J Physiol 596.15 (2018) pp 3007–3019

The Journal of Physiology

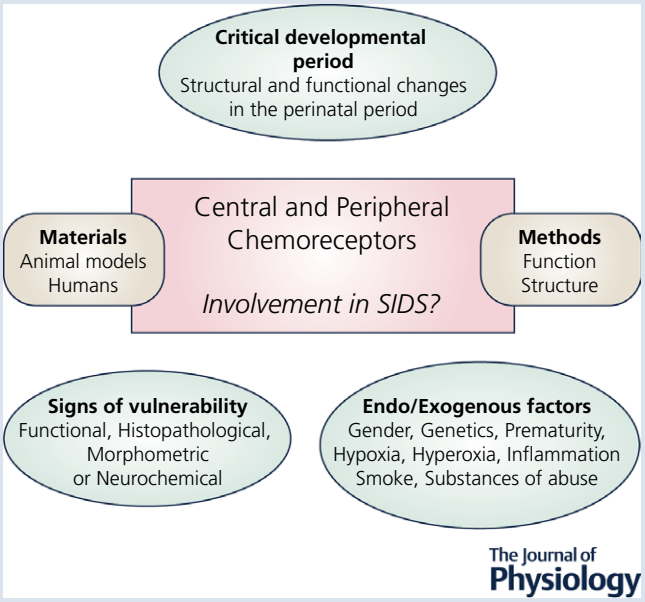
SYMPOSIUM REVIEW

Central and peripheral chemoreceptors in sudden infant death syndrome

Andrea Porzionato, Veronica Macchi and Raffaele De Caro

Section of Anatomy, Department of Neuroscience, University of Padova, Italy

Edited by: Harold Schultz & Estelle Gauda



Abstract The pathogenesis of sudden infant death syndrome (SIDS) has been ascribed to an underlying biological vulnerability to stressors during a critical period of development. This paper reviews the main data in the literature supporting the role of central (e.g. retrotrapezoid nucleus, serotoninergic raphe nuclei, locus coeruleus, orexinergic neurons, ventral medullary surface, solitary tract nucleus) and peripheral (e.g. carotid body) chemoreceptors in the pathogenesis of SIDS. Clinical and experimental studies indicate that central and peripheral chemoreceptors

Andrea Porzionato is a specialist in legal medicine and achieved a PhD in neuroscience. **Veronica Macchi** specialized in radiology and achieved PhD in neuroscience. **Raffaele De Caro** specialized in pathological anatomy and legal medicine. All the authors are Professors of Human Anatomy in the University of Padua and they are involved in judicial autopsies. Professor De Caro is the Director of the Institute of Human Anatomy, Past-President of the European Association of Clinical Anatomy and President of the Italian College of Human Anatomists. The research interests of the group include the central and peripheral structures of respiratory control, investigated through histopathological and morphometric/stereological approaches in human autoptic material (e.g. drug-related deaths, sudden infant death syndrome, abusive head trauma) and experimental animal studies (e.g. exposure to hypoxic, hyperoxic or inflammatory stimuli).

This review was presented at the symposium 'Advances in cellular and integrative control of oxygen and carbon dioxide homeostasis', which took place at the XX ISAC meeting, Baltimore, MD, USA, 23–27 July 2017.

DOI: 10.1113/JP274355

undergo critical development during the initial postnatal period, consistent with the age range of SIDS (<1 year). Most of the risk factors for SIDS (gender, genetic factors, prematurity, hypoxic/hyperoxic stimuli, inflammation, perinatal exposure to cigarette smoke and/or substance abuse) may structurally and functionally affect the developmental plasticity of central and peripheral chemoreceptors, strongly suggesting the involvement of these structures in the pathogenesis of SIDS. Morphometric and neurochemical changes have been found in the carotid body and brainstem respiratory chemoreceptors of SIDS victims, together with functional signs of chemoreception impairment in some clinical studies. However, the methodological problems of SIDS research will have to be addressed in the future, requiring large and highly standardized case series. Up-to-date autopsy protocols should be produced, involving substantial, and exhaustive sampling of all potentially involved structures (including peripheral arterial chemoreceptors). Morphometric approaches should include unbiased stereological methods with three-dimensional probes. Prospective clinical studies addressing functional tests and risk factors (including genetic traits) would probably be the gold standard, allowing markers of intrinsic or acquired vulnerability to be properly identified.

(Received 30 September 2017; accepted after revision 20 March 2018; first published online 12 April 2018)
Corresponding author A. Porzionato: Section of Anatomy, Department of Neuroscience, University of Padova, Via A. Gabelli 65, 35121 Padova, Italy. Email: andrea.porzionato@unipd.it

Abstract figure legend Main findings (derived from autoptic and experimental studies) supporting a role for central and peripheral chemoreceptors in the pathogenesis of SIDS.

Introduction

According to the so-called 'triple-risk model', the pathogenesis of sudden infant death syndrome (SIDS) is considered to be the result of underlying biological vulnerability to stressors during a period of critical development (Filiano & Kinney, 1994). Many data in the literature suggest that central and peripheral chemoreceptors are involved in the pathogenesis of some SIDS cases. This paper reviews clinical and experimental data indicating that chemoreceptors undergo critical development during the first postnatal period (when the risk of SIDS is higher), are structurally/functionally exposed to most factors triggering SIDS, and may also show structural/functional changes in SIDS victims.

Peripheral chemoreceptors

Postnatal development. The work described here mainly examines the carotid body (CB) as the main peripheral chemoreceptor. However, some authors also believe that other peripheral chemoreceptors, such as aortic bodies, pulmonary neuroendocrine cells and adrenomedullary chromaffin cells, play a role in the pathogenesis of SIDS (Porzionato *et al.* 2008, 2013).

The CB undergoes structural and functional development during the first postnatal period (the first three postnatal weeks in the rat) (reviewed in Porzionato *et al.* 2008; De Caro *et al.* 2013), gradually increasing in hypoxic sensitivity. The period of CB postnatal maturation corresponds to the age range in which the risk of SIDS is highest, indicating that 'something going wrong' in this phase may explain at least some cases of SIDS.

Signs of intrinsic vulnerability in SIDS victims.

Functional signs. SIDS victims and their siblings have been reported to show prolonged sleep apnoea, excessive periodic breathing, impaired sleep breathing pattern, reduced evoked arousability, and reduced ventilatory sensitivity to hypoxia and hypercapnia (e.g. Valdes-Dapena, 1980; Slotkin *et al.* 1995; Gaultier, 2000; Kahn *et al.* 2003). Such changes may involve peripheral and/or central chemoreceptors. In particular, as regards the arousal response, various central and peripheral components are involved, such as the brainstem respiratory rhythm generator, central chemoreceptors, brainstem and mid-brain arousal centres, cortically mediated motor responses, and peripheral chemo-, baro- and mechanoreceptors.

In order to evaluate in detail the possible consequences of functional abnormalities, the consequences of CB denervation in the first postnatal period have been studied in several experimental animals. CB denervation within this period causes significant decreases in minute ventilation, respiratory rate, arterial oxygen pressure (P_{O_2}) and arousal responses to hypoxia and to water stimulation of the laryngeal reflex, together with increased frequency of apnoeas and higher mortality (e.g. Bowes *et al.* 1981*a*, 1981*b*; Bureau *et al.* 1985; Grogaard *et al.* 1986; Fewell *et al.* 1989; Donnelly & Haddad 1990). These findings

support the hypothesis that abnormal or impaired CB development plays a role in the pathogenesis of SIDS.

Structural and neurochemical changes. Obviously, identification of specific pathological findings in the CB of SIDS victims would be of the utmost importance in demonstrating the role played by the CB. Morphometric and neurochemical changes have been reported in the CB of SIDS victims (reviewed in Porzionato *et al.* 2013). As described in this review, morphometric findings include reduced or increased CB volume, reduced volume and number of glomic cells, higher number of progenitor cells, and reduction and hypoplasia of dense-cored granules. Conversely, other authors have not found changes in CB volume, mean combined weight, total surface area, functional parenchyma area, number or size of granules, or cytoplasmic argyrophilia in type I cells. Although higher concentrations of dopamine (tenfold) and noradrenaline (threefold) have also been reported in the CB of SIDS victims (Perrin *et al.* 1984), this finding was not confirmed by another research group (Lack *et al.* 1986). Some changes are highly intriguing, although at present the lack of recent unbiased studies of confirmation leaves some doubt about the possibility of identifying reliable findings associated with SIDS.

Intrinsic and extrinsic risk factors.

