Invited Review

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HIGHLIGHTED TOPIC | Neural Control of Perinatal Respiration

Long-term effects of the perinatal environment on respiratory control

Ryan W. Bavis1 and Gordon S. Mitchell2

1Department of Biology, Bates College, Lewiston, Maine; and 2Department of Comparative Biosciences, University of Wisconsin, Madison, Wisconsin

Bavis RW, Mitchell GS. Long-term effects of the perinatal environment on respiratory control. J Appl Physiol 104: 1220–1229, 2008. First published January 10, 2008; doi:10.1152/japplphysiol.01086.2007.—The respiratory control system exhibits considerable plasticity, similar to other regions of the nervous system. Plasticity is a persistent change in system behavior triggered by experiences such as changes in neural activity, hypoxia, and/or disease/injury. Although plasticity is observed in animals of all ages, some forms of plasticity appear to be unique to development (i.e., “developmental plasticity”). Developmental plasticity is an alteration in respiratory control induced by experiences during “critical” developmental periods; similar experiences outside the critical period will have little or no lasting effect. Thus complementary experiments on both mature and developing animals are generally needed to verify that the observed plasticity is unique to development. Frequently studied models of developmental plasticity in respiratory control include developmental manipulations of respiratory gas concentrations (O2 and CO2). Environmental factors not specifically associated with breathing may also trigger developmental plasticity, however, including psychological stress or chemicals associated with maternal habits (e.g., nicotine, cocaine). Despite rapid advances in describing models of developmental plasticity in breathing, our understanding of fundamental mechanisms giving rise to such plasticity is poor; mechanistic studies of developmental plasticity are of considerable importance. Developmental plasticity may enable organisms to “fine tune” their phenotype to optimize the performance of this critical homeostatic regulatory system. On the other hand, developmental plasticity could also increase the risk of disease later in life. Future directions for studies concerning the mechanisms and functional implications of developmental plasticity in respiratory motor control are discussed.

EARLY LIFE EXPERIENCES PROFOUNDLY influence the development of vertebrate neural systems. Although an individual’s genetic blueprint sets its developmental trajectory, internal and external environmental factors shape the phenotype that is ultimately realized. Thus experiences during either the prenatal or early postnatal periods often have long-term effects on neural systems whereas similar experiences have only transient effects in mature animals. It is hypothesized that this developmental plasticity enables organisms to fine tune their phenotype to prevailing and/or predicted environmental conditions, potentially serving a key role in normal developmental processes. This flexibility may also lead to harmful phenotypes, however, if environmental cues do not accurately predict future environments or if individuals respond inappropriately to stimuli not anticipated by evolutionary history (e.g., disease, environmental toxins, or medical interventions). Indeed, mismatches between the resulting phenotype and subsequent environment may increase the risk of disease in later life (i.e., the paradigm of developmental origins of adult health and disease; 39). Therefore, it is essential to understand the impact of early life experiences on critical homeostatic control systems, such as the neural control of breathing.

Research on developmental plasticity in respiratory neural control has intensified over the last two decades, and here we review some of what has been revealed through these studies. We begin by establishing what constitutes “developmental plasticity.” Next we review several specific examples of developmental plasticity in respiratory control, focusing on animal models for which complementary studies are available in adults. These studies suggest that diverse stimuli can elicit developmental responses, but the underlying mechanisms are largely unknown. Thus we end by highlighting potential directions for future research.

WHAT IS DEVELOPMENTAL PLASTICITY?

It is now widely acknowledged that the respiratory neural control system exhibits considerable plasticity, similar to other regions of the nervous system. Plasticity is a persistent change in the morphology and/or function of the respiratory control system based on prior experience (e.g., neural activity, hypoxia, injury, or disease) (66). However, this broad definition belies the complexity of plasticity and the reality that there are...
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Different types. For example, plasticity may occur in varying time domains (seconds to minutes, hours to days, or even weeks to years) that reflect distinctly different mechanisms (e.g., rapid transcriptional and translational events, hormonal regulation, or developmental cascades).

Although plasticity is observed in all age groups, some forms of respiratory plasticity appear to be unique to developing animals. This subset of plasticity is commonly described as developmental plasticity. Carroll (19) defined developmental plasticity, as it relates to the control of breathing, as the process “whereby long-standing or permanent alterations in mature respiratory control are induced by experience during “critical” periods of development. The same experience occurring outside of the critical period has little or no lasting effect, indicating that the plasticity depends on time windows during ontogeny when development can be altered in response to the external environment.” Although this definition encompasses most of the accepted examples of developmental plasticity in respiratory control reviewed below, researchers in other fields apply this term more broadly to include plastic responses that are unique to development regardless of their duration, such as environmentally induced changes in the timing of key developmental events (e.g., Ref. 21). In this review, we will apply this more inclusive definition of developmental plasticity to respiratory neural control.

