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Differential changes in thalamic and cortical excitatory synapses onto striatal spiny projection neurons in a Huntington disease mouse model

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ABSTRACT

Huntington disease (HD), a neurodegenerative disorder caused by CAG repeat expansion in the gene encoding huntingtin, predominantly affects the striatum, especially the spiny projection neurons (SPN). The striatum receives excitatory input from cortex and thalamus, and the role of the former has been well-studied in HD. Here, we report that mutated huntingtin alters function of thalamostriatal connections. We used a novel thalamostriatal (T-S) coculture and an established corticostriatal (C-S) coculture, generated from YAC128 HD and WT (FVB/NJ background strain) mice, to investigate excitatory neurotransmission onto striatal SPN. SPN in T-S coculture from WT mice showed similar mini-excitatory postsynaptic current (mEPSC) frequency and amplitude as in C-S coculture; however, both the frequency and amplitude were significantly reduced in YAC128 T-S coculture. Further investigation in T-S coculture showed similar excitatory synapse density in WT and YAC128 SPN dendrites by immunostaining, suggesting changes in total dendritic length or probability of release as possible explanations for mEPSC frequency changes. Synaptic N-methyl-D-aspartate receptor (NMDAR) current was similar, but extrasynaptic current, associated with cell death signaling, was enhanced in YAC128 SPN in T-S coculture. Employing optical stimulation of cortical versus thalamic afferents and recording from striatal SPN in brain slice, we found increased glutamate release probability and reduced AMPAR/NMDAR current ratios in thalamostriatal synapses, most prominently in YAC128. Enhanced extrasynaptic NMDAR current in YAC128 SPN was apparent with both cortical and thalamic stimulation. We conclude that thalamic afferents to the striatum are affected early, prior to an overt HD phenotype; however, changes in NMDAR localization in SPN are independent of the source of glutamatergic input.

1. Introduction

Huntington disease (HD) is an autosomal dominant neurodegenerative disorder caused by an expansion of CAG repeats in the gene encoding huntingtin (Htt). The brain region most affected is the striatum, an area critical for cognition and motor control, where GABAergic spiny projection neurons (SPN) make up >90% of all neurons and show severe loss in late-stage HD. The striatum receives excitatory inputs from the cortex and thalamus, and the role of the former has been extensively studied in HD models. In particular, increased cortical excitability is one of the early features of HD (Cepeda et al., 2003; Graham et al., 2009; Joshi et al., 2009), and coordinated firing in corticostriatal circuits is disrupted (Miller et al., 2011). Although the magnitude of thalamic volume loss has been shown to closely correlate with degree of cognitive impairment in HD patients (Kassubek et al., 2005), the role of excitatory thalamostriatal connections and their functional changes in HD remain under-explored.

Impaired synaptic transmission and cellular signaling, leading to cell damage and death, have been reported at the pre-manifest stages of disease, in HD patients and animal models (Raymond et al., 2011). SPN carrying the HD mutation are more sensitive to NMDA-induced toxicity and express a larger proportion of NMDAR at the cell surface (Fan et al., 2007). Specifically, NMDAR expression was shown to be increased at extrasynaptic sites, where their activation triggers cell death signaling (Milnerwood et al., 2010, 2012). Blocking extrasynaptic NMDAR (exNMDAR) with the low-affinity, use-dependent antagonist memantine exerted a neuroprotective effect and restored levels of exNMDAR in YAC128 striatum to those of wild-type (WT) FVB/NJ mice (Okamoto et al., 2009; Milnerwood et al., 2010, 2012; Dau et al., 2014). However, it is not known whether thalamic input contributes to progressive striatal synaptic changes, or to what extent any thalamostriatal synaptic alterations replicate those occurring at the corticostriatal synapse.

