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Perinatal maturation of the respiratory rhythm generator in mammals: from experimental results to computational simulation

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Abstract

The survival of neonatal mammals requires a correct function of the respiratory rhythm generator (RRG), and therefore, the processes that control its prenatal maturation are of vital importance. In humans, lambs and rodents, foetal breathing movements (FBMs) occur early during gestation, are episodic, sensitive to bioamines, central hypoxia and inputs from CNS upper structures, and evolve with developmental age. In vitro, the foetal rodent RRG studied in preparations where the upper CNS structures are lacking continuously produces a rhythmic command, which is sensitive to hypoxia and bioaminergic inputs. The rhythm is slow with variable periods 4 days before birth. It becomes faster 2 days before birth, similar to the postnatal rhythm. Compelling evidence suggests that a region of the RRG called the preBötzinger complex (PBC) contains respiratory pacemaker neurones which play a primary role in perinatal rhythmogenesis. Although the RRG functions during early gestation, no pacemakers are found in the putative PBC area and its electrical stimulation and lesion do not affect the early foetal rhythm. To know whether the early foetal and perinatal rhythms originate from either pacemaker neurones or network connection properties, and to know which maturational processes might explain the appearance of PBC pacemakers and the rhythm increase during perinatal development, we computationally modelled maturing RRG. Our model shows that both network noise and persistent sodium conductance are crucial for rhythmogenesis and that a slight increase in the persistent sodium conductance can solve the pacemaker versus network dilemma in a noisy network.

Keywords: PreBötzinger complex; Pacemaker neurones; Respiratory network; Synaptic noise

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1. Introduction

In mammals, survival at birth imperatively requires a correct function of the respiratory system to allow rhythmic airflow to and from the lungs, blood oxygenation and oxygen delivery to all tissues. Therefore, within the very first minutes of extra-uterine life, the numerous respiratory muscles of the chest wall pump and those of the upper airway valve must rhythmically and co-ordinately contract to efficiently induce and control airflow, respectively (Monteau and Hilaire, 1991; Hilaire and Duron, 1999). This means that the neural respiratory network controlling these muscles must elaborate its vital rhythmic command and adapt it to environmental and behavioural cues at delivery. In adults and neonates, the respiratory rhythm generator (RRG) is located within the brainstem and compelling evidence exists that a small region of the rostral ventrolateral medulla called the preBötzinger complex (PBC) plays a crucial role in respiratory rhythmogenesis. In foetuses in utero, the RRG already functions and produces foetal breathing movements (FBMs) early during development without apparent reason since they are not responsible for gas exchange although they may be important for the normal development of the lungs (Rigatto, 1992; Blanco, 1994; Harding, 1995).

Here, we review the main steps of the respiratory network maturation, focusing on the RRG and more specifically on the PBC. Reports in humans, lambs and rats show similar maturational trends, with episodic FBMs in utero and a foetal RRG in vitro which first produces a slow and variable rhythm during the early gestation and then a faster rhythm during the late gestation. Whether the PBC already has a primary role in rhythmogenesis during the early gestation is questioned. If not, this means that at least under some circumstances, the RRG may produce a respiratory rhythm without a functional PBC. In mice, we report similar maturational trends and we propose a computational simulation that mimics the RRG perinatal maturation. This simulation shows that the synaptic noise in the network plays a crucial role in the functional maturation of the RRG and that a slight increase in the persistent sodium conductance in some network cells is sufficient both to mimic the respiratory rhythm increase and to switch a pacemaker-free RRG into a pacemaker-driven one (network versus pacemaker dilemma), as observed from early to late gestation.

2. The foetal respiratory rhythm in humans

Ultrasonographic studies in human foetuses report the appearance of occasional abdominal movements by midgestation, before 20 weeks, and of chest movements during the following week, inducing significant nasal fluid movements at 22 weeks (Cosmi et al., 2003). At these early developmental stages, FBMs are rare, episodic and variable but they become more frequent from 21 to 25 weeks of gestation although they remain sporadic and variable. At 28–39 weeks, FBMs still episodically appear with a mean frequency ranging around 40 breaths/min and show circadian variations (Roberts et al., 1979). Despite the early occurrence of FBMs in utero, the RRG and all the respiratory apparatus are far from being mature at these developmental stages and in the case of an early delivery, survival in extra-uterine conditions is highly improbable. When the delivery occurs between 20 and 22 weeks of gestation, the signs of life are weak and survival duration is short (around 1 h). It does not increase until 23 weeks (around 6 h) although 4.5% of those infants can survive up to 1-year-old (Macfarlane et al., 2003). Survival requires the co-ordination between sucking, swallowing and breathing and this coordination is not achieved in preterm infants born between 28 and 31 weeks of gestation. It matures later on between 33 and 36 weeks after conception (Mizuno and Ueda, 2003). In infants born at 31 weeks of gestation, periodic and regular breathing epochs are mixed and persist during 1 month; these infants are most probably breathing near the apnoea threshold (Pereira et al., 1995).

