

Primordial respiratory-like rhythm generation in the vertebrate embryo

Jean Champagnat and Gilles Fortin

Address : Biologie Fonctionnelle du Neurone, Institut Alfred Fessard, CNRS., 1, av. de la Terrasse, 91198 Gif-sur-Yvette France.

Running head: Rhythmogenesis and segmentation in embryonic hindbrain

Key words: Hindbrain, Rhombomeres, Development, Respiration, Central pattern generators, Transcription factor, *Kreisler*, *Krox-20*, *Hoxa-1*.

Correspondence: J. Champagnat, Institut Alfred Fessard, C.N.R.S.,
91198, Gif-sur-Yvette, France.

Phone: 33 (1) 69 82 34 04

Fax: 33 (1) 69 07 05 38

Number of : pages, 11

main text : words, 2369 ; figures, 5 ;

box : words 757 ; figure, 1

Summary

Respiration is a rhythmic motor behavior that appears in the fetus and acquires a vital importance at birth. It is generated centrally, within neuronal networks of the hindbrain. This region of the brain is of particular interest since it is the best understood with respect to the cellular and molecular mechanisms that underlie its development. Examination of hindbrain activities in the chick embryo has revealed that the central rhythm generator is active before foetal maturation and conforms to the rhombomeric organization of the embryonic hindbrain. Inactivation of genes required for the normal formation of rhombomeres in mice leads to perturbations of the reticular formation that affect respiration after birth and compromise the probability of survival. From studies of hindbrain development we may gain an understanding of how genes govern the early embryonic development of neuronal

networks and how this might specify patterns of motor activities operating throughout life.

How the respiratory rhythm is generated is a fascinating problem that has interested cellular neurobiologists, medical scientists and comparative physiologists. The rhythmic respiratory-related neuronal network has been located within the hindbrain, in the pons and the medulla. In mammals, it is active before birth and controls respiratory-like rhythmic movements that are among the earliest detectable behaviors of the fetus (box 1). It is vital after birth and its impairment might contribute to sudden infant death syndromes that are the primary cause of death between one month and one year of age in developed countries. The genetic analysis of human diseases with aberrant ventilatory control led to the hypothesis of a genetic control of breathing which, however, remains difficult to differentiate from confounding environmental effects. Genetic mechanisms postulated from studies of human twins¹ and inbred mice strains² remain to be elucidated. The basis of variation of respiratory parameters in mammalian species³ is also unknown. The present review specifically examines genes that affect respiratory parameters such as frequency, by controlling the organization of the central rhythmic neuronal network. Study of the evolution of the network in vertebrates suggests that such genes exist and are expressed during the early development of the hindbrain.

Conserved features of the hindbrain in vertebrates

The ability to produce rhythmic motor behaviors generated centrally and linked to respiratory function is a property of the brainstem reticular formation which has been remarkably conserved during the phylogeny of vertebrates^{4,5,6} including agnathians⁷, teleostean fishes^{4,6}, lungfishes⁸, amphibians⁹, reptiles¹⁰ and birds^{11,12}. Therefore, conservative developmental mechanisms orchestrating the organogenesis of the brainstem in all vertebrates¹³ are probably crucial for breathing. In all vertebrates, the hindbrain is one of the vesicles that appears towards the anterior end of the neural tube. The hindbrain neuroepithelium becomes partitioned into an iterated series of cellular compartments along the antero-posterior axis, which are called rhombomeres (r, Figure 1A). This segmentation process is transient (during the second half of the first month in humans, between E8 and E12 in mice, between stages¹⁴ 9 and 24 in chicks). It persists afterwards in the germinative zone (until stage 36 in chicks)¹⁵ and it is believed to determine neuronal fates by encoding segment-specific positional information¹³. For example, differentiation and spatial distribution of branchiomotor neurons conform to this rhombomeric pattern with a two-segment periodicity¹⁶. A wealth of data have been accumulated on genes which are expressed in the embryo and govern the hindbrain segmentation. Hox homeobox genes form four conserved clusters encoding transcription factors that orchestrate ontogenesis along the rostro-caudal axis of the body, including hindbrain segmentation and limb formation¹⁷. Hox expression in the hindbrain is also directed by conserved regulatory genes, such as the r3/r5 enhancer Krox-20¹⁸.

