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Loss of corticostriatal and thalamostriatal synaptic terminals precedes striatal projection neuron pathology in heterozygous Q140 Huntington's disease mice

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ABSTRACT

Motor slowing, forebrain white matter loss, and striatal shrinkage have been reported in premanifest Huntington's disease (HD) prior to overt striatal neuron loss. We carried out detailed LM and EM studies in a genetically precise HD mimic, heterozygous Q140 HD knock-in mice, to examine the possibility that loss of corticostriatal and thalamostriatal terminals prior to striatal neuron loss underlies these premanifest HD abnormalities. In our studies, we used VGLUT1 and VGLUT2 immunolabeling to detect corticostriatal and thalamostriatal (respectively) terminals in dorsolateral (motor) striatum over the first year of life, prior to striatal projection neuron pathology. VGLUT1+ axospinous corticostriatal terminals represented about 55% of all excitatory terminals in striatum, and VGLUT2+ axospinous thalamostriatal terminals represented about 35%, with VGLUT1+ and VGLUT2+ axodendritic terminals accounting for the remainder. In Q140 mice, a significant 40% shortfall in VGLUT2+ axodendritic thalamostriatal terminals and a 20% shortfall in axospinous thalamostriatal terminals were already observed at 1 month of age, but VGLUT1+ terminals were normal in abundance. The 20% deficiency in VGLUT2+ thalamostriatal axospinous terminals persisted at 4 and 12 months in Q140 mice, and an additional 30% loss of VGLUT1+ corticostriatal terminals was observed at 12 months. The early and persistent deficiency in thalamostriatal axospinous terminals in Q140 mice may reflect a development defect, and the impoverishment of this excitatory drive to striatum may help explain early motor defects in Q140 mice and in premanifest HD. The loss of corticostriatal terminals at 1 year in Q140 mice is consistent with prior evidence from other mouse models of corticostriatal disconnection early during progression, and can explain both the measurable bradykinesia and striatal white matter loss in late premanifest HD.

Introduction

Although considerable attention has focused on the role of differential striatal projection neuron loss in the progression of HD motor symptoms (Deng et al., 2004; Glass et al., 2000; Reiner et al., 1988; Richfield et al., 1995), little attention has focused on the neuronal pathology underlying the emerging motor symptoms reported in premanifest HD individuals. For example, numerous studies have noted that premanifest HD individuals are slowed in the initiation and/or execution of a variety of motor tasks involving the eyes, hands or lower limbs (Bechtel et al., 2010; Biglan et al., 2009; Blekher et al., 2004; de Boo et al., 1997; Delval et al., 2011; Kirkwood et al., 1999, 2000; Rao et al., 2008, 2011; Siemers et al., 1996; Tabrizi et al., 2011; Turner et al., 2011). This defect is mild in premanifest cases not yet near clinical onset, but more severe in those near onset (Bechtel et al., 2010; Kirkwood et al., 2000; Rao et al., 2008, 2011; Rupp et al., 2010). Diverse types of imaging studies (computed tomography, magnetic resonance imaging, positron emission tomography, and diffusion tensor imaging) show that these growing motor symptoms in premanifest HD occur in parallel with a slowly progressive loss of cerebral and striatal white matter (Aylward et al., 2011; Ciarmiello et al., 2006; Dumas et al., 2012; Hobbs et al., 2010a; Kipps et al., 2005; Paulsen et al., 2006; Reading et al., 2005; Rosas et al., 2006), striatal hypometabolism (Ciarmiello et al., 2006; Grafton et al., 1992), and reduced striatal activation during behavioral tasks (Paulsen et al., 2004; Wolf et al., 2012). Nonetheless, the limited neuropathological studies of premanifest striatum have reported little or no neuronal loss, particularly in the motor striatum (i.e. putamen) (Albin et al., 1991; Vonsattel and DiFiglia, 1998; Vonsattel et al., 1985).