Briefly, in humans, compelling evidences exist that FBMs appear rather soon during development but are episodic. When present, they show two different patterns: a predominant one with irregular FBMs (in rate and amplitude), interrupted by long lasting apnoeas and a less frequent one of sporadic deep respiratory efforts, like sighs and gasps, occurring at a low rate. The first pattern only occurs during rapid eye movements (REM) sleep, is depressed by central hypoxia (that stimulates postnatal breathing), is not directly related to sudden changes in blood

gases, pH values and afferent inputs although they may contribute to FBM occurrences (Walker, 1986; Rigatto, 1992, 1996; Cosmi et al., 2001; Waters and Gozal, 2003). Epidemiological follow-up studies suggest that sub-optimal conditions during gestation (maternal smoking, preterm birth and foetal lung compression) cause respiratory alterations that may persist during postnatal life (Harding, 1995). Altered prenatal maturation of the RRG may result in respiratory dysfunction after birth that may facilitate and/or contribute to Sudden Infant Death Syndrome. In addition, alterations of either the serotonergic or catecholaminergic systems have been reported in victims of Sudden Infant Death Syndrome, suggesting that these neuromodulators play a role in the perinatal RRG maturation (Hilaire and Duron, 1999; Ozawa and Okado, 2002; Viemari et al., 2003; Hilaire et al., 2004).

3. The foetal respiratory rhythm in lambs

Although breathing movements have been demonstrated in the foetuses of every mammalian species, for technical reasons, they have mainly been studied in chronically instrumented foetal lambs (Jansen and Chernick, 1991). Diaphragmatic EMG recordings show episodes of rhythmic contractions during the first third of gestation (gestation: 147 days). First, they often occur as doublets whose frequency of occurrence decreases with age. Both frequency and shape of the diaphragmatic bursts evolve from variable and episodic during the early gestational period to stable over the second half of gestation (Cooke and Berger, 1990, 1996). FBMs are affected by circadian rhythm, mainly occurring during REM sleep and are depressed by hypoxia (Dalton et al., 1977; Worthington et al., 1978).

Inputs from CNS upper structures affect FBMs. First, sections performed through either the caudal hypothalamus or the upper pons dissociate FBMs from electrocortical activity but FBMs remain episodic after caudal hypothalamus sections whereas FBMs tend to become continuous after upper pons sections (Dawes et al., 1983). Second, transection and lesion experiments show that the long lasting depression induced by central hypoxia implicates some pontine structures (Dalton et al., 1977; Walker, 1995). Third, administration of morphine induces apnoea followed by hyperpnoea but midcollicular transection lengthens the morphine-induced apnoea and abolished the hyperpneic response (Hasan et al., 1990). Fourth, electrical stimulation of the posteromedial thalamus inhibits FBMs (Koos et al., 2004). In addition, bioaminergic neurones are likely to play a significant role in FBM occurrences which may be facilitated by a neuronal release of central catecholamines (Bamford et al., 1986; Joseph and Walker, 1993) although this facilitation is not sufficient to maintain sustained and continuous FBMs (Joseph and Walker, 1990). At birth, the surge of noradrenaline during delivery may contribute to continuous breathing, but is probably not the primary mechanism for its establishment (Weintraub et al., 1998; Endo et al., 1999). It has been suggested that inhibitory influences are more prominent before than after birth and that conservation of energy is more important in foetuses than any advantage FBMs impart to the foetus (Jansen and Chernick, 1991).

4. The foetal and neonatal respiratory rhythm in rats

4.1. In utero data

In foetal rats, ultrasonic recordings reveal the occurrences of FBMs with rather similar characteristics to those reported in lambs and humans (Kobayashi et al., 2001). The first FBMs are detected during the second week of gestation (embryonic day 16, E16; gestation: 21 days). They appear as single events occurring at a very low frequency and their incidence increases with age. Episodes of clustered FBMs start to occur by E18 and become more frequent and long lasting by E20, although they are still interrupted by apnoeas. Exposing the dams to hypoxia induces a rapid and marked suppression of FBMs in E20 foetuses.