Plasticity expressed during development is not always developmental plasticity: developing animals may express forms of respiratory plasticity that are more-or-less indistinguishable from those observed in mature animals, and these cases should not be classified as developmental plasticity. With the exception of characteristics that are by definition specific to development (e.g., the timing of developmental events), complementary experiments on both immature and mature animals are needed to verify that the observed plasticity is unique to development; however, care must be taken to ensure that the stimulus used to elicit plasticity is comparable between age groups. Because the magnitude of plastic responses can vary in adults as they age (12), developmental plasticity is most reliably detected if the plasticity differs qualitatively from that of the adult (i.e., present vs. absent, increase vs. decrease, permanent vs. transient). Unfortunately, such approaches may overlook valid cases of developmental plasticity. For example, similar phenotypes observed at the whole animal level may have very different underlying mechanisms. As one example, it is conceivable that an environmental stimulus may enhance a ventilatory reflex in both neonates and adults while acting peripherally in one group and centrally in the other. Thus studies at multiple levels of the respiratory control system may be needed to adequately describe the full range of early life experiences that elicit developmental plasticity.

DEVELOPMENTAL PLASTICITY IN RESPIRATORY CONTROL: EXAMPLES

Many environmental factors impinge on the developing nervous system and, therefore, have the potential to durably alter respiratory control. Not surprisingly, numerous studies have investigated the impact of changes in respiratory gas concentrations (O₂ and CO₂) during development on adult respiratory control. These studies generally reveal that O₂ and CO₂ can elicit plasticity throughout the respiratory control system and that persistent changes in breathing are not merely the result of chronic (nonspecific) increases in descending respiratory drive during development. Indeed, experimental treatments that increase perinatal respiratory drive without changing blood gases (e.g., chronic changes in metabolic rates) appear to have no persistent effect on respiratory control (89, 90). Moreover, environmental factors not traditionally or specifically associated with breathing may also have profound effects on the development of respiratory control, including psychological stress and various chemical agents (e.g., nicotine, cocaine). In the following sections, we highlight developmental plasticity in vertebrate respiratory control elicited by respiratory and nonrespiratory stimuli. Because space does not permit an exhaustive review of all potential examples of developmental plasticity, we focus on animal models for which there is sufficient evidence to address whether the observed plasticity is indeed unique to development.

Prenatal hypoxia. The effects of prenatal hypoxia on respiratory control are complex and depend on experimental conditions as well as the species studied. In rats, prenatal exposure to chronic hypoxia (pregnant dams exposed to 10–12% O₂) caused persistent hyperventilation in newborns (38) and in young rats studied at 1 and 3, but not 9, wk of age (76). These rats had augmented acute hypoxic ventilatory responses (HVR) at 1 and 3 wk of age, but the HVR was actually reduced compared with age-matched controls at 9 wk of age (76). Although the mechanism of this developmental plasticity is unknown, prenatal hypoxia reduces postnatal carotid body dopamine expression and norepinephrine turnover in noradrenergic brain stem cell groups (A1, A2, and A5) at similar ages (1 and 3 wk) (76); whether these changes are causally linked to the enhanced HVR requires further study. Importantly, rats and most other mammals exposed to chronic hypoxia as adults also exhibit increased resting ventilation and HVR (i.e., ventilatory acclimatization), but breathing normalizes within a period of hours to days posthypoxia (78); no subsequent decrease in HVR has been reported. Thus plasticity induced by prenatal hypoxia exhibits characteristics unique to development.

Prenatal hypoxia has also been studied in other vertebrates. Newly hatched chicks hyperventilate under normoxic conditions after hypoxia (15–16% O₂) from the fifth day of incubation through hatching (101). These chicks also exhibit reduced ventilatory responses to hypoxia and hypercapnia. In contrast, adult birds generally show a modest increase in parabronchial ventilation during acclimatization to altitude, suggesting increased hypoxic sensitivity (79). These data indicate that chronic hypoxia has age-dependent effects on respiratory control in birds, although it is unknown whether prenatal hypoxia has truly long-lasting effects on breathing. In another recent study, zebrafish (Danio rerio) were raised for the first 7 days postfertilization (P₂ of 30–40 Torr) (107); hatching occurred 2–3 days postfertilization, so this exposure included the very early postnatal period. This treatment had no effect on resting ventilation or ventilatory responses (respiratory frequency or opercular displacement) to acute hypoxia, hypercapnia, or cyanide when fish were studied 3 mo later. Thus there does not appear to be a consistent effect of prenatal hypoxia across vertebrate classes.

Neonatal (postnatal) hypoxia. The mammalian HVR is generally biphasic in neonates, with an initial increase in ventilation (early or augmentation phase) followed by a secondary response. The mammalian HVR is generally biphasic in neonates, with an initial increase in ventilation (early or augmentation phase) followed by a secondary
decline toward, or below, baseline levels (late or depressive phase) (15, 44). Over the first few postnatal days, there is a gradual increase in peak ventilation during the early phase and a decrease in the ventilatory roll-off during the late phase of the response (15) such that the HVR soon resembles the sustained adult response. This postnatal maturation of the HVR may be triggered by the rapid rise in arterial Po2 at birth. Consistent with this hypothesis, the HVR is absent or greatly diminished in young rats, cats, and sheep maintained in hypoxia (10–15% O2) from birth until 0–24 h before study (5 days to 10 wk of age) (28, 42, 43, 94, 113). These effects reflect reduced carotid body responses to hypoxia (42, 43, 99, 113). Indeed, the normal postnatal increase in the early phase of the HVR is largely attributable to resetting of carotid body O2 sensitivity, and this maturation is blocked at the level of the carotid body type I cells when rats are maintained in hypoxia from birth (99). Chronic postnatal hypoxia may also alter central neural development because maturation of the late phase of the HVR, thought to reflect central neural depression (15), is delayed in chronically hypoxic rats (28). Both the delayed timing of developmental events and the direction of the changes in HVR (i.e., decreased in neonates vs. increased in most mammals exposed as adults; Ref. 78) are consistent with developmental plasticity.