We are only now beginning to understand how the rhythmic hindbrain network appears in the embryo. Insights into this system are coming from the investigation of rhythmic networks in the embryo and postnatal respiration of mice in which genes controlling segmentation of the hindbrain have been knocked out.

Rhythm generation starts in embryo and conforms to the rhombomeric pattern of the hindbrain

Electrophysiological recordings performed on an isolated preparation of chick embryo hindbrain, revealed that by the end of the segmentation period (stage 24) the hindbrain neuronal network starts to exhibit a consistent and organized activity in the form of recurring episodes composed of burst discharges that occur simultaneously in the different cranial nerves¹⁹ (Figure 1A). At this stage the neuronal network is already organized with distinct reticular and motor neurons. Cross-correlation analysis of primordial activities recorded in the trigeminal, facial and glossopharyngeal rootlets demonstrated that the generated rhythm was transmitted to the different motoneuronal pools through multisynaptic reticular relationships²⁰. This system was named « intersegmental coactivator » (C in Figure 1) to emphasize the difference with the adult network in which paucisynaptic relationships exist between respiratory activities in cranial (and spinal) nerves (see ref. 20 for a discussion). When intersegmental relationships are interrupted by transverse sectioning of the hindbrain rostral and caudal to the exit of the branchiomotor nerve (Figure 1B), the ability to generate the rhythmic pattern is preserved in each transverse slice²⁰. Each pair of rhombomeres thus contains its own rhythm generator (G in Figure 1), an organization that contrasts with the restricted location of respiratory neuronal groups in the fetus or in the adult (box1). The rostrocaudal patterns of rhythm present before and after surgical isolation are different²⁰, with, for example, faster rhythms in more rostral hindbrain segments (Figure 1B). Thus, neurons in different rhombomeres probably differ in membrane electrical properties or network organization, in agreement with the proposed role of segmentation in the supply of neurobiological substrates with both redundant elements and discrete variations in local properties^{13,16}. Long duration recordings of the isolated hindbrain (Figure 2A) also demonstrate that the primordial rhythm generator matures after stage 31 in a relative autonomy with respect to extrinsic factors¹⁹. Altogether analysis of the chick embryo shows that the embryonic hindbrain contains reticular co-activators and rhythm generators which, like motoneurons, seem to be organized in register with the rhombomeric organization.

Early specifications in the embryonic hindbrain are vital after birth

At stage 18, a set of eight reticular neuronal phenotypes has been characterised in chick rhombomeres from their pattern of axonal trajectories²¹, providing anatomical evidence for the co-activating system. When active after stage 24, these cells belong to the rhythmic network (Figure 2B). Clonal analysis after intracellular labelling of single cells at stages 7-8 revealed that determination of ultimate neuronal phenotypes may occur in precursor cells, before the end of mitotic expansion and dispersal²². Such early phenotypic choices would also be determinant in conferring electrophysiological properties, such as the ability to generate a rhythm. Therefore, specification of future rhythm generators, co-activators or motoneurons would take place within the yet unsegmented hindbrain and until cells are segregated into the different rhombomeres (Figure 3). Specification might also take place during cell migration within the stereotyped environment of individual rhombomeres.

Because hindbrain segmentation is transient and followed by a dramatic reconfiguration of neurons and synapses during the foetal and neonatal brainstem maturation (box 1), the advent of mutant mice will help to understand whether and