4.2. In vitro neonatal data

The perinatal maturation of the rat RRG and of the PBC has been studied in in vitro conditions where the isolated RRG continues to rhythmically function, either in ‘en bloc’ preparations (Suzue, 1984) or in brainstem slices (Smith et al., 1991; Pena and Ramirez, 2002).

In 'en bloc' preparations containing the medulla and the cervical cord (medullary preparations), the rat RRG produces regular bursts recorded from the phrenic roots at a frequency of about 10/min and compelling evidence suggests that the PBC is playing a key role (Di Pasquale et al., 1994a,b; Hilaire and Duron, 1999). Briefly, electrical stimulation delivered as a single shock during expiration within the PBC area triggers an inspiration that resets the respiratory rhythm. In this area, the application of bioamines alters the in vitro respiratory rhythm while electrolytic lesion abolishes it. Indeed, the PBC is the kernel of the neonatal RRG since it contains respiratory neurones among which about 7% possess intrinsic bursting properties. They are defined as bursting pacemaker neurones because they continue to produce rhythmic bursts of spikes when all their synaptic connections have been blocked. The PBC neurones express NK1 receptors to substance P (SP) which provide a good histological marker to localise the PBC area. The PBC mediates numerous neuromodulatory processes affecting the neonatal respiratory rhythm (hypoxia; serotonin, 5-HT; noradrenaline, NA; acetylcholine; SP, etc.). Its activity is depressed and facilitated by endogenous NA released from the pontine A5 and A6 neurones, respectively (Hilaire et al., 2004), and is increased by 5-HT and SP (Hilaire and Duron, 1999).

The neonatal RRG has also been studied in brainstem slices containing the main neural elements necessary for respiratory rhythmogenesis, i.e. the neurones from the ventral respiratory group and from the PBC, and the motor output neurones from XII nucleus. All these neurones synchronously fire rhythmic bursts of spikes and their rhythms are sensitive to bioamines (Pena and Ramirez, 2002). Most of the results from slices are consistent with those obtained from 'en bloc' preparations although a higher concentration of K^+ (8–9 mM) is necessary in the former. The data obtained from slices further argue for the presence of bursting pacemakers in the PBC (Smith et al., 1991; Johnson et al., 1994).

Both in 'en bloc' and slice preparations, the bursting pacemaker neurones of the PBC likely impose their rhythmic command to the other neurones of the respiratory network (Feldman and Smith, 1989; Smith et al., 1991, 2000; Johnson et al., 1994; Rekling and Feldman, 1998; Butera et al., 1999b; Hilaire and Duron, 1999; Koshiya and Smith, 1999; Del Negro et al., 2001). However, the blockade of the pacemaker properties by the application of riluzole silences the PBC pacemakers but does not abolish the respiratory rhythm, suggesting that PBC pacemakers are not essential for rhythmogenesis (Del Negro et al., 2002a) and that the respiratory rhythm may emerge from network connection properties.

4.3. *In vitro foetal data*

In rat foetuses, the RRG of 'en bloc' preparations functions early during development (Greer et al., 1992; Di Pasquale et al., 1992, 1994a,b). From E16 to E18, medullary preparations produce rhythmic phrenic bursts of short duration at low frequency (2–3 bursts/min) with variable periods. The phrenic rhythm is not episodic, probably because the CNS upper structures have been removed during the dissection, and is not affected by central hypoxia and alkalosis. The RRG is not yet inhibited by the pontine A5 neurones, it is strongly excited by exogenous 5-HT, and endogenous 5-HT plays a crucial facilitatory role since the blockade of 5-HT receptors highly reduces or even abolishes the RRG activity (Di Pasquale et al., 1994a). At these early stages, the PBC is likely to be immature since both electrical stimulation and lesion of the rostral ventrolateral medulla have no drastic respiratory effects. Although the PBC area contains inspiratory neurones, none of them display bursting pacemaker properties. At E20, the rhythm of 'en bloc' preparations increases and stabilises around 8 bursts/min. The RRG now responds, as it does in neonates, to central hypoxia and alkalosis, to pontine A5 inhibition and 5-HT facilitation. In the PBC area, some inspiratory neurones displaying pacemaker properties are present (10% of the tested neurones) and electrical stimulation and lesion triggers and abolishes the rhythm, respectively.

The rat foetal RRG has also been studied in brainstem slices (Pagliardini et al., 2003). An elegant combination of electrophysiological, immunohistochemical and pharmacological approaches shows that respiratory rhythm starts at E16.5 and that although NK1 receptors appear early in the developing medulla (E13), they mark the PBC area only by E17 when SP has powerful effects raising the in vitro rhythm by three- to four-fold. As in 'en bloc' preparations, the frequency and amplitude of the bursts produced in slices increase

with age. However, the stabilisation of the respiratory period and the lengthening of the bursts reported in 'en bloc' preparations are not confirmed in slices.