Sustained neonatal hypoxia also produces long-term effects on respiratory control in mammals. Whereas two studies found that rats hyperventilate for several weeks after return to normoxia (72, 73), other studies in rats (8) and sheep (94) report no lasting effects on normoxic ventilation. On the other hand, studies consistently report a blunted HVR for at least 5–8 wk in rats and sheep exposed to 1–2 wk of neonatal hypoxia (8, 73, 94); it is possible that these effects are permanent. This form of plasticity may also be sex specific: the HVR is blunted in male rats at 7–9 wk of age, whereas age-matched females exhibit a normal HVR (8). As previously mentioned, HVR is generally augmented after adult mammals are exposed to chronic hypoxia, and these effects rapidly reverse on return to normoxia (78), quite different than the blunted HVR following neonatal hypoxia in rats and sheep. Interestingly, chronic exposure to hypobaric hypoxia does blunt the HVR of adult cats, but these centrally mediated effects reverse within a week on return to normoxia (103); there is no evidence of central nervous system (CNS) involvement in blunted HVR of rats exposed to neonatal hypoxia (see below). Thus the long-lasting changes in HVR observed after neonatal hypoxia appear to be unique to development.

These persistent changes in the HVR of rats and sheep after neonatal hypoxia do not reflect altered function of peripheral chemoreceptors. Although hypoxia delays carotid body maturation, carotid body O2 sensitivity rapidly increases and approaches that of age-matched controls within days after return to normoxia (94, 99). Moreover, Eden and Hanson (28) observed that carotid sinus nerve responses to hypoxia are normal in 5- to 10-wk-old rats maintained in hypoxia (13–15% O2) from birth, even though these rats exhibited a blunted HVR. If changes in carotid body sensitivity are transient, what causes the attenuated HVR after neonatal hypoxia? Phrenic nerve responses to isocapnic hypoxia are normal in anesthetized, adult male rats after neonatal hypoxia, despite blunting of the HVR (8). These data suggest that blunting arises downstream of the phrenic motoneuron, perhaps resulting from changes in respiratory muscles or mechanics. Given that hypercapnic ventilatory responses (HCVR) are normal following neonatal hypoxia (73), our laboratory previously proposed that neonatal hypoxia induces physiological changes in respiratory mechanics that occur specifically during hypoxic breathing (e.g., changes in airway resistance) (8), but this hypothesis has not yet been tested directly.

Recent studies concerning the effects of chronic intermittent hypoxia (CIH) on the development of respiratory control in rats provide additional evidence for long-lasting developmental plasticity following perinatal hypoxia. In rats, the most striking effect of neonatal CIH (10 and 21% O2 alternating at 90-s intervals, 12 h/day for 30 days beginning on the first postnatal day) is an increase in normoxic ventilation that persists into adulthood, and it may be permanent (84, 85); similar effects on normoxic ventilation have been reported following prenatal CIH (40). The magnitude of these effects, and their persistence, decreases with age at onset of exposure and are most pronounced when CIH commences during the first 2 postnatal wk (83, 84). Perinatal CIH also decreases ventilatory and phrenic nerve responses to acute hypoxia, even when the latter is expressed as a percentage of the maximal response to account for differences in baseline respiratory drive (85); CIH may attenuate the acute HVR in neonatal piglets as well (110).

Mechanisms underlying long-term alterations in respiratory control following neonatal CIH are unknown. However, CIH has profound effects on both central and peripheral nervous systems during development. For example, postnatal CIH induces anatomic changes in brain stem nuclei implicated in cardiorespiratory control, specifically those receiving projections from the vagus and glossopharyngeal nerves (86). In particular, CIH enhanced neuronal proliferation within the nucleus ambiguous and reduced the number of neurons in the nucleus tractus solitarius (NTS), the first central relay ofafferent information from peripheral chemoreceptors, labeled following microinjection of a neuronal tracer into the nodose ganglion. Interestingly, CIH has also been shown to enhance carotid body function in neonates. In contrast to reduced carotid body sensitivity in rats exposed to sustained hypoxia from birth (see above), CIH (16 h; 15 s of 5% O2 followed by 5 min of 21% O2) on the day of birth increased the carotid body and ventilatory responses to acute hypoxia measured the following day (75); the HCVR was not affected by this treatment. CIH affects carotid body function more rapidly in neonates than in adult rats (hours vs. days), and these effects do not reverse readily on return to normoxia (80). Although increased baseline carotid body activity could contribute to increased normoxic ventilation in adult rats following neonatal CIH, these results seem at odds with persistent blunting of the HVR (85). These discrepancies could relate to differences in the experimental design (e.g., duration of CIH) or to changes in CNS processing of carotid chemoefferent inputs (e.g., anatomic changes in the NTS).