These findings in premanifest human HD raise the possibility that the very earliest motor defects in HD victims may be related to the loss of afferent connectivity of motor striatum with its major sources of excitatory input — cerebral cortex and thalamus. Both inputs mainly end as terminals that make asymmetric synaptic contact with dendritic spines of striatal projection neurons, which make up the vast majority of striatal neurons (Albin et al., 1989; Gerfen, 1992; Reiner and Anderson, 1990; Smith et al., 2004; Wilson et al., 1982). The input from cerebral

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cortex, which arises from all cortical regions to a greater or lesser extent, is the more substantial of the two, and is thought to provide striatum with an instructive signal for its role in motor control (Gerfen, 1992; Wilson, 1992). The thalamic input, which arises heavily from intralaminar, mediodorsal and midline thalamic nuclei (Berendse and Groenewegen, 1990; Groenewegen and Berendse, 1994), however, also provides drive to striatum that is critical to its role in movement selection and facilitation (Kato et al., 2011; Smith et al., 2004, 2009, 2011). In symptomatic HD, neuronal loss occurs in cortical layers 3, 5, and 6, and is prominent by grade 4 (Byers et al., 1973; Cudkowicz and Kowall, 1990; De La Monte et al., 1988; Passani et al., 1997; Selemon et al., 2004; Sotrel et al., 1991; Vonsattel et al., 1985). Similarly, more than 50% neuronal loss in the intralaminar nuclei has been reported for advanced HD (Heinsen et al., 1996), and thalamic shrinkage in HD correlates with cognitive decline (Kassubek et al., 2004a,b). Consistent with their vulnerability in symptomatic HD, regional cortical thinning and volume loss, and thalamic volume loss have been seen in premanifest HD (Aylward et al., 2011; Rosas et al., 2005), although again without evidence of neuron loss (Vonsattel et al., 1985).

Thus, although available data suggest that early loss of excitatory cortical and/or thalamic input could be a major contributor to the striatal hypoactivity and motor abnormalities seen in premanifest HD, direct neuropathological evidence for such input loss is lacking. However, given the difficulties in obtaining EM grade fixation in human brain tissue and given the difficulty in obtaining premanifest HD brains for neuropathological study, it would be challenging to address this issue by detailed EM study of human striatum. As an alternative approach, we examined thalamostriatal and corticostriatal input loss over the first year of life in a precise genetic mimic of human HD, the heterozygous Q140 knock-in mouse, in which expression of the mutant protein occurs from the native gene (Hickey et al., 2008). Although homozygous Q140 mice show motor and behavioral abnormalities beginning by 6 months of age, and HD-like striatal neurochemical abnormalities and neuron loss by 12 months, the behavioral phenotype in heterozygous Q140 mice is more attenuated and mice are not yet overtly symptomatic at 1 year of age (Rising et al., 2011). To identify corticostriatal and thalamostriatal terminals in Q140 heterozygotes, we used immunolabeling for VGLUT1 and VGLUT2, respectively. Excitatory thalamic projection neurons use the vesicular glutamate transporter VGLUT2 for packaging glutamate in synaptic vesicles, while excitatory cortical neurons use VGLUT1 (Fremeau et al., 2001, 2004; Fujiyama et al., 2004; Herzog et al., 2001; Lei et al., 2013; Varoqui et al., 2002). We confirmed the absence of striatal pathology at 12 months in Q140 heterozygotes. Our results indicate deficiencies in thalamic input to the spines and dendrites of striatal neurons already at one month, with substantial loss of cortical input to the spines of striatal neurons evident at 1 year. Our results suggest that loss of thalamostriatal and corticostriatal terminals may, in fact, underlie motor impairments in premanifest HD.

