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Epigenetic silencing by early life hypoxic stress programmes respiratory motor control

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Preterm birth is a risk factor for the development of cardiorespiratory complications. Infants that are born prematurely face myriad challenges due to physiological immaturity. Respiratory control impairments in early life including apnoea of prematurity with resultant disruption to systemic oxygen status can provoke long-term disability, including increased propensity to develop morbidities in later life such as sleep-disordered breathing. In rodents, exposure to intermittent hypoxia mimicking recurrent episodes of oxygen desaturation that are characteristic of apnoeas, provokes plasticity at multiple sites of the respiratory control network culminating in breathing instabilities, altered chemoreflex control of breathing and impaired respiratory motor nerve and muscle function. Persistent effects of stressors presenting during critical periods of early development may be sustained by epigenetic mechanisms. Such changes may be especially relevant to perinatal exposure to intermittent hypoxia since it is established that hypermethylation of genes encoding antioxidant enzymes underlies carotid body chemoreceptor sensitization and respiratory instability following exposure to intermittent hypoxia during postnatal development (Nanduri et al., 2017).

In this issue of *Experimental Physiology*, Bittencourt-Silva et al. (2019) extend work in this important and topical area of investigation focussing on the lasting consequences of exposure to intermittent hypoxia during postnatal development on respiratory motor activity and breathing. Male rat pups (together with their dams) were exposed to intermittent hypoxia for the first 10-15 days of life. Phrenic, hypoglossal, abdominal and cervical vagus nerve recordings were subsequently made in juvenile rats (postnatal day 21-25) using the established *in situ* working heart-brainstem preparation. Whole-body plethysmography was used to determine breathing in conscious juvenile and adult rats (postnatal day 90-99). Rat pups exposed to normoxia (sham) or intermittent hypoxia received either vehicle or the DNA methyltransferase inhibitor decitabine to prevent putative DNA

methylation-dependent epigenetic programming.

In juvenile and adult rats exposed to antecedent intermittent hypoxia there was evidence of enhanced variability of the respiratory period with an increased incidence of apnoeas. Postnatal exposure to intermittent hypoxia caused long-lasting increases in ventilation due to elevated tidal volume. In addition, there was evidence of altered respiratory motor output manifest as enhanced phrenic motor activity. Increased neural drive in spinal motor pathways such as the phrenic nerve is consistent with enhanced tidal volume, suggesting a persistent facilitation of inspiratory drive eliciting hyperventilation and blood gas disturbances. Interestingly, abdominal nerve activity was unchanged suggesting that active expiration was not persistently provoked by postnatal exposure to intermittent hypoxia. Hypoglossal motor nerve activity was unchanged, a surprise in the light of observations of reduced hypoglossal motor responsiveness in adult rats following exposure to intermittent hypoxia (Veasey et al. 2004). However, consistent with the potential for impaired control of upper airway patency following exposure to intermittent hypoxia, Bittencourt-Silva et al. (2019) observed reduced vagal motor inspiratory and post-inspiratory activity in rats exposed to neonatal intermittent hypoxia compared with sham animals.

Noteworthy, respiratory timing indices in the working heart-brainstem preparation were equivalent between sham and intermittent hypoxia groups, suggesting that instability is not necessarily a representation of aberrant respiratory rhythmogenesis per se, although such changes have been documented by others, but rather is reflective of perturbed integrative control of breathing revealed in the study of conscious animals. However, central network plasticity is suggested by the observation of divergent outcomes in respiratory motor pathways, which suggests site-specific changes in circuitry, neuromodulation and/or motor neuron excitability. Remarkably, chronic administration of decitabine, administered systemically during the period of gas exposures, prevented the elaboration of altered respiratory motor responses to postnatal exposure to intermittent hypoxia. Moreover, in juvenile animals, decitabine prevented respiratory timing instability and attenuated the ventilatory effects of intermittent hypoxia although differences in body mass (additive decreases due to intermittent hypoxia and drug) complicate interpretation of the ventilatory data, since volume-related parameters are expressed per unit body mass, which may not be reflective of somatic growth. Also, ventilatory data were not expressed relative to oxygen consumption or carbon dioxide production. Assessments of metabolic parameters as well as ventilatory responsiveness are required to provide a comprehensive assessment of the control of breathing in this experimental model, and these are now clearly worthy of pursuit. Early life administration of decitabine affected pulmonary ventilation in adult animals (with restored body mass), which also requires further consideration.

As such, the study confirms that hypoxic stress during the postnatal period of respiratory network development has the capacity to induce respiratory motor deficits that persist into later life. The differential effects of exposure to intermittent hypoxia on motor control of pump (diaphragm) and laryngeal (abductor and adductor) muscles could prove deleterious for the control of laryngeal calibre, which is critical during the highly co-ordinated functions of swallowing and breathing. Sex-specific outcomes in cardiorespiratory responses to early life stressors with evidence of increased adversity in males compared with females is well documented in basic science and clinical studies. Whether sex differences exist in the epigenetic programming of breathing is unclear, but this would be interesting to explore in various models of early life stress with implications for the neuroendocrine control of cardiorespiratory homeostasis (Rousseau et al. 2017).

In conclusion, Bittencourt-Silva et al. (2019) confirm and extend previous work drawing focus to epigenetic modulation of respiratory motor control arising from postnatal exposure to intermittent hypoxia. An increased understanding of the complex relationship between DNA methylation and site-specific modifications in respiratory neural control networks will advance our appreciation of the notably deleterious impact of early life exposures to hypoxic stress on cardiorespiratory control. Additional translational studies of oxygen dysregulation (intermittent hypoxia and hyperoxia) combined with other recognised risk factors such as infection/inflammation (Hocker et al. 2019; McDonald et al., 2019) are required to fully characterize the portfolio of potential drivers of epigenetic dependent and independent mechanisms of respiratory morbidity across the lifespan. Such translational studies will further shape our understanding of the pivotal role of oxygen dysregulation as a driver of cardiorespiratory malaise with the potential to inform interventional strategies in the clinical setting.

Competing Interests

None.

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