Gender and genetic factors. The incidence of SIDS is higher in male than in female infants; conversely, gender and sex steroids are well-known to influence central and peripheral chemoreception. In the cat, oestrogen and/or progesterone stimulate the responsiveness of hypoxic ventilation and CB neural output. Dopamine and noradrenaline turnover is higher in the CB of female rats; turnover is also increased by orchidectomy and reduced by ovariectomy (Pequignot *et al.* 1997). Sex-specific effects on the developmental plasticity of peripheral chemoreception have also been reported. For instance, neonatal exposure to chronic caffeine treatment affects the adult respiratory pattern in male but not female rats, with increased respiratory frequency in the early hypoxic response and increased tidal volume in the late phase (Montandon *et al.* 2008). High inter-individual variability has also been reported in the peripheral chemoreceptor function of infants subjected to alternate breath and hyperoxic tests (Bouferrache *et al.* 2002) and has been ascribed, at least partly, to genetic factors (Thomas *et al.* 1993; Gaultier & Gallego, 2005). The hypoxic ventilatory response is specifically impaired in newborn mice lacking genes for endothelin-converting enzyme-1 (Renolleau *et al.* 2001) and Nurr1 (Nsegbe *et al.* 2004).

Prematurity. Prematurity is the main intrinsic factor increasing infant vulnerability to SIDS, and there is much clinical evidence about its detrimental effects on peripheral chemoreception. Premature infants show a higher contribution of peripheral arterial chemoreceptors to baseline ventilation. This parameter is defined by measuring ventilation changes in response to brief hyperoxic stimuli, which rapidly suppress afferents from peripheral arterial chemoreceptors (Gauda *et al.* 2004). Premature infants have been reported to reduce ventilation by 40% after a brief 100% hyperoxic stimulus during sleep, in comparison with a 14% reduction in full-term infants (Aizad *et al.* 1984). The hypersensitivity of peripheral arterial chemoreceptors may precipitate unstable respiration due to apnoeic responses after sighs or brief arousals, and premature infants also show a higher frequency of apnoea. These data support the hypothesis that prematurity may also increase the incidence of SIDS due to its effects on chemoreception. Another intriguing fact is that many conditions associated with prematurity functionally affect chemoreception (see below).

Continuous and/or intermittent hypoxia. The development of the peripheral and central mechanisms of respiratory control may be affected by a series of environmental factors (developmental plasticity), which are known to play a role in SIDS. Premature infants are frequently exposed to hypoxic events, which may further modify the maturation of peripheral arterial chemoreceptors, increasing the risk of disorders of breathing and of SIDS. The literature stresses that various types of hypoxic exposure (continuous or intermittent) may induce differing changes in the structure and function of the CB (Gauda *et al.* 2004, 2007; Porzionato *et al.* 2009*a*).

A reduced ventilatory response to hypoxia has been reported in newborn rats and lambs exposed to chronic continuous hypoxia after birth (e.g. Eden & Hanson, 1987; Sladek *et al.* 1993) and in infants with bronchopulmonary dysplasia (Calder *et al.* 1994). Postnatal chronic hypoxia also suppresses the developmental increase of the $[Ca^{2+}]_i$ response to hypoxia of rat type I cells (Sterni *et al.* 1999).

In premature infants, intermittent hypoxia may be more frequent than continuous hypoxia, due to recurrent episodes of apnoea (Peng *et al.* 2004), and preterm infants with recurrent apnoea show an increased hypoxic ventilatory response, which is correlated with the number of apnoeic episodes (Nock *et al.* 2004). In preterm infants, the rate of intermittent hypoxia is also correlated with the O_2 saturation target range: preterm infants randomized to a low range (85–89%) have a higher

incidence of intermittent hypoxaemia (Di Fiore *et al.* 2012). In addition, lower 90-day survival has been reported for infants born small for gestational age with an increased incidence of intermittent hypoxia during the first three postnatal days (Di Fiore *et al.* 2017).

Postnatal intermittent hypoxia has been reported to increase the hypoxic ventilatory response in piglets (Waters & Tinworth, 2001) and rats (Peng *et al.* 2004). Significant response differences have also been reported in the exposure of newborn and adult rats to chronic intermittent hypoxia. The increased hypoxic ventilatory response develops with shorter periods of intermittent hypoxia and lasts for longer in newborn than in adult rats (Prabhakar *et al.* 2007). Thus, a quite convincing body of evidence indicates that prematurity exposes infants to various types of chronic hypoxia (continuous, intermittent, or both) and that these stimuli affect chemoreception.

Hyperoxia. Premature newborns are frequently exposed to supplemental oxygen, which may cause higher peripheral oxygen saturation (S_{pO_2}) levels, and hyperoxaemia may also have a deleterious effect on peripheral chemoreception.

In the rat, various patterns of postnatal hyperoxia exposure have been reported to produce functional changes, such as reduced ventilatory and phrenic nerve hypoxic response (e.g. Ling *et al.* 1997), reduced single-unit axonal spiking rates in normoxia and acute hypoxia, lengthening in nerve conduction time (Donnelly *et al.* 2005), and smaller hypoxia-induced $[Ca^{2+}]_i$ responses (Carroll *et al.* 2009). Again in the rat, Erickson *et al.* (1998) reported CB hypoplasia and reduction in the number of carotid sinus nerve axons after one postnatal week of 30% or 60% hyperoxia, and hyperoxia-induced reduction in CB volume has been reported to persist until adulthood (Bisgard *et al.* 2003; Bavis *et al.* 2008).

The probable roles of oxidative stress, functional inactivity, and changes in the expression of trophic factors have been emphasized in hyperoxia-induced CB hypoplasia. In particular, postnatal hyperoxia exposure upregulates the glomic expression of cerebellin 1 precursor (Dmitrieff *et al.* 2011) and spexin (Porzionato *et al.* 2012) in the rat.

However, although there are no doubts about the detrimental effects of hyperoxia on CB development, there is also the fact that hyperoxia is usually far lower in the clinical context than in experimental conditions. In general, it is reasonable to surmise that hypoxic stimuli are more important for maldevelopment and the pathogenesis of SIDS than hyperoxic stimuli.

Exposure to smoke. Maternal cigarette smoking is closely correlated with SIDS. Infants born to mothers who smoke show altered hypoxic arousal (Lewis & Bosque, 1995) and ventilatory responses (Ueda *et al.* 1999).

Experimental data also show that prenatal and perinatal exposure to nicotine alters the function of the CB. Increased mortality after severe continuous hypoxia has been reported in rats prenatally exposed to nicotine (Slotkin *et al.* 1995). In newborn rats, prenatal or perinatal nicotine exposure reduces ventilation in normoxia and hypoxia (St-John & Leiter, 1999) and impairs autoresuscitation after repeated asphyxial stimuli (Fewell & Smith, 1998).

Prenatal (Bamford & Carroll, 1999) or postnatal (Holgert *et al.* 1995) nicotine administration produces a decreased ventilatory response to hyperoxia in newborn rats. Higher frequencies of spontaneous and post-hypoxic apnoeas have also been observed in newborn mice (Robinson *et al.* 2002). In newborn lambs prenatally exposed to nicotine, Hafstrom *et al.* (2002) observed reduced ventilatory and arousal responses to hypoxia during sleep and a lower ventilatory response to hyperoxia during sleep and wakefulness.

In the carotid bodies at postnatal day (PN) 3 in rats, reduced dopamine and increased expression of tyrosine hydroxylase have been reported after injections of nicotine (Holgert *et al.* 1995). In the CB of newborn rats, increases in tyrosine hydroxylase and dopamine β-hydroxylase mRNA have been found after perinatal nicotine exposure (Gauda *et al.* 2001). Prenatal and early postnatal nicotine exposure also increases the number of cells in the petrosal ganglion expressing tyrosine hydoxylase mRNA at PN3 (Gauda *et al.* 2001). Prenatal exposure to nicotine also augments mRNA levels of the substance P-precursor preprotachykinin A in carotid bodies and in the petrosal ganglia of newborn rats at PN1, indicating perturbation of the CB–petrosal ganglion pathway (Berner *et al.* 2008). The experimental body of evidence of the functional and neurochemical effects of nicotine on the CB is quite sound and consistent, although further confirmation in humans is necessary.

