Postnatal developmental expressions of neurotransmitters and receptors in various brain stem nuclei of rats

Qiuli Liu and Margaret T. T. Wong-Riley
Department of Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, Wisconsin

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Liu, Qiuli, and Margaret T. T. Wong-Riley. Postnatal developmental expressions of neurotransmitters and receptors in various brain stem nuclei of rats. J Appl Physiol 98: 1442–1457, 2005. First published December 23, 2004; doi:10.1152/japplphysiol.01301.2004.—Previously, we reported that the expression of cytochrome oxidase in a number of brain stem nuclei exhibited a plateau or reduction at postnatal day (P) 3–4 and a dramatic decrease at P12, against a general increase with age. The present study examined the expression of glutamate, N-methyl-D-aspartate receptor subunit 1 (NMDAR1), GABA, GABA<sub>B</sub> receptors, glycine receptors, and glutamate receptor subunit 2 (GluR2) in the ventrolateral subnucleus of the solitary tract nucleus, nucleus ambiguus, hypoglossal nucleus, medial accessory olivary nucleus, dorsal motor nucleus of the vagus, and cuneate nucleus, from P2 to P21 in rats. Results showed that 1) the expression of glutamate increased with age in a majority of the nuclei, whereas that of NMDAR1 showed heterogeneity among the nuclei; 2) GABA and GABA<sub>B</sub> expressions decreased with age, whereas that of glycine receptors increased with age; 3) GluR2 showed two peaks, at P3–4 and P12; and 4) glutamate and NMDAR1 showed a significant reduction, whereas GABA, GABA<sub>B</sub> receptors, glycine receptors, and GluR2 exhibited a concomitant increase at P12. These features were present but less pronounced in hypoglossal nucleus and dorsal motor nucleus of the vagus and were absent in the cuneate nucleus. These data suggest that brain stem nuclei, directly or indirectly related to respiratory control, share a common developmental trend with the pre-Bötzinger complex in having a transient period of imbalance between inhibitory and excitatory drives at P12. During this critical period, the respiratory system may be more vulnerable to excessive exogenous stressors.

gamma-aminobutyric acid; gamma-aminobutyric acid B receptors; glutamate; N-methyl-D-aspartate receptor subunit 1; glycine receptors

PREVIOUSLY, WE REPORTED THAT the rat pre-Bötzinger complex, postulated as the center of respiratory rhythmogenesis (12, 33, 34, 39), exhibited reduced cytochrome oxidase (CO) activity [a marker of neurons’ metabolic capacity and levels of functional activity (46)] at postnatal days (P) 3–4 and especially at P12, against a general increase with age (22). During those periods, there was a concomitant decrease in glutamate and N-methyl-D-aspartate receptor subunit 1 (NMDAR1), and an increase in GABA, GABA<sub>B</sub> receptor, and glycine receptor expression (22). Glutamate receptor subunit 2 (GluR2), which reduces the permeability of DL-α-amino-3-hydroxy-5-methylisoxazole-propionic acid (AMPA) receptors to Ca<sup>2+</sup>, thereby reducing neuronal excitation, also exhibited an increase during those two periods (22). These results suggest that decreased CO activity at P3–4 and P12 might be associated with an increase in inhibitory drive (mediated by GABA and glycine, their receptors, and possibly by GluR2 through a blockage of Ca<sup>2+</sup> entry) and a decrease in excitatory drive (mediated by glutamate and its receptors).

We also reported that, in several brain stem nuclei, such as the ventrolateral subnucleus of the solitary tract nucleus (NTSVL), nucleus ambiguus (Amb), hypoglossal nucleus (XII), nucleus raphe obscurus, dorsal motor nucleus of the vagus nerve (DMNX), medial accessory olivary nucleus (IOma), spinal nucleus of the trigeminal nerve, and medial vestibular nucleus (MVe), CO activity exhibited a general increase with age from P0 to P21, with MVe having the slowest rise. Notably, in all of the nuclei examined except for MVe, there was a plateau or decrease at P3–4 and a prominent rise-fall-rise pattern at P11–13, similar to that observed in the PBC (23). The data suggest that the two postnatal periods with reduced CO activity, P3–4 and especially at P12, may represent common sensitive periods for most of the brain stem nuclei with known or suspected respiratory control functions.