Excitatory thalamic input onto striatal SPN is less abundant than cortical input (Lacey et al., 2005; Lei et al., 2013; Zhang et al., 2013), with approximately 40% of excitatory terminals being formed by the

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thalamus and 60% by the cortex (Deng et al., 2013; Lei et al., 2013; but see also: Doig et al., 2010; Ellender et al., 2013). Additionally, axodendritic connections, as opposed to axospinous ones, are more commonly formed on striatal SPN by thalamic afferents (especially those from parafascicular nuclei: Raju et al., 2006; Lacey et al., 2007) than by cortical ones (Lacey et al., 2005; Moss and Bolam, 2008; Doig et al., 2010; Lei et al., 2013; Zhang et al., 2013). Together, these anatomical findings suggest distinct functional properties of thalamic- and cortical-SPN synapses; indeed, electrophysiological recordings in slices from rat brain have shown marked differences in NMDAR/AMPAR current ratio and NMDAR subunit composition (Ding et al., 2008; Smeal et al., 2008), as well as differences in release probability and short-term synaptic plasticity (Ding et al., 2008) between corticostriatal and thalamostriatal synapses. The two major studies, however, significantly differed in their final conclusions (Ding et al., 2008; Smeal et al., 2008). Notably, a recent study has shown thalamostriatal connections are preferentially lost in an HD mouse model before phenotype development, preceding the loss of corticostriatal connections (Deng et al., 2013, 2014); however, whether the functional properties of these connections are significantly altered was not known.

The aim of this study was to investigate the properties of thalamostriatal synaptic transmission and their possible changes in the early stages of Huntington disease, comparing them to corticostriatal synapses. We used a novel thalamostriatal coculture system to characterize basic electrophysiological features of thalamic inputs to striatal SPN, including miniature excitatory events, synapse formation and NMDAR currents. We also applied optical stimulation of thalamic afferents in slice, to measure changes in thalamostriatal synapses in a more physiological environment. We conclude that thalamostriatal synapses in HD undergo marked early changes and may therefore significantly contribute to the pathogenesis of the disease.

2. Materials and methods

2.1. Transgenic mice

In all experiments, the YAC128 HD mouse model was used. YAC128 is a yeast artificial chromosome (YAC) transgenic mouse model of HD that expresses the full-length human *HTT* gene with 128 CAG repeats on the FVB/NJ background (Slow et al., 2003). For coculture preparation, WT (FVB/NJ strain) and YAC128 mice (line 55, which produces viable homozygotes) of both sexes were used; for viral injections and slice electrophysiology, only male WT (FVB/NJ strain) and YAC128 mice (line 53, which expresses higher levels of human Htt in heterozygotes: Slow et al., 2003) were used. All the animals were bred and maintained according to the Canadian Council on Animal Care regulations at the University of British Columbia (UBC) Faculty of Medicine Animal Resource Unit.

2.2. Coculture preparation

Embryonic cocultures were prepared, based on a protocol established previously in the lab (Kaufman et al., 2012; Milnerwood et al., 2012). Briefly, brains of male and female fetuses (E17–18) of time-pregnant mice were removed and placed on ice in Hank's Balanced Salt Solution (HBSS, Gibco), followed by the dissection of striatum, cortex and thalamus. For electrophysiological recordings and double immunostaining, striatal cells (of which 80–90% were SPN, see: Shehadeh et al., 2006) were then nucleofected at time of plating with enhanced yellow fluorescent protein (YFP)-encoding plasmid; for immunocytochemical localization of surface GluN2B-containing NMDAR, YFP-GluN2B plasmid was used, as previously described (Kaufman et al., 2012; Milnerwood et al., 2012). In each case, 2 million striatal cells were suspended in 100 μl of electroporation buffer (Mirus Bio) with 2 μg of DNA, placed in a cuvette and nucleofected (AMAXA nucleofector I). The cells were then resuspended in D-minimum essential medium (DMEM, Gibco) with 10% fetal bovine serum (to make DMEM+) and plated with non-transfected thalamic or cortical neurons at a 1:1 ratio at 1.6×10^5/well density. After 3–5 h, DMEM+ was replaced with 500 μl of plating medium (2% B27, Invitrogen; 1% penicillin/streptomycin, Gibco; 2 mM α-glutamine, Gibco; in Neurobasal medium, Gibco). At 4 days *in vitro* (DIV), another 500 μl of plating medium was added; thereafter, half of the media was exchanged every 3–4 days. Cocultures were maintained in a humidified 37 °C incubator with 5% CO_2 until 13–15 DIV, when the experiments were performed.