how segmentation influences the respiratory network after birth. Three genes in particular, *Kreisler*, *Krox-20* and *Hoxa-1* (Figure 3, middle), have been shown to play roles in the early steps of the hindbrain segmentation process: after disruption of these genes, the developing hindbrain is shortened by loss or severe reduction of rhombomeres corresponding to their expression domain (r5 and r6 for *Kreisler*^{23,24}, r3 and r5 for *Krox-20*^{25,26}) or within the rostral part of their expression domain (r4, r5 and r6 for *Hoxa-1*^{27,28,29,30}). Life-threatening deficiency of a rhythm promoting system has been described in *Krox-20*^{-/-} mice³¹. Neurons of this system have been located in the caudal pontine reticular formation by recording rhythmic activity from the isolated hindbrain. In vivo, all the *Krox-20*^{-/-} animals show an abnormally low respiratory frequency (Figure 4B). In addition, during the first day after birth, apnoeas last 10-times longer than normal and about two-thirds of the animals die. The remaining third survives for several weeks. In contrast, *Kreisler*^{-/-} mice are viable and fertile while all *Hoxa-1*^{-/-} die from anoxia shortly after birth and central respiratory deficits have been proposed a possible cause of the death²⁷. Malformation of glossopharyngeal and vagal sensory afferents^{27,28} (yellow arrow in Figure 4) contributes to hypoventilation of *Hoxa-1*^{-/-} animals ; however observations in other mutants (*BDNF*^{-/-}) show that respiratory deficits due to the exclusive impairment of sensory afferents are not lethal during the first day after birth³². Observations of transgenic mice therefore confirm that the fate of neurons greatly varies in different rhombomeres. After birth, systems specified in r3 and r4 (violet and blue in Figure 4) retain a vital importance for breathing after birth while those specified in r5 and r6 (green in Figure 4) do not.

Early rhythm maturation in the hindbrain

Network organization in the embryo hindbrain is reminiscent of the primitive rhythmic motor control of the buccal cavity by trigeminal and hypoglossal nerves co-active with facial, glossopharyngeal and vagal control of the opercular cavity in lower vertebrates^{5,6}. In contrast, respiratory pump muscles in mammals control thoracic and abdominal cavities (box 1) while trigeminal or facial motoneurons have only accessory respiratory function. Thus, reconfiguration of redundant rhythmogenic circuits present in the embryo appears to be operated by a regressive scheme by which the major respiratory neuronal groups (box 1) are located rostrally and caudally in the brainstem within territories probably derived from r1 or the isthmus region (PRG) and r6-r8 (VRG, DRG), according to fate maps^{33,34}. This is probably why *Kreisler* mice preserve viability despite rather extensive defects of the embryonic network in r5. Segment-specific regression of rhombomeric rhythm generators also explains observation in vitro of a rhythm generator located near to the trigeminal nucleus in lamprey⁷ and much more caudally in mammals (box 1).

New control systems might also emerge in the foetal hindbrain somehow independently of the hindbrain segmentation. In the caudal pontine reticular formation (figure 4A), a population of noradrenergic reticular neurons, the A5 group, exerts a depressant effect on the respiratory frequency that appears during foetal gestation (see box 1). This cell population is unaffected in *Krox-20*^{-/-} although a large number of non-noradrenergic neurons are eliminated in the same area³¹: the A5 neurons are densely packed (Figure 5) and the rhythm depressant systems predominate functionally in the caudal pons. The depressant controls might therefore have developed late, in the unsegmented hindbrain of the fetus or, alternatively, been specified outside r3 or r5 and migrated after the elimination of spatial restriction and boundaries between rhombomeres.

The mechanisms by which rhythm controlling properties are lost or respecified in some part of the primordial network are unclear at present. Experiments in chick indicate that maturation may result from the incorporation into the neuronal networks of cells deriving from mitotic precursor cells that remain confined within rhombomeric domains of the ventricular zone until stage 36¹⁵. Alternatively, neurons might have escaped spatial restriction, after having acquired positional specification³⁵. Finally, during gestation as well as after birth, the network is probably influenced by a variety of neurotrophic factors that control neuron number in selective populations. Recently, transgenic mice lacking BDNF have been shown to exhibit chemoafferent neuron losses distinct from mice lacking NT4 and to display a severe deficit in the control of breathing³².

Concluding remarks

Respiratory frequency in mice and probably many aspects of respiratory patterns in vertebrates appear to be partly specified by genes controlling segmentation of the hindbrain and organization of a primordial rhythm generator. Analysis of this generator supports the hypothesis of an early specification of cell properties that are involved in the rhythm generation. Therefore, an immediate concern of future research is to take advantage of the embryonic hindbrain preparation to identify neurons and membrane molecules critical for rhythmogenesis and gene mutations that would affect the early maturational steps in the hindbrain rhythmic network.

