons test.

Figure 1-video 1. Movie showing the glutamate release (VGLUT-pHluorin) in WT neurons after
the stimulation.

Source data legends

Figure 1-source data 1. Data analysed for anterograde velocity
Figure 1-source data 2. Data analysed for retrograde velocity
Figure 1-source data 3. Data analysed for net flux
Figure 1-source data 4. Data analysed for VGLUT1 pHluorin exocytosis number of events
Figure 2-source data 1. Data analysed for accelerating rotarod over 8 days
Figure 2-source data 2. Data analysed for accelerating rotarod during Day 1
Figure 2-source data 3. Data analysed for accelerating rotarod during Day 8
Figure 3-source data 1. Data analysed for short-term plasticity
Figure 4-source data 1. Data analysed for the number of the corticostriatal synapses (i)
Figure 4-source data 2. Data analysed for the number of the synaptic vesicles (ii)
Figure 4-source data 3. Data analysed for the number of the axon terminal size in the presynaptic zone
Figure 4-source data 4. Data analysed for the density of synaptic vesicles
Figure 4-source data 5. Data analysed for the number of vesicles per zone
Figure 5-source data 1. Data analysed for HTT-KIF1A co-localization in axons
Figure 5-source data 2. Data analysed for the proximity ligation assay performed between HTT and
KIF1A
Figure 5-source data 3. Data analysed for the protein content of KIF1A and VAMP2 levels in vesicular
fractions
Figure 5—source data 4. Western blot scans for the data presented in Figure 5E (KIF1A and VAMP2
levels in brain vesicular fractions). Shown in red are the cropped regions presented in Figure 5E. Films
containing the second batch of samples (Gel 2) are shown.
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Figure 6-source data 2. Data analysed for retrograde velocity
Figure 6-source data 3. Data analysed for net flux
Figure 7-source data 1. Data analysed for the number of the synaptic vesicles
Figure 8-source data 1. Data analysed for accelerating rotarod over 8 days
Figure 8-source data 2. Data analysed for accelerating rotarod during Day 1
Figure 8-source data 3. Data analysed for accelerating rotarod during Day 8

Figure supplement - Source data legends

Figure 1-figure supplement 1-source data 1. Data analysed for number of anterograde vesicles
Figure 1-figure supplement 1-source data 2. Data analysed for number of retrograde vesicles
Figure 1-figure supplement 1-source data 3. Data analysed for the linear flow rate
Figure 1-figure supplement 1-source data 4. Data analysed for VGLUT1 pHluorin exocytosis
amplitude of events
Figure 2—figure supplement 1-source data 1. Data analysed for accelerating rotarod 1st trial at Day 1
Figure 2—figure supplement 1-source data 2. Data analysed for accelerating rotarod during Day 1 at 18 months
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Figure 4—figure supplement 1-source data 2. Data analysed for the number of the corticostriatal synapses (i)
Figure 4—figure supplement 1-source data 3. Data analysed for the number of the synaptic vesicles (ii)
Figure 5—figure supplement 1-source data 1. Data analysed for HTT-VAMP2 co-localization in axons
Figure 5—figure supplement 1-source data 2. Data analysed for KIF1A-VAMP2 co-localization in axons
Figure 5—figure supplement 1-source data 3. Data analysed for the proximity ligation assay performed between VAMP2/HTT
Figure 5—figure supplement 1-source data 4. Data analysed for the proximity ligation assay performed between VAMP2/KIF1A
Figure 5—figure supplement 1-source data 5. Data analysed for the protein content of KIF1A and VAMP2 levels in total fractions
Figure 5—figure supplement 1-source data 6. Western blot scans for the data presented in Figure 5—figure supplement 1D (KIF1A levels in whole brain lysates). Shown in red are the cropped regions presented in Figure 5—figure supplement 1D. Films containing the first batch of samples (Gel 1) are shown.
Figure 6—figure supplement 1-source data 1. Western blot scans for the data presented in Figure 6—figure supplement 1A (KIF1A levels in cortical neurons). Shown in red are the cropped regions presented in Figure 6—figure supplement 1A. Films containing the samples are shown.
Figure 6—figure supplement 1-source data 2. Data analysed for number of anterograde vesicles
Figure 6—figure supplement 1-source data 3. Data analysed for number of retrograde vesicles
Figure 6—figure supplement 1-source data 4. Data analysed for the linear flow rate
Figure 6—figure supplement 2-source data 1. Data analysed for for anterograde velocity
Figure 6—figure supplement 2-source data 2. Data analysed for for retrograde velocity
Figure 6—figure supplement 2-source data 3. Data analysed for the linear flow rate
Figure 6—figure supplement 2-source data 4. Data analysed for the net flux
Figure 1

