

Modeling Neural Mechanisms for Genesis of Respiratory Rhythm and Pattern. II. Network Models of the Central Respiratory Pattern Generator

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Rybak, Ilya A., Julian F. R. Paton, and James S. Schwaber. Modeling neural mechanisms for genesis of respiratory rhythm and pattern. II. Network models of the central respiratory pattern generator. *J. Neurophysiol.* 77: 2007–2026, 1997. The present paper describes several models of the central respiratory pattern generator (CRPG) developed employing experimental data and current hypotheses for respiratory rhythmogenesis. Each CRPG model includes a network of respiratory neuron types (e.g., early inspiratory; ramp inspiratory; late inspiratory; decrementing expiratory; postinspiratory; stage II expiratory; stage II constant firing expiratory; preinspiratory) and simplified models of lung and pulmonary stretch receptors (PSR), which provide feedback to the respiratory network. The used models of single respiratory neurons were developed in the Hodgkin-Huxley style as described in the previous paper. The mechanism for termination of inspiration (the inspiratory off-switch) in all models operates via late-I neuron, which is considered to be the inspiratory off-switching neuron. Several two- and three-phase CRPG models have been developed using different accepted hypotheses of the mechanism for termination of expiration. The key elements in the two-phase models are the early-I and dec-E neurons. The expiratory off-switch mechanism in these models is based on the mutual inhibitory connections between early-I and dec-E and adaptive properties of the dec-E neuron. The difference between the two-phase models concerns the mechanism for ramp firing patterns of E2 neurons resulting either from the intrinsic neuronal properties of the E2 neuron or from disinhibition from the adapting dec-E neuron. The key element of the three-phase models is the pre-I neuron, which acts as the expiratory off-switching neuron. The three-phase models differ by the mechanisms used for termination of expiration and for the ramp firing patterns of E2 neurons. Additional CRPG models were developed employing a dual switching neuron that generates two bursts per respiratory cycle to terminate both inspiration and expiration. Although distinctly different each model generates a stable respiratory rhythm and shows physiologically plausible firing patterns of respiratory neurons with and without PSR feedback. Using our models, we analyze the roles of different respiratory neuron types and their interconnections for the respiratory rhythm and pattern generation. We also investigate the possible roles of intrinsic biophysical properties of different respiratory neurons in controlling the duration of respiratory phases and timing of switching between them. We show that intrinsic membrane properties of respiratory neurons are integrated with network properties of the CRPG at three hierarchical levels: at the cellular level to provide the specific firing patterns of respiratory neurons (e.g., ramp firing patterns); at the network level to provide switching between the respiratory phases; and at the systems level to control the duration of inspiration and expiration under different conditions (e.g., lack of PSR feedback).

INTRODUCTION

This is the second in a series of papers presenting the results of our investigations into the neural mechanisms for

respiratory rhythm and pattern generation at the cellular, network, and system levels employing modeling methods. The preceding paper (Rybak et al. 1997a) described models of single respiratory neurons developed in the Hodgkin-Huxley style. Using these neuron models, we have constructed several network models of the central respiratory pattern generator (CRPG). The main objective of the present paper is to explore possible neural mechanisms that provide the genesis and control of both respiratory oscillations and specific firing patterns of respiratory neurons.

Our CRPG models are based on a ‘network paradigm’ for neurogenesis of the respiratory rhythm that suggests that the respiratory oscillations results from reciprocal inhibitory interactions between respiratory neurons (Balis et al. 1994; Botros and Bruce 1990; Geman and Miller 1976; Gottschalk et al. 1994; Ogilvie et al. 1992; Richter and Ballantyne 1983; Richter et al. 1986a; Rubio 1972). However, in our models, the genesis of the respiratory rhythm especially depends on the intrinsic membrane properties of respiratory neurons, although the neurons do not have pacemaker properties. A role of pacemaker neurons for respiratory rhythmogenesis (see Feldman and Cleland 1982; Smith et al. 1991) is not considered here.

In contrast to previous network models of CRPG, which were based on relatively simple, nonspecific models of single neurons (Botros and Bruce 1990; Duffin 1991; Duffin et al. 1995; Geman and Miller 1976; Gottschalk et al. 1994; Ogilvie et al. 1992; Rubio 1972), we have used more realistic neuron models developed in the Hodgkin-Huxley style using existing experimental data on their intrinsic biophysical properties (Rybak et al. 1997a). This allowed us to incorporate distinct models of different respiratory neurons into network models, compare firing patterns and membrane trajectories of modeled respiratory neurons with those recorded in vivo, and analyze the contribution of intrinsic properties of different respiratory neurons to CRPG performance at both the network and system levels.

