Brainstem network pathology and impaired respiratory drive as successive signatures in a rat model of Parkinson's disease

Parkinson's disease (PD) is a devastating neurodegenerative disorder. Progressive destruction of midbrain dopaminergic neurons and axonal projections of the nigrostriatal pathway disrupts circuitry within the basal ganglia controlling movement. Consequently, PD is characterised by motor impairment culminating in resting tremor, impaired voluntary movement, muscle rigidity and abnormal balance and posture, symptoms markedly affecting the quality of life of people with PD. Non-motor impairments also present in PD, including deficits in autonomic, sensory and cognitive function. Sleep is disturbed in PD and sleep-disordered breathing is a common comorbidity. Indeed, there appears to be a reciprocal association between PD and sleep-disordered breathing, particularly an increased risk of developing PD in people (and perhaps especially women) with obstructive sleep apnoea (Sheu *et al*., 2015). Dysphagia and poor upper airway control are features of PD, with aspiration pneumonia recognised as the leading cause of mortality. There is evidence of abnormal ventilatory control in the absence of lung disease in PD, but ventilatory dysfunction in PD is understudied despite recognition that respiratory insufficiency is a hallmark feature of other neurodegenerative diseases associated with increased morbidity and mortality. Increased awareness of early brainstem involvement in pre-motor manifestations of PD has awoken interest in the need for full characterisation of the extent of disability in PD. The search is on for prodromal signatures of clinical relevance.

In this issue of *Experimental Physiology*, Fernandes-Junior *et al*. (2018) describe studies using an established experimental model of PD, which aimed to determine the temporal relationship between neuroanatomical changes in the brainstem and functional alterations in breathing. Adult male rats received bilateral injections of 6-hydroxydopamine (6-OHDA, a selective neurotoxin), or vehicle (control) into the caudate putamen of the striatum, which was confirmed after 30 days to produce >70% persistent loss of tyrosine hydroxylase-expressing neurons of the substantia nigra pars compacta of the midbrain. Neuroanatomical assessments of key brainstem sites governing respiratory control as well as measures of basal ventilation and ventilatory responsiveness to carbon dioxide in conscious animals were assessed 30, 40 and 60 days after induction of the model.

Using immunohistochemistry, Fernandes-Junior *et al*. (2018) revealed progressive widespread brainstem neuron and astrocytic glial cell loss in rhythmogenic, integrative and chemosensitive sites of the central respiratory network. Portrayed in the study is evidence, by day 30 in 6-OHDA rats compared with control rats, of fewer phox2b immuno-reactive neurons both in the nucleus tractus solitarii (NTS, a key integrative, chemosensitive and relay hub of the respiratory network) and the retrotrapezoid nucleus (RTN, a key site of central chemoreception). Subsequently, by day 40, the density of neurokinin-1 receptors of the rostral ventrolateral respiratory group (rVRG) and pre-Bötzinger complex (the principal rhythmogenic site of the medulla oblongata) was lower in 6-OHDA rats. In addition, the relative area of glial fibrillary acidic protein immuno-reactive astrocytes, which are implicated in respiratory control, was less in the rVRG (day 30), RTN (day 40), NTS (day 60) and pre-Bötzinger complex (day 60) of 6-OHDA rats.

Using whole-body plethysmography, Fernandes-Junior *et al*. (2018) demonstrated reduced basal minute ventilation (day 40 and 60) in 6-OHDA rats owing to reductions in respiratory frequency (day 40 and 60) and tidal volume (day 60). Similarly, in 6-OHDA rats, ventilation was depressed during hypercapnic breathing. Surprisingly, in the light of substantial cell loss in brainstem chemosensitive