Carbon monoxide (CO) is another constituent of smoke which passes from mother to fetus, leading to tissue hypoxia (Hutter & Blair, 1996). Although CO mainly exerts its effects at the level of the central nervous system, *in vitro* and *in vivo* effects on peripheral arterial chemoreceptors have also been reported. Exogenous CO exposure reduces the hypoxic ventilatory response in rats, and this effect has been ascribed to the peripheral chemoreceptors (Zhuang *et al.* 2006), based upon evidence that CO exposure inhibits CB sensory discharge in hypoxia in the cat (Lahiri *et al.* 1993) and reverses hypoxic inhibition

of the K^+ channels of CB chemoreceptors in the rat (Riesco-Fagundo *et al.* 2001).

Substance abuse. An increased risk of SIDS has been reported in infants perinatally exposed to marijuana (e.g. Scragg *et al.* 2001) or prenatally exposed to opiates or cocaine during pregnancy (Kandall *et al.* 1993), although one confounding factor may be maternal cigarette smoking.

Experimental studies have been carried out on the effects of specific substances on chemoreception, although associations in human studies are obviously more difficult to demonstrate.

Cannabinoids have been reported to modulate arousal from sleep in the rat (Carley *et al.* 2002). In this species, cannabinoid type 1 receptor is expressed in the nodose–petrosal–jugular ganglia complex, superior cervical ganglia and, to a lesser extent, the CB (McLemore *et al.* 2004).

Infants of opiate- or cocaine-abusing mothers have abnormal sleeping ventilatory patterns (greater duration of apnoea, more periodic breathing) (Ward *et al.* 1990). Prenatal cocaine blunts the hypoxic response (Lipton *et al.* 1996*a*) and decreases the levels of CB dopamine (Lipton *et al.* 1996*b*) in PN5 rat pups, and reduces the expression of glial cell line-derived neurotrophic factor in the CB of rat fetuses at E20.5 (Lipton *et al.* 1999).

Chronic ethanol exposure is another risk factor for SIDS by reason of studies identifying an association between 'binge drinking' during pregnancy and SIDS. Experimental studies in rats and bullfrog tadpoles have identified various effects on breathing, including changes in adaptation to chemosensory challenges (reviewed in Dubois *et al.* 2013).

Infections and inflammatory events. Infections and inflammatory events are also risk factors for SIDS. One-third of premature human infants are exposed to intrauterine infection and inflammation. Acute inflammation in newborns is associated with increased frequency and severity of apnoea and intermittent hypoxia (Stock *et al.* 2010).

As regards the possibility of infective/inflammatory agents exerting their effects through the CB, experimental studies in animals have shown that pre- and postnatal lipopolysaccharide (LPS) administration may affect peripheral chemoreception (Fernandez *et al.* 2008; Samarasinghe *et al.* 2015; Master *et al.* 2016). In particular, maternal intraperitoneal LPS administration produces lower baseline ventilation, increased apnoea frequency, and hypersensitivity to hypoxia/hypercapnia in mouse pups (Samarasinghe *et al.* 2015).

In the rat, intraperitoneal postnatal LPS administration causes more frequent desaturation episodes (hypoxic events, apnoea), attenuated ventilatory responses to changes in oxygen tension, and attenuated hypoxic chemosensitivity of the carotid sinus nerve in *in vitro* recordings. Carotid bodies show increases in inflammatory cytokines (IL1β, IL6) and the volume fraction occupied by type II cells, together with significant reductions in dopamine content and ultrastructural changes (swelling of mitocondria and Golgi bodies, irregular chromatin condensation) (Master *et al.* 2016). These experimental studies (although not very numerous) are highly suggestive of the possible detrimental effect of infection/inflammation on ventilatory control through effects on the CB.

Breastfeeding is a well-known SIDS protective factor and its possible beneficial immunomodulatory role has been proposed. According to the above findings, beneficial anti-inflammatory and immunomodulatory effects may also be hypothesized in the CB.

Central respiratory chemoreceptors

Postnatal development. The main central respiratory chemoreceptors are considered to be the retrotrapezoid nucleus and subsets of serotoninergic neurons, mainly located in the raphe magnus nucleus. Other structures with possible chemoreceptive functions are some noradrenergic (locus coeruleus) and orexinergic neuronal populations, the ventral medullary surface, and the solitary tract nucleus (Guyenet & Bayliss, 2015). Their differing roles and integration are not yet fully understood. For instance, the various central chemoreceptors may be involved in different ways in sleep and wakefulness (Feldman *et al.* 2003). Some changes have been reported during development. The parafacial respiratory group has been postulated to be the precursor of retrotrapezoid nucleus in the fetal and early neonatal period (Guyenet *et al.* 2009).

The CB also projects through the solitary tract nucleus to the retrotrapezoid nucleus (Guyenet & Bayliss, 2015). Inputs from peripheral chemoreceptors can modify the sensitivity of central chemoreceptors, and central stimuli can affect peripheral chemoreceptor sensitivity. Reduced afferent inputs from peripheral arterial chemoreceptors during maturation may also cause maldevelopment of central respiratory centres, leading to further alterations in cardiorespiratory regulation (Gauda *et al.* 2007).

Newborns are also characterized by immature respiratory control, which includes breathing rhythmicity and its modulation by central/peripheral chemoreceptors and suprapontine influences (Gaultier & Gallego, 2005). Episodes of apnoea (usually central) decrease with

advancing postnatal age (Gaultier, 1999) and the hypercapnic ventilatory response increases with postnatal age (Gaultier & Gallego, 2005).

In the rat, although central respiratory centres undergo progressive development during the first two/three postnatal weeks, a specifically critical period has been identified at the end of the second week (PN12–PN13), when a series of neurochemical, metabolic, ventilatory and electrophysiological changes occur (reviewed in Wong-Riley *et al.* 2013). During this critical period, the ventilatory response to acute hypoxia is at its weakest, and the expression of tryptophan hydroxylase is also at its lowest in the ventrolateral medullary surface, raphe obscurus and raphe magnus. These changes are particularly suggestive, because the peak incidence of SIDS occurs between the second and fourth months, a period which corresponds to the end of the second postnatal week in the rat in terms of brain development (Wong-Riley *et al.* 2013).

Again in the rat, the initial reduction in hypercapnic ventilatory response during the critical period (although not confirmed by all authors) is followed by an increase (Stunden *et al.* 2001; Wickstrom *et al.* 2002). *In vitro* studies have also shown that rat medullary raphe neurons increase their CO_2 chemosensitivity with postnatal age (Richerson, 2004).

In humans, the developmental expression of serotonin receptors has been studied by autoradiography, and shows a progressive reduction in ^{3}H-LSD binding from fetal to infant to mature age in the nucleus raphe dorsalis, median raphe nucleus and nucleus raphe obscurus (Duncan *et al.* 2010). ^{3}H-Nicotine binding also decreases in most brainstem nuclei with increasing age, in both cases of SIDS and controls (Duncan *et al.* 2008).

On the whole (similar to the above information about peripheral chemoreceptors), the period of critical postnatal development of central respiratory chemoreceptors supports the hypothesis that some structural/functional maldevelopment plays a role in the pathogenesis of SIDS.

Signs of intrinsic vulnerability. According to the above considerations, a series of histopathological, morphometric and neurochemical studies have been performed to examine the possible presence of markers of intrinsic vulnerability in the main respiratory centres. Focusing on central respiratory chemoreceptors, we can refer to the following findings.

Lavezzi *et al.* (2012) reported hypoplasia or agenesis of the retrotrapezoid nucleus in SIDS and cases of sudden intrauterine death, but some concerns have been raised regarding the true anatomical location of the nucleus in that study (Rudzinski & Kapur, 2013).

Some structures of the medullary serotonin system show significantly lower serotonin 1A receptor binding in SIDS cases than in controls, especially in the paragigantocellularis lateralis and the intermediate reticular zone. The raphe obscurus and paragigantocellularis also show reduced serotonin contents and the raphe obscurus reduced expression of tryptophan hydroxylase (Duncan *et al.* 2010). Conversely, significant differences in ^{3}H-nicotine binding between SIDS and controls were not reported in a series of brainstem nuclei, including solitary tract nucleus, locus coeruleus and raphe dorsalis (Duncan *et al.* 2008).

Another nucleus which has been considered in the past is the arcuate nucleus, because of the presumed homology with the chemosensory area in the ventral medullary surface of lower mammals, such as cats or rodents. In particular, morphometric changes have been reported in SIDS cases, e.g. reduced volume, neuronal density and neuronal (nuclear–cytoplasmic) areas, higher neuronal form factor, and reduced astrocyte density (Matturri *et al.* 2000; Biondo *et al.* 2003). Reduced binding to various receptors (muscarinic, kainate, serotoninergic and PACAP receptor 1) has also been reported (Kinney *et al.* 1995, 2003; Panigrahy *et al.* 1997, 2000; Huang *et al.* 2017). In reality, the chemosensory function of the human arcuate nucleus is believed to be speculative by some authors, since it has not yet been demonstrated (Guyenet, 2011).