5. The foetal respiratory rhythm in mice

In mice, ultrasonic recordings of FBMs are not yet available but the ability of C-section delivered foetuses to breathe has been studied (Viemari et al., 2003). At E16 (gestation: 20 days), exteriorised foetuses only produce gasps at a very low frequency (Fig. 1A E16) and die within few minutes. At E18, after gasping for a few minutes, they nearly all survive and display normal respiratory movements (Fig. 1A E18) similar to that observed in early postnatal days (Fig. 1A P4). In 'en bloc' preparations (Viemari et al., 2003), the RRG of the E16 foetal mice continuously produces low frequency phrenic bursts of variable periods (Fig. 1B E16) as in E18 foetal rats. The rhythm is highly increased by the application of either SP or NA but is affected neither by a short lasting central hypoxia nor electrical stimulation nor lesion of the 'assumed' PBC area. By E18, the rhythm has increased (Fig. 1B E18) and now presents the neonatal responses to SP, NA, central hypoxia and PBC lesion and stimulation. After birth, the burst duration and the period stability increase.

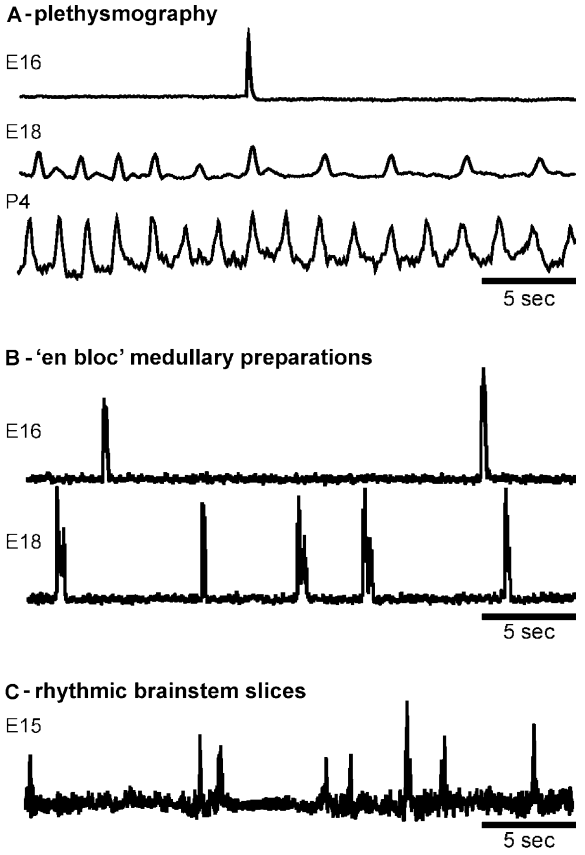

Fig. 1. In vivo and in vitro respiratory activity in maturing mice. (A) Plethysmographic recordings of breathing in foetal mice delivered at embryonic days 16 (E16; one gasp) and E18 and in neonatal mice 4 days after birth (P4; inspiration upward). (B) Integrated activity of rhythmic phrenic bursts recorded from 'en bloc' medullary preparations at E16 and E18 in foetal mice. Note the increased rhythm at E18. (C) Integrated activity of multiunitary recorded neurones from the ventral respiratory group in a brainstem slice of foetal mouse at E15.

In slices from foetal mice, no published data are available yet. However, on-going experiments performed in our laboratory show maturational processes that are rather close to those reported in rats, with a variable respiratory command (rhythm and amplitude) produced as early as E15 (Fig. 1C).

Although the NK1 receptors to SP are expressed by the PBC neurones and mark the PBC area, NK1 receptor-knockout mice have a normal prenatal maturation of the RRG, a normal breathing at birth and survive (Ptak et al., 2002). Studies performed on mutant mice with genetically-induced alterations of their bioaminergic systems confirm that bioamines such as NA play a role in RRG prenatal maturation. Indeed, loss of neurones in the NA structures alters the prenatal maturation of the RRG. At E18, the mutant RRG produces a rhythm which is abnormal in vitro and unsuitable for survival in vivo (Shirasawa et al., 2000; Hilaire et al., 2004; Viemari et al., 2004). However, transgenic mice lacking the transcription factor gene *MafB* have a disorganised PBC area but normal bioaminergic systems, display only gasps in vivo and do not survive more than a few minutes (Blanchi et al., 2003). In vitro, their RRG produces a slow and variable rhythm at E18 (1–2 burst/min) and does not respond to SP, central hypoxia, electrical stimulation or lesion of the PBC area. From these results, two main conclusions may be reached. First, the PBC is indeed crucial for a normal respiratory rhythm and survival at birth. Second, the RRG without a functional PBC, and probably without respiratory pacemaker neurones, can produce a rhythm even if this rhythm is slow, variable and not suitable for survival. Indeed, consistently with riluzole results

in neonatal rats (Del Negro et al., 2002a), the RRG can produce a respiratory rhythm without active PBC pacemakers.