During transition from fish to tetrapod, a major evolutionary step in the respiratory system^{6,7,8,12}, there has been a rapid (ca. 10 million years during the Upper Devonian) transformation of both the hindbrain neurocranium and the limbs, while many other characters have evolved more slowly³⁶. Such co-evolution argues in favour of a link between genetic or developmental processes that govern the brainstem and limb anatomy, and the respiratory function. Combined neurobiological and genetic analysis of signalling pathways might lead in the near future to the understanding of how maturation of hindbrain rhythmogenesis is correlated with maturation in distant systems such as the limbs. Atavistic traits of the rhythm generation might be revealed, as recently described for the limbs³⁷.

Selected references

- 1 Kobayashi, S.,M. et al.** (1993) *Am. Rev. Respir. Dis.* 139, 1192-1198
- 2 Tankersley, C.G., Fitzgerald, R.S., Kleeberger, S.R.** (1994) *Am. J. Physiol.* 267, R1371-R1377
- 3 Mortola, J.P.** (1987) *Physiol. Rev.* 67, 187-234
- 4 Adrian, E.D. and Buytendijk, F.J.J.** (1931) *J. Physiol.* 71, 121-135
- 5 Milsom, W.K.** (1991) *Annu. Rev. Physiol.* 53, 87-105
- 6 Ballintijn, C.M.** (1982) in: *The Neurobiology of the Cardiorespiratory System*, E.W. Taylor ed., Manchester Univ. Press, Manchester, pp 3-27

- 7 **Rovainen, C.M.** (1983) *Neuroscience* 10, 875-882
- 8 **Pack A.I. et al.** (1993) in: *Respiratory Control*, Speck, D.F., Dekin, M.S., Revelette, W.R. and Frazier, D.T. eds, Univ. Press of Kentucky, Lexington, pp 52-57
- 9 **McLean, H.A., Perry S.F. and Remmers J.E.** (1995) *J. Comp. Physiol. A* 177, 135-144
- 10 **Takeda, R. et al.** (1986) *Respiration Physiology* 64, 149-160
- 11 **Wild, J.M.** (1993) *Brain Research* 606, 319-324
- 12 **Fortin, G. Foutz A.S. and Champagnat J.** (1994) *NeuroReport* 5, 1137-1140
- 13 **Lumsden A.** (1990) *T.I.N.S.* 13, 329-335
- 14 **Hamburger, V. and Hamilton, H.L.** (1951) *J. Morphol.* 88, 49-92
- 15 **Wingate R.J.T. and Lumsden A.** (1996) *Development* 122, 2143-2152
- 16 **Lumsden, A. And Keynes, R.** (1989) *Nature* 337, 424-428
- 17 **Krumlauf, R.** (1994) *Cell* 78, 191-201
- 18 **Nonchef S. et al.** (1996) *P.N.A.S.* 93, 9339-9345
- 19 **Fortin, G., Champagnat, J. and Lumsden, A.** (1994) *NeuroReport* 5, 1149-1152
- 20 **Fortin et al.** (1995) *J. Physiol.* 486, 735-744
- 21 **Clarke, J.D.W. and Lumsden, A.** (1993) *Development* 118, 151-162
- 22 **Lumsden A. et al.** (1994) *Development* 120, 1581-1589
- 23 **Cordes S.P. and Barsh G.S.** (1994) *Cell* 79, 1025-1034
- 24 **McKay I.J. et al.** (1995) *Development* 120, 2199-2211
- 25 **Schneider-Maunoury et al.,** (1993) *Cell* 75, 1199-1214
- 26 **Swiatek P.J. and Gridley T.** (1993) *Genes and Development* 7, 2071-2084
- 27 **Lufkin, T. et al.** (1991) *Cell* 66, 1105-1119
- 28 **Chisaka, O. Musci T.S. and Capecchi M.R.** (1992) *Nature*, 355, 516-520
- 29 **Mark, M. et al.** (1993) *Development* 119, 319-338
- 30 **Dollé, P. et al.** (1993) *P. N. A. S.* 90, 7666-7670
- 31 **Jacquin T.D. et al.** (1996) *Neuron*, in press
- 32 **Erickson, J.T. et al.** (1996) *J. Neurosci*, in press
- 33 **Tan, K. and Le Douarin, N.M.** (1991) *Anat. Embryol.* 183, 321-343
- 34 **Marin, F. and Puelles, L.** (1995) *European J. of Neurosci.* 7, 1714-1738