Phases of the respiratory cycle and types of respiratory neurons

According to Richter’s theory, the respiratory cycle consists of three phases: inspiratory, postinspiratory (stage I expiration), and late expiratory (stage II expiration) (Richter and Ballantyne 1983; Richter et al. 1986a; see Fig. 1A in Rybak et al. 1997a). Some previous CRPG models were based on this theory (Botros and Bruce 1990; Gottschalk et

al. 1994; Ogilvie et al. 1992). An alternative point of view is that the respiratory cycle comprises just two phases—inspiration and expiration (Balis et al. 1994; Duffin 1991; Duffin et al. 1995; Ezure 1990; Geman and Miller 1976; Rubio 1972). The difference between a two- and three-phase CRPG hinges on the control of expiration. For example, does it consist of independently controlled postinspiratory and late (stage II) expiratory phases or is it controlled as a single phase? We have investigated these possibilities in simulation and found fundamental differences in the interpretation and explanation of neural mechanisms underlying: rhythm generation per se, the expiratory off-switch, and ramp firing patterns of expiratory neurons.

Respiratory neurons were classified into types depending on their firing pattern and phase of firing relative to the phrenic cycle (Richter 1996; Richter and Ballantyne 1983; Richter et al. 1986a; see Fig. 1A in Rybak et al. 1997a). These types included the following: early inspiratory (early-I); ramp inspiratory (ramp-I); late inspiratory (late-I); postinspiratory (post-I); stage II expiratory (E2); preinspiratory (pre-I). Early-I and post-I neurons were characterized by a decrease in spike frequency during their bursts whereas ramp-I and E2 neurons demonstrate ramp firing patterns (i.e., spiking frequency increased with time). We used this classification but incorporated two additional types of neurons not present in Richter's classification: decrementing expiratory (dec-E) and stage II constant firing expiratory (con-E2) neurons. In contrast to post-I neurons, which are active and adapt during the postinspiratory phase only, dec-E neurons are active and adapt throughout both postinspiratory and late (stage II) expiratory phases (Balis et al. 1994; Bryant et al. 1993; Cohen 1979; Duffin 1991; Duffin et al. 1995; Ezure 1990; Feldman 1986; von Euler 1986). Con-E2 neurons in our models are similar to E2 neurons: they are also active during the stage II of expiration, but show continuous rather than a ramp firing pattern.

Phase switching mechanisms

The respiratory rhythm occurs because of sequential phase switching. von Euler (1977, 1986) proposed a conceptual model of the functional organization of the respiratory phase switching but did not consider detailed neural mechanisms. A plausible neural mechanism for the inspiratory off-switch (switch between inspiration and expiration) was described by Cohen and Feldman (Cohen 1979; Cohen and Feldman 1977; Feldman 1986) and used in some previous CRPG models (Botros and Bruce 1990; Gottschalk et al. 1994; Ogilvie et al. 1992). The mechanism operates via late-I neurons, which are considered to be inspiratory off-switching neurons. We have incorporated this mechanism into all our CRPG models.

According to earlier models (Gottschalk et al. 1994; Ogilvie et al. 1992), the switch between postinspiration and expiration results from the reciprocal inhibitory interaction between post-I and E2 neurons and the adaptation of post-I neurons. However, because post-I neurons are not active during the late expiratory phase (stage II), they cannot contribute to the ramp firing patterns of E2 neurons. Thus the mechanism for ramp firing patterns of E2 neurons becomes unexplained.

The switching between expiration and inspiration (expira-

tory off-switch) is understood poorly, and there is no accepted point of view for this mechanism. In the two-phase CRPG models proposed recently (Duffin 1991; Duffin et al. 1995), this mechanism was based on mutual inhibition between the dec-E and early-I neurons and adaptation in dec-E firing patterns. In the models based on Richter's theory, the expiratory off-switch occurs because of the reciprocal interaction between E2 and early-I neurons (Gottschalk et al. 1994; Ogilvie et al. 1992). However, for this to occur, E2 neurons would be expected to adapt and not display ramp firing patterns. Consequently, these models were unable to reproduce the expiratory off-switch and ramp firing pattern of E2 neurons simultaneously.

Our simulations have explored the importance of the following issues for respiratory rhythmogenesis: 1) the number of respiratory phases (2 vs. 3) that are controlled independently; 2) the expiratory off-switch mechanism; and 3) the mechanism for ramp firing patterns of late-expiratory neurons (E2). Correspondingly, we have developed and analyzed CRPG models based on different possible mechanisms. In addition, we have investigated the specific roles of intrinsic membrane properties of different respiratory neurons for CRPG performance at the network and system levels in simulation.

METHODS

Models of single respiratory neurons

Two distinct model types of single respiratory neurons—type I and type II—were developed and described in our previous paper (Rybak et al. 1997a). They were single compartment neuron models developed in the Hodgkin-Huxley style. The maximal conductances used for these channels in the two neuron types are shown in Table 1.¹

A combination of $K_{AHP}(Ca)$ and Ca_L channels in neuron type I provided an adaptive frequency response to excitatory synaptic stimulation. This neuron model was used² to simulate adapting (early-I, post-I, and dec-E) and switching (late-I, pre-I) neurons.