In the present study, we wished to investigate if the various brain stem nuclei, including the NTSVL, Amb, XII, IOma, and DMNX, undergo a postnatal developmental change in their expressions of several neurotransmitters and receptors (glutamate, NMDAR1, GluR2, GABA, GABA<sub>B</sub> receptors, and glycine receptors), similar to those in the PBC (22). The NTSVL is related to peripheral chemosensitive afferents (10), as well as plays a role in respiratory regulation (4). The Amb, DMNX, and XII contribute to the control of upper airway muscles, the vagus, and the tongue, respectively, during respiration (16, 42). The inferior olivary nucleus is associated mainly with the cerebellum (37), which may be involved in coordinating ventilatory skeletal muscles with upper airway muscles (18). Since these nuclei, as well as spinal nucleus of the trigeminal nerve, nucleus raphe obscurus, and MVe, all showed a prominent rise-fall-rise pattern in CO activity between P11 and P13 (23) as that found in the PBC (22), they may share a common trend in neurochemical development. Thus, for the present study, we chose the cuneate nucleus (CN) as a negative control, as this nucleus is known for its relay function in somatosensory transduction but is not generally regarded as having any respiratory function.

MATERIALS AND METHODS

Tissue preparation. A total of 66 Sprague-Dawley rats from six litters were used in accordance with the National Institutes of Health and Medical College of Wisconsin regulations. At P2, P3, P4, P5, P7, P10, P11, P12, P13, P14, and P21, rats were deeply anesthetized with 4% chloral hydrate (0.1 ml/10 g ip; Fisher Scientific, Fair Lawn, NJ).
and perfused through the aorta with 4% paraformaldehyde in 0.1 M sodium PBS, pH 7.4, with 4% sucrose. Six rats from six different litters were used at each time point. After perfusion, brain stems were removed and postfixed by immersion in the same fixative for 3 h at 4°C. They were then cryoprotected in increasing concentrations of sucrose (10, 20, and 30%) in 0.1 M PBS at 4°C, frozen on dry ice, and stored at −80°C until use.

Coronal sections of frozen brain stems were cut at 12-μm thickness with a cryostat. For each time point, eight sets of serial sections were mounted on gelatin-coated slides. One set was processed for CO histochemistry, whereas the other seven sets were for immunohistochemistry.

Immunohistochemistry. Sections were blocked overnight at 4°C with 5% nonfat dry milk-5% normal goat serum-1% Triton X-100 in 0.1 M PBS (pH 7.4). They were then incubated at 4°C for 36–48 h in the primary antibodies diluted at the appropriate concentrations in the blocking solution. The dilutions were 1:10,000 for neurokinin-1 receptor (Sigma), 1:500 for GABA (Sigma), 1:100 for glutamate (Sigma), 1:1,000 for NMDAR1 (Chemicon), 1:400 for glycine receptors (Chemicon), 1:300 for GluR2 (Chemicon), and 1:400 for GABAB receptors (Chemicon; against a sequence common to both the GABAaR1α and GABAaR1β receptors). Sections were then incubated in the appropriate secondary antibodies: goat anti-rabbit IgG-horseradish peroxidase (HRP; Bio-Rad Laboratories) for neurokinin-1 receptor, GABA, NMDAR1, glycine receptors, and GluR2; goat anti-mouse IgG-HRP (Bio-Rad Laboratories) for glutamate; or goat anti-guinea pig IgG-HRP (Chemicon) for GABAa receptors, GABA, NMDAR1, glycine receptors, and GluR2; goat horseradish peroxidase (HRP; Bio-Rad Laboratories) for neurokinin-1 receptor, GABA, NMDAR1, glycine receptors, and GluR2; goat anti-rabbit IgG-horseradish peroxidase (HRP; Bio-Rad Laboratories) for neurokinin-1 receptor, GABA, NMDAR1, glycine receptors, and GluR2; goat anti-mouse IgG-HRP (Bio-Rad Laboratories) for glutamate; or goat anti-guinea pig IgG-HRP (Chemicon) for GABAa receptors, at 1:100 dilution in the modified blocking solution (without Triton X-100) for 4 h at room temperature. Immunoreactivity was detected with 0.05% 3,3′-diaminobenzidine-0.004% H2O2 in PBS (pH 7.4) for 5 min, and the reaction was stopped with cold PBS (pH 7.4). The sections were then washed three times with cold 0.1 M PBS (pH 7.4), dehydrated, and coverslipped.