In addition to changes in normoxic and hypoxic ventilation, neonatal CIH has also been shown to alter the expression of another form of respiratory plasticity, long-term facilitation (LTF). LTF is a long-lasting increase in respiratory motor output following repeated episodes of hypoxia (see Ref. 66 for review), and previous studies have shown that pretreatment with CIH enhances phrenic and ventilatory LTF in adult rats (58, 64). McGuire and Ling (63) observed that neonatal CIH
(11–12% \text{O}_2 and 21% \text{O}_2 alternating at 5-min intervals, 12 h/day for 7 days beginning 2 days after birth) also enhanced ventilatory LTF, although the enhancement lasted considerably longer (>3 wk vs. <1 wk in adults; 64). In contrast, Reeves et al. (85) found that their neonatal CIH protocol (10% and 21% \text{O}_2 alternating at 90-s intervals, 12 h/day for 30 days beginning on the first postnatal day) reduced phrenic LTF subsequently measured in young adult rats (7–9 wk of age). The reason for the opposing results of these two studies is unclear, but potential explanations include differences in the CIH protocol and differences in how LTF was measured (i.e., poikilocapnic ventilation in awake animals vs. isocapnic phrenic motor output in anesthetized and vagotomized animals). In any case, these data suggest that CIH can have prolonged, age-specific effects on the expression of LTF.

Hyperoxia. Just as postnatal hypoxia from birth appears to delay postnatal resetting of carotid body \text{O}_2 sensitivity in mammals, brief prenatal hyperoxia hastens this maturation. Blanco et al. (16) ventilated the lungs of late-gestation fetal sheep in utero to increase arterial \text{PO}_2 above normal (i.e., \sim 180 vs. \sim 28 Torr). Sheep receiving hyperoxic ventilation (>24 h) exhibited increased carotid body sensitivity to hypoxia relative to sheep receiving normoxic ventilation. In contrast, prolonged exposure to hyperoxia during the early postnatal period causes life-long impairment of hypoxic responses (4, 33, 57). In kittens raised in 30% \text{O}_2 for the first 12–13 days of life, the acute HVR is abolished immediately following the hyperoxic exposure (43). Ling et al. (56) subsequently demonstrated that rats exposed to 60% \text{O}_2 for the first month of life have dramatically reduced HVR as adults (3–5 mo old). In that study, hyperoxia-treated rats increased ventilation only one-third as much as the untreated control group at similar arterial \text{PO}_2. Similar observations have now been made for adult rats previously exposed to only 1–2 wk of 30 or 60% \text{O}_2 (7, 10) or to 2 wk of intermittent exposure to 60% \text{O}_2 (21 or 60% \text{O}_2 at 1-h intervals; 10) in the early postnatal period; this plasticity does not differ between sexes (10, 33). The HCVR of adult rats is not altered by perinatal hyperoxia (56), indicating that this plasticity is specific to the hypoxic chemoreflex vs. a more general impairment of the respiratory system. Furthermore, these effects of hyperoxia are unique to development because exposure to similar levels and durations of hyperoxia have no lasting effect on respiratory control if presented after the second postnatal week (6, 56, 57). The effects of developmental hyperoxia on normoxic ventilation are not consistent across studies, with different groups of rats showing either mild hyperventilation (10, 56) or no persistent changes as adults (7, 10).

Prolonged neonatal or perinatal hyperoxia impairs carotid body development, thereby attenuating the HVR (4, 57). Hyperoxia-treated rats develop smaller carotid bodies with fewer \text{O}_2-sensitive (type I) cells and fewer neurons in the carotid sinus nerve (CSN) to carry afferent information to the brain stem (14, 29, 81). Carotid body hypoplasia appears to result from a failure of the carotid body to grow during the hyperoxic exposure rather than death of existing cells (109, S. E. Piro, E. K. Dmitrieff, and R. W. Bavis, unpublished observations); chemosensory neuron degeneration may be secondary to the carotid body hypoplasia (29, 47). Fewer type I cells and primary afferent neurons likely explain much of the reduction in HVR, but hyperoxia may also impair the \text{O}_2 sensitivity of the surviving cells. Immediately after a 2-wk exposure to 60% \text{O}_2, single-unit chemoreceptor \text{O}_2 responsiveness is reduced, and the conduction time of action potentials between the carotid body and petsosal ganglion \text{O}_2 responsiveness is increased (24); some of these effects may be mediated by reduced expression of \text{O}_2-sensitive \text{K}^+ channels (TASK-1, TASK-2, and TASK-5) in the type I cells following perinatal hyperoxia (50). Similarly, Prieto-Lloret et al. (81) report that \sim 75% of the CSN fibers and carotid body preparations that respond to increasing extracellular \text{K}^+ fail to respond to hypoxia in adult rats exposed to developmental hyperoxia, suggesting a long-lasting loss of \text{O}_2 sensitivity in these cells. As a result, adults previously exposed to perinatal hyperoxia exhibit severely attenuated CSN responses to hypoxia (14, 33, 57, 81).

There currently is no evidence suggesting that impaired function of the central nervous system contributes to the attenuated HVR of adult rats after perinatal hyperoxia. Phrenic nerve responses to electrical stimulation of the carotid sinus nerve are virtually identical between hyperoxia-treated and control rats (33, 57), suggesting that central integration of chemosensory inputs is not impaired. On the other hand, normal phrenic responses to carotid sinus nerve stimulation are somewhat surprising because the number of chemosensory neurons stimulated is reduced in hyperoxia-treated rats (29).