Reduced tyrosine hydroxylase immunoreactivity and increased numbers of GFAP-positive glial cells have been reported in the locus coeruleus of SIDS cases. Similar changes have also been described for other structures with noradrenergic neurons, i.e. the area reticularis superficialis ventrolateralis and dorsal vagal nucleus (Obonai *et al.* 1998).

Although the above morphometric and neurochemical changes further support a pathogenetic role for central respiratory chemoreceptors, they are still difficult to apply for diagnosis in the forensic context, due to the need for specific methodological experience and proper controls for comparison. Of particular interest is a recent paper which reported higher serum serotonin levels in SIDS victims than in controls, with a subset of 31% SIDS cases with serotonin levels higher than 2 standard deviations above the mean of controls, supporting peripheral anomalies in serotonin metabolism and proposing a simple marker (Haynes *et al.* 2017).

Few studies have examined experimental models of impaired central chemoreception, due to methodological problems, but Cummings *et al.* (2011) showed that lesions of the lower brainstem serotoninergic neurons compromise autoresuscitation during repeated hypoxia episodes.

Intrinsic and extrinsic risk factors.

Gender and genetic factors. Gender effects have also been observed on central respiratory chemoreceptors.

Brainstem noradrenergic cell groups (A1, A2, A5) receiving chemosensory fibres show higher dopamine and noradrenaline turnover in female rats; orchidectomy and ovariectomy also respectively increase and reduce dopamine/noradrenaline turnover in these structures (Pequignot *et al.* 1997). The retrotrapezoid nucleus in female mice shows a higher threshold for c-fos upregulation in response to CO_2 (10% *versus* 5%) (Niblock *et al.* 2012). In the guinea-pig, oestrogens regulate the neuronal expression of potassium channels contributing to the chemosensitivity of retrotrapezoid cells, such as Kv4.1 (Roepke *et al.* 2007). The number of potassium channel-immunoreactive neurons in the medullary raphe also differs between males and females in some inbred rat strains (Riley *et al.* 2010).

Genetic studies have also reported associations between SIDS and gene variations in the serotonin transporter gene *SLC6A4* (e.g. Weese-Mayer *et al.* 2003; although their results were not confirmed by Paterson *et al.* 2010) and serotonin 1A receptor gene HTR1A (Morley *et al.* 2008), with possible implications in central chemoreception on the part of the serotoninergic system.

Genetic factors are also probably involved in inter-individual variability in hypercapnic ventilatory responses (Gaultier & Gallego, 2005). In inbred adult mouse strains, the hypercapnic ventilatory response is genetically determined (Tankersley, 2003). In addition, an abnormal hypercapnic ventilatory response has been reported in mice lacking specific genes such as *Htr1a* and the *Mash-1-Ret-Phox2b* signalling pathways as well as genes for endothelin-1 and PACAP (e.g. Burton *et al.* 1997; Kuwaki *et al.* 1999; Cummings *et al.* 2004; Baccini *et al.* 2012).

Prematurity. Clinical and experimental data indicate reduced central chemoreception in prematurity. Preterm newborns show breathing irregularity, periodic breathing, frequent episodes of apnoea and weaker hypercapnic ventilatory response (Zhang *et al.* 2003; Darnall, 2010). The incidence of periodic breathing and apnoea decreases from 30 to 40 weeks post-menstrual age (Parmelee *et al.* 1972). Periodic breathing has been correlated to a decrease in ventilation and CO_2 sensitivity (Rigatto & Brady, 1972), with the apnoeic threshold closer to eupnoic CO_2 levels (Khan *et al.* 2005).

Hypoxic and hyperoxic stimuli. Hypoxia and hyperoxia mainly affect the developmental plasticity of peripheral arterial chemoreception, although some studies also report a few minor central effects. For instance, in an integration of experimental data and a computational model, Molkov *et al.* (2011) proposed that chronic intermittent hypoxia increases the CO_2 sensitivity of the retrotrapezoid nucleus/parafacial respiratory group in the rat.

In the rat, exposure to sustained hypoxia at PN11–PN15 (critical period) reduces the ventilatory response to acute hypoxia and increases mortality (Mayer *et al.* 2014; MacFarlane *et al.* 2016). Although a possible role played by the CB cannot be excluded, increased numbers of microglial cells and decreased serotonin immunoreactivity have been reported in the solitary tract nucleus and dorsal vagal nucleus. The above changes can be prevented by minocycline treatment, thus supporting the role played by microglial cells (MacFarlane *et al.* 2016).

Perinatal hyperoxial exposure of rat pups has also been reported to reduce the frequency of phrenic bursting generated by the central respiratory network, at baseline and in response to hypoxia (Bierman *et al.* 2014).

Exposure to smoke. Many experimental studies have shown that prenatal/perinatal exposure to nicotine affects the postnatal development of central breathing patterns and chemoreception. In the rat, prenatal nicotine exposure is correlated with a higher incidence of apnoea in the first two postnatal days, and higher breathing frequency and lower tidal volume in later periods, together with blunting of ventilatory responses to hypercapnia and/or hypoxia (Huang *et al.* 2004, 2010). In the mouse, prenatal to early postnatal nicotine exposure reduces basal minute volume and ventilatory responses to hypercarbia and hypoxia in the first postnatal days (Eugenin *et al.* 2008). In the same study, isolated brainstem–spinal cord preparations from PN0–PN3 nicotine-exposed newborns showed longer and more irregular fictive respiratory cycles, together with a lower response to acidification. A switch from muscarinic to nicotinic receptor-based mechanisms has also been reported in the cholinergic component of central respiratory chemoreception (Eugenin *et al.* 2008). Lei *et al.* (2015) report that, in medullary slices sampled from prenatally exposed rats, responses to acidification (increased burst frequency and reduced integrated amplitude) are lower than in controls, indicating a blunting effect on central chemoreception. In mouse, prenatal to early postnatal nicotine exposure also causes specific changes in the raphe obscurus nucleus (decreased number of serotoninergic neurons and increased expression of $5\text{-}HT_{1A}$ autoreceptors) (Cerpa *et al.* 2015).

Reduced serotoninergic binding has been reported in the arcuate nucleus of infants exposed to perinatal maternal smoking (Kinney *et al.* 2003). Reduced ^{3}H-nicotinic receptor binding in the locus coeruleus, periacqueductal grey matter and raphe dorsalis has been reported in control cases exposed to maternal smoking *in utero* with respect to other controls, although similar

differences were not found in SIDS cases (Duncan *et al.* 2008).

As regards other constituents of cigarette smoke, newborn guinea-pigs prenatally exposed to CO have been reported to show significantly greater tidal volume and minute ventilation during steady-state hypercapnia, indicating increased sensitivity to CO_2 (McGregor *et al.* 1998). Newborn guinea-pigs prenatally exposed to CO also showed a significant decrease in tyrosine hydroxylase expression in the solitary tract nucleus, dorsal vagal nucleus, area postrema, intermediate reticular nucleus and ventrolateral medulla, and significantly increased choline acetyltransferase expression in the dorsal vagal nucleus and hypoglossal nucleus (Tolcos *et al.* 2000).

Infections and inflammatory events. Infections and inflammatory events may also have negative effects on chemoreception and respiratory control through central mechanisms, as attenuated hypoxic ventilatory responses following intratracheal LPS have also been reported in the rat after carotid sinus nerve transection (Balan *et al.* 2011). In addition, vulnerability to LPS is highest in the critical period for the development of central respiratory centres. In the rat, intraperitoneal injection of LPS attenuates the early and late phases of the acute hypoxia ventilatory response if performed at PN10, but not at PN5 or PN20; increased mortality and increased expression of TNFα and iNOS in the brainstem have also been reported (Rourke *et al.* 2016).

Methodological considerations and future perspectives

In conclusion, the studies reviewed here strongly support the role of central and peripheral respiratory chemoreceptors in the pathogenesis of SIDS. It has been amply demonstrated that respiratory chemoreceptors undergo critical development during the postnatal period, when SIDS typically occurs, and that this development may be structurally/functionally affected by most risk factors for SIDS. In spite of this, conclusive demonstration of chemoreception impairment in SIDS cases is still difficult, due to a series of methodological problems concerning human material.