CO histochemistry. Mounted sections were incubated in 0.05% 3,3′-diaminobenzidine (Sigma)-0.02% cytochrome c (type III; Sigma)-4% sucrose in 0.1 M PBS (pH 7.4) at 37°C in the dark for 3 h (45). After incubation, sections were washed with cold 0.1 M PBS (pH 7.4) three times for 5 min each, air-dried, and coverslipped.

Quantitative densitometry and statistical analysis. Optical densitometric measurements of reaction product of immunohistochemistry or CO histochemistry were performed with a Zeiss Zonax MPM 03 photometer, a ×25 objective, and a 2-μm-diameter measuring spot. White (tungsten) light was used for illumination, and all lighting conditions were held constant for all measurements. The white matter was used as an internal standard for measurements because of its very low levels of immunoreactivity and CO activity. Thus the white matter was set at zero for each section measured. The optical densitometric value of a single neuron in the various nuclei was an average reading of two to four spots in the cytoplasm. Thirty to eighty neurons in each brain stem nucleus for each rat, and a total of 150–350 neurons for each marker at each age, were measured. The mean optical density values and standard deviations of each marker at each age were then obtained. Each time point for each graph generated for each neurochemical represented the mean value from six animals derived from six different litters. Statistical comparisons were made by using one-way ANOVA (to control for the type I comparison-wise error rate) and Tukey’s Studentized range test (between successive age groups, e.g., P2 vs. P3 and P3 vs. P4, to control for the type I experiment-wise error rate). Significance was set at $P < 0.01$ for one-way ANOVA and $P < 0.05$ for Tukey’s test.

The part of the Amb chosen for quantitative measurements was the rostral loose, semicompact formation innervating upper airway muscles and representing pharyngolaryngomotor and laryngomotor functions (3).

RESULTS

CO activity in the CN. CO activity in the CN showed a relatively high and stable level during the first 3 wk of postnatal development, with just a minor up-down-up fluctuation between P4 and P7 (Fig. 1). Notably, there was not a rise-fall-rise pattern at P11–13 as in the other brain stem nuclei reported previously (22, 23).

Glutamate-immunoreactive neurons in the brain stem nuclei. At P21, glutamate immunoreactivity was observed in ~90% of the neuronal population in the IOa, Amb, CN, and IO. Glutamate-immunoreactive (Glut-ir) neurons were mainly multipolar and oval in shape, with a few fusiform neurons, and mainly small in size (except for the Amb and XII, in which

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**Fig. 1.** A: neurons in the cuneate nucleus (CN) histochemically reacted for cytochrome oxidase (CO) at postnatal day (P) 2 (A1), P7 (A2), P12 (A3), and P21 (A4). Scale bar: 20 μm for all. B: optical densitometric measurements of CO reaction product in the CN during postnatal development. ANOVA showed significant differences among the ages examined ($P < 0.01$). Tukey’s Studentized range tests were done between successive age groups. Significance between one age group and its adjacent younger age group: *$P < 0.05$, ***$P < 0.001$. **
Fig. 2. Glutamate-immunoreactive (ir) neurons in the ventrolateral subnucleus of the solitary tract nucleus (NTSvL; A), nucleus ambiguus (Amb; B), medial accessory olivary nucleus (IOma; C), hypoglossal nucleus (XII; D), dorsal motor nucleus of the vagus nerve (DMNX; E), and cuneate nucleus (CN; F) at P2 (1), P7 (2), P12 (3), and P21 (4).
Glu-ir neurons were mainly medium in size. Glutamate immunoreaction product was not evenly distributed in the cytoplasm, and Glu-ir processes were not distinct (except for the DMNX). In the NTSVL, Amb, and IOma, there was a general increase in glutamate immunoreactivity with age, but with a significant decrease at P12 (Figs. 2 and 3). Glutamate expression in the XII and DMNX showed a statistically significant but less dramatic drop at P12. On the other hand, the CN maintained a relatively steady level of Glu-ir throughout postnatal development, with a slight increase, rather than a decrease, at P12.

