ELSEVIER

Neurobiology of Aging 34 (2013) 873–886

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Trophic factors differentiate dopamine neurons vulnerable to Parkinson's disease

Stefanie Reyes[a], Yuhong Fu[a], Kay L. Double[a], Veronica Cottam[a], Lachlan H. Thompson[b], Deniz Kirik[c], George Paxinos[a], Charles Watson[a,d], Helen M. Cooper[e], Glenda M. Halliday[a,*]

[a] *Neuroscience Research Australia and the School of Medical Sciences, The University of New South Wales, Randwick, Sydney, New South Wales, Australia*
[b] *Florey Neuroscience Institutes, Melbourne, Australia*
[c] *Brain Repair and Imaging in Neural Systems, Department of Experimental Medical Science, Lund University, Lund, Sweden*
[d] *Faculty of Health Science, Curtin University, Perth, Australia*
[e] *Queensland Brain Institute and The University of Queensland, Brisbane, Australia*

Received 22 March 2012; received in revised form 22 March 2012; accepted 22 March 2012

Abstract

Recent studies suggest a variety of factors characterize substantia nigra neurons vulnerable to Parkinson's disease, including the transcription factors pituitary homeobox 3 (Pitx3) and orthodenticle homeobox 2 (Otx2) and the trophic factor receptor deleted in colorectal cancer (DCC), but there is limited information on their expression and localization in adult humans. Pitx3, Otx2, and DCC were immunohistochemically localized in the upper brainstem of adult humans and mice and protein expression assessed using relative intensity measures and online microarray data. Pitx3 was present and highly expressed in most dopamine neurons. Surprisingly, in our elderly subjects no Otx2 immunoreactivity was detected in dopamine neurons, although Otx2 gene expression was found in younger cases. Enhanced DCC gene expression occurred in the substantia nigra, and higher amounts of DCC protein characterized vulnerable ventral nigral dopamine neurons. Our data show that, at the age when Parkinson's disease typically occurs, there are no significant differences in the expression of transcription factors in brainstem dopamine neurons, but those most vulnerable to Parkinson's disease rely more on the trophic factor receptor DCC than other brainstem dopamine neurons.

Keywords: Deleted in colorectal cancer; Dopamine neurons; Orthodenticle homeobox 2; Pitx3; Substantia nigra

1. Introduction

By the onset of the motor symptoms of Parkinson's disease (PD) neuronal degeneration is still largely confined to a very restricted region of the substantia nigra, the ventral tier (Dickson et al., 2009). This is despite a more widespread deposition of α-synuclein in Lewy bodies and neurites at this early disease stage (Dickson et al., 2009). Identifying the cellular proteins and mechanisms that underlie this vulnerability is a priority. A number of studies on laboratory animals have identified candidate cellular proteins that appear to be expressed in restricted populations of brainstem dopamine neurons (Double et al., 2010) but confirmation of their expression in adult humans is generally lacking.

Dopamine neurons in the brainstem are not confined to the substantia nigra pars compacta (SNC or A9 cell group) but are also located in the ventral tegmental area (VTA or A10 cell group) (Fig. 1). Approximately two-thirds are found in the SNC and one-third in the VTA (Halliday et al., 2012; Smidt and Burbach, 2007) with these 2 types of dopamine neurons known to have molecular and genetic differences (Chung et al., 2005; Greene et al., 2005). Within the SNC only the ventral subset of neurons degenerates by the onset of motor symptoms (Fearnley and Lees, 1991; Gibb and Lees, 1991), the dorsal tier neurons being resistant to PD at this stage (Dickson et al., 2009).

* Corresponding author at: Neuroscience Research Australia, Randwick, NSW 2031, Australia. Tel.: +61 2 93991704; fax: +61 2 93991105.
E-mail address: g.halliday@neura.edu.au (G. Halliday).