35 Birgbauer and Fraser (1994) *Development* 120, 1347-1356

36 Ahlberg, P.E., Clack, J.A. and Luksevics, E. (1996) *Nature* 381, 61-64

37 Sordino, P., Van der Hoeven, F. And Duboule, D. (1995) *Nature* 375, 678-681

38 Chavrier P. et al. (1988) *EMBO J.* 7, 29-35

39 Wilkinson, D.G. et al. (1989) *Nature* 337, 461-464

40 Murphy, P. and Hill, R.E. (1991) *Development* 111, 61-74

Legends of the figures

Fig. 1. Rhythmic activity of the embryonic hindbrain. The hindbrain is isolated in a recording chamber constantly superfused with an artificial cerebrospinal fluid. Activity of cranial rootlets is recorded close to their exit point (in this figure, trigeminal (5n) and facial (7n) rootlets in r2 and r4). The nerve activities are integrated as illustrated in figure 2B: upward deflections indicate bursts of activity. **(A)** The segmented hindbrain at the Hamburger-Hamilton¹⁴ (HH) developmental stage 24 (left, fp: floor plate, r2-r8: rhombomeres) generates a permanent rhythmic activity (right). Note the co-activation of different rhombomeres (simultaneous bursts of 5n and 7n activities indicated by the yellow rectangle). **(B1)** Rhythm is generated in trigeminal and facial segments isolated by hindbrain transections (blue arrows) at stage (HH32). The metameric organization of rhythm generators persists after disappearance of segmentation (which at this stage is restricted dorsally to the proliferative zone¹⁵, not shown). The rhythmic pattern exhibits segment-specific variations²⁰: that generated by the isolated facial segment remains similar to that recorded before sectioning, suggesting a somehow dominant role of the facial coactivator. Comparing this facial rhythm with that recorded from trigeminal (and glossopharyngeal or hypoglossal) nerves also revealed a rostrocaudal trend in which the more rostral levels generate a faster rhythm than the more caudal ones; Fortin, Lumsden, Champagnat unpublished recordings. **(B2)** Schematic presentation of three mechanisms operating in the rhythmic hindbrain. Co-activator units (C, yellow) are responsible for the simultaneous activity of efferent units (motor nuclei and nerves, red) in different hindbrain segments (anterior to the top). The rhythmic activity results from segmental generator units (G, blue) and not from a single common generator as demonstrated in B1 by acute isolation of hindbrain segments (blue arrows).

Fig. 2. Pattern of burst formation in the chick embryonic hindbrain. **(A)** Factors intrinsic to the hindbrain are responsible for rhythm maturation at stage 31. The integrated glossopharyngeal nerve activity (9n) is recorded ex-ovo at the beginning of the developmental stage 31, for 5.5 h (time after isolation of the hindbrain is indicated above traces). Rapid changes of rhythmic motor patterns provide valuable criteria in discerning the detail of maturation states¹⁹: the rate at which additional discharges are acquired (one by one every 2 hours at stage 31) agrees well with the average number of discharges at stages 24-30 (compare recording at 0.5 h with Figure 1A) and 32 (compare recordings at 5.5h with Figure 1B1, top). **(B)** Bursting activity of a reticular neuron at stage 32. The neuron is

anatomically identified (left) by the axon trajectory towards the medial longitudinal fasciculus (mlf ; a : anterior, m : medial). Whole-cell patch clamp recording of the reticular neuron (right, Em) showing membrane depolarization and firing of action potentials during bursts of nerve activity. Four successive bursts from one episode are shown. Nerve activity (7n, middle) is integrated with a time constant of 50 ms (top, arbitrary units on ordinate) ; Fortin, Lumsden, Champagnat unpublished recordings.