$K_{AHP}(Ca)$ and Ca_T channels together provided ramp frequency firing patterns in neuron type II after release from inhibition. We used neuron type II for simulation of ramp-I, E2, and con-E2 neurons to obtain rebound excitation and frequency ramp firing patterns, which are typical for these neuronal types.

Synaptic inputs to the neuron

According to our basic single neuron model (Rybak et al. 1997a) (Eq. 1), the main neuronal equation of the Hodgkin-Huxley style for the membrane potential V_i of the neuron can be represented as follows

$$c \cdot \frac{d}{dt} V_i = \sum_p g_{pi} \cdot (E_p - V_i) + g_{SynEi} \cdot (E_{SynE} - V_i) + g_{SynIi} \cdot (E_{SynI} - V_i) \quad (1)$$

where c is the neuron membrane capacitance; t is time; g_{pi} and E_p are, respectively, the conductance and reversal potential of the

¹ The maximal conductance for the transient potassium-A channels (\bar{g}_A) was reduced to 0.07 μS compared with that used in our previous paper (see Table 2 in Rybak et al. 1997a) to match experimental measurements (0.02–0.07 μS in Champagnat et al. 1986).

² To provide the required adaptation time constant in neuron type I the activation time constant of $K_{AHP}(Ca)$ channel (τ_{mAHP} in Rybak et al. 1997a, Table 1) in some cases (e.g., for dec-E neuron) was multiplied by a coefficient $1 \leq k_\tau \leq 9$.

TABLE 1. *Maximal conductances of ionic channels in the two models of single respiratory neurons*

Type of Neuron	Maximal Conductance, \bar{g}_i , μS						
	\bar{g}_{Na}	\bar{g}_{DR}	\bar{g}_A	\bar{g}_{AHP}	\bar{g}_{CaL}	\bar{g}_{CaT}	\bar{g}_L
Type I*	3.0	0.9	0.07	0.15	0.0015		0.01
Type II†	3.0	0.9	0.07	0.15		0.0025	0.01

* Early inspiratory (early-I), postinspiratory (post-I), decrementing expiratory (dec-E), late inspiratory (late-I), and preinspiratory (pre-I) neurons.
 † Ramp inspiratory (ramp-I), stage II expiratory (E2), and stage II constant firing expiratory (con-E2) neurons.

ionic channel p in the neuron; E_{SynE} and E_{SynI} are the reversal potentials of excitatory and inhibitory synaptic channels, respectively; g_{SynEi} and g_{SynIi} are the conductances of these channels in the neuron i .

Synaptic interactions between the neurons in the network were simulated in the following way. At rest the conductances of excitatory and inhibitory synaptic channels were 0. Each spike from the neuron j to the excitatory or inhibitory synapse of the neuron i at the time t_{kj} changed the conductance of excitatory or inhibitory synaptic channels. Dynamics of these changes for $t \geq t_{kj}$ can be described as follows

$$g_{SynEi}(t) = [g_{SynEi}(t_{kj}) + k_E \cdot S\{W_{ji}\}] \cdot \exp\left(-\frac{t - t_{kj}}{\tau_E}\right)$$

$$g_{SynIi}(t) = [g_{SynIi}(t_{kj}) + k_I \cdot S\{-W_{ji}\}] \cdot \exp\left(-\frac{t - t_{kj}}{\tau_I}\right) \quad (2)$$

where W_{ij} is the weight of the synaptic connection from the neuron j to the neuron i ($W_{ij} > 0$ for excitatory synapses and $W_{ij} < 0$ for inhibitory synapses); the function $S\{\cdot\}$ separates excitatory and inhibitory synaptic drives (allows each drive to affect only the corresponding synaptic conductance)

$$S\{x\} = \max(0, x) \quad (3)$$

k_E and k_I are the coefficients which define the values of increase in synaptic conductance resulting from a single spike for the excitatory and inhibitory synapses, respectively, when $\text{abs}(W_{ij}) = 1$; τ_I and τ_E are the inactivation time constants of synaptic conductances for the excitatory and inhibitory synapses, respectively.

The above dynamic model of synaptic conductances produced synaptic potentials of regular shape in the postsynaptic neuron. The rising edge time constant of synaptic potentials was short and was defined by the membrane time constant; the falling edge time constant was defined by the inactivation time constant of the synaptic conductance.