As regards studies on autopsy material, the main problem is difficulty in obtaining large standardized series of cases and controls. Particularly in a rare and (probably) heterogeneous entity such as SIDS, reliable results can only derive from large and highly standardized series. In case-control studies, we know that many confounding factors may bias the statistical results. In the available literature on the matter, possible biases due to under-matching, over-matching or other mismatching of controls are not always adequately considered. Higher attention should be paid in sampling controls and justifying the case:control ratio.

Studies on morphometric parameters in the comparative analysis of central and peripheral chemoreceptors between SIDS victims and controls are described here. However, most of these approaches show methodological problems, usually involving parameters calculated on two-dimensional planes, such as surface areas or cell densities per surface area. Counting on a two-dimensional plane produces intrinsic bias in estimating cell numbers, because the probability of objects being hit by a single section is proportional to their size and form, and does not depend only on their number. Thus, stereological methods should be applied, with three-dimensional probes (disectors or fractionators) which permit sampling of objects with a probability which is proportional only to their number (West, 1993). Some authors have reported differences of as much as 40% between two-dimensional (biased) methods and three-dimensional (unbiased) methods (Pakkenberg *et al.* 1991; Coggeshall, 1992). Obviously, design and measurement take precedence over statistics and biased measures cannot be rescued by statistical analysis. In a previous study, we performed stereological analysis of the main medullary nuclei of adults and infants according to the optical dissector method. The infant series contained both cases of SIDS and controls, but significant differences were not found, although this may have been due to the small number of cases (Porzionato *et al.* 2009*b*). Further unbiased stereological studies will be necessary to verify possible morphometric changes in central and peripheral respiratory chemoreceptors of SIDS victims.

One of the main problems of working with human postmortem material is tissue degradation, which may affect results in unpredictable ways and which is difficult to standardize between cases and controls.

The above problems have particularly affected research on the CB, as this structure is still rarely sampled in SIDS autopsies. Standardized collection of the CB in SIDS would be necessary to develop large tissue banks for analysis, but international standardized autopsy protocols for SIDS do not usually include CB sampling. For instance, some intriguing aspects of the CB in SIDS have not yet been investigated, e.g. the expression of several neurotransmitters/neuromodulators and their corresponding receptors and transporters. Nevertheless, findings on experimental animals clearly indicate modulation of neurotransmission/neuromodulation in response to SIDS risk factors.

Apart from autopsy studies, further functional studies on prematures and full-term newborns would be useful to identify infants at risk of SIDS. In fact, most experimental studies on animals have identified functional (rather than structural) effects as the result of intrinsic/extrinsic risk factors for SIDS. Prospective studies addressing some

simple functional tests on large infant series would be the only way of specifically identifying and correlating central and/or peripheral chemoreceptor impairment with the risk of SIDS. Some authors have also suggested evaluation of chemoreceptor function (hyperoxia and alternate breath tests) in preterm infants before hospital discharge (Gaultier & Gallego, 2005). Genetic factors have also been mentioned as playing a role in breathing instability; thus, integration of functional and genetic data in prospective clinical studies would be the gold standard. Obviously, the design of such studies would need consideration of technical, clinical and ethical issues of particular importance and difficulty.

References

Aizad T, Bodani J, Cates D, Horvath L & Rigatto H (1984). Effect of a single breath of 100% oxygen on respiration in neonates during sleep. *J Appl Physiol* **57**, 1531–1535.

Baccini G, Mlinar B, Audero E, Gross CT & Corradetti R (2012). Impaired chemosensitivity of mouse dorsal raphe serotonergic neurons overexpressing serotonin 1A (Htr1a) receptors. *PLoS One* **7**, e45072.

Balan KV, Kc P, Hoxha Z, Mayer CA, Wilson CG & Martin RJ (2011). Vagal afferents modulate cytokine-mediated respiratory control at the neonatal medulla oblongata. *Respir Physiol Neurobiol* **178**, 458–464.

Bamford OS & Carroll JL (1999). Dynamic ventilatory responses in rats: normal development and effects of prenatal nicotine exposure. *Respir Physiol* **117**, 29–40.

Bavis RW, Wenninger JM, Miller BM, Dmitrieff EF, Olson EB Jr, Mitchell GS & Bisgard GE (2008). Respiratory plasticity after perinatal hyperoxia is not prevented by antioxidant supplementation. *Respir Physiol Neurobiol* **160**, 301–312.

Berner J, Ringstedt T, Brodin E, Hökfelt T, Lagercrantz H & Wickström R (2008). Prenatal exposure to nicotine affects substance P and preprotachykinin-A mRNA levels in newborn rat. *Pediatr Res* **64**, 621–624.

Bierman AM, Tankersley CG, Wilson CG, Chavez-Valdez R & Gauda EB (2014). Perinatal hyperoxic exposure reconfigures the central respiratory network contributing to intolerance to anoxia in newborn rat pups. *J Appl Physiol* **116**, 47–53.

Biondo B, Lavezzi A, Tosi D, Turconi P & Matturri L (2003). Delayed neuronal maturation of the medullary arcuate nucleus in sudden infant death syndrome. *Acta Neuropathol* **106**, 545–551.

Bisgard GE, Olson EB Jr, Wang ZY, Bavis RW, Fuller DD & Mitchell GS (2003). Adult carotid chemoafferent responses to hypoxia after 1, 2, and 4 wk of postnatal hyperoxia. *J Appl Physiol* **95**, 946–952.

Bouferrache B, Filtchev S, Leke A, Freville M, Gallego J & Gaultier C (2002). Comparison of the hyperoxic test and the alternate breath test in infants. *Am J Respir Crit Care Med* **165**, 206–210.

Bowes G, Townsend ER, Bromley SM, Kozar LF & Phillipson EA (1981*a*). Role of the carotid body and of afferent vagal stimuli in the arousal response to airway occlusion in sleeping dogs. *Am Rev Respir Dis* **123**, 644–647.

Bowes G, Townsend ER, Kozar LF, Bromley SM & Phillipson EA (1981*b*). Effect of carotid body denervation on arousal response to hypoxia in sleeping dogs. *J Appl Physiol* **51**, 40–45.

Bureau MA, Lamarche J, Foulon P & Dalle D (1985). The ventilatory response to hypoxia in the newborn lamb after carotid body denervation. *Respir Physiol* **60**, 109–119.

Burton MD, Kawashima A, Brayer JA, Kazemi H, Shannon DC, Schuchardt A, Costantini F, Pachnis V & Kinane TB (1997). RET proto-oncogene is important for the development of respiratory CO_2 sensitivity. *J Auton Nerv Syst* **63**, 137–143.

Calder NA, Williams BA, Smyth J, Boon AW, Kumar P & Hanson MA (1994). Absence of ventilatory responses to alternating breaths of mild hypoxia and air in infants who have had bronchopulmonary dysplasia: implications for the risk of sudden infant death. *Pediatr Res* **35**, 677–681.

Carley DW, Paviovic S, Janelidze M & Radulovacki M (2002). Functional role for cannabinoids in respiratory stability during sleep. *Sleep* **25**, 391–398.

Carroll JL, Kim I, Dbouk H, Yang DJ, Bavis RW & Donnelly DF (2009). Time-dependence of hyperoxia-induced impairment in peripheral chemoreceptor activity and glomus cell calcium response. *Adv Exp Med Biol* **648**, 299–306.

Cerpa VJ, Aylwin Mde L, Beltrán-Castillo S, Bravo EU, Llona IR, Richerson GB & Eugenín JL (2015). The alteration of neonatal raphe neurons by prenatal-perinatal nicotine. Meaning for sudden infant death syndrome. *Am J Respir Cell Mol Biol* **53**, 489–499.

Coggeshall RE (1992). A consideration of neural counting methods. *Trends Neurosci* **15**, 9–13.

Cummings KJ, Hewitt JC, Li A, Daubenspeck JA, Nattie EE (2011). Postnatal loss of brainstem serotonin neurones compromises the ability of neonatal rats to survive episodic severe hypoxia. *J Physiol* **589**, 5247–5256.

Cummings KJ, Pendlebury JD, Sherwood NM & Wilson RJ (2004). Sudden neonatal death in PACAP-deficient mice is associated with reduced respiratory chemoresponse and susceptibility to apnoea. *J Physiol* **555**, 15–26.

Darnall RA (2010). The role of CO_2 and central chemoreception in the control of breathing in the fetus and the neonate. *Respir Physiol Neurobiol* **173**, 201–212.

De Caro R, Macchi V, Sfriso MM & Porzionato A (2013). Structural and neurochemical changes in the maturation of the carotid body. *Respir Physiol Neurobiol* **185**, 9–19.

