Neurobiology of Disease 62 (2014) 160–171

Contents lists available at ScienceDirect

Neurobiology of Disease

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

Hyperactivity and cortical disinhibition in mice with restricted expression of mutant huntingtin to parvalbumin-positive cells ☆,☆☆

S.E. Dougherty [a,d], J.J. Hollimon [d], L.J. McMeekin [d], A.S. Bohannon [d], A.B. West [b], M. Lesort [d], J.J. Hablitz [c], R.M. Cowell [d,*]

[a] Neuroscience Graduate Program, University of Alabama at Birmingham, 35294, USA
[b] Department of Neurology, University of Alabama at Birmingham, 35294, USA
[c] Department of Neurobiology, University of Alabama at Birmingham, 35294, USA
[d] Department of Psychiatry & Behavioral Neurobiology, University of Alabama at Birmingham, 35294, USA

ARTICLE INFO

Article history:
Received 26 April 2013
Revised 20 September 2013
Accepted 2 October 2013
Available online 11 October 2013

Keywords:
GABAergic
Interneuron
Huntington Disease
Parvalbumin
Cortical electrophysiology

ABSTRACT

Recent evidence suggests that interneurons are involved in the pathophysiology of Huntington Disease (HD). Abnormalities in the function of interneurons expressing the calcium buffer parvalbumin (PV) have been observed in multiple mouse models of HD, although it is not clear how PV-positive interneuron dysfunction contributes to behavioral and synaptic deficits. Here, we use the cre-lox system to drive expression of mutant huntingtin (mthtt) in parvalbumin (PV)-positive neurons and find that mutant mice exhibit diffuse mthtt immunoreactivity in PV-rich areas at 10 months of age and mthtt aggregates in PV-positive processes at 24 months of age. At midlife, mutant mice are hyperactive and display impaired GABA release in the motor cortex, characterized by reduced miniature inhibitory events and severely blunted responses to gamma frequency stimulation, without a loss of PV-positive interneurons. In contrast, 24 month-old mutant mice show normalized behavior and responses to gamma frequency stimulation, possibly due to compensatory changes in pyramidal neurons or the formation of inclusions with age. These data indicate that mthtt expression in PV-positive neurons is sufficient to drive a hyperactive phenotype and suggest that mthtt-mediated dysfunction in PV-positive neuronal populations could be a key factor in the hyperkinetic behavior observed in HD. Further clarification of the roles for specific PV-positive populations in this phenotype is warranted to definitively identify cellular targets for intervention.

Introduction

Huntington Disease (HD) is a devastating neurological disorder characterized by motor, psychiatric, and cognitive disturbances. HD is caused by an aberrant expansion of the CAG repeat domain within exon one of the huntingtin (htt) gene (Group, H. s. D. C. R, 1993). At the cellular level mutant htt (mthtt) interferes with various functions including transcriptional regulation (Bithell et al., 2009; Hodges et al., 2006; Luthi-Carter et al., 2002), the maintenance of calcium homeostasis (Giacomello et al., 2011; Perry et al., 2010), and synaptic physiology (Cummings et al., 2009; Klapstein et al., 2001; Milnerwood and Raymond, 2007).

Though the mutant protein is ubiquitously expressed, specific neuronal populations are especially vulnerable to the toxic effects of mthtt. Within the striatum, projection neurons undergo neurodegeneration while some regional interneurons are spared (Hodgson et al., 1999; Shelbourne et al., 2007). Studies have indicated that the cerebral cortex is affected as well (Gray et al., 2013; Gu et al., 2005; Spampanato et al., 2008) and that reduced trophic support from the cortex may contribute to striatal neuron vulnerability (Zuccato et al., 2001). Interestingly, pan-neuronal expression of mthtt is sufficient to cause hypoactivity and cortical alterations, while pyramidal neuron-specific expression has no impact on behavior or cortical dysfunction, leading investigators to hypothesize that cortical pathology requires the involvement of interneurons (Gu et al., 2005). In support of this hypothesis, early alterations in responses of parvalbumin (PV)-positive interneurons to excitatory neurotransmission are observed in the BACHD mouse model, implicating involvement of this subpopulation in the development of symptoms (Spampanato et al., 2008).