NMDAR1-ir neurons in the brain stem nuclei. At P21, NMDAR1 immunoreactivity was expressed in ≈80–90% of neurons in the IOma, XII, and DMNX, and 50–60% of neurons in the NTSVL, Amb, and CN. These neurons were multipolar, fusiform, oval, or pyramidal in shape and mainly small to medium in size. The expression of NMDAR1 in the NTSVL showed fluctuations during development, with a notable drop at P12 and a final rise at P21 (Figs. 4 and 5). In the Amb and IOma, the highest expression of NMDAR1 was at P5–7, and the lowest was at P12. In the XII and DMNX, NMDAR1 immunoreactivity was maintained at a relatively steady level with minor fluctuations throughout development, but the drop at P12 was, nevertheless, notable. This was not the case for the CN, where NMDAR1 immunoreactivity essentially remained the same between P7 and P14.

GluR2-ir neurons in the brain stem nuclei. At P21, GluR2 immunoreactivity was evident in 70–90% of neurons in the IOma, XII, and DMNX, and 55–60% in the Amb, and 40% in the NTSVL. GluR2-ir neurons were mainly multipolar and pyramidal in shape and mainly small to medium in size, with some large neurons in the Amb and XII at P21. The expression of GluR2 in the NTSVL showed fluctuations during development, with a slight increase from P2 to P21, whereas that in the Amb and XII had a slight decrease with age. However, all four nuclei exhibited a small increase at P3 and a dramatic rise at P12 (Figs. 6 and 7). In the DMNX and CN, the expression of GluR2 was maintained at a plateau throughout development, with a minor increase on P11 for DMNX, but no fluctuations between P4 and P13 for the CN.

![Optical densitometric measurements of immunoreaction product for glutamate in the NTSVL, Amb, IOma, XII, DMNX, and CN during postnatal development. ANOVA showed significant differences among ages in each nucleus examined (P < 0.01). Tukey's Studentized range tests were done between successive age groups. Significance between one age group and its adjacent younger age group: *P < 0.05, **P < 0.01, and ***P < 0.001.](http://jap.physiology.org/)
Fig. 4. N-methyl-D-aspartate receptor subunit 1 (NMDAR1)-ir neurons in the NTSVL (A), Amb (B), IOma (C), XII (D), DMNX (E), and CN (F) at P2 (1), P7 (2), P12 (3), and P21 (4).
**GABA-ir neurons in the brain stem nuclei.** At P21, GABA immunoreactivity was found in 30–40% of neurons in most brain stem nuclei examined and in ~20% of neurons in the DMNX. These neurons were mainly small in size and multipolar, oval, or fusiform in shape. The expression of GABA in the NTSVL, Amb, IOma, and XII showed a general decline with age, with a distinct rise at P3 and another rise at P12 (Figs. 8 and 9). The DMNX showed a smaller peak at P3–4 and a larger peak at P12, but against a gradual rise of GABA-ir with age. In the CN, the expression was generally maintained at a steady level, with a smaller rise at P11.