6. The foetal and neonatal RRG in computo

Maturational studies in humans, lambs and rodents show that the RRG starts to function early during gestation, first producing a slow and variable rhythm that evolves with age towards a neonatal pattern with a faster and stable rhythm. In rodents, RRG maturational studies have brought into light what we call the pacemaker versus network dilemma. On the one hand, the mature RRG contains PBC pacemakers, and *MafB* mutant mice with altered PBC do not produce a normal rhythm, which suggest a pacemaker-driven RRG. On the other hand, silencing the PBC pacemakers in neonates by riluzole does not abolish the rhythm and no pacemakers are detected in the early foetal PBC although the RRG produces a rhythm, which suggest a pacemaker-free network. Therefore, one can wonder whether the RRG function arises from PBC pacemaker activity or from network connection properties. If pacemaker neurones are not involved, does the rhythm necessarily emerge from reciprocal inhibitions between inspiratory and expiratory neurones? If pacemaker neurones are involved, which maturational processes can explain their appearance within 2 days? How do the perinatal rhythm increase and the period stabilise? We have tried to answer these questions by using a computational model.

6.1. The RRG computational model

Bursting respiratory neurones have been already modelled with single compartments equipped with three ionic currents (Butera et al., 1999a): potassium current (I_K), fast sodium current (I_{Na}) and persistent sodium current (I_{NaP}). The last one has an inactivation variable (h) with a very slow time constant ($\tau(h) = 10$ s), and thus, the membrane potential requires a long time to depolarise up to its firing threshold. The kinetics of I_{NaP} is responsible for both the burst and the interburst durations while these of I_{Na} and I_K are responsible for the spike generation. From these neurones, neonatal RRG have been modelled where the kernel is composed of a layer of such pacemakers, fully connected to each other, driving a layer of "followers" which have the same parameters but no NaP current (Butera et al., 1999a; Smith et al., 2000).

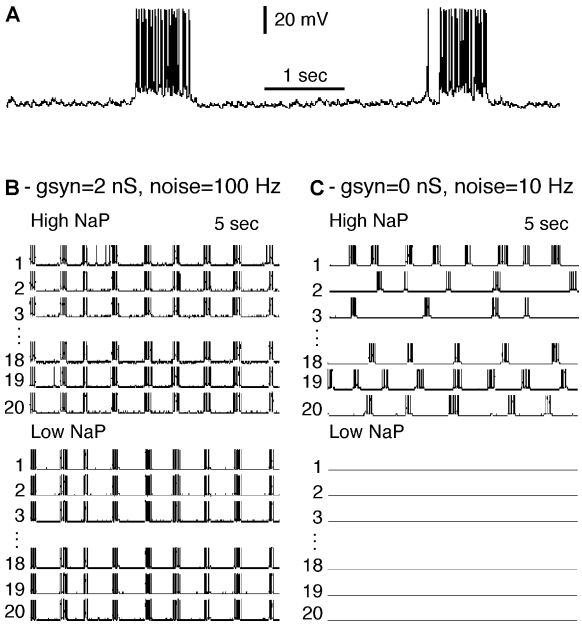

Fig. 2. Computational simulation of the "mature" neonatal RRG. (A) The trace shows the membrane potential variations of one of high-NaP neurones used to simulate the RRG. (B) The traces show the membrane potential variations of 6 out of 20 high-NaP artificial pacemaker neurones ($g_{NaP} = 2.8 \pm 0.8$ nS) and 6 out of 20 of low-NaP follower ones ($g_{NaP} = 0.56 \pm 0.16$ nS) in a "mature" neonatal network. Each of the 40 neurones constituting the "mature" network is fully connected to the others ($g_{syn} = 2$ nS) and receives a high level of noise (100 Hz). The figure shows the network activity during 20 s when in a steady state. All the neurones synchronously fire rhythmic bursts of spikes, with occasional ectopic potentials in the high-NaP neurones. (C) When g_{syn} is set to zero (disconnected neurones) and the level of noise lowered to 10 Hz, the low-NaP neurones become silent while the high-NaP ones fire desynchronised bursts of spikes.