Fig. 3. Possible developmental steps leading to the formation of the hindbrain rhythmic network. Assignment of the future neuronal functions is made in precursor cells of the neuroepithelium (middle). Synaptic relationships and membrane properties differentiate within the segmented hindbrain thus establishing rhombomere-related networks that eventually generate the rhythmic activity (bottom). Early phenotypic choices leading to motor effectors (red) or coactivators (orange) are supported by clonal analysis²²; those leading to generators (blue) are hypothetical. Only branchiomotor nuclei are shown with segmental organization found in chick^{13,16}. Top: transcription factors (among which Kreisler, Krox-20 and Hoxa-1 are documented by a loss-of-function mutation of the gene) control the hindbrain segmentation process, by which cell populations are segregated within polyclonal compartments¹³ (r1-r8) along the rostro-caudal axis of the hindbrain (caudal is on the left). The extent of Krox-20, Hoxa-1 and Kreisler gene expression within rhombomeres is indicated in gray. The Krox-20 gene encodes a protein with three C2H2-type zinc fingers expressed in two stripes with sharp edges corresponding to rhombomeres r3 and r5^{18,25,26}. The gene is expressed first at the r3 level in days E 7.75-8 in mice⁴¹. The Hoxa-1 gene is located at the most 3' end of the Hoxa cluster¹⁷ with expression extending from the caudal end of the embryo to a rostral boundary that coincides with the limit between the not yet formed rhombomeres 3 and 4 in days 7.75-8.25^{27,28}. By day 8.5 this expression retreats caudally from the hindbrain. The Kreisler mutation is a X-ray induced recessive mutation: this gene encodes a basic domain-leucine zipper transcription factor that is expressed in the future rhombomeres r6 and r5 in days E 8-9.5, with a sharp rostral edge coincident with the r4/r5 boundary^{23,24}.

Fig. 4 Anatomical phenotypes in transgenic mice exhibiting rhombomere elimination. (A) Persistence of rhombomere-related anatomical boundaries in the caudal pons after birth: insets showing the relative position of trigeminal (5) and abducens (6) nuclei and intracranial facial nerve (7n) in wild type (left, see location in **B**) and Krox-20^{-/-} mice in which r5 (green) and r3 (violet) are eliminated. Colour code gives origin of motoneurons in rhombomere¹⁶: part of the 5th nucleus originates in r2. Nuclei 5, 6 but not 7 are affected by the mutation²⁵. Blueprints set in r3 are also required for the development of a portion of the rostral parvocellular reticular formation normally intercalated between 7n and 5. **(B)** Parasagittal view (as in box 1) showing location of nuclei found to be hypoplastic in the following mutants: in both Krox-20^{-/-} and Hoxa-1^{-/-} (green, deletion of r5); in Hoxa-1^{-/-} and not Krox-20^{-/-} (blue, deletion of r4); in Krox-20^{-/-} and not Hoxa-1^{-/-} (violet, deletion of r3). Arrow (yellow) indicates abnormal sensory inputs to the DRG (caudal to r5) in Hoxa-1^{-/-} animals. The rostral domain of defects in Krox-20^{-/-} (nucleus 5, caudal pontine reticular formation (see Fig. 5) and pontine nuclei, Pn) forms a dorso-ventral column³¹ consistent with the progeny of r3 forming a strip of cells in the dorso-ventral axis of the postsegmental hindbrain^{15,34}. Caudally, the anatomical organization is more complex: the 7th nucleus, reduced in Hoxa-1^{-/-27,28} not Krox-20^{-/-25,26} migrates caudally to the superior olive (SO, reduced in both Krox-20^{-/-31} and Hoxa-1^{-/-28}. Note

also that respiratory groups are located outside the territories effected in *Krox-20^{-/-}* : the respiratory phenotype in these mutants (**C**) results from modification of the caudal pontine reticular formation³¹. **(C):** Plethysmographic recording of respiration in a *Krox-20^{-/-}* mice and a wild-type littermate (+/+) during the 9th day after birth; an upward deflection indicates inspiration; the respiratory frequency is about half normal in the mutant mice³¹; Jacquin, Borday, Schneider-Maunoury, Topilko, Kato, Ghilini, Charnay, Champagnat, unpublished recordings.