Although this finding could reflect technical limitations of the approach, it may indicate that central neural integration of chemosensory activity is actually enhanced in adult rats raised in hyperoxia. This possibility awaits further study, but it is known that hyperoxia can directly influence central neural function in adult mammals (22).

Although there has been much progress toward understanding the carotid body’s role in the adult HVR after perinatal hyperoxia, mechanisms whereby hyperoxia influences carotid body development are just beginning to be revealed. Three non-mutually exclusive hypotheses include \text{O}_2 toxicity mediated by reactive oxygen species (ROS), hyperoxia-induced suppression of carotid body activity, and gene regulation by \text{O}_2 or ROS (reviewed in Ref. 4). There is little support for the \text{O}_2 toxicity hypothesis, however, because two antioxidants, vitamin E and the superoxide anion scavenger manganese (III) tetrakis (1-methyl-4-pyridyl) porphyrin pentachloride, failed to reduce the effects of perinatal hyperoxia on carotid body size, CSN responses to hypoxia, and/or the acute HVR (11). Recent experiments are consistent with a role for hyperoxia-induced suppression of carotid body activity in this plasticity, however. Activity modulates development in many neural systems by altering gene expression and releasing neurotrophic factors (68), and activity is known to regulate the maturation of dopaminergic carotid chemosensory neurons (47). If hyperoxia alters development of the carotid body by silencing its normal activity, it should be possible to rescue the HVR by experimentally increasing carotid body activity during the hyperoxic exposure. Indeed, rats given intermittent hypercapnia (0 or 7.5% \text{CO}_2 at 1-h intervals) throughout their exposure to perinatal hyperoxia achieved a greater adult HVR than rats exposed to hyperoxia alone (10). Because intermittent hypercapnia had no effect on the HVR in normoxia-reared rats, it is likely that hypercapnia increased carotid body activity and permitted normal, activity-dependent development. Further studies are needed to verify this conclusion and to identify the relevant pathways by which changes in activity might alter...
carotid body development. Moreover, inasmuch as low O₂ regulates the expression of genes linked to cardiorespiratory function (92), additional (direct) effects of high O₂ levels on O₂-sensitive gene expression cannot be ruled out (e.g., Ref. 25).

Other vertebrates have peripheral chemoreceptors thought to be homologous with the mammalian carotid body (65), raising the question of whether hyperoxia has similar effects on peripheral chemoreceptor development and the HVR in these groups. In zebrafish, exposure to hyperoxia (P0₂ = 350–400 Torr) for the first 7 days postfertilization, the period when gill O₂ chemoreceptors are innervated, alters respiratory control into adulthood (107). Specifically, hyperoxia-reared zebrafish (>3 mo of age) exhibit increased respiratory frequency during normoxia and attenuated respiratory responses to acute hyperoxia, cyanide, and hypercapnia. Respiratory control was unchanged when adult fish were exposed to an equivalent 7-day exposure (107); longer hyperoxic exposures (28 days) decrease ventilatory chemosensitivity in adult zebrafish but do not alter normoxic ventilation (108). Japanese quail (Coturnix japonica) also exhibit attenuated HVR as adults following developmental hyperoxia (2–4 wk of 60% O₂) (93). The critical period for this developmental plasticity spans the late prenatal and early postnatal period in quail, and, in contrast to rats (6), equivalent exposures initiated in the postnatal period (i.e., no prenatal exposure) are ineffective at eliciting long-term effects on the HVR (R. W. Bavis and J. C. Simons, unpublished data). The earlier critical period in Japanese quail vs. rats may reflect the earlier maturation of peripheral O₂ sensitivity in precocial birds (69).

Hypercapnia. Perinatal hypercapnia influences postnatal maturation of the HCVR in rats, although there are minimal long-term changes in respiratory control in this species. The HCVR has a complex pattern of maturation in mammals, with an initial decrease in responsiveness and a subsequent increase back toward adult levels (reviewed in Ref. 82). Rats normally reach a minimal HCVR between 6 and 8 days of age, but continuous exposure to 7.5% CO₂ from 5 days before birth delays maturation so that the nadir occurs between 9 and 12 days of age (82). Various studies have also shown that chronic hypercapnia from birth (5–7.5% CO₂ for up to 2 wk) attenuates the acute HCVR and that these effects may last for nearly 2 wk after return to room air (9, 82, 87); the HVR does not appear to be altered by chronic neonatal hypercapnia (87). However, our laboratory and others have shown that these effects do not persist into adulthood (9, 13), and the HVR may normalize after only 2 wk of normocapnic recovery (9). Thus there are no apparent differences between the transient blunting of the HCVR following neonatal hypercapnia and the ventilatory acclimation to chronic hypercapnia in adult rats and other mammals (23). Intermittent hypercapnia (0 or 7.5% CO₂ at 1-h intervals) for the first 2 postnatal wk has no effect on the HCVR at either 2 or 7 wk of age in rats (K. E. R. Russell, J.C. Simons, and R. W. Bavis, unpublished observations); the adult HVR is also unchanged following this intermittent hypercapnia protocol (10).

In contrast to rats, perinatal hypercapnia does elicit long-lasting, if not permanent, developmental plasticity in birds. In female Japanese quail exposed to 2% CO₂ throughout embryonic development, the adult HCVR was reduced by ~25% with no change in baseline ventilation (5). In contrast, the HCVR of male quail was unaffected by the identical treatment in this study, indicating that the expression of this plasticity is sex specific. Reanalysis of an earlier study (112) revealed the same pattern for zebra finches (Taeniopygia guttata), with attenuated HCVR in female, but not male, finches following embryonic CO₂ exposure (5). Sex had no effect on the expression of respiratory plasticity in rats following chronic neonatal hypercapnia (9). The critical period for this developmental plasticity appears to be lengthy in birds because both embryonic (i.e., prenatal; 2% CO₂) and nestling (i.e., postnatal; 5% CO₂) exposures produce qualitatively similar effects on the HCVR in zebra finches (112). The mechanisms underlying this plasticity have not yet been investigated. Although changes in metabolism, gas exchange, and blood buffering do not appear to be involved in the altered HCVR in quail (5), rats exhibit persistent changes in respiratory mechanics following chronic neonatal hypercapnia (88). Therefore, changes in the HCVR could reflect changes in respiratory mechanics rather than CO₂ chemosensitivity per se.

Mild hypercapnia (P CO₂ = 9 Torr) during early development (first 7 days postfertilization) has no effect on respiratory control of adult zebrafish (107).

Neonatal maternal separation. Psychologically stressful conditions, such as restraint, alter ventilatory control in adult mammals (51). To study the long-term consequences of nonrespiratory neonatal stress on respiratory control, Kinkead and colleagues (52) have been using a model of neonatal maternal separation (NMS) in which rat pups are taken from their mother and isolated in an incubator (35°C, 45% relative humidity) for 3 h each day on postnatal days 3–12. Mother-pup interactions have important influences on neural development in mammals, and disrupting this interaction may cause lifelong changes in behavior and the physiological response to stress (reviewed in Ref. 52).

As adults (>2 mo of age), male rats that experienced NMS exhibit normal baseline ventilation but with an increased HVR (35, 36, 52, 53). The effect of NMS on the HCVR of male rats is equivocal: the change in minute ventilation during hypercapnia is somewhat reduced, but the change in ventilation-to-metabolism ratio is not altered (i.e., the animals do not hyperventilate less than controls) (37). The enhanced HVR of male rats appears to involve both peripheral and central neural plasticity. For example, their carotid bodies express more mRNA for tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, and dopamine D₂ receptors after NMS (54); mRNA expression for these genes did not change in the superior cervical ganglion or adrenal gland. Although the role of dopamine in carotid body function is controversial, the changes observed after NMS resemble those that occur during other forms of excitatory carotid body plasticity (e.g., acclimation to hypoxia) (77) and point toward enhanced peripheral chemosensitivity. Furthermore, NMS increases the phrenic nerve response to electrical stimulation of the CSN in anesthetized male rats, indicating that increased gain of the central neural integration of carotid chemosensitive inputs may also contribute to the enhanced HVR (53). In male rats, NMS also disrupts GABAergic modulation of the paraventricular nucleus (PVN) of the hypothalamus, a region that may influence respiratory control through its interactions with key respiratory structures (e.g., NTS, phrenic motoneurons). Indeed, studies of the PVN in these rats reveal l) increased expression of...
GABA_A receptors and 2) greater respiratory responses to pharmacological activation and inactivation of GABA receptors (36). Genest and colleagues (36) suggest that increased expression of GABA_A receptors may (partially) compensate for deficient GABA release or for enhanced excitatory input to the PVN via direct projections from the NTS. Thus NMS effects on respiratory control may involve a disruption of the balance between excitatory and inhibitory processes within cardiorespiratory regions of the CNS in male rats.

In contrast to the enhanced HVR in males after NMS, female rats exhibit a modest decrease in the acute HVR as adults due to a smaller increase in respiratory frequency during hypoxia (35). Consistent with the sex specificity of this plasticity, female rats also showed no change in tyrosine hydroxylase mRNA expression in the carotid body after NMS (54), further suggesting a potential link between increased tyrosine hydroxylase expression and enhanced HVR in male rats. The effects of NMS on the HCVR also differed between the sexes, with NMS females exhibiting a clear increase in the HCVR as adults (37).

The persistent nature of changes in respiratory control after NMS suggests that this plasticity is unique to development, but this point is difficult to address experimentally because NMS is, by definition, specific to development: it is not possible to precisely replicate this treatment in adult animals. If psychological stress (i.e., activation of the hypothalamic-pituitary-adrenal axis) contributes to the long-term respiratory effects of NMS, one surrogate for NMS in adult animals is immobilization stress. Placing male rats in a restrainer for 90 min for 1 or 2 days has no effect on baseline ventilation when studied 24 h later, but these rats do exhibit an attenuated HCVR (51). Thus the effects of immobilization stress on HCVR are qualitatively similar to those produced by NMS, although their time course and underlying mechanisms could still differ. In contrast to NMS, immobilization appears to have no lasting effect on the HVR (51). The different outcomes from these experimental protocols could reflect differences in the specific stressors or the duration of the treatment (2 days in immobilization vs. 10 days for NMS) rather than the age of exposure, but these data are nevertheless suggestive of developmental plasticity in the HVR.

One potential link between stress and changes in respiratory control is the release of stress hormones. Chronic elevation of plasma corticosterone in adult rats enhances the HVR in a sex-specific manner: only males show an increased HVR 14 days after subcutaneous implantation of corticosterone pellets (32). It is noteworthy, then, that the NMS protocol durably enhances the basal activation of the hypothalamic-pituitary-adrenal axis in male rats, but not females, and that this results in increased plasma levels of corticosterone and ACTH (35). Thus the different effects of NMS and immobilization stress on the HVR may be traceable to the age-dependent capacity for neonatal stress to cause long-term changes in stress hormone levels (106), which is itself a form of developmental plasticity. Similarly, differences in stress hormone levels between males and females after NMS may help to explain the sexually dimorphic effects of NMS on respiratory control.

Nicotine, cocaine, and other pharmacological agents. Certain pharmacological agents alter the development of respiratory control, including those sometimes experienced by human fetuses through maternal use or via therapeutic administration in newborns. For example, nicotine binds to nicotinic acetylcholine receptors throughout the nervous system and alters the neurochemistry of structures implicated in the control of breathing (41). The cardiorespiratory effects of prenatal nicotine exposure vary considerably among studies, depending on the species and method of exposure (e.g., cigarette smoke vs. nicotine), among other factors (reviewed in Ref. 41). Based on studies on human newborns and various animal models, a few generalizations are possible. During normoxic breathing, the respiratory pattern may be altered following prenatal nicotine (e.g., rapid, shallow, and variable breathing, more frequent apneas) with little change in overall ventilation. Some of these effects may represent neuromodulation within the CNS, including enhanced synaptic inhibition in the brain stem pre-Botzinger complex (41, 61, 62). The HVR may also be blunted in the very early postnatal period, but this result varies considerably among studies and does not appear to persist beyond the neonatal period (41). Although there is a paucity of data on the effects of chronic nicotine exposure in adults, there is some indication that the effects of nicotine are opposite those observed in neonates. In monozygotic human twins, for example, smokers may have an augmented HVR compared with nonsmoking siblings, with no change in the HCVR (49); acute exposure to nicotine-containing smoke also increases the HVR in humans (114). Thus prenatal nicotine does influence respiratory control development, at least transiently, and some of these effects may be age-dependent.

Cocaine is another drug that influences respiratory control development, perhaps by impairing dopaminergic processes in the CNS and carotid body (60, 105, 111). With respect to normoxic ventilation, there is a tendency toward increased ventilation in guinea pigs and rabbits for several days after birth when exposed to cocaine prenatally (reviewed in Ref. 74) but no persistent change in rats (59) or mice (1); prenatal cocaine exposure also increased the frequency of apneas and periodic breathing during normoxia in piglets (70). Whereas the HCVR appears to be augmented in neonatal guinea pigs (74), no change was reported in the HCVR of mice following prenatal cocaine exposure (1). In young rats (1–2 or 5 days old; 59, 98), mice (2 days old; 1), and piglets (~4 days old; 100), however, HVR was significantly attenuated in cocaine-exposed groups; in rats, at least, these effects may be short lived (i.e., days; 98). Interestingly, St.-John (98) reported that prenatal cocaine exposure delayed the postnatal maturation of the HVR in neonatal rats and that this was responsible for the observed reduction in HVR at 1–2 days of age. In that study, the HVR increased over the first few postnatal days in control pups, but this did not occur in cocaine-exposed pups until day 3 (98); delayed maturation of the eupneic breathing pattern and the HVR response has also been suggested in piglets after prenatal cocaine exposure (70, 71). Studies investigating chronic cocaine exposure on respiratory control in adult animals are unfortunately limited, but in rats the respiratory effects of chronic cocaine administration do not differ noticeably from those observed in neonates (i.e., attenuated HVR with no change in normoxic ventilation or HCVR; 105). Thus, developmentally specific effects of cocaine on respiratory control may be limited to changes in the timing of developmental events.

Although the duration and developmental specificity of cardiorespiratory effects are poorly understood for drugs other
than nicotine and cocaine, other common agents appear to have lasting effects on respiratory control when experienced during the perinatal period (e.g., caffeine, an adenosine receptor antagonist; 17, 67). These observations suggest that the developing respiratory control system may be susceptible to a wide range of environmental chemicals.

FUTURE DIRECTIONS FOR THE STUDY OF DEVELOPMENTAL PLASTICITY

The examples described above provide compelling evidence that environmental factors influence the development of respiratory control, affecting the timing of key developmental events as well as the respiratory phenotype carried into adulthood. Although this review focused on animal models, for which the perinatal environment can be manipulated in controlled experiments, developmental plasticity is expected to have considerable relevance to human health. Indeed, animal models support a causal link between hypoxia in early postnatal life and reduced HVR in some high-altitude populations (55) and in patients with cyanotic heart disease (27, 97) or bronchopulmonary dysplasia (18, 20). Similarly, long-term O2 therapy in infants is correlated with attenuated hypoxic responses (20, 48), much as in rats and other vertebrates exposed to developmental hyperoxia. These studies raise additional questions about the impact of maternal drug use on respiratory control and the long-term implications of pharmacological therapies in neonates or treatments that disrupt normal mother-offspring interactions. Although little is known about long-term health consequences of developmental plasticity in respiratory control, environmentally induced changes in respiratory control likely contribute to cases of sudden infant death syndrome and may predispose infants to a variety of cardiorespiratory diseases such as sleep-disordered breathing, hypertension, and heart failure later in life (e.g., 34, 46, 95). Longitudinal studies in human patients are needed to adequately assess these potential risks.

Considerable progress has been made in cataloging examples of developmental plasticity, and these descriptive studies are critical for understanding the potential impact of the perinatal environment on respiratory control in health and disease. As evidenced by the examples of developmental plasticity discussed above, however, much less is known about the mechanisms underlying such plasticity. Although it is clear that both the peripheral chemoreceptors and the CNS can be involved in respiratory plasticity, systematic evaluations of both peripheral and central contributions in models of developmental plasticity are rare. The importance of such systematic evaluations is considerable. Respiratory plasticity in the intact animal may (indeed is likely to) reflect the combined effects of plasticity at multiple levels of the respiratory control system. Identifying the sites of plasticity will facilitate future studies on the link between the environment and the change in phenotype at the cellular and molecular level.

Even for perinatal hyperoxia and neonatal maternal separation, arguably the best understood examples of developmental plasticity at this point (4, 52), the underlying cellular and molecular mechanisms remain elusive. Indeed, how can relatively brief perturbations of the developmental environment cause long-term changes in the neural control of breathing? Given the enduring nature of developmental plasticity, structural changes are likely to be involved in many cases. For example, as described above, perinatal hyperoxia (29, 109) and neonatal CIH (86) are associated with hypoplasia in the peripheral nervous system and CNS, respectively. The perinatal period is characterized by high rates of synaptogenesis, cellular proliferation, and apoptosis in structures associated with respiratory control (e.g., 104, 109, 115); these processes generally occur during narrow developmental windows, thereby defining potential critical periods for plasticity. Interestingly, neurotrophic factors regulate neuronal survival, growth, target innervation, and maturation throughout the developing nervous system (45), including multiple cell types associated with the control of breathing (e.g., carotid chemosensitive neurons, pontine A5 neurons, motoneurons; 2, 47). To the extent that neurotrophin expression is sensitive to environmental stimuli (e.g., changes in activity of chemosensory neurons; 3), these molecules are particularly attractive candidates for mediating long-term structural plasticity.

Long-term changes in gene expression appear to mediate developmental plasticity within the respiratory control system as well, as evidenced by changes in the expression of neurotransmitters and their receptors in the carotid body and CNS following prenatal hypoxia (76) or exposure to neonatal maternal separation (54). Although environmental regulation of transcription factors is often reversible, their effects may have long-term consequences by altering the trajectory of developmental cascades. Moreover, some transcription factors are expressed only transiently during specific developmental periods, thereby creating a critical period during which the environment might influence the mature phenotype. For example, expression of Phox2 transcription factors is developmentally regulated in petrosal ganglion neurons, being greatest at embryonic day 16.5 and decreasing thereafter in rats, and the expression of Phox2 proteins is highly correlated with the ability of depolarizing stimuli to induce the mature dopaminergic phenotype of these neurons (reviewed in Ref. 47). Environmentally induced changes in neural activity during this window of Phox2 expression may therefore alter the functional phenotype of these neurons. In addition to the interaction between environmental factors and specific transcription factors, durable changes in gene expression can be achieved via epigenetic regulation (e.g., DNA methylation, histone modifications, and chromatin remodeling). Epigenetic regulation is emerging as a major contributor to neural plasticity (31). By regulating whether genes are accessible to transcriptional machinery, these processes are uniquely suited for long-term changes in gene expression. Importantly, epigenetic regulation is responsive to environmental stimuli, particularly during development, and factors such as maternal behavior, diet, and chemical toxins are known to alter DNA methylation and chromatin structure (30, 31, 102). The extent to which these epigenetic processes contribute to developmental plasticity in respiratory control is ripe for study.

Hormonal regulation of respiratory control development is another area that warrants further investigation. There is strong evidence, for example, that stress hormones contribute to respiratory plasticity after NMS (52). Given the variety of environmental factors known to activate the hypothalamic-pituitary-adrenal axis, including hypoxia (26) and hypercapnia (91), stress hormones have the potential to exert widespread influence on respiratory control development. However, the
tremendous diversity of plastic responses (e.g., magnitude and direction of change, affected respiratory variables, etc.) argues against a unifying pathway for developmental plasticity in respiratory control. Given that many of the examples of developmental plasticity reviewed above are sex-specific (rats: neonatal hypoxia, maternal separation and caffeine; birds: prenatal hypercapnia), sex hormones may also influence the expression of developmental plasticity. Although not all sexual dimorphism is directly linked to circulating levels of sex hormones, sex hormones are important modulators of ventilation and metabolism (96) and influence the expression of respiratory plasticity in adults (12). Indeed, perinatal manipulation of sex hormones can itself elicit respiratory plasticity (reviewed in Ref. 96). Therefore, environmental influences on sex hormone levels and the interaction between sex hormones and other pathways that produce plasticity are important areas for future research in respiratory developmental plasticity.

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GRANTS

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