Materials and methods

Animals

Results from 35 WT and heterozygous Q140 mice (obtained from JAX, Bar Harbor, Maine) are presented here, and all animal use was carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals, Society for Neuroscience Guidelines, and University of Tennessee Health Science Center Guidelines. Heterozygous HD mutants were studied because the human disease most commonly occurs due to a single allelic defect. It should be noted that the repeat length in the Q140 mice that we used had undergone a spontaneous reduction during breeding at JAX, and the average repeat length in our eight 1-month old Q140 mice was 127.8, our five 4-month old Q140 mice was 128.6, and our five 12-month old Q140 mice was 135.0. Seven 1-month old WT mice, five 4-month old WT mice, and five 12-month WT mice were studied. For histological analysis, mice were deeply anesthetized with 0.2 ml of 35% chloral hydrate in saline, and then exsanguinated by transcardial perfusion with 30 ml of 6% dextran in sodium phosphate buffer (PB), followed by 200 ml of 3.5% paraformaldehyde–0.6% glutaraldehyde–15% saturated picric acid in PB (pH 7.4). The brain of each mouse was removed, postfixed overnight in 3.5% paraformaldehyde–15% saturated picric acid in PB. The right side of the brain was used for light microscopic (LM) and the left for electron microscopic (EM) analysis. For LM studies, forebrain was section at 35 μm and collected as 12 parallel series, #1 and #7 of which were mounted on slides at the time for cresyl violet staining. For EM studies, forebrain was sectioned at 50 μm on a vibratome.

Light microscopic visualization of VGLUT

Single-label immunofluorescence was carried out to examine the localization of VGLUT1 and VGLUT2 in striatal axons and terminals. For these studies, we used either a guinea pig VGLUT2 antibody (AB5907, Chemicon Temecula, CA) or a rabbit VGLUT2 antibody (V2514, Sigma), and a guinea pig VGLUT1 antibody (AB5905, Chemicon Temecula, CA). Sections were incubated overnight at room temperature either in the guinea pig anti-VGLUT2 (1:1000), rabbit anti-VGLUT2 (1:2000), or guinea pig anti-VGLUT1 (1:1000). After incubation in primary antibody at room temperature with gentle agitation, the tissue was rinsed three times, and the secondary antibody incubation carried out. The sections were incubated for 2 h at room temperature (with gentle agitation) in an Alexa 594-conjugated goat anti-guinea pig IgG (to detect the guinea pig anti-VGLUT1 or guinea pig anti-VGLUT2) or an Alexa 594-conjugated goat anti-rabbit IgG (to detect the rabbit anti-VGLUT2). Secondaries were from Molecular Probes (Eugene, OR), and were diluted at 1:200. Sections were rinsed three times in 0.1 M PB after incubation in secondaries, mounted on gelatin-coated slides, and coverslipped with ProLong® antifade medium (Molecular Probes, Eugene, OR). Sections were viewed and images were captured using a Nikon D-Eclipse C1 confocal laser scanning microscope (CLSM), using a 40× oil objective. Z-stack serial images were collected at 1 μm (40× oil) steps from dorsolateral striatum. Note that some single-label tissue was also prepared using the peroxidase-antiperoxidase method as detailed in prior studies (Deng et al., 2006, 2007). Three striatal images from each of 3 sections at the level of the anterior commissure for each mouse were analyzed (thus totaling nine images per mouse) using the public-domain software NIH ImageJ (http://rsbweb.hih.gov/ij/download.html). For each image, five areas were selected for detailed measurement, four from each image quadrant and one from the center. Areas selected for measurement were 300–500 μm^2 in size, and free of perikarya or fiber bundles. Three types of measurements were made: 1) the mean labeling intensity of the selected area; 2) the intensity of the immunolabeled fibers in the selected area; and 3) the percent of the selected area covered by immunolabeled fibers. Immunolabeled fibers were highlighted by auto-thresholding to allow their selective measurement.

Light microscopic visualization of striatal neuron markers

LM immunohistochemical analysis was carried out to determine the effects of the Q140 mutation on the neurochemical integrity of striatal projection neurons, as evaluated by their outputs to their target areas. The peroxidase-antiperoxidase (PAP) procedure as described previously (Reiner et al., 2007, 2012a) was used for substance P (SP) immunolabeling to study SP + striatal projection systems, and for enkephalin (ENK) immunolabeling to study ENK + striatal projection systems. The anti-SP was a rabbit polyclonal antibody (ImmunoStar, Hudson, WI) whose specificity has been documented previously (Figueredo-Cardenas et al., 1994). The anti-ENK used was a rabbit polyclonal antibody against leucine-enkephalin (ImmunoStar, Hudson, WI) whose specificity has also been shown previously (Reiner, 1987; Reiner et al., 2007).