Our model is focused on persistent sodium current-based pacemaker neurones because compelling evidence suggests that these neurones have a key role in respiratory rhythmogenesis. Calcium currents that produce pacemaker properties might participate in neonates but this is not yet documented in foetuses. To mimic the RRG, we use a network of 40 neurones *à la* Butera (Fig. 2A), each with a resting potential value set at −59 mV (Butera et al., 1999b) and a persistent sodium conductance (g_{NaP}). However, the 40 neurones are segregated into two groups of 20 in function of their g_{NaP} value: one group, the high-NaP neurones, has a mean g_{NaP} five-fold higher than the other group, the

low-NaP neurones. As in the original model (Butera et al., 1999a), the low-NaP neurones can be considered as followers since they need to be connected to high-NaP neurones (pacemakers) to be able to produce either individual or bursts of spikes. In addition in our model, we add a poissonian noise generator impinging on each cell. We use a single noise generator which corresponds to a summary of the three types of noise that each RRG neurone receives in vivo, i.e. (1) the network noise generated by the synaptic excitations arising from neighbouring neurones (Smith et al., 2000), (2) the synaptic noise due to the ionic environment of the synapse and the random processes of the synaptic inputs, (3) the channel noise which is the stochastic opening and closing of the ionic channels affecting the pacemaker neurone activity (Rowat and Elson, 2004). The last one is the only remaining noise when synaptic connections are blocked.

Each cell of the RRG network is fully connected to all the others by fast excitatory synapses with a 0.1 ms synaptic delay and a 0 mV reversal potential. The random synaptic conductances follow an exponential distribution with a fixed mean (g_{syn}). Values for g_{NaP} and g_{syn} were taken from previously published values for the mature network which proved to reproduce correctly the mature RRG behavior (Butera et al., 1999a; Smith et al., 2000). For each cell, g_{NaP} and g_{syn} values are randomly drawn from a gaussian distribution and an exponential distribution, respectively, with fixed mean and variance. Noise was estimated by comparisons of in computo and in vitro recordings. We used the free GENESIS software [http://www.genesis-sim.org/GENESIS] to compute the evolution of the RRG network activity against time.

6.2. The pacemaker versus network dilemma

We use two sets of g_{NaP} values to mimic the neonatal and foetal RRGs. First to mimic the "mature" neonatal RRG, the mean g_{NaP} is set at 2.8 ± 0.2 nS (mean ± variance) for the high-NaP neurones (pacemakers) and 0.56 ± 0.1 nS for the low-NaP neurones (followers). Second to mimic the "immature" foetal RRG, the mean g_{NaP} is set at 1.8 ± 0.2 nS (high-NaP neurones) and 0.36 ± 0.1 nS (low-NaP neurones). One must be aware that membrane potentials of "immature" and "mature" artificial neurones are driven by the same mechanism, which is the slow inactivation variable h.

In the "mature" RRG with fully connected neurones ($g_{syn} = 2$ nS) and a certain amount of noise (100 Hz), all the neurones synchronously fire bursts of spikes, giving a regular rhythm to the network (Fig. 2B) with high-NaP neurones firing occasional ectopic spikes as experimentally observed (Del Negro et al., 2002b). After disconnecting the neurones by lowering the mean synaptic conductance ($g_{syn} = 0$ nS) and decreasing the noise level (10 Hz), all the followers become silent while the high-NaP pacemakers still produce rhythmic bursts of spikes that are desynchronised (Fig. 2C). Our simulation closely mimics the experimental observation in 'en bloc' neonatal rat preparations of the loss of synchronisation in two pacemakers when perfused by low Ca^{2+} solution which blocks the synaptic relations (Onimaru et al., 1989).

In the "immature" foetal RRG placed in similar conditions as above ($g_{syn} = 0$ nS and noise level = 10 Hz), all the neurones are silent, even the high-NaP neurones. Keeping $g_{syn} = 0$ nS (no synaptic connections) but increasing the noise up to 100 Hz induces sporadic individual spikes in all the neurones but no bursts of spikes (Fig. 3A). Then, increasing g_{syn} up to 2 nS to fully in-