**GABAB receptor-ir neurons in the brain stem nuclei.** At P21, ~80–95% of neurons in the DMNX and XII, 65% in the IOma, and 30–45% in the NTSVL, Amb, and CN expressed GABAB receptor immunoreactivity. These neurons were mainly multipolar or pyramidal in shape and small to medium in size. The patterns of expression of GABAB receptors were somewhat similar to those of GABA (compare Figs. 10 and 11 with Figs. 8 and 9). That is, there was a general decline from P2 to P21, but with a peak at P3 and P12 in almost all of the nuclei examined. Notable features for this receptor were that there was a much more prominent rise in immunoreactivity at P21 in the NTSVL and Amb, and the early peak was more prominent on P4 for DMNX and CN.

**Glycine receptor-ir neurons in the brain stem nuclei.** At P21, ~40–50% of neurons in most brain stem nuclei examined and ~20% in the NTSVL showed glycine receptor immunoreactivity. These neurons were mainly multipolar or oval in shape and small to medium in size. The expression of glycine receptors generally increased with age in all of the nuclei examined, with the most significant increase occurring in the CN (Figs. 12 and 13). In the NTSVL, Amb, IOma, and XII, a small peak was found at P3 and a more prominent one at P12. In the DMNX, the peak occurred at P10, whereas in the CN the highest expression was at P21.

**DISCUSSION**

The present data showed that the general developmental trend for each neurochemical tested is shared by most of the
Fig. 6. Glutamate receptor subunit 2 (GluR2)-ir neurons in the NTSVL (A), Amb (B), IOma (C), XII (D), DMNX (E), and CN (F) at P2 (1), P7 (2), P12 (3), and P21 (4).
brain stem nuclei examined, with the clear exception of the CN, which exhibited a fundamentally different trend. Specific findings regarding the other five nuclei (NTSVL, Amb, IOma, XII, and DMNX) can be summarized as follows. 1) The expression of glutamate increased with age, with a major reduction at P12. 2) NMDAR1 exhibited some developmental heterogeneity among the nuclei, but the distinct decrease in its expression at P12 was shared by all five nuclei. 3) Except for DMNX, GABA expression declined with age, but all five nuclei showed a marked increase in GABA expression at P12 and another increase at P3 or P3–4. 4) The expression of GABAB receptors also declined slightly with age, but there was a distinct peak at P12 in all five nuclei, and a minor rise in all but IOma at P3 or P3–4. 5) On the other hand, glycine receptors showed an increased expression with age, with a major peak expression at P12 and a minor peak at P3 for four of the five nuclei. The exception was DMNX, which showed a major peak at P10 rather than at P12. 6) GluR2 showed a slight increase in expression with age for NTSVL and IOma and a slight decrease in expression with age for the other nuclei; however, in four out of five nuclei tested, there were two peaks: a minor one at P3–4 and a major one at P12. The exception was DMNX, which showed only one peak, and it occurred at P11 rather than at P12.

Thus, in all but the CN, there was a reduction in the expression of excitatory neurotransmitters and receptors and an increase in the expression of inhibitory neurotransmitters and receptors at P12. GluR2 is a glutamate receptor, but it reduces the permeability of AMPA receptors to Ca2+ and therefore reduces neuronal excitation, and its expression paralleled that of inhibitory rather than excitatory neurotransmission receptors. These trends are highly reminiscent of those reported previously by us for the PBC (22). They also correlate well with the reduction in CO activity occurring in these nuclei (NTSVL, Amb, IOma, XII, and DMNX) on P12 (23). Thus nuclei that are more directly involved with respiratory control, especially the first four nuclear groups plus the PBC, exhibited development of neurochemical and metabolic patterns that are quite comparable with each other. The trends in DMNX are similar, although not identical, to those of the above nuclei.