The complete dynamics of synaptic conductances of the neuron i in our models can be described as follows

$$g_{SynEi}(t) = k_E \cdot \left[\sum_j S\{W_{ji}\} \cdot \sum_{t_{kj} < t} \exp\left(-\frac{t - t_{kj}}{\tau_E}\right) + S\{W_{oi}\} \cdot I_o + S\{W_{PSRi}\} \cdot I_{PSR}(t) \right]$$

$$g_{SynIi}(t) = k_I \cdot \left[\sum_j S\{-W_{ji}\} \cdot \sum_{t_{kj} < t} \exp\left(-\frac{t - t_{kj}}{\tau_I}\right) + S\{-W_{oi}\} \cdot I_o + S\{-W_{PSRi}\} \cdot I_{PSR}(t) \right] \quad (4)$$

The first term in both the above equations describes the temporal summation of the effects of all trains of action potentials coming to the neuron i from all other neurons. The tonic input drive I_o (from a reticular activation system, peripheral and central chemoreceptors, etc.) and feedback drive from pulmonary stretch receptors (PSR) I_{PSR} are considered continuous values. Their effects on synaptic conductances of the neuron i are expressed by the second

and third terms respectively in both above equations. W_{oi} and W_{PSRi} define the synaptic weights of these inputs to the neuron i .

The following values of parameters were chosen to provide realistic amplitudes and shapes of synaptic potentials in the postsynaptic neuron for the used values of synaptic weights: $k_E = 0.006 \mu S$; $k_I = 0.02 \mu S$; $\tau_E = \tau_I = 25$ ms; $I_o = 1$.

Tonic input to the CRPG

It generally is accepted that most neurons participating in the generation of the central respiratory rhythm receive a tonic input. This input includes drive from both peripheral and central chemoreceptors and from different medullary (e.g., reticular activating system) and supramedullary (e.g., pons, hypothalamus) sources (Cohen 1979; Feldman 1986; Richter et al. 1986a; von Euler 1986). In our models, all types of respiratory neurons received a net excitatory synaptic input except for late-I and pre-I neurons (all W_{oi} except those for late-I and pre-I neurons were positive). The two latter neurons received tonic inhibitory drive. Tonic inhibitory drive to late-I neurons has an experimental evidence (Lawson et al. 1989) and was incorporated in previous models (Botros and Bruce 1990). No information of a tonic (excitatory or inhibitory) input to pre-I neurons was found in the literature. We arbitrarily set the tonic input synaptic weights in pre-I neurons to be negative and equal to the inhibitory input weights in late-I neurons. However, the negative weights of tonic drive to late-I and/or pre-I neurons are not critical for our CRPG models: they can operate as well at positive weights of input to both or any of these neurons if the weights provide weak (suprathreshold) depolarization.

Pulmonary feedback loop

We included a pulmonary feedback loop into our CRPG models. In the respiratory system, this feedback is provided by PSR afferents via the vagus nerves. The frequency of PSR discharges is related to lung volume, which, in turn, is controlled by CRPG motor output via the diaphragm movements (Cohen 1979; Feldman 1986; von Euler 1986). The integrated phrenic (diaphragmatic nerve) activity reflects the motor output of CRPG. While developing CRPG models, we made the following simplifications: integrated phrenic activity was obtained by weighted integration of ramp-I and post-I neuron activities and PSR afferent inputs to CRPG neurons were direct [like the models of Geman and Miller (1976) and Botros and Bruce (1990)], although PSR's influence on the real respiratory network is most likely indirect.

Pulmonary feedback loop is described as follows

$$\tau_P \cdot \frac{d}{dt} P = -P + W_{IP} \cdot \sum_{t_{kr} < t} \exp\left(-\frac{t - t_{kr}}{\tau_E}\right) + W_{PP} \cdot \sum_{t_{kp} < t} \exp\left(-\frac{t - t_{kp}}{\tau_E}\right)$$

$$\tau_L \cdot \frac{d}{dt} L = -L + k_P \cdot P + L_{min}; \quad I_{PSR} = k_{PSR} \cdot S\{L - L_{min}\} \quad (5)$$

where P is the integrated phrenic nerve activity; W_{IP} and W_{PP} are the weights that define the contributions of ramp-inspiratory and post-inspiratory discharges, respectively, to the integrated phrenic output; L is the lung volume; I_{PSR} is the activity of PSR related to

the lung volume; k_P and k_{PSR} are positive coefficients; τ_P and τ_L are time constants.

In our simulations, we set $k_P = 1$; $k_{PSR} = 1$; $L_{min} = 0$. The time constants and phrenic weights were chosen to match the form of integrated phrenic bursts obtained from experimental recording (Fig. 1A in Rybak et al. 1997a): $\tau_P = 200$ ms if $(d/dt)P \geq 0$, otherwise $\tau_P = 10$ ms; $W_{ip} = 0.8$; $W_{pp} = 0.2$. The lung inflation-deflation time constant was chosen to be $\tau_L = 200$ ms so that L decayed to its baseline during the first half of expiration to match the lung volume trajectory shown by von Euler (1986, p. 44, Fig. 24).

