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Alterations of striatal indirect pathway neurons precede motor deficits in two mouse models of Huntington's disease

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ABSTRACT

Striatal neurons forming the indirect pathway (iSPNs) are particularly vulnerable in Huntington's disease (HD). In this study we set out to investigate morphological and physiological alterations of iSPNs in two mouse models of HD with relatively slow disease progression (long CAG repeat R6/2 and zQ175-KI). Both were crossed with a transgenic mouse line expressing eGFP in iSPNs. Using the open-field and rotarod tests, we first defined two time points in relation to the occurrence of motor deficits in each model. Then, we investigated electrophysiological and morphological properties of iSPNs at both ages. Both HD models exhibited increased iSPN excitability already before the onset of motor deficits, associated with a reduced number of primary dendrites and decreased function of Kir- and voltage-gated potassium channels. Alterations that specifically occurred at symptomatic ages included increased calcium release by back-propagating action potentials in proximal dendrites, due to enhanced engagement of intracellular calcium stores. Moreover, motorically impaired mice of both HD models showed a reduction in iSPN spine density and progressive formation of huntingtin (Htt) aggregates in the striatum.
Our study therefore reports iSPN-specific alterations relative to the development of a motor phenotype in two different mouse models of HD. While some alterations occur early and are partly non-progressive, others potentially provide a pathophysiological marker of an overt disease state.

1. Introduction

Striatal atrophy is a characteristic pathologic feature of Huntington's disease (HD). Being part of the basal ganglia, the striatum is involved in motor control and action selection. Key to its function is the organization into two distinct pathways ((Gerfen and Surmeier, 2011); (Calabresi et al., 2014)) originating from two categories of striatal spiny projection neurons (SPNs). Direct pathway SPNs (dSPNs) express the dopamine D1 receptor and project directly to the basal ganglia output nuclei. Indirect pathway SPNs (iSPNs) express the D2 receptor and send their projections to the globus pallidus pars externa, modulating the basal ganglia output structures via the subthalamic nucleus (Albin et al., 1989). Post mortem investigations have indicated that these two SPN populations are not equally susceptible in HD patients, as iSPN markers are the first to disappear during the disease process (Reiner et al., 1988). Functionally, iSPNs are often associated with a "no-go" signal (Durieux et al., 2009) and their activation can indeed stop or slow down ongoing motor behavior (Alcacer et al., 2017; Kravitz et al., 2010). This motor suppressive action has lead to the hypothesis that iSPN dysfunction is a primary cause of chorea in HD (Galvan et al., 2012; Reiner et al., 1988). Loss of D1 receptor expression and dSPN degeneration on the other hand has been suggested to underlie the development of rigidity and bradykinesia in the late stages of HD (Berardelli et al., 1999).

Various alterations of SPNs have been studied in HD mouse models, however without distinguishing between dSPNs and iSPNs. Fortunately, the generation of genetic reporter mice of SPN subtype has made this distinction possible (Gong and Yang, 2005). Studies using fluorescence reporter mice showed how crucially the two SPN subpopulations differ in health (Cepeda et al., 2008; Gertler et al., 2008; Planert et al., 2013) and disease, e.g. Parkinson's and L-DOPA-induced dyskinesia (Fieblinger et al., 2014; Kreitzer and Malenka, 2007). Few studies have so far looked at SPN-type specific deficits in HD, supporting the notion of iSPN-selective vulnerability that had emerged from histopathological examinations. For example, iSPNs but not dSPNs show early deficits in corticostriatal synaptic plasticity (Plotkin et al., 2014). Moreover, evoked glutamate currents are larger in iSPNs than dSPNs (Andre et al., 2011) and there is an increase of GABAergic inputs onto iSPNs, but not dSPNs (Cepeda et al., 2013). While clearly highlighting the role of iSPNs, these reports remained largely focused on synaptic transmission. Other important physiological parameters, such as intrinsic properties and morphology are still to be investigated with knowledge of the

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specific SPN type (Cepeda et al., 2007; Raymond et al., 2011). Changes in iSPN dendrites are of high interest, as dendritic alterations have been observed in brains of HD patients and some mouse models (Ferrante et al., 1991; Graveland et al., 1985; Klapstein et al., 2001).