0197-4580/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2012.07.019

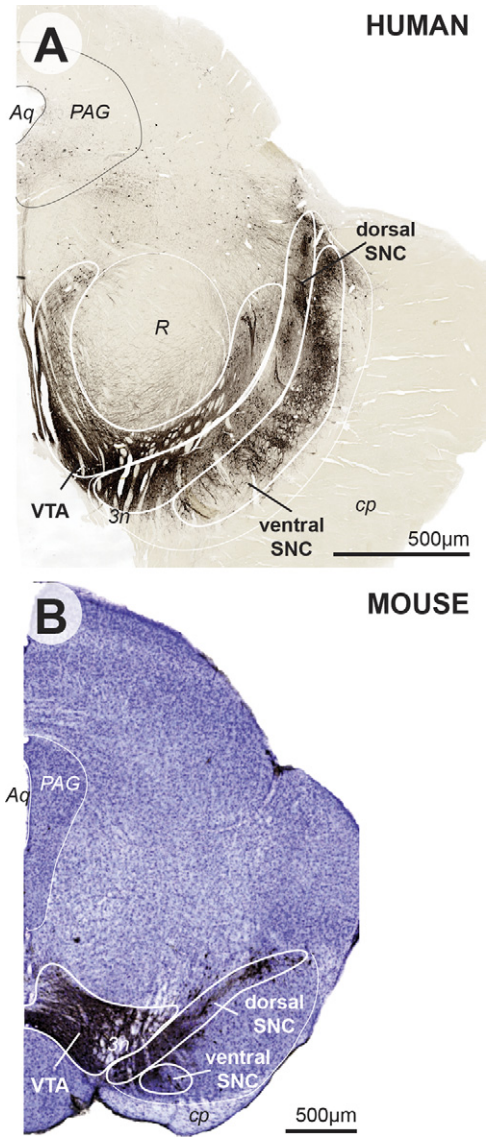

Fig. 1. Photomicrographs showing the comparative location of dopamine neurons in the ventral and dorsal substantia nigra pars compacta (SNC) and ventral tegmental area (VTA) in the human (A) and mouse (B) brainstem. The representative sections are immunohistochemically stained for tyrosine hydroxylase (TH), the rate-limiting enzyme for dopamine, and the mouse sections were counterstained with cresyl violet. Surrounding landmarks including the periaqueductal gray (PAG), red nucleus (R), cerebral peduncle (cp), and the exiting third nerve (3n) are shown.

The 2 main types of brainstem dopamine neurons (A9 and A10) are regulated by important transcription factors that determine their phenotype, with several of these transcription factors maintaining their expression into adulthood (Orme et al., 2009). These include Nurr1 (Bäckman et al., 1999; Saucedo-Cardenas et al., 1998), pituitary homeobox 3 (Pitx3) (Smidt et al., 1997), the engrailed genes (Simon et al., 2001), and orthodenticle homeobox 2 (Otx2). Nurr1 and the engrailed genes are ubiquitously expressed in brainstem dopamine neurons (Simon et al., 2001), while Pitx3 and Otx2 have been implicated in establishing the 2 main dopamine cell phenotypes, the A9 SNC and the A10 VTA neurons (Simeone et al., 2011; Simon et al., 2003). Pitx3 expression continues to be expressed in adult mice but only in ventral SNC neurons (Van Den Munckhof et al., 2003). There have been no descriptions of Pitx3 expression in the adult human brainstem. In detailed work in mice with some confirmation in primates, Otx2 expression has been found to be restricted to VTA neurons (Simeone et al., 2011) with animal studies supporting the concept that its expression contributes to the differential vulnerability between the SNC and VTA in PD (Di Salvio et al., 2010a). For cell phenotype maintenance, trophic factors are also vital, with factors like glial cell derived neurotrophic factor (GDNF) which supports all dopamine neurons (Sullivan and Toulouse, 2011) currently undergoing therapeutic trials in PD (Rangasamy et al., 2010). A trophic factor receptor with restricted expression to the ventral part of the SNC has been identified in adult mice (Osborne et al., 2005) and may be important for the maintenance of these most vulnerable neurons. This receptor, known as deleted in colorectal cancer (DCC), recognizes the guidance and neurite outgrowth factor netrin-1 (Bradford et al., 2009). Confirmation of the restricted adult expression of transcription and trophic factors in human dopamine neurons vulnerable to PD may provide better targets for early therapeutic interventions.

2. Methods

2.1. Human and mouse brain tissue

Formalin-fixed human brain tissue was obtained from the Sydney Brain Bank (tissue collection approved by the Human Research Ethics Committee of The University of New South Wales, Sydney, Australia) following approvals by their Scientific Advisory Committee (study approval number 0118) and the Human Research Ethics Advisory Committee of The University of New South Wales, Sydney, Australia (ethics approval number 090030). Formalin-fixed blocks of the entire upper part of the brainstem from 5 aged (85 ± 3 years, 3 males, 2 females without neuropsychiatric, neurological or neurodegenerative disease) and 3 middle-aged (58 ± 16 years, 2 males, 1 female without brainstem neurodegenerative disease) brain donors were obtained. In addition, the online public resources of the Allen Institute for Brain Science was used to assess regional gene expression in the public datasets for a young (24 years, male) and a middle-aged (39 years, male) human. C57BL/6J mice at different ages (male at 4 weeks, 3 males at 8–10 weeks, male at 10 months, female at 2 years) were obtained from the Animal Resource Centre, Perth, Australia and the study protocols approved by the Animal Care and Ethics Committee of The University of New South Wales, Sydney, Australia (ACEC 11/75A). Following transcardial perfusion of the mice with 4% paraformaldehyde (4 °C, pH 7.4), the brains were postfixed in the same fixative for 4 hours and then cryoprotected in 30% sucrose buffer.