Two plausible configuration of PSR feedback to the respiratory network were analyzed previously and used in simulation (Botros and Bruce 1990): first, in which PSR afferents excited the post-I and late-I neurons, and second, in which they inhibited the early-I and excited the late-I neurons. We used a combination of these configurations as a basis. In all our CRPG models, PSR afferents inhibited the early-I neurons and excited the post-I and dec-E neurons. The inhibitory inputs to early-I neurons are supported by the observation that these neurons are inhibited by lung inflation (von Euler 1986). Excitatory inputs to post-I neurons are based on the finding of excitation of post-I neurons after electrical stimulation of the vagus nerve (Remmers et al. 1986; Richter et al. 1987; von Euler 1986). The excitatory inputs to dec-E neurons are based on data that these neurons are excited by lung inflation (Cohen 1979; Ezure 1990; Feldman 1986).

Choosing the types and weights of synaptic interactions between the respiratory neurons

The following qualitative criteria have been taken into account while assigning synaptic connections between the respiratory neurons and tuning their weights: a CRPG model should generate a steady respiratory rhythm and show realistic firing patterns and membrane trajectories in all respiratory neurons with and without PSR feedback and a model should respond with physiologically plausible changes in the rhythm and neuronal firing patterns to different perturbations (e.g., disconnection of PSR feedback or different types of external stimulation). The model performances during different perturbations (related to the second criterion) are described in the next paper (Rybak et al. 1997b). However, the network architectures and weights of synaptic connections presented in this paper fulfilled both the above criteria.

The simulation of CRPG models was carried using the MacGregor's (1987) integration method with the integration step of 0.1 ms.

RESULTS

Two-phase CRPG models

Our two-phase CRPG models employ the expiratory off-switch mechanism based on mutual inhibitory connections between the early-I and dec-E neurons and adaptive properties of the dec-E neuron as proposed by Duffin (1991). All other expiratory neurons (post-I, con-E2, E2) do not participate in rhythmogenesis but provide postinspiratory (post-I neurons) and ramp expiratory (E2 neurons) motor output patterns. Therefore, the duration of expiration in these models is independent of the activity of post-I or other expiratory neurons. It is defined completely by the adapting pattern of dec-E neuron and interactions between the dec-E and early-I neurons.

Two-phase CRPG model 1 includes early-I, ramp-I, late-I, dec-E, post-I, con-E2, and E2 neurons. The schematic of

this model is shown in Fig. 1A. Table 2A shows the types of neurons and weights of synaptic connections between them. Model behavior including trajectories of membrane potentials of all neurons and phrenic output is shown in Fig. 2A. The ramp firing pattern of the E2 neuron is based on intrinsic properties of neuron type II used for simulation of this neuron. This neuron type generates the ramp firing pattern when it is released rapidly from a hyperpolarized state (Rybak et al. 1997a). Slow disinhibition from the adapting post-I neuron does not produce this rapid release. To provide an abrupt release from inhibition of the E2 neuron, we have used an additional neuron, con-E2, which has the same membrane properties as the E2 neuron but is less inhibited by the post-I neuron (see Table 2A). As the activity of the post-I neuron decreases, the con-E2 neuron fires earlier than the E2 neuron and abruptly inhibits the post-I neuron, which, in turn, rapidly disinhibits the E2 neuron.