Fig. 7. Optical densitometric measurements of immunoreaction products for GluR2 in the NTSVL (A), Amb (B), IOma (C), XII (D), DMNX (E), and CN (F) during postnatal development. ANOVA showed significant differences among ages in each nucleus examined (P < 0.01). Tukey’s Studentized range tests were done between successive age groups. Significance between one age group and its adjacent younger age group: *P < 0.05, **P < 0.01, and ***P < 0.001.
Fig. 8. GABA-ir neurons in the NTSVL (A), Amb (B), IOrna (C), XII (D), DMNX (E), and CN (F) at P2 (1), P7 (2), P12 (3), and P21 (4).
On the other hand, the expression pattern in the CN is quite different from that in the other nuclei tested. It maintained a relatively high level of expression throughout development for most of the neurochemicals tested, except for a distinct reduction in GABA<sub>B</sub> receptors and a marked rise in glycine receptors with development. Except for a minor rise at P12 against a relatively stable and high expression of glutamate throughout development, there were no statistically significant peaks or valleys on P12 for the other neurochemicals (the rise at P12 for GABA<sub>B</sub> receptors was not statistically different from the level at P11). Likewise, CO activity in the CN also showed a maintained high level throughout development, with no distinct reduction at P12 as in the other nuclei. Thus the CN may already be quite mature at birth, at least with regard to the enzyme and neurochemicals tested in this study (with the exception of the glycine receptors).

As in the case of the PBC, the positive correlation between CO activity and excitatory neurochemicals and the negative correlation between this enzyme and inhibitory neurochemicals in the respiratory-related brain stem nuclei during development, and especially at P12, indicate that the metabolic activity of these nuclei is dictated more by excitatory than inhibitory neurotransmission. This is also true for other regions of the brain tested (46). The progression of metabolic development, which is dependent on the maturation of synaptic organization, may be genetically determined, as carotid body denervation can only delay or prolong, but not eliminate, the overall pattern of metabolic development in the PBC (21). Such developmental trends are likely to exist in other systems, such as the sleep-arousal system or the cardiovascular control system.

The present data also suggest that glutamate may play a more important role with age in the respiratory-related nuclear groups tested. Because the developmental trend of NMDAR1 does not parallel exactly that of glutamate, other glutamate receptors (such as AMPA, kainate receptors, metabotropic glutamate receptors) may also be involved in glutamate neurotransmission in these nuclei. With regard to inhibitory neurotransmission, GABA<sub>B</sub> receptors may play a more important role in the neonate than in the adult, whereas glycine receptors may become more prominent with age.

Fig. 9. Optical densitometric measurements of immunoreaction products for GABA in the NTSvl (A), Amb (B), IOMa (C), XII (D), DMNX (E), and CN (F) during postnatal development. ANOVA showed significant differences among ages in each nucleus examined (P < 0.01). Tukey’s Studentized range tests were done between successive age groups. Significance between one age group and its adjacent younger age group: *P < 0.05, **P < 0.01, and ***P < 0.001.
Fig. 10. GABA<sub>B</sub> receptor-ir neurons in the NTS<sub>NL</sub> (A), Amb (B), IOma (C), XII (D), DMNX (E), and CN (F) at P2 (1), P7 (2), P12 (3), and P21 (4).
The neurotransmitters and receptors examined in this study may play different roles in the neonate and in the adult. Neurons in the NTS are immature at birth, because they possess growth cones and have transient potassium currents, and their dendrites undergo modifications during the first week of life (9). The mechanisms of respiratory rhythmogenesis may also be different between birth and the adult. NMDA receptors mediate particular phasic components of the gasping response during early postnatal life, but not at later stages of development (14). NMDA receptors in the NTS play an essential role in developing the mature expression of the hypoxic ventilatory response (29), but they may not be essential for respiratory rhythm generation or drive transmission in the neonate (13). The AMPA receptor mechanisms modulate components of respiratory pattern generation in the immature, but not in the mature, rat (44). In the spinal cord, GluR2 has a high expression in the early postnatal period, but it declines as the rats mature to adulthood (6). On the other hand, synaptic AMPA receptors in neocortical pyramidal neurons incorporate more GluR2 subunits as neurons undergo a switch in their functional properties (20). Blockade of synaptic inhibition (GABAergic and glycineric transmission) both in vivo and in vitro only disrupts respiratory rhythmic activity after P15 and not before (30). In the spinal cord, GABA synthesizing enzyme glutamic acid decarboxylase isoforms 65 and 67 mRNA declined about threefold in the first 2 postnatal wk (41), comparable to our findings that GABA expression in four of the six nuclei examined declined during the first 2 wk, albeit a peak occurred on P12. GABA<sub>B</sub>, rather than GABA<sub>A</sub> or glycine, receptor-mediated postsynaptic modulation may play a more important role in the respiratory network in neonatal rodents than in the adult (47). Several central systems, such as the auditory and hypoglossal systems, may undergo a functional shift in inhibitory transmission from GABAergic to glycineric during postnatal development (19, 38).