Di Fiore JM, Martin RJ, Li H, Morris N, Carlo WA, Finer N, Walsh M; SUPPORT Study Group of the Eunice Kennedy Shriver National Institute of Child Health, and Human Development Neonatal Research Network (2017). Patterns of oxygenation, mortality, and growth status in the surfactant positive pressure and oxygen trial cohort. *J Pediatr* **186**, 49–56.

Di Fiore JM, Walsh M, Wrage L, Rich W, Finer N, Carlo WA, Martin RJ; Support Study Group of Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network (2012). Low oxygen saturation target range is associated with increased incidence of intermittent hypoxemia. *J Pediatr* **161**, 1047–1052.

Dmitrieff EF, Wilson JT, Dunmire KB & Bavis RW (2011). Chronic hyperoxia alters the expression of neurotrophic factors in the carotid body of neonatal rats. *Respir Physiol Neurobiol* **175**, 220–227.

Donnelly DF & Haddad GG (1990). Prolonged apnea and impaired survival in piglets after sinus and aortic nerve section. *J Appl Physiol* **68**, 1048–1052.

Donnelly DF, Kim I, Carle C & Carroll JL (2005). Perinatal hyperoxia for 14 days increases nerve conduction time and the acute unitary response to hypoxia of rat carotid body chemoreceptor. *J Appl Physiol* **99**, 114–119.

Dubois CJ, Kervern M, Naassila M & Pierrefiche O (2013). Chronic ethanol exposure during development: disturbances of breathing and adaptation. *Respir Physiol Neurobiol* **189**, 250–260.

Duncan JR, Paterson DS, Hoffman JM, Mokler DJ, Borenstein NS, Belliveau RA, Krous HF, Haas EA, Stanley C, Nattie EE, Trachtenberg FL & Kinney HC (2010). Brainstem serotonergic deficiency in sudden infant death syndrome. *JAMA* **303**, 430–437.

Duncan JR, Randall LL, Belliveau RA, Trachtenberg FL, Randall B, Habbe D, Mandell F, Welty TK, Iyasu S & Kinney HC (2008). The effect of maternal smoking and drinking during pregnancy upon ^{3}H-nicotine receptor brainstem binding in infants dying of the sudden infant death syndrome: initial observations in a high risk population. *Brain Pathol* **18**, 21–31.

Eden GJ & Hanson MA (1987). Effects of chronic hypoxia from birth on the ventilatory response to acute hypoxia in the newborn rat. *J Physiol* **392**, 11–19.

Erickson JT, Mayer C, Jawa A, Ling L, Olson EB Jr, Vidruk EH, Mitchell GS & Katz DM (1998). Chemoafferent degeneration and carotid body hypoplasia following chronic hyperoxia in newborn rats. *J Physiol* **509**, 519–526.

Eugenín J, Otárola M, Bravo E, Coddou C, Cerpa V, Reyes-Parada M, Llona I & von Bernhardi R (2008). Prenatal to early postnatal nicotine exposure impairs central chemoreception and modifies breathing pattern in mouse neonates: a probable link to sudden infant death syndrome. *J Neurosci* **28**, 13907–13917.

Feldman JL, Mitchell GS & Nattie EE (2003). Breathing: rhythmicity, plasticity, chemosensitivity. *Annu Rev Neurosci* **26**, 239–266.

Fernández R, González S, Rey S, Cortés PP, Maisey KR, Reyes EP, Larraín C & Zapata P (2008). Lipopolysaccharide-induced carotid body inflammation in cats: functional manifestations, histopathology and involvement of tumour necrosis factor-alpha. *Exp Physiol* **93**, 892–907.

Fewell JE, Kondo CS, Dascalu V & Filyk SC (1989). Influence of carotid denervation on the arousal and cardiopulmonary response to rapidly developing hypoxemia in lambs. *Pediatr Res* **25**, 473–477.

Fewell JE & Smith FG (1998). Perinatal nicotine exposure impairs ability of newborn rats to autoresuscitate from apnea during hypoxia. *J Appl Physiol* **85**, 2066–2074.

Filiano JJ & Kinney HC (1994). A perspective on neuropathologic findings in victims of sudden infant death syndrome: the triple risk model. *Biol Neonate* **65**, 194–197.

Gauda EB, Cooper R, Akins PK & Wu G (2001). Prenatal nicotine affects catecholamine gene expression in newborn rat carotid body and petrosal ganglion. *J Appl Physiol* **91**, 2157–2165.

Gauda EB, Cristofalo E & Nunez J (2007). Peripheral arterial chemoreceptors and sudden infant death syndrome. *Respir Physiol Neurobiol* **157**, 162–170.

Gauda EB, McLemore GL, Tolosa J, Marston-Nelson J & Kwak D (2004). Maturation of peripheral arterial chemoreceptors in relation to neonatal apnoea. *Semin Neonatol* **9**, 181–194.

Gaultier C (1999). Sleep apnoea in infants. *Sleep Med Rev* **3**, 303–312.

Gaultier C (2000). Development of the control of breathing: implications for sleep-related breathing disorders in infants. *Sleep* **23**, S136–S139.

Gaultier C & Gallego J (2005). Development of respiratory control: evolving concepts and perspectives. *Respir Physiol Neurobiol* **149**, 3–15.

Grogaard J, Kreuger E, Lindstrom D & Sundell H (1986). Effects of carotid body maturation and terbutaline on the laryngeal chemoreflex in newborn lambs. *Pediatr Res* **20**, 724–729.

Guyenet PG (2011). Loss of brainstem serotonergic neurons impairs autoresuscitation in neonate rats: is this relevant to the sudden infant death syndrome? *J Physiol* **589**, 5343–5344.

Guyenet PG & Bayliss DA (2015). Neural control of breathing and CO_2 homeostasis. *Neuron* **87**, 946–961.

Guyenet PG, Bayliss DA, Stornetta RL, Fortuna MG, Abbott SB & DePuy SD (2009). Retrotrapezoid nucleus, respiratory chemosensitivity and breathing automaticity. *Respir Physiol Neurobiol* **168**, 59–68.

Hafstrom O, Milerad J & Sundell HW (2002). Prenatal nicotine exposure blunts the cardiorespiratory response to hypoxia in lambs. *Am J Respir Crit Care Med* **166**, 1544–1549.

Haynes RL, Frelinger AL 3rd, Giles EK, Goldstein RD, Tran H, Kozakewich HP, Haas EA, Gerrits AJ, Mena OJ, Trachtenberg FL, Paterson DS, Berry GT, Adeli K, Kinney HC & Michelson AD (2017). High serum serotonin in sudden infant death syndrome. *Proc Natl Acad Sci USA* **114**, 7695–7700.

Holgert H, Hokfelt T, Hertzberg T & Lagercrantz H (1995). Functional and developmental studies of the peripheral arterial chemoreceptors in rat: effects of nicotine and possible relation to sudden infant death syndrome. *Proc Natl Acad Sci USA* **92**, 7575–7579.

Huang J, Waters KA & Machaalani R (2017). Pituitary adenylate cyclase activating polypeptide (PACAP) and its receptor 1 (PAC1) in the human infant brain and changes in the Sudden Infant Death Syndrome (SIDS). *Neurobiol Dis* **103**, 70–77.

Huang YH, Brown AR, Costy-Bennett S, Luo Z & Fregosi RF (2004). Influence of prenatal nicotine exposure on postnatal development of breathing pattern. *Respir Physiol Neurobiol* **143**, 1–8.

Huang YH, Brown AR, Cross SJ, Cruz J, Rice A, Jaiswal S & Fregosi RF (2010). Influence of prenatal nicotine exposure on development of the ventilatory response to hypoxia and hypercapnia in neonatal rats. *J Appl Physiol* **109**, 149–158.

Hutter CD & Blair ME (1996). Carbon monoxide - does fetal exposure cause sudden infant death syndrome? *Med Hypotheses* **46**, 1–4.

Kahn A, Groswasser J, Franco P, Scaillet S, Sawaguchi T, Kelmanson I & Dan B (2003). Sudden infant deaths: stress, arousal and SIDS. *Early Hum Dev* **75**, S147–166.

Khan A, Qurashi M, Kwiatkowski K, Cates D & Rigatto H (2005). Measurement of the CO_2 apneic threshold in newborn infants: possible relevance for periodic breathing and apnea. *J Appl Physiol* **98**, 1171–1176.

Kandall SR, Gaines J, Habel L, Davidson G & Jessop D (1993). Relationship of maternal substance abuse to subsequent sudden infant death syndrome in offspring. *J Pediatr* **123**, 120–126.