PV+ interneurons are critical in synchronizing the output of pyramidal neurons (Du et al., 1996; Perney et al., 1992), with the activation of PV+ interneurons alone being sufficient to drive cortical oscillations

☆ The authors declare no competing financial interests.
☆☆ This work was funded by: National Institutes of Health (NIH) Grant 5R01NS070009-04 (R.M.C.), 5R21NS071168-02 (M.L.), 5P30NS047466-09 (J.J.H.).
* Corresponding author at: Department of Psychiatry & Behavioral Neurobiology, 1720 7th Avenue South SC 729, Birmingham, AL 35294, USA. Fax: +1 205 975 4879.
E-mail address: rcowell@uab.edu (R.M. Cowell).
Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nbd.2013.10.002

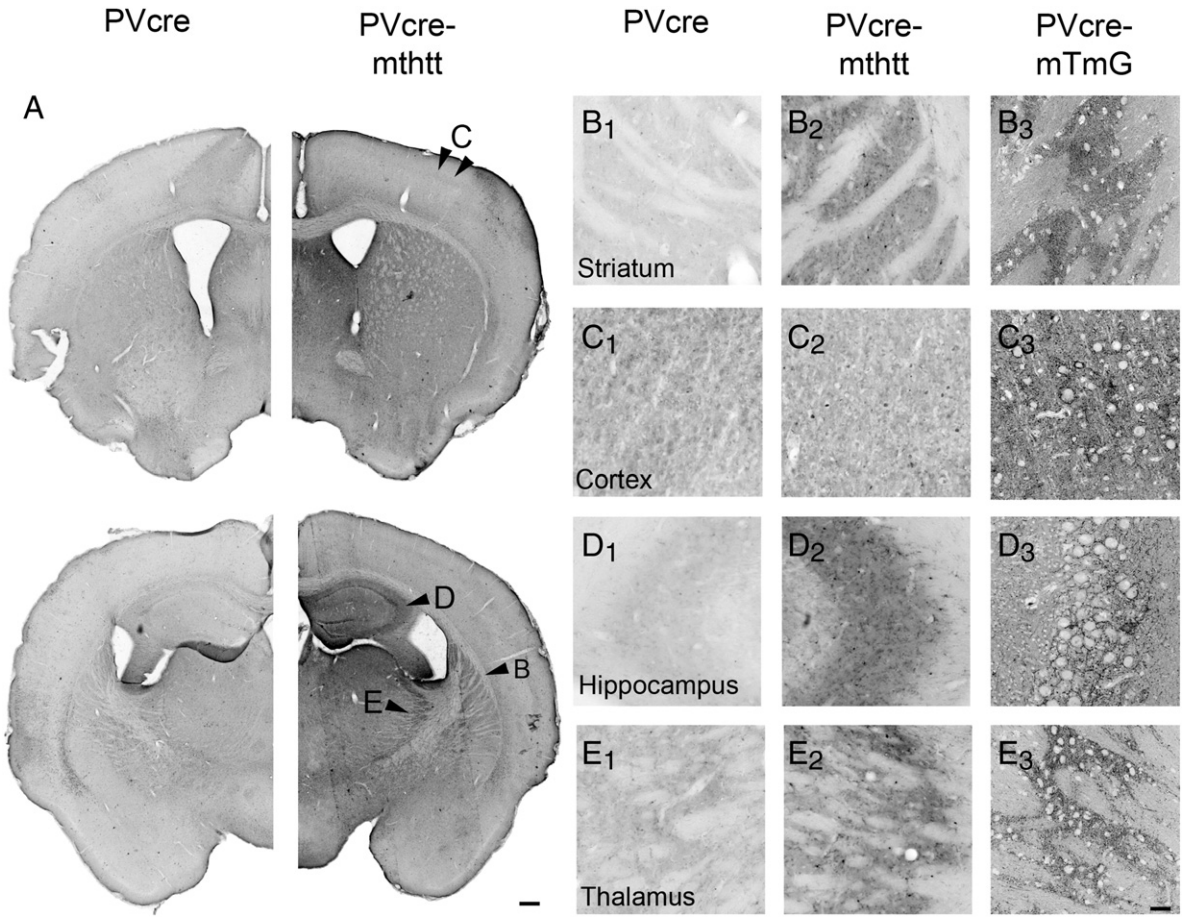

Fig. 1. *Diffuse immunoreactivity for huntingtin is detected throughout parvalbumin-rich regions in PVcre-mthtt mice at 10 months of age*. Brains from 10 month-old PVcre-mthtt mice exhibit diffuse huntingtin immunoreactivity throughout the brain as compared to PVcre (littermate control) mice, as revealed by chromogenic immunohistochemistry (A). Immunoreactivity is detectable in the striatum (B), cortex (C), hippocampus (D), and reticular nucleus of the thalamus (E). Similar patterns of labeling are noted in a PVcre mouse crossed with the mTmG reporter mouse (right column in B–E); green fluorescence images were collected on a confocal microscope, converted to grayscale, and inverted to allow for comparison to chromogenic staining. Scale bars: A: 250 μm. B–E: 10 μm.

(Sohal et al., 2009). Synchronization is disrupted in a number of neurological disorders (Gonzalez-Burgos and Lewis, 2008; Lodge et al., 2009), including HD (Thiruvady et al., 2007; Walker et al., 2008), making it critical to elucidate the contribution of PV+ interneuron dysfunction to the pathogenesis of HD.

To investigate the role of the PV+ subclass of GABAergic neurons in HD-associated motor and synaptic dysfunction, we utilized a cre-lox system of conditional gene expression (Gu et al., 2005). We bred mice with expression of a floxed stop codon preceding the mthtt gene to mice with a PV promoter-driven cre recombinase enzyme (Hippenmeyer et al., 2005). The resultant mice had expression of mthtt only in PV+ cells and exhibited hyperactivity and reduced GABA release in response to gamma frequency stimulation at midlife. However, 24 month-old mutant mice no longer exhibited behavioral differences or impairments in gamma frequency-stimulated GABA release, potentially due to post-synaptic compensatory changes. These results suggest that mthtt can drive hyperactivity by influencing PV+ neuron function.