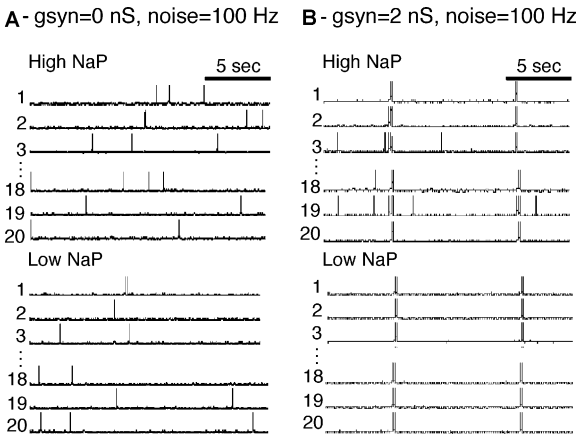

Fig. 3. Computational simulation of the "immature" foetal RRG. Same representation as in Fig. 2 (B and C) but for an "immature" foetal RRG, i.e. when g_{NaP} is set at 1.8 ± 0.2 and 0.36 ± 0.1 nS for the high- and the low-NaP neurones, respectively. (A) None of the neurones can be classified as bursting pacemakers since they only produced single spikes when $g_{syn} = 0$ nS and the level of noise = 100 Hz (when the level of noise is 10 Hz, the neurones are silent; not shown). (B) When the neurones are fully connected ($g_{syn} = 2$ nS), they all generate synchronous rhythmic bursts of spikes as in the "mature" network but the frequency is lower than that is seen in a "mature" network (see Fig. 2B).

terconnect the neurones induces synchronous rhythmic bursts in all the neurones (Fig. 3B). So when the "immature" RRG does not contain bursting pacemaker neurones (no rhythmic bursts of spikes when $g_{syn} = 0$ nS), it can produce a rhythm when the neurones are fully connected and receive enough noise. It is worth noting that this rhythm is about three to four times slower than that observed in the "mature" RRG.

Thus, a simple solution to solve the pacemaker versus network dilemma is to speculate a slight increase in g_{NaP} or g_{NaP}/g_l ratio in which g_l represents the leakage conductance related to the ion pump currents (Del Negro et al., 2002b). In a noisy network, an increase of g_{NaP} is sufficient to explain the emergence of respiratory pacemaker neurones from the early to the late gestational periods and, at the same time, to induce the experimentally observed increase in rhythm (Di Pasquale et al., 1994b; Viemari et al., 2003).

However, the blockade of NaP channels in neonatal rats by riluzole application silences the PBC pacemakers but does not abolish the respiratory rhythm, suggesting that NaP currents are not essential for rhythmogenesis (Del Negro et al., 2002a). We think that riluzole only reduces g_{NaP} but never totally abolishes it. Indeed, the NaP current is due for about 50% to the "window" current, i.e. a current generated by the fast Na channels which are not blocked by riluzole (Rybak et al., 2003). Therefore, the experimental situation of riluzole application to the "mature" neonatal RRG leads to situation which is similar to that of our simulated foetal RRG, i.e. bursting pacemaker neurones are not seen when NaP values are low and the neurones disconnected ($g_{syn} = 0$ nS) but the network is able to produce synchronised rhythmic bursts when the neurones are connected despite their low NaP values.

The "immature" high-NaP neurones must be viewed as conditional pacemakers. When they do not receive enough noise, even if they are connected to each other, the NaP current is not sufficient for the membrane potential to reach its spiking threshold and all the neurones remain silent (not shown). When the noise level is increased, they are able to fire individual spikes even if they are synaptically isolated (Fig. 3A). The reciprocal synaptic excitations are then necessary to maintain their membrane potentials above firing threshold to elicit bursts of spikes and to synchronise them (Fig. 3B). Therefore, noise alone or synaptic connections alone are not sufficient to change the "immature" high-NaP neurones from conditional bursters into true pacemakers but it requires both. There is no need to introduce either calcium currents (Kosmidis et al., 2004) or reciprocal inhibitions (Rybak et al., 2004) to generate a rhythm. This does not mean that reciprocal inhibitions do not exist and do not play a role in rhythmogenesis but suggests that they are not required to generate a rhythm, in agreement with results obtained in vitro on 'en bloc' preparations and rhythmic brainstem slices suggesting that Cl^--mediated inhibition is not essential for neonatal RRG activity (Onimaru et al., 1990; Feldman et al., 1991; Paton and Richter, 1995; Ramirez et al., 1996).

Our computational study suggests a role of the noise in the RRG function. This may be related to an increase in afferent inputs to the RRG occurring during development. In addition, noise may be required for rhythm appearance since FBMs are lacking during quiet sleep but present during REM sleep and awake state. The RRG activity produced during the early gestation is drastically facilitated by endogenous bioamines which may act specifically on PBC neurones and/or non-specifically on all the neurones of the entire network, leading to an "enhancement of emergent bursting by noise" (De Vries and Sherman, 2000).