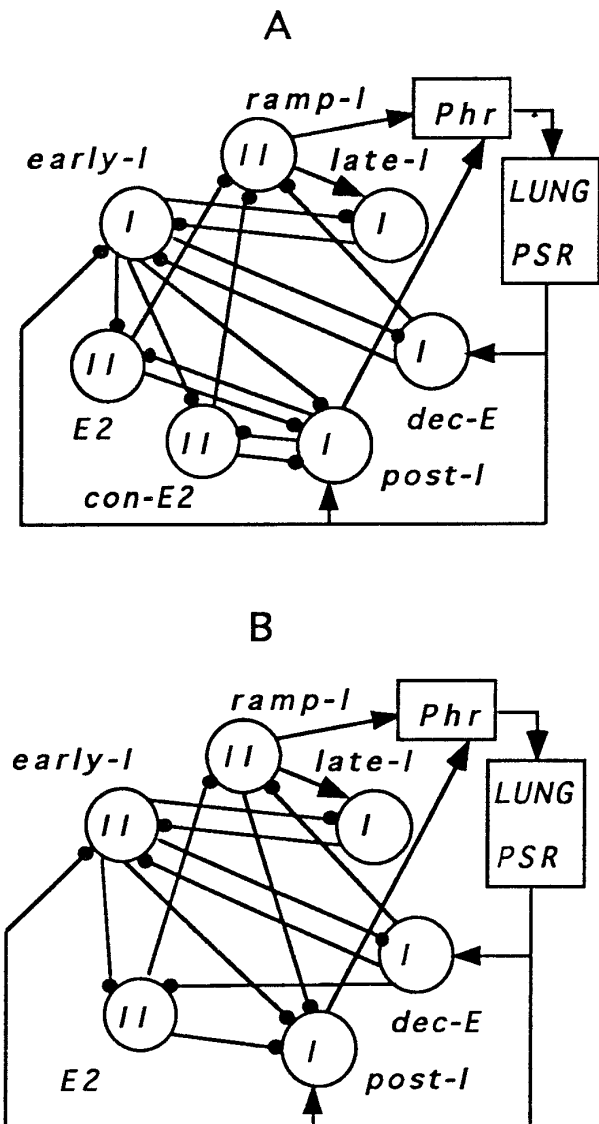


FIG. 1. Schematics of 2-phase central respiratory pattern generator (CRPG) models: model 1 (A) and model 2 (B). Large circles represent neurons. Numbers inside circles indicate neuron types (see Rybak et al. 1997a). Filled circles, inhibitory synapses; arrows, excitatory synapses. Tonic inputs to neurons are not shown.

TABLE 2. *Two-phase models: types of neurons and weights of synaptic inputs*

Neuron	Type	Synaptic Weights of Inputs From Other Neurons, W_{ij}							Tonic W_{oi}	PSR W_{PSRi}
		early-I	ramp-I	late-I	dec-E	post-I	con-E2	E2		
<i>A. Two-phase model 1</i>										
early-I	I			-0.15	-0.54				+1.00	-0.02
ramp-I	II				-4.50		-0.25	-0.25	+1.00	
late-I	I	-0.60	+2.45						-0.50	
dec-E	I	-3.00							+1.00	+0.24
post-I	I	-4.20							+1.00	+0.56
con-E2	II	-4.00				-0.45		-0.30	+1.00	
E2	II	-4.00				-3.60			+1.00	
<i>B. Two-phase model 2</i>										
Neuron	Type	early-I	ramp-I	late-I	dec-E	post-I	E2	Tonic W_{oi}	PSR W_{PSRi}	
early-I	I			-0.15	-0.38			+1.00	-0.02	
ramp-I	II				-4.20		-0.50	+1.00		
late-I	I	-0.60	+2.45					-0.50		
dec-E	I	-3.30						+1.00	+0.56	
post-I	I	-0.20	-0.70				-1.00	+1.00	+0.40	
E2	II	-3.20			-0.27			+1.00		

The two-phase model 1 demonstrates a stable respiratory rhythm and physiologically plausible membrane trajectories and firing patterns under normal conditions and when PSR feedback is disconnected (Fig. 2, A and B). Disconnection of PSR feedback in the model (see Fig. 2B) causes an increase in duration and amplitude of ramp-I neuron and phrenic discharges reflecting the loss of the Hering-Breuer inspiration-inhibiting reflex (Cohen 1979; Feldman 1986; von Euler 1986); a decrease in duration of the post-I neuron burst and postinspiratory phase that corresponds to data obtained by Richter et al. (1986a); and an increase in the expiratory phase duration following the increase in duration of preceding inspiration as reported by Clark and von Euler (1972).

Interestingly, the E2 and con-E2 neurons show rebound spiking at the beginning of the expiratory phase (Fig. 2B), which is analogous to that recorded experimentally (Richter et al. 1993).

The two-phase CRPG model 2 contains the same neurons as the previous model without con-E2 (Fig. 1B; Table 2B). Model 2 differs from model 1 by the mechanism used for the ramp firing patterns of E2 neurons; this mechanism is based here on disinhibition from the slowly adapting dec-E neuron. Figure 3 shows the performance of this model with and without PSR feedback. Disconnecting PSR feedback in model 2 causes the same effects as in model 1 (Fig. 3B).

The interesting points about the two-phase model 2 are that the E2 neuron shows rebound excitation at the beginning of the expiratory phase (Richter et al. 1993) and that the post-I neuron demonstrates a short second burst at the end of expiration (Klages et al. 1993; Remmers et al. 1986; Richter 1996; Richter et al. 1987).

Three-phase models of CRPG

In our three-phase models, the expiratory off-switch mechanism is based on the pre-I neuron, which depolarizes slowly during the late expiratory phase (because of synaptic

excitation from the ramp firing E2 neuron) and generates a short discharge to terminate expiration. The duration of the postinspiratory phase is defined by the properties of post-I, dec-E, and E2 (or con-E2) neurons and their synaptic interactions. The duration of the late expiratory phase (stage II) depends on the ramp firing pattern of the E2 neuron and excitatory synaptic input from E2 to the pre-I neurons. Thus these two phases can be regulated independently unlike the two-phase models. The three-phase models described below differ mainly by the mechanisms used for ramp firing patterns of E2 neurons and for the termination of expiration.