2.3. Electrophysiology in coculture system

Whole-cell patch-clamp recordings were performed on WT or YAC128 YFP-transfected striatal SPN at 13–15 DIV, at a holding potential of −70 mV, as described previously (Kaufman et al., 2012; Milnerwood et al., 2012). Briefly, neurons were superfused at room temperature (RT) with extracellular solution containing (in mM): 167 NaCl, 2.4 KCl, 0.01 $MgCl_2$, 10 glucose, 10 HEPES, 2 $CaCl_2$, pH 7.3, and 300 mOsm. Glycine (10 μM, Sigma-Aldrich; co-agonist of NMDAR) and picrotoxin (PTX, 100 μM, Tocris; $GABA_A$ receptor blocker) were added just before recording. For the mEPSC and NMDAR current recordings, tetrodotoxin (TTX, 0.3 μM, Affix Scientific; sodium channel blocker) was also present in the solution. NMDA application (1 mM for 3 s; Sigma-Aldrich) was performed using a perfusion valve-control system (VC-6; Warner Instruments Corporation, CT, USA) with sweeps repeated 3–5 times at 30 s inter-pulse intervals. To evoke spontaneous synaptic bursting, 4-aminopyridine (4-AP, 10 μM, Tocris; potassium channel blocker) was applied for 2–5 min, followed by MK-801 (10 μM, Tocris; open-channel blocker of NMDAR) for 3–5 min to irreversibly block synaptic NMDAR. All signals, apart from mEPSC recordings, were filtered at 10 kHz, digitized at 20 kHz and analysed in Clampfit10.2 (Axon Instruments, CA, USA). mEPSCs were filtered at 1 kHz, digitized at 20 kHz and analysed with Clampfit10.2 event analysis function with a detection threshold set at −8 pA. Intracellular recording solution contained (in mM): 130 Cesium methanesulfonate, 5 CsCl, 4 NaCl, 1 MgCl, 10 HEPES, 5 EGTA, 5 lidocaine, 0.5 GTP, 10 Na-phosphocreatine, 5 MgATP, pH 7.2, and 290 mOsm. Pipette resistance (Rp) was 3–5 MΩ. Series resistance (Rs) was <30 MΩ and uncompensated; the data were not included in the analysis if Rs changed by >20% by the end of the experiment. The numbers in figures represent the number of cells examined from at least 3 independent coculture batches. For mEPSC experiments, 20 coculture batches of C-S WT, 14 of C-S YAC128, 11 of T-S WT and 10 of T-S YAC128 were used. For synaptic NMDAR current experiments, 4 coculture batches of T-S WT and 4 of T-S YAC128 were used. For extrasynaptic NMDAR current experiments, 9 coculture batches of T-S WT and 11 of T-S YAC128 were used.

2.4. Immunocytochemistry

Immunostaining of YFP-transfected WT and YAC128 striatal SPN cocultured with cortical or thalamic neurons was performed at 13–15 DIV as described previously (Gladding et al., 2012; Kaufman et al., 2012; Milnerwood et al., 2012; Parsons et al., 2014). Briefly, for double staining of YFP and VGLUT1/2, coverslips were fixed with 4% paraformaldehyde with sucrose (PFA–sucrose, 15 min) at room temperature (RT), washed with phosphate buffered saline (PBS; 3×, RT), permeabilized with ice-cold methanol (5 min, −20 °C), washed (PBS, 3×, RT) and incubated in PBS containing 0.03% Triton X-100 (PBST, 5 min, RT). Then, coverslips were blocked in 10% normal goat serum (NGS) in PBS (30 min, RT), followed by an incubation with primary antibodies: chicken anti-green fluorescent protein antibodies (GFP, cross reactive with eYFP; AbCam, 1:1000) and either guinea-pig anti-vesicular glutamate transporter 1 antibodies (VGLUT1, AB5905, Chemicon, 1:1000, for corticostriatal coculture) or guinea pig anti-vesicular glutamate transporter 2 antibodies (VGLUT2, AB2251, Millipore,