Another point to consider is that neurotransmitters and receptors may undergo developmental transformations, including a switch in receptor subunit composition and subtype shifts that may result in changes in functional properties and kinetics of transmission (1, 27). In the PBC, GABA<sub>A</sub> α<sub>3</sub>-subunit was...
Fig. 12. Glycine receptor-ir neurons in the NTSvL (A), Amb (B), I0ma (C), XII (D), DMNX (E), and CN (F) at P2 (1), P7 (2), P12 (3), and P21 (4).
expressed at relatively high levels at P0 but declined with age, whereas GABA$_A$ $\alpha_1$-subunit was expressed at relatively low levels at P0 but increased with age, and the two trends intersected at P12 (24). These switches may be associated with possible changes in GABA$_A$ receptor subtypes that would mediate different functional properties of GABA transmission (5). This mechanism may contribute partially to the dramatic reduction in CO activity within the PBC at P12 (22). In the murine PBC, the reversal potential of GABA$_A$ receptor-mediated current switched from depolarizing to hyperpolarizing within the first postnatal week (35). This may or may not contribute to neurochemical changes presently observed at P3–4 in various brain stem nuclei; such a current switch has not yet been reported in the rat. Besides GABA, other neurotransmitter systems, such as glycine, glutamate, and serotonin, may also play important roles in synaptic and functional maturation, as they also undergo marked changes in gene expression during the development of the rat brain (7, 11, 20, 25, 26, 32, 41, 43).

The central respiratory network of the mouse may appear to be mature at P15 (31), and the hypoglossal motoneurons may have adultlike features by P16 (38). Our data suggest that neurochemical composition underlying synaptic organization in a number of respiratory-related nuclei is still undergoing major adjustments at P12 and that the mature state is not reached until after this date, perhaps at P21 or later, in the rat. A number of the brain stem nuclei examined are functionally connected. The NTSVL receive peripheral chemosensitive afferents (10) and send their axons to the ventral respiratory group (including the PBC and Amb) (28). The propriobulbar neurons in the PBC are interconnected with bulbospinal neurons and other brain stem cardiorespiratory groups (such as parabrachial nucleus, Kölliker-Fuse nucleus, and others) (2). The bulbospinal I neurons in the ventral respiratory group also have connections with other medullary respiratory neurons, such as the DMNX, XII, and NTSVL (2). The inferior olivary nucleus may be involved in the respiratory network via the cerebellum, which exerts a substantial influence on breathing.
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Thus these nuclei may be functionally interconnected during postnatal development as well as in the adult.

In addition to respiratory-related functions, several nuclei examined are also involved in nonrespiratory roles. For example, the solitary tract nucleus (17) and the XII may be involved in the weaning process, the Amb is known to play an important role in cardiovascular regulation (36), the dorsomotor nucleus of the vagus is involved mainly in the parasympathetic system (8), and the inferior olive is intimately connected with the cerebellum (37). Those functions or behaviors may also undergo significant changes during postnatal development.

In summary, our previous and present data strongly suggest that a critical period exists in the respiratory control system of the rat. During this period (around P12 in the rat), the system is dominated by inhibitory drive due to a transient imbalance between excitatory and inhibitory neurotransmission, leading to suppressed metabolic activity in a number of respiratory-related nuclei in the brain stem. If such periods exist in the human, and if a vulnerable infant is exposed to some external respiratory stressors during this sensitive period, the infant may not be able to respond adequately, and catastrophic events, such as sudden infant death syndrome, may occur.

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