THREE-PHASE CRPG MODEL 1. Three-phase model 1 includes early-I, ramp-I, late-I, post-I, con-E2, E2, and pre-I neurons. The schematic of this model, its behavior with and without PSR feedback, and weights of synaptic connections are shown in Figs. 4A and 5 and Table 3A, correspondingly.

Similar to the two-phase model 1, the ramp firing pattern of E2 neuron in the three-phase model 1 is based on intrinsic neuronal properties. The expiratory off-switch operates in the same way as the inspiratory off-switch. The pre-I neuron is excited by the increasing activity of E2 neuron and then terminates the firing bursts of con-E2 neuron by inhibitory feedback (Figs. 4B and 5A; Table 3A). During the late expiratory phase, the early-I neuron is inhibited by both E2 and con-E2 neurons. Termination of con-E2 neuron discharge by the pre-I neuron disinhibits the early-I neuron. The early-I neuron fires, inhibits post-I, E2 and con-E2, neurons, and initiates inspiration.

The post-I neuron shows a second burst (spike) during the transition from expiration to inspiration. (Fig. 5, A and B). Removal of PSR feedback causes a rebound burst in both E2 and con-E2 neurons at the beginning of the expiratory phase (Fig. 5B). Disconnection of PSR feedback (Fig. 5B) produces an increase in the duration and amplitude of ramp-I neuron and phrenic discharges; a decrease in the duration of post-I neuron bursts and postinspiratory phase; and a decrease in the duration of expiration resulting