Fig. 5. Reorganization of the caudal pontine reticular formation in *Krox-20^{-/-}* mice. Parasagittal sections of the caudal pons showing tyrosine hydroxylase immunoreactivity of A5 noradrenergic neurons and fibres. In the homozygous mutant (-/-) compared to the wild-type animal, the cell bodies of the respiratory rhythm depressant A5 neurons are more densely packed and form clusters because intermingled non-noradrenergic (rhythm promoting) reticular neurons are suppressed. The total volume of the pontine reticular formation is also greatly reduced by the mutation, but shape of the A5 neurons and their total number are not changed³¹. Therefore, early specification of non-noradrenergic neurons requires proper *Krox-20* expression in the embryo ; that of noradrenergic neurons does not. Differential suppression of neurons controlling rhythm frequency is particularly effective in modifying the respiratory network function ; Jacquin, Borday, Schneider-Maunoury, Topilko, Kato, Ghilini, Charnay, Champagnat, unpublished observations.

Box 1 :

The foetal and mature respiratory networks in mammals

Differences between foetal and adult breathing behavior in mammals reveal the extent of maturational changes that continue after birth and eventually result in the adult respiratory pattern. Foetal breathing is part of a global stereotyped movement pattern including startle, hiccuping, limb movements and head retroflexion. In human, this pattern starts at the beginning of the third month of pregnancy and increases in complexity and frequency afterward^a. In mice, co-ordinated rhythmic movements of the rib cage, opening of the mouth and flexion of the neck and body start at E 15.5^b. The respiratory rhythm in adult mammals (reviewed in ref. c) characterises permanent, bilaterally synchronized and coordinated activities in spinal motor axons that innervate the diaphragm, intercostal and abdominal pump muscles as well as in cranial motoneurons that determine the flow resistance of the airways. The principal regulatory loops for breathing involve sensory inputs from peripheral (e.g. lungs) mecanoreceptors and (e.g. carotid bodies) chemoreceptors and intracranial chemosensors (Fig. A). The generator responsible for the balanced sequence of inspiration, postinspiration (that slows lung deflation) and expiration comprises a network of rhythmic « respiratory » reticular neurons (Fig. B), all located within the hindbrain, which sequentially activate motoneurons during inspiration (e.g. phrenic motoneurons, Fig. B), post inspiration (e.g. expiratory recurrent laryngeal motoneurons) and expiration (e.g. internal intercostal motoneurons). The required neuronal patterns are generated by the synaptic relationships and electrical membrane properties of respiratory neurons^c.

The adult respiratory network exhibits a rostro-caudal organization first recognized in 1923^d. A pontine respiratory group (PRG, Fig. A) located in the nucleus parabrachialis medialis and in the Kölliker-Fuse nuclei, is distinct from the other

respiratory groups located caudal to the facial nucleus^c. The network is also organized along the dorso-ventral axis, with the bulbar dorsal respiratory group (DRG) distinguished from the (ventral) VRG by Bianchi in 1974 and located in the nucleus of the solitary tract where vagal and glossopharyngeal sensory afferents terminate (large arrow in Fig. A). In the hindbrain isolated in vitro, the persisting rhythm is generated in a subdivision of the VRG in newborn rodents^e (star in Fig. A). The dorsal part of the adult network (DRG, PRG) plays a role in the transition from inspiration to post-inspiration via the activation of membrane NMDA receptors in new-born cats^f and adult mammals^c in-vivo but does not function normally in vitro^g.

In the late foetal period, such an organization of the respiratory networks forming discrete cell groups is also found. The use of foetal lamb in vivo has revealed rostrally, powerful pontine inhibitory mechanisms that are brought into play by maturation of sleep states and causes periodic breathing until birth^{h,i}. Study of rat hindbrain in-vitro has revealed that the rostrocaudal organization of the ventral part of the network is established in the fetus at E18-E20^{j,k}. Rostral noradrenergic neurons of the ventral pons (the A5 group, Fig. A) exert a depressant effect upon the caudal VRG rhythm generator^j. The VRG is also responsible for the respiratory-like activity at all levels of the spinal cord^k.