6.3. The rhythm maturational changes

In rodent 'en bloc' preparations, the mean respiratory rhythm, the mean duration of the inspiratory bursts and the period stability increase from foetal to neonatal ages (Viemari et al., 2003; Di Pasquale et al., 1994b). To explain these experimentally observed changes, we vary g_{NaP} by steps of 0.1 nS from 1 to 4 nS in high-NaP neurones. No rhythmic bursts occur until g_{NaP} reaches 1.8 nS. Then, increasing g_{NaP} raises the rhythm from 4.2 ± 0.1 bursts/min (1.8 nS) to 21 ± 1 bursts/min (2.3 nS) and 32 ± 2 bursts/min (4 nS) while the burst duration lengthens. The period stability remains almost unaffected by g_{NaP} changes.

To identify the factors other than g_{NaP} which may contribute to the experimentally observed changes, we fix g_{NaP} (2.8 ± 0.2 nS) and impose a step-by-step variation of only one of the other parameters. As the inputs to the RRG are likely to increase during development, we increase the noise from 10 to 320 Hz. This increases the rhythm but decreases the period stability. As the synaptic weight w affects the mean synaptic conduc-

tance ($g_{syn} = w \times 0.2$ nS), we increase w from 0.51 to 5.9. Until w reaches 2.8, the rhythm increases but the period stability decreases. Then for w value above 2.8, both rhythm and stability remain unchanged. As the cell area of PBC neurones increases with age (Pagliardini et al., 2003), thus affecting their membrane resistance and capacitance (Onimaru and Homma, 2002), we increase the area of our artificial cells from 115 to 215 μm^2. This decreases both the rhythm and the period stability. Increasing the number of neurones in the network from 8 to 160 in steps of 8 (without changing the ratio pacemakers/followers) shows that the rhythmogenesis requires at least 24 cells. From 24 to 112 cells, the rhythm increases and then remains unchanged for more cells. The period stability increases up to 48 cells and then decreases regularly with the increase in cell number. The influence of the ratio pacemakers/followers was studied by increasing the number of pacemakers, keeping the total number of cells of the network equal to 40. A minimum of 10 pacemakers (25%) is required for a rhythm to occur. A further increase in their number has no marked effects on the rhythm or on the period stability but lengthens the burst duration.

In summary, none of the tested parameters alone can satisfactorily increase both the rhythm and period stability. Nevertheless, it is possible to construct a model where both rhythm, inspiratory duration and period stability increase by combining the variations in two parameters. This can be achieved by increasing both g_{NaP} (from 1.7 to 2.8 nS) and the number of high- and low-NaP cells (from 20 high- plus 20 low-NaP cells to 57 high- plus 20 low-NaP cells). However, it is quite arbitrary and, to better understand the maturation of RRG, the developmental changes of all these parameters should be controlled experimentally.

7. Conclusions

In utero, episodic FBMs occur early during gestation in humans, lambs and rats and evolve with age. In vitro, studies of rodent preparations where the CNS upper structures are eliminated reveal that the RRG is continuously active 4 days before birth when it produces a slow rhythm with variable period. Two days before birth, the foetal rhythm becomes faster and stable, similar to the neonatal rhythm. Studies of wild type and mutant mice suggest that a region of the RRG called the PBC contains bursting pacemakers which play a primary role in rhythmogenesis during the late gestation and the postnatal period. However, conflicting data show that the mouse foetal RRG of *MafB* mutants lacking functional PBC and the rat neonatal RRG under blockade of pacemaker properties can both produce a rhythm. Our computational model shows (1) that a noisy and fully connected network can generate a rhythm without true pacemakers and inhibitory connections and (2) that an increase in g_{NaP} is sufficient to explain the emergence of bursting pacemakers in the network from the early to the late gestational periods, and the experimentally observed increase in rhythm (Di Pasquale et al., 1994b; Viemari et al., 2003). Both the experimentally observed data and the computational simulation reveal that inputs that sustain the network noise within the RRG contribute to its rhythmic activity, and therefore, its normal maturation. Here, we wish to further underline that bioaminergic inputs during the perinatal period, and more specifically NA inputs, may have a crucial role in the RRG maturation. Therefore, any genetic and epigenetic factors altering the bioaminergic systems during pregnancy may alter the RRG maturation leading to postnatal respiratory sequelae (Shirasawa et al., 2000; Hilaire et al., 2004; Viemari et al., 2004).

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