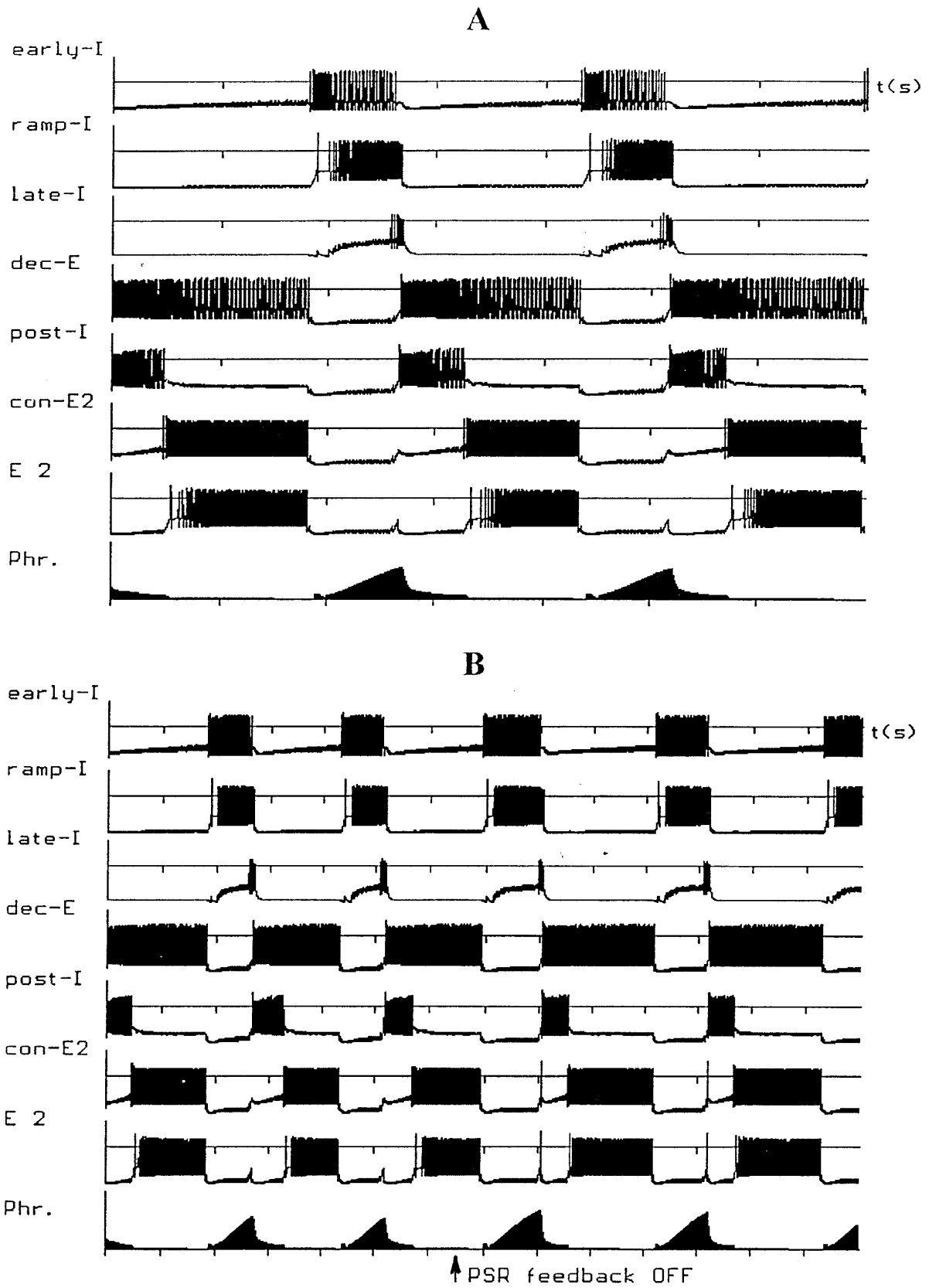


FIG. 2. Performances of the 2-phase model 1 (A and B). In B, pulmonary stretch receptors (PSR) feedback was disconnected at time marked (\uparrow).

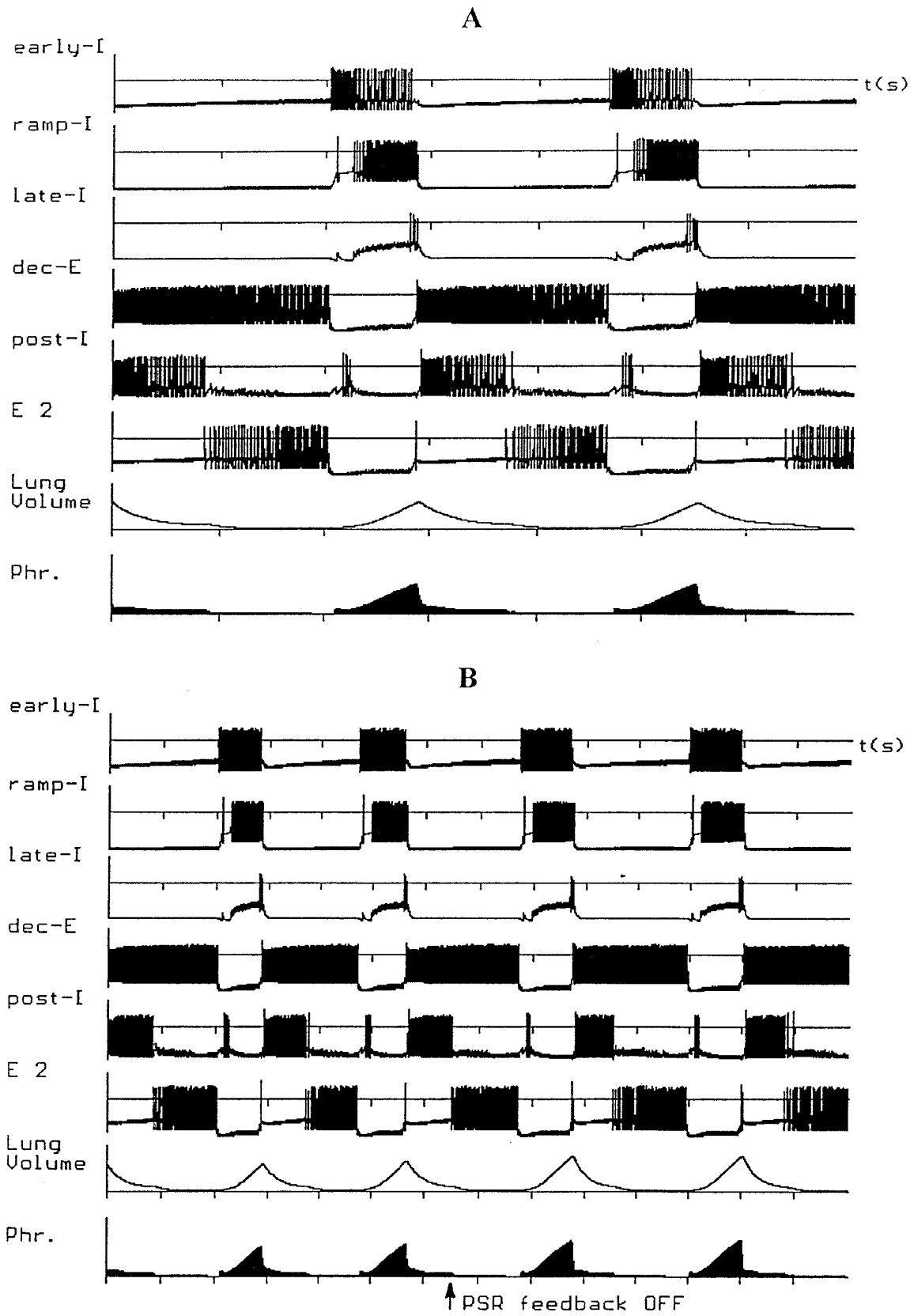


FIG. 3. Performances of the 2-phase model 2 (A and B). In B, PSR feedback was disconnected at time marked (\dagger).