Earlier in gestation, the foetal breathing activity in vivo is present almost continuously and associated with the activity in the postural muscles of the neck and limbs^{h,i}, also seen in the mice at E15.5^b (and in the chick at an equivalent stage of development ca. 10 days after laying, stage 35-37). Before E18 in rats, typical features of the rhythmic hindbrain in vitro are lacking^{j,k}, particularly the predominance of hindbrain over spinal rhythms, the effect of the A5 group and the central sensitivity of the hindbrain to CO₂. Preliminary recordings of the isolated hindbrain of the mouse embryo at these early stages of gestation has revealed generation of a co-activated rhythm (Fig. C) comparable to that found originally in the chick embryo.

Selected references

- a De Vries, J.I.P. Visser, G.H.A. and Precht, H.F.R. (1982) *Early Hum. Dev.* 7, 301-322
- b Suzue, T. (1994) *Neuroscience Research* 21, 173-176
- c Bianchi, A.L., Denavit-Saubié, M. and Champagnat, J. (1995) *Physiol. Rev.* 75, 1-45
- d Lumsden T. (1923) *J. Physiol.* 57 : 153-160
- e Smith et al. (1991) *Science* 254, 726-729
- f Schweitzer, P. et al. (1990) *Developmental Brain Res.* 56, 290-293
- g Morin-Surun, M.P. et al. (1995) *J. Neurophysiol.* 74 : 770-778
- h Blanco, C.E. (1994) *Biol. Neonate* 65, 182-188
- i Jansen, A. and Chernick, V. (1991) *J. Applied Physiol.* 70 : 1431-1446
- j Greer, J.J., Smith, J.C. and Feldman, J.L. (1992) *J. Neurophysiol.* 67 : 996-999
- k Di Pasquale, E., Monteau, R. and Hilaire, G. (1992) *Exp. Brain Res.* 89 : 459-464

Box Fig. Respiratory rhythm and neurons in mammals

(A)

Schematic presentation of respiratory groups (red), premotor controls (blue) and cranial motor nuclei (green) in a parasagittal view of the brainstem and mesencephalon (forebrain on the right, cerebellum on top and spinal cord on the lower left are not drawn). The facial nucleus is shown with its ascending and descending roots. The ambiguous nucleus, within the VRG is not shown. Large red arrow dorsally : sensory inputs (to the DRG) ; thin red arrows : DRG and PRG projections to other respiratory groups ; red arrowheads ventrally : central chemosensitivity. The VRG is defined as in ref. c; others^{e,j} define from rostral to caudal a Böttinger or retrofacial complex, a pre-Böttinger complex (star) and a VRG sensu stricto ; 3-12: motor nuclei; A5 : noradrenergic cell group ; DRG : dorsal respiratory group ; fr : fasciculus retroflexus ; Hb, habenular nucleus, IC: inferior colliculus, IO: inferior olive, IP: interpeduncular nucleus, mlf: medial longitudinal fasciculus; Pn: pontine nuclei; PRG : pontine respiratory group ; SC: superior colliculus; scp: superior cerebellar peduncle; SO: superior olive; Ve: vestibular nuclei ; VRG : ventral respiratory group.

(B) Membrane potential of an inspiratory neuron of the VRG (upper trace) and inspiratory activity of the phrenic nerve innervating the diaphragm (lower trace) in an adult cat. Both activities are time-locked despite suppression of sensory inputs from the lung by vagotomy ; (Haji, Pierrefiche, Takeda, Foutz, Champagnat, Denavit-Saubié, unpublished recording).

(C) Integrated activity of facial (7n) and glossopharyngeal (9n) nerves in a mouse embryo before maturation of sleep states (the hindbrain is isolated in vitro at E 14). Note the slow frequency of the rhythm, the co-activation of the motor activities and the tendency of bursts to form episodes, as in the chick embryo at a comparable stage on development (Fortin et al. unpublished).