A

VAMP2-mCh trafficking

B

VGLUT1-pHluorin exocytosis

C

Exocytic events (#/30s)/#wild-type events

-4 0 2 4 6

ns

ns

ns

ns

ns

ns
Figure 2

A Experimental procedure

B Accelerating rotarod over 8 days

C Accelerating rotarod during Day 1

D Accelerating rotarod during Day 8
Figure 3

A. Diagram of Mice (2-3 months) and Brain Slices with Cortical Neurons in Layer V (S2) paired/pulse stimulations and Evoked EPSC recording in MSN of DLS.

B. Graph showing paired-pulse ratio with inter-stimulus intervals of 20 ms, 50 ms, and 25 pA.

C. Bar graph illustrating short-term plasticity with different conditions: Wild-type (n=13), HTT-SD (n=12), and HTT-SA (n=12) with indicated significance levels.* ns = P < 0.05, ** ns = P < 0.01, *** ns = P < 0.001, **** ns = P < 0.0001.
**Figure 4**

**A**

Wild-type HTT-SD

**B**

i) # corticostriatal synapses

ii) # synaptic vesicles

ns

****

**C**

Presynaptic zones

Density of SVs

ns

**D**

Zone 1 (0-40nm)

Zone 2 (40-80nm)

Zone 3 (80-120nm)

ns

****
Figure 5

A  Vesicular HTT interaction with KIF1A

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<tr>
<th>Molecular motor complexes</th>
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<td>1: Dyn1c1</td>
<td>1: Syn1</td>
</tr>
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<td>2: Dyn1c1</td>
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<td>11: Snap47</td>
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<tr>
<td>10: Kif1a</td>
<td>17: Dyn1c1</td>
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</table>

LFQ intensity (log2)

B  Free cultured neurons (2D-STED)

C  Isolated axons (Airyscan)

D  Proximity ligation assay (HTT-KIF1A)

E  KIF1A levels on vesicles
Figure 6
VAMP2-mCherry axonal transport

A

B

Velocity

Net Flux

<table>
<thead>
<tr>
<th></th>
<th>Wild-type sh-Scr</th>
<th>Wild-type sh-Kif1a</th>
<th>HTT-SD sh-Scr</th>
<th>HTT-SD sh-Kif1a</th>
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<tr>
<td>Velocity (µm/sec)</td>
<td>ns</td>
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<td>Net flux (µm/s)</td>
<td>-1.5</td>
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</table>

Cortical
Striatal
sh-scr
sh-KIF1A
B

Vamp2-mCherry
sh-Scr-GFP or sh-Kif1a-GFP
Wild-type HTT-SD
sh-scr
sh-KIF1A

Synaptic
Figure 7

(A) Stereotaxic injections of lentiviruses

(B) Neuronal projections

(C) Quantification of synaptic vesicles/synapse

- WT HTT-SD
- sh-Scr
- sh-Kif1a
- ns
- ***
- ****
A. Experimental procedure

B. Accelerating rotarod over 8 days

C. Accelerating rotarod

- Day 1
- Day 8

Legend:
- Wild-type sh-Scr
- Wild-type sh-Kif1a
- HTT-SD sh-Scr
- HTT-SD sh-KIF1A
Figure 1-figure supplement 1

**A** Vamp-2-mCherry axonal transport

<table>
<thead>
<tr>
<th># vesicles (/100µm)</th>
<th>Wild-type</th>
<th>HTT-SD</th>
<th>HTT-SA</th>
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<td>25</td>
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</table>