Kinney HC, Filiano JJ, Sleeper LA, Mandell F, Valdes-Dapena M & White WF (1995). Decreased muscarinic receptor binding in the arcuate nucleus in sudden infant death syndrome. *Science* **269**, 1446–1450.

Kinney HC, Randall LL, Sleeper LA, Willinger M, Belliveau RA, Zec N, Rava LA, Dominici L, Iyasu S, Randall B, Habbe D, Wilson H, Mandell F, McClain M & Welty TK (2003). Serotonergic brainstem abnormalities in Northern Plains Indians with the sudden infant death syndrome. *J Neuropathol Exp Neurol* **62**, 1178–1191.

Kuwaki T, Ling GY, Onodera M, Ishii T, Nakamura A, Ju KH, Cao WH, Kumada M, Kurihara H, Kurihara Y, Yazaki Y, Ohuchi T, Yanagisawa M & Fukuda Y (1999). Endothelin in the central control of cardiovascular and respiratory functions. *Clin Exp Pharmacol Physiol* **26**, 989–994.

Lack EE, Perez-Atayde AR & Young JB (1986). Carotid bodies in sudden infant death syndrome: a combined light microscopic, ultrastructural, and biochemical study. *Pediatr Pathol* **6**, 335–350.

Lahiri S, Iturriaga R, Mokashi A, Ray DK & Chugh D (1993). CO reveals dual mechanisms of O_2 chemoreception in the cat carotid body. *Respir Physiol* **94**, 227–240.

Lavezzi AM, Weese-Mayer DE, Yu MY, Jennings LJ, Corna MF, Casale V, Oneda R & Matturri L (2012). Developmental alterations of the respiratory human retrotrapezoid nucleus in sudden unexplained fetal and infant death. *Auton Neurosci* **170**, 12–19.

Lei F, Yan X, Zhao F, Zhang S, Zhang Q, Zhou H & Zheng Y (2015). Impairment of central chemoreception in neonatal rats induced by maternal cigarette smoke exposure during pregnancy. *PLoS One* **10**, e0137362.

Lewis KW & Bosque EM (1995). Deficient hypoxia awakening response in infants of smoking mothers: possible relationship to sudden infant death syndrome. *J Pediatr* **127**, 691–699.

Ling L, Olson EB Jr, Vidruk EH & Mitchell GS (1997). Integrated phrenic responses to carotid afferent stimulation in adult rats following perinatal hyperoxia. *J Physiol* **500**, 787–796.

Lipton JW, Davidson TL, Carvey PM & Weese-Mayer DE (1996*a*). Prenatal cocaine: effect on hypoxic ventilatory responsiveness in neonatal rats. *Respir Physiol* **106**, 161–169.

Lipton JW, Ling Z, Vu TQ, Robie HC, Mangan KP, Weese-Mayer DE & Carvey PM (1999). Prenatal cocaine exposure reduces glial cell line-derived neurotrophic factor (GDNF) in the striatum and the carotid body of the rat: implications for DA neurodevelopment. *Brain Res Dev Brain Res* **118**, 231–235.

Lipton JW, Yuengsrigul A, Ling ZD, Weese-Mayer DE & Carvey PM (1996*b*). Prenatal cocaine exposure and postnatal hypoxia independently decrease carotid body dopamine in neonatal rats. *Neurotoxicol Teratol* **18**, 283–287.

MacFarlane PM, Mayer CA & Litvin DG (2016). Microglia modulate brainstem serotonergic expression following neonatal sustained hypoxia exposure: implications for sudden infant death syndrome. *J Physiol* **594**, 3079–3094.

McGregor HP, Westcott K & Walker DW (1998). The effect of prenatal exposure to carbon monoxide on breathing and growth of the newborn guinea pig. *Pediatr Res* **43**, 126–131.

McLemore GL, Cooper RZ, Richardson KA, Mason AV, Marshall C, Northington FJ & Gauda EB (2004). Cannabinoid receptor expression in peripheral arterial chemoreceptors during postnatal development. *J Appl Physiol* **97**, 1486–1495.

Master ZR, Porzionato A, Kesavan K, Mason A, Chavez-Valdez R, Shirahata M & Gauda EB (2016). Lipopolysaccharide exposure during the early postnatal period adversely affects the structure and function of the developing rat carotid body. *J Appl Physiol* **121**, 816–827.

Matturri L, Biondo B, Mercurio P & Rossi L (2000). Severe hypoplasia of medullary arcuate nucleus: quantitative analysis in sudden infant death syndrome. *Acta Neuropathol* **99**, 371–375.

Mayer CA, Di Fiore JM, Martin RJ & MacFarlane PM (2014). Vulnerability of neonatal respiratory neural control to sustained hypoxia during a uniquely sensitive window of development. *J Appl Physiol* **116**, 514–521.

Molkov YI, Zoccal DB, Moraes DJ, Paton JF, Machado BH & Rybak IA (2011). Intermittent hypoxia-induced sensitization of central chemoreceptors contributes to sympathetic nerve activity during late expiration in rats. *J Neurophysiol* **105**, 3080–3091.

Montandon G, Bairam A & Kinkead R (2008). Neonatal caffeine induces sex-specific developmental plasticity of the hypoxic respiratory chemoreflex in adult rats. *Am J Physiol Regul Integr Comp Physiol* **295**, R922–R934.

Morley ME, Rand CM, Berry-Kravis EM, Zhou L, Fan W & Weese-Mayer DE (2008). Genetic variation in the HTR1A gene and sudden infant death syndrome. *Am J Med Genet A* **146A**, 930–933.

Niblock MM, Lohr KM, Nixon M, Barnes C, Schaudies M & Murphy M (2012). Cells in the female retrotrapezoid region upregulate c-fos in response to 10%, but not 5%, carbon dioxide. *Brain Res* **1433**, 62–68.

Nock ML, Difiore JM, Arko MK & Martin RJ (2004). Relationship of the ventilatory response to hypoxia with neonatal apnea in preterm infants. *J Pediatr* **144**, 291–295.

Nsegbe E, Wallén-Mackenzie A, Dauger S, Roux JC, Shvarev Y, Lagercrantz H, Perlmann T & Herlenius E (2004). Congenital hypoventilation and impaired hypoxic response in Nurr1 mutant mice. *J Physiol* **556**, 43–59.

Obonai T, Yasuhara M, Nakamura T & Takashima S (1998). Catecholamine neurons alteration in the brainstem of sudden infant death syndrome victims. *Pediatrics* **101**, 285–288.

Pakkenberg B, Møller A, Gundersen HJ, Mouritzen Dam A & Pakkenberg H (1991). The absolute number of nerve cells in substantia nigra in normal subjects and in patients with Parkinson's disease estimated with an unbiased stereological method. *J Neurol Neurosurg Psychiatry* **54**, 30–33.

Panigrahy A, Filiano J, Sleeper LA, Mandell F, Valdes-Dapena M, Krous HF, Rava LA, Foley E, White WF & Kinney HC (2000). Decreased serotonergic receptor binding in rhombic lip-derived regions of the medulla oblongata in the sudden infant death syndrome. *J Neuropathol Exp Neurol* **59**, 377–384.

Panigrahy A, Filiano JJ, Sleeper LA, Mandell F, Valdes-Dapena M, Krous HF, Rava LA, White WF & Kinney HC (1997). Decreased kainate receptor binding in the arcuate nucleus of the sudden infant death syndrome. *J Neuropathol Exp Neurol* **56**, 1253–1261.

Parmelee AH, Stern E & Harris MA (1972). Maturation of respiration in prematures and young infants. *Neuropadiatrie* **3**, 294–304.

Paterson DS, Rivera KD, Broadbelt KG, Trachtenberg FL, Belliveau RA, Holm IA, Haas EA, Stanley C, Krous HF, Kinney HC & Markianos K (2010). Lack of association of the serotonin transporter polymorphism with the sudden infant death syndrome in the San Diego Dataset. *Pediatr Res* **68**, 409–413.

Peng YJ, Rennison J & Prabhakar NR (2004). Intermittent hypoxia augments carotid body and ventilatory response to hypoxia in neonatal rat pups. *J Appl Physiol* **97**, 2020–2025.

Pequignot JM, Spielvogel H, Caceres E, Rodriguez A, Semporé B, Pequignot J & Favier R (1997). Influence of gender and endogenous sex steroids on catecholaminergic structures involved in physiological adaptation to hypoxia. *Pflugers Arch* **433**, 580–586.

Perrin DG, Cutz E, Becker LE, Bryan AC, Madapallimatum A & Sole MJ (1984). Sudden infant death syndrome: increased carotid-body dopamine and noradrenaline content. *Lancet* **2**, 535–537.

Porzionato A, Macchi V & De Caro R (2009*a*). Sudden infant death syndrome. *N Engl J Med* **361**, 2580.

Porzionato A, Macchi V, Parenti A & De Caro R (2009*b*). Morphometric analysis of infant and adult medullary nuclei through optical disector method. *Anat Rec* **292**, 1619–1629.

Porzionato A, Macchi V, Parenti A, Matturri L & De Caro R (2008). Peripheral chemoreceptors: postnatal development and cytochemical findings in Sudden Infant Death Syndrome. *Histol Histopathol* **23**, 351–365.

Porzionato A, Macchi V, Stecco C & De Caro R (2013). The carotid body in Sudden Infant Death Syndrome. *Respir Physiol Neurobiol* **185**, 194–201.

Porzionato A, Rucinski M, Macchi V, Stecco C, Sarasin G, Sfriso MM, Di Giulio C, Malendowicz LK & De Caro R (2012). Spexin is expressed in the carotid body and is upregulated by postnatal hyperoxia exposure. *Adv Exp Med Biol* **758**, 207–213.

Prabhakar NR, Peng YJ, Kumar, GK & Pawar A (2007). Altered carotid body function by intermittent hypoxia in neonates and adults: relevance to recurrent apneas. *Respir Physiol Neurobiol* **157**, 148–153.

Renolleau S, Dauger S, Vardon G, Levacher B, Simonneau M, Yanagisawa M, Gaultier C & Gallego J (2001). Impaired ventilatory responses to hypoxia in mice deficient in endothelin-converting-enzyme-1. *Pediatr Res* **49**, 705–712.

Richerson GB (2004). Serotonergic neurons as carbon dioxide sensors that maintain pH homeostasis. *Nat Rev Neurosci* **5**, 449–61.

Riesco-Fagundo AM, Pérez-García MT, González C & López-López JR (2001). O_2 modulates large-conductance Ca^{2+}-dependent K^+ channels of rat chemoreceptor cells by a membrane-restricted and CO-sensitive mechanism. *Circ Res* **89**, 430–436.

Rigatto H & Brady JP (1972). Periodic breathing and apnea in preterm infants. I. Evidence for hypoventilation possibly due to central respiratory depression. *Pediatrics* **50**, 202–218.

Riley D, Dwinell M, Qian B, Krause KL, Bonis JM, Neumueller S, Marshall BD, Hodges MR & Forster HV (2010). Differences between three inbred rat strains in number of K^+ channel-immunoreactive neurons in the medullary raphe nucleus. *J Appl Physiol* **108**, 1003–1010.

Robinson DM, Peebles KC, Kwok H, Adams BM, Clarke LL, Woollard GA & Funk GD (2002). Prenatal nicotine exposure increases apnoea and reduces nicotinic potentiation of hypoglossal inspiratory output in mice. *J Physiol* **538**, 957–973.

Roepke TA, Malyala A, Bosch MA, Kelly MJ & Rønnekleiv OK (2007). Estrogen regulation of genes important for K^+ channel signaling in the arcuate nucleus. *Endocrinology* **148**, 4937–4951.

Rourke KS, Mayer CA & MacFarlane PM (2016). A critical postnatal period of heightened vulnerability to lipopolysaccharide. *Respir Physiol Neurobiol* **232**, 26–34.

Rudzinski E & Kapur R (2013). Comment on developmental alterations of the human retrotrapezoid nucleus homologue by Lavezzi et al. *Auton Neurosci* **179**, 170–171.

Samarasinghe TD, Sands SA, Skuza EM, Joshi MS, Nold-Petry CA & Berger PJ (2015). The effect of prenatal maternal infection on respiratory function in mouse offspring: evidence for enhanced chemosensitivity. *J Appl Physiol* **119**, 299–307.

Scragg RK, Mitchell EA, Ford RP, Thompson JM, Taylor BJ & Stewart AW (2001). Maternal cannabis use in the sudden death syndrome. *Acta Paediatr* **90**, 57–60.

Sladek M, Parker RA, Grŏgaard JB & Sundell HW (1993). Long-lasting effect of prolonged hypoxemia after birth on the immediate ventilatory response to changes in arterial partial pressure of oxygen in young lambs. *Pediatr Res* **34**, 821–828.

Slotkin TA, Lappi SE, McCook EC, Lorber BA & Seidler FJ (1995). Loss of neonatal hypoxia tolerance after prenatal nicotine exposure: implications for sudden infant death syndrome. *Brain Res Bull* **38**, 69–75.

Sterni LM, Bamford OS, Wasicko MJ & Carroll JL (1999). Chronic hypoxia abolished the postnatal increase in carotid body type I cell sensitivity to hypoxia. *Am J Physiol Lung Cell Mol Physiol* **277**, L645–L652.

St-John WM & Leiter JC (1999). Maternal nicotine depresses eupneic ventilation of neonatal rats. *Neurosci Lett* **267**, 206–208.

Stock C, Teyssier G, Pichot V, Goffaux P, Barthelemy JC & Patural H (2010). Autonomic dysfunction with early respiratory syncytial virus-related infection. *Auton Neurosci* **156**, 90–95.

Stunden CE, Filosa JA, Garcia AJ, Dean JB & Putnam RW (2001). Development of in vivo ventilatory and single chemosensitive neuron responses to hypercapnia in rats. *Respir Physiol* **127**, 135–55.

Tankersley CG (2003). Genetic aspects of breathing: on interactions between hypercapnia and hypoxia. *Respir Physiol Neurobiol* **135**, 167–178.

Thomas DA, Swaminathan S, Beardsmore CS, McArdle EK, MacFadyen UM, Goodenough PC, Carpenter R & Simpson H (1993). Comparison of peripheral chemoreceptor responses in monozygotic and dizygotic twin infants. *Am Rev Respir Dis* **148**, 1605–1609.

Tolcos M, McGregor H, Walker D & Rees S (2000). Chronic prenatal exposure to carbon monoxide results in a reduction in tyrosine hydroxylase-immunoreactivity and an increase in choline acetyltransferase-immunoreactivity in the fetal medulla: implications for Sudden Infant Death Syndrome. *J Neuropathol Exp Neurol* **59**, 218–228.

Ueda Y, Stick SM, Hall G & Sly PD (1999). Control of breathing in infants born to smoking mothers. *J Pediatr* **135**, 226–232.

Valdés-Dapena MA (1980). Sudden infant death syndrome: a review of the medical literature 1974–1979. *Pediatrics* **66**, 597–614.

Ward SL, Bautista D, Chan L, Derry M, Lisbin A, Durfee MJ, Mills KS & Keens TG (1990). Sudden infant death syndrome in infants of substance-abusing mothers. *J Pediatr* **117**, 876–881.

Waters KA & Tinworth KD (2001). Depression of ventilatory responses after daily, cyclic hypercapnic hypoxia in piglets. *J Appl Physiol* **90**, 1065–1073.

Weese-Mayer DE, Zhou L, Berry-Kravis EM, Maher BS, Silvestri JM & Marazita ML (2003). Association of the serotonin transporter gene with sudden infant death syndrome: a haplotype analysis. *Am J Med Genet A* **122A**, 238–245.

West MJ (1993) New stereological methods for counting neurons. *Neurobiol Aging* **14**, 275–285.

Wickström R, Hökfelt T & Lagercrantz H (2002). Development of CO_2-response in the early newborn period in rat. *Respir Physiol Neurobiol* **132**, 145–158.

Wong-Riley MT, Liu Q & Gao XP (2013). Peripheral-central chemoreceptor interaction and the significance of a critical period in the development of respiratory control. *Respir Physiol Neurobiol* **185**, 156–169.

Zhang L, Wilson CG, Liu S, Haxhiu MA & Martin RJ (2003). Hypercapnia-induced activation of brainstem GABAergic neurons during early development. *Respir Physiol Neurobiol* **136**, 25–37.

Zhuang J, Xu F, Campen M, Hernandez J, Shi S & Wang R (2006). Transient carbon monoxide inhibits the ventilatory responses to hypoxia through peripheral mechanisms in the rat. *Life Sci* **78**, 2654–2661.

Additional information

Competing interests

None of the authors has any conflict of interests.

Author contributions

All the authors have contributed to writing the paper. All authors approved the final version of the manuscript. All persons designated as authors qualify for authorship and all those who qualify for authorship are listed.

Funding

This work was funded through a Research Project of the University of Padova (PRAT 2014 Andrea Porzionato: CPDA141382/14).