Our study investigates iSPN physiology and morphology in the dorsolateral striatum in two mouse models of HD. The first is the widely used R6/2 mouse, overexpressing exon 1 of the gene coding for huntingtin (Htt) (Mangiarini et al., 1996). We utilized a R6/2 line with longer CAG expansion and less aggressive disease progression (Morton et al., 2009), making it easier to segregate different disease stages. The second model is the zQ175 knock-in mouse (zQ175-KI). This model has a slowly progressing behavioral phenotype and recapitulates many of the synaptic and anatomical pathologies present in the more aggressive models (Heikkinen et al., 2012; Menalled et al., 2012). We crossed both HD models with a reporter line expressing eGFP in iSPNs (BAC-*adora2a*-eGFP). After defining comparable pre-symptomatic and symptomatic stages, we assessed iSPN pathophysiology in each model "before" and "after" the onset of motor deficits. In both models, iSPNs show an early increase in excitability, driven by potassium channel dysfunction and a non-progressive loss of primary dendrites. With the occurrence of motor deficits, HD mice additionally showed loss of iSPN dendritic spines and altered dynamics of calcium release evoked by back-propagating action potentials (bAPs) in proximal dendrites. These data provide novel insights into progressive and non-progressive iSPNs pathophysiology in HD models.

2. Material and methods

2.1. Animals

All mice in this study were housed under a 12 h light/dark cycle, with free access to food and water. We used two different models, with complementary validity: the R6/2 with prolonged CAG repeats (326 ± 8, $n = 20$) and the zQ175-KI mouse. These mice were crossed with a reporter line expressing eGFP in striatal iSPNs (BAC-*adora2a*-eGFP). All mice were on the strain background C57BL/6 J. We used hemizygotic R6/2 and heterozygotic zQ175-KI mice of both genders. Littermates that carried the eGFP-, but not the huntingtin transgene were used as controls throughout the study. All procedures and conditions had been approved by the Malmö-Lund Ethical Committee for Animal Research.

2.2. Open field behavior

General motor activity (horizontal and vertical activity) was evaluated during the light phase of the diurnal cycle in an open field (50 × 50 cm arena, framed by transparent Plexiglas walls) and recorded using the "ANYmaze" video tracking system (Stoelting, USA). R6/2 mice and littermate controls were tested at 12 and 20 weeks of age, while zQ175-KI and respective controls at 3 and 12 months of age. At the beginning of the test the mice were placed in the centre of the arena and recorded for 2 h.

2.3. Rotarod test

Rotarod test (Rotamex 4/8, Columbus Instruments; diameter of rotating rod: 3 cm) was used to assess motor coordination and motor learning in mice (Murmu et al., 2013). Tests were performed in both R6/2 and controls at 12 and 20 weeks of age, and in zQ175-KI and controls at 3 and 12 months of age. Mice were tested for 6 consecutive days on an accelerating rotarod (4–40 rpm) for a maximum of 300 s. Mice underwent three trials per day, separated by 15 min. The very first trial on the first day was used for habituation and not included in the analysis.

2.4. Brain slice preparation

Acute brain slice were prepared as described previously (Fieblinger et al., 2014). In brief, mice were deeply anesthetized with Pentobarbital (65 mg/kg, i.p.) and shortly perfused with ice-cold artificial cerebro-spinal fluid (aCSF). The aCSF contained (in mM): 124.0 NaCl, 3.0 KCl, 1.0 $CaCl_2$, 2.0 $MgCl_2$, 26.0 $NaCO_3$, 1.0 NaH_2PO_4 and 16.66 glucose. The osmolarity was typically 300–310 mOsm/L and pH 7.4. The aCSF was gassed at all times with 5%/95% CO_2/O_2, to maintain oxygenation and pH. After brain removal, parasagittal slices (275 μm) were cut on a vibratome (VT1200s, Leica, Germany). Slices were transferred to a holding chamber an incubated at 34 °C for 30 min. Afterwards the temperature was allowed to return to room temperature for electrophysiological experiments.