- **Linear flow rate (µm/s)**

  - Wild-type: ns
  - HTT-SD: ns
  - HTT-SA: ns

**B** VGLUT1-pHluorin exocytosis

- **4 AP-Bic DIV11**
- **Cortical**
- **Striatal**

- **VGLUT1-pHluorin**

- **Synaptic**

- **Amplitude of events**

- **Cortical**

- **Striatal**

- **VGLUT1-pHluorin**
Figure 2-figure supplement 1

A
Accelerating rotarod - 1st trial of Day 1

B
Accelerating rotarod during Day 1 (18months)
**Figure 3-figure supplement 1**

**A**
- Mice (2-3 months) → Brain slices → sEPSCs registration in MSN of DLS

**B**
**mEPSC recordings**
- **Wild-type**
- **HTT-SD**
- **HTT-SA**

![Graphs showing cumulative probability distributions for mEPSC recordings](image-url)

- **Cumulative probability**
  - Amplitude (pA)
  - Inter-event interval (ms)

- **Bar graphs** for amplitude and inter-event interval with comparisons and annotations.

- **ns** indicates non-significant differences.
Figure 4-figure supplement 1

A

Wild-type HTT-SD

<table>
<thead>
<tr>
<th>Zone 1</th>
<th>Zone 2</th>
<th>Zone 3</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.04</td>
<td>0.02</td>
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</tbody>
</table>

Area of presynaptic zones (μm²)

ns

B

Wild-type HTT-SA

# vesicles (4 months)

F

# corticostriatal synapses

ns

F

0 10 20 30 40 50 60 70 80 90 100

# vesicles/synapses

****
**Figure 5-figure supplement 1**

A **Isolated axons (Airyscan)**

Wild-type HTT-SD

- mCherry/HTT
- KIF1A/HTT
- mCherry/KIF1A

HTT-SD

- mCherry/HTT
- KIF1A/HTT
- mCherry/KIF1A

B **Proximity ligation assay**

Wild-type

- DAPI
- VAMP2-HTT

HTT-SD

- DAPI
- VAMP2-HTT

Wild-type

- DAPI
- VAMP2-KIF1A

HTT-SD

- DAPI
- VAMP2-KIF1A

C **VAMP2-HTT**

PLA signal/cell (100µm²)

- Wild-type
- HTT-SD

D **VAMP2-KIF1A**

PLA signal/cell (100µm²)

- Wild-type
- HTT-SD

**D KIF1A levels in total fraction**

Wild-type HTT-SD

- MW
- pS421
- HTT
- KIF1A
- Glu
- p150
- Vinculin
- VAMP2

KIF1A/p150

- Wild-type
- HTT-SD

VAMP2/p150

- Wild-type
- HTT-SD

ns
Figure 6-figure supplement 1

A. KIF1A levels in wild-type cortical neurons

- Untransfected
- sh-Scr
- sh-Kif1a

**MW**
- UNTR
- sh-Kif1a
- sh-Scr

**Tubulin**
- KIF1A

B. VAMP2-mCherry axonal transport

**# vesicles**
- Linear Flow

- Vesicles (#/100 µm)
- Linear flow (µm/s)

- Wild-type sh-Scr
- Wild-type sh-Kif1a
- HTT-SD sh-Scr
- HTT-SD sh-Kif1a

**Linear Flow**

- ns
- ****

**VAMP2-mCherry axonal transport**

- Linear Flow

- Wild-type sh-Scr
- Wild-type sh-Kif1a
- HTT-SD sh-Scr
- HTT-SD sh-Kif1a

**Linear Flow**

- ns
- ****

**VAMP2-mCherry axonal transport**

- Linear Flow

- Wild-type sh-Scr
- Wild-type sh-Kif1a
- HTT-SD sh-Scr
- HTT-SD sh-Kif1a

**Linear Flow**

- ns
- ****
Figure 6-figure supplement 2

A

BDNF-mCherry transport with KIF1A silencing

Wild-type HTT-SD

B

BDNF-mCherry transport with KIF1A silencing

- Wild-type sh-Scr
- Wild-type sh-Kif1a
- HTT-SD sh-Scr
- HTT-SD sh-Kif1a

Velocity

Linear Flow

Net Flux

Velocity (µm/sec)

Linear flow (µm/s)

Net flux (µm/s)