sites, ventilatory responsiveness to 7% inspired CO_2 (Δ V_E from respective baseline) was preserved at each time point of the study; although notably, 60 days after neurotoxic insult this was achieved by way of a different pattern of respiration compared with control animals, with significant blunting of the tidal volume response to CO_2 in 6-OHDA rats. The preservation of hypercapnic ventilatory responsiveness is surprising given the fundamental roles of the RTN (Kumar *et al*., 2015) and NTS (Fu et al., 2017) in ventilatory responsiveness to CO_2, but presumably relates to considerable redundancy in central mechanisms of chemosensitivity, which appear intact in this progressive model (as is the capacity to raise respiratory frequency), at least at this stage of the disease. Indeed, this group has demonstrated a key role for catecholaminergic neurons of the pontine locus coeruleus in driving hypercapnic breathing in PD animals. The new observations extend previous work by this research group, which collectively and convincingly demonstrate aberrant control of respiratory frequency under basal resting conditions and in response to chemosensory challenge. Interestingly, prior work by this group has established that indices of motor behaviour, including spontaneous locomotion, and motor coordination and balance are unaffected in 6-OHDA rats at the time of the emergence of respiratory impairments, revealing that altered respiratory control is a prodromal signature, a prophecy of the impending pathology of the pernicious disease. Fernandes-Junior *et al*. (2018) demonstrate that neuroanatomical changes in the brainstem precede the emergence of functional changes in respiratory homeostasis, but it is also worth noting that dysregulated breathing, blood gas derangements and disrupted cerebrovascular function in PD might well compound neurodegeneration establishing a vicious cycle and spiral of disability. Of interest, it is established that exposure to chronic intermittent hypoxia, modelling human sleep apnoea, causes oxidative stress and inflammation in the substantia nigra of rats (Synder *et al*., 2017).

The work of Fernandes-Junior *et al*. (2018) reveals the need for comprehensive assessments of cardiorespiratory control in translational animal models of PD. Assessments of ventilation, metabolism and arterial blood gas and pH status are needed to fully characterize respiratory deficits in PD animals. Studies of respiratory control and plasticity during wakefulness and sleep are warranted. Further characterization of motor control of breathing, especially motor control of the upper airway, and respiratory muscle form and function is required, given the evidence of pharyngeal muscle remodelling in people with PD with confirmed dysphagia. It is also plausible that there are changes in modulatory circuits projecting to respiratory nuclei, which needs to be explored. Importantly, whereas the 6-OHDA model has utility, there is recognition of the need to better model the pathology of PD so as to recapitulate the destructive and debilitating qualities of the disease. The growing interest in nigrostriatal pathway degeneration, with preclinical evidence suggesting that early axonal loss may be especially important in early stage PD symptoms (O'Keeffe & Sullivan, 2018), highlights the importance of α-synuclein accumulation and Lewy pathology. Assessments of respiratory and autonomic control in α-synuclein models of PD will be an important extension to current experimental approaches and should provide insights directly relevant to human PD pathology.

The elegant study of Fernandes-Junior *et al*. (2018), which adds to an impressive portfolio of progress in this area by this research group, provides impetus for the careful screening and characterisation of non-motor manifestations including aberrant respiratory control in PD and other neurodegenerative conditions. Onward!

Competing interests.

None

David P. Burns and Ken D. O'Halloran*

Department of Physiology, School of Medicine, College of Medicine & Health, University College Cork, Cork, Ireland.

*Correspondence

Professor Ken D. O'Halloran

Department of Physiology, University College Cork, Western Gateway Building, Western Road, Cork, Ireland.

k.ohalloran@ucc.ie

References

Fernandes-Junior SA, Carvalho KS, Moreira TS, Takakura AC. Correlation between neuroanatomical and functional respiratory changes observed in an experimental model of Parkinson's disease. *Exp Physiol*. **XXX**, XXX-XXX.

Fu C, Xue J, Wang R, Chen J, Ma L, Liu Y, Wang X, Guo F, Zhang Y, Zhang X & Wang S (2017). Chemosensitive Phox2b-expressing neurons are crucial for hypercapnic ventilator response in the nucleus tractus solitarius. *J Physiol*. **595**, 4973-4989.

Kumar NN, Velic A, Soliz J, Shi Y, Li K, Wang S, Weaver JL, Sen J, Abbott SB, Lazarenko RM,Ludwig MG, Perez-Reyes E, Mohebbi N, Bettoni C, Gassmann M, Suply T, Seuwen K, Guyenet PG, Wagner CA & Bayliss DA (2015). Regulation of breathing by CO_2 requires the proton-activated receptor GPR4 in retrotrapazoid nucleus neurons. *Science* **348**, 1255-1260.

O'Keeffe GW, Sullivan AM. Evidence for dopaminergic axonal degeneration as an early pathological process in Parkinson's disease. *Parkinsonism Relat Disord*. 2018. doi:10.1016/j.parkreldis.2018.06.025.

Sheu JJ, Lee HC, Lin HC, Kao LT & Chung SD (2015). A 5-year follow-up study on therelationship between obstructive sleep apnea and Parkinson disease. *J Clin Sleep Med*. 11, 1403-1408.

Synder B, Shell B, Cunningham JT & Cunningham RL (2017).Chronic intermittent hypoxia induces oxidative stress and inflammation in brain regions associated with early-stage neurodegeneration. *Physiol Rep*. **5**, 9.