2.5. Patch-clamp recordings

iSPNs in the dorsolateral striatum were identified by somatic eGFP expression. Patch pipettes with a resistance of typically 3–5 mOhm were pulled from thick-walled borosilicate glass on a Sutter P-97 puller. Recordings were sampled at 10–20 kHz using a Multiclamp 700B amplifier (Molecular Devices, USA) and converted using a Digidata 1440 (Molecular Devices, USA). Data were analyzed in pClamp (v.10, Molecular Devices, USA). For measurements of intrinsic excitability, the internal recording solution contained (in mM): 135 $KMeSO_4$, 5 KCl, 10 HEPES-K, 0.16 $CaCl_2$, 2 ATP-Mg, 0.5 GTP-Na, 5 phosphocreatine-Tris and 5 phosphocreatine-Na. The pH was adjusted to 7.25–7.3 with 1 M KOH and osmolarity was approx. 270–280 mOsm/L. Cells were held in current-clamp and intrinsic excitability was assessed with 500 ms somatic current step injections. SPN properties were calculated from these recordings as described previously (Fieblinger et al., 2014).

For K-channel recordings, the internal recording solution contained (in mM): 135 K-gluconate, 5 KCl, 0.5 $CaCl_2$, 5 HEPES-K, 2 ATP-Mg, 0.5 GTP-Na and 5 EGTA-K. The pH was adjusted to 7.25–7.3 with 1 M KOH and osmolarity was approx. 270–280 mOsm/L. Kir-Channels were recorded as described previously (Ariano et al., 2005a; Cazorla et al., 2012). In brief, SPNs were held at −50 mV in voltage-clamp and then probed with 300 ms steps (−10 mV per step) ranging from −50 mV to −150 mV. Kir-currents were measured before and after application of 3 mM cesium chloride (CsCl). The recordings were performed in the presence of TTX (1 μM) and cadmium chloride ($CdCl_2$, 0.1 mM), to block sodium- and voltage-gated calcium channels, respectively.

Depolarization-activated K^+-currents were recorded under the same conditions (TTX, $CdCl_2$, CsCl) and as described previously (Hernandez et al., 2015). SPNs were held in voltage-clamp at −70 mV and prepulsed to −40 or −110 mV for 100 ms, followed by a test pulse (500 ms, −80 to 0 mV) and channel currents were defined by the amplitude of the yielded difference current. Display traces in Fig. 9 were recorded with an internal solution containing (in mM): 120 CsMeSO3, 5 NaCl, 10 TEA-Cl, 10 HEPES, 5 QX-314, 4 ATP-Mg and 0.3 GTP-Na. All recordings were performed at room temperature.

2.6. 2-Photon imaging

2-Photon imaging was performed on a Zeiss 710 NLO with MaiThai laser (Spectra Physics), as in (Fieblinger et al., 2014). To reveal iSPN morphology, 50 μM of AlexaFluor-568 was added to $KMeSO_4$-based internal recording solution (see above). The excitation wavelength was set to 780 nm and the beam was focused through a 63× water-immersion objective (1.0 NA, Zeiss). For spine analysis, dendritic regions of interests were imaged. These were taken in the mid-distance from the soma (typically 50–100 μm), avoiding the aspiny proximal and the very distal regions (>100 μm). Dendritic stretches of 25–35 μm length were optically sectioned in a z-stack, with a x-y pixel size of 0.053 × 0.053 μm^2 and z-sections were spaced 0.65 μm apart. Typically 2–3