Methods

Animals

The Institutional Animal Care and Use Committee of the University of Alabama at Birmingham approved all experimental protocols. The "PVcre" and "mthtt" mouse lines were obtained from Jackson Laboratories and maintained through breeding to WT hybrid (B6CBA) animals. The "mthtt" strain was B6.129-*Gt(ROSA)26Sor*$^{tm1(HD*103Q)Xwy}$/J (JAX# 007708); these mice harbor a transgene at the *Rosa26* locus composed of a stop codon bounded by lox-p sites, followed by exon 1 of human huntingtin with 103 CAG repeats. When crossed with a mouse expressing cre recombinase driven by a cell-specific promoter, mthtt is expressed in the specific cell of interest. The "PVcre" mouse line used was B6; 129P2-*Pvalb*$^{tm1(cre)Arbr}$/J (JAX #008069); these mice express cre recombinase in almost all PV-positive cells in the body (Hippenmeyer et al., 2005) and exhibit maximum recombination by 3 months of age (Carlen et al., 2012). To confirm the neuroanatomical location of recombination with this mouse line, we crossed PVcre mice with the mTmG reporter mouse (JAX# 007676; (Muzumdar et al., 2007)). All experiments were conducted with both male and female animals at all ages. For all experiments, PVcre littermate mice were used as controls.

The R6/2 mouse line was obtained from Jackson Laboratories and maintained through breeding male F1 generation offspring to WT hybrid (B6CBA) females (B6CBA-Tg (HDexon1)62Gpb/3J). The mice were obtained from Jackson Laboratories by way of an ovarian transplant female with ovaries from a sexually unviable R6/2 +/− female. These mice were used at 12 weeks of age, exclusively as positive controls for immunodetection experiments. R6/2 mice carried a repeat length of 167 as genotyped by Laragen, Inc (Culver City, CA). All animals were housed in groups of up to 5 animals per cage with food and water *ad libitum*.

Behavioral assessment

Behavioral analyses were conducted on littermates at six (n = 9–14/group), eight (n = 6–8/group), ten (n = 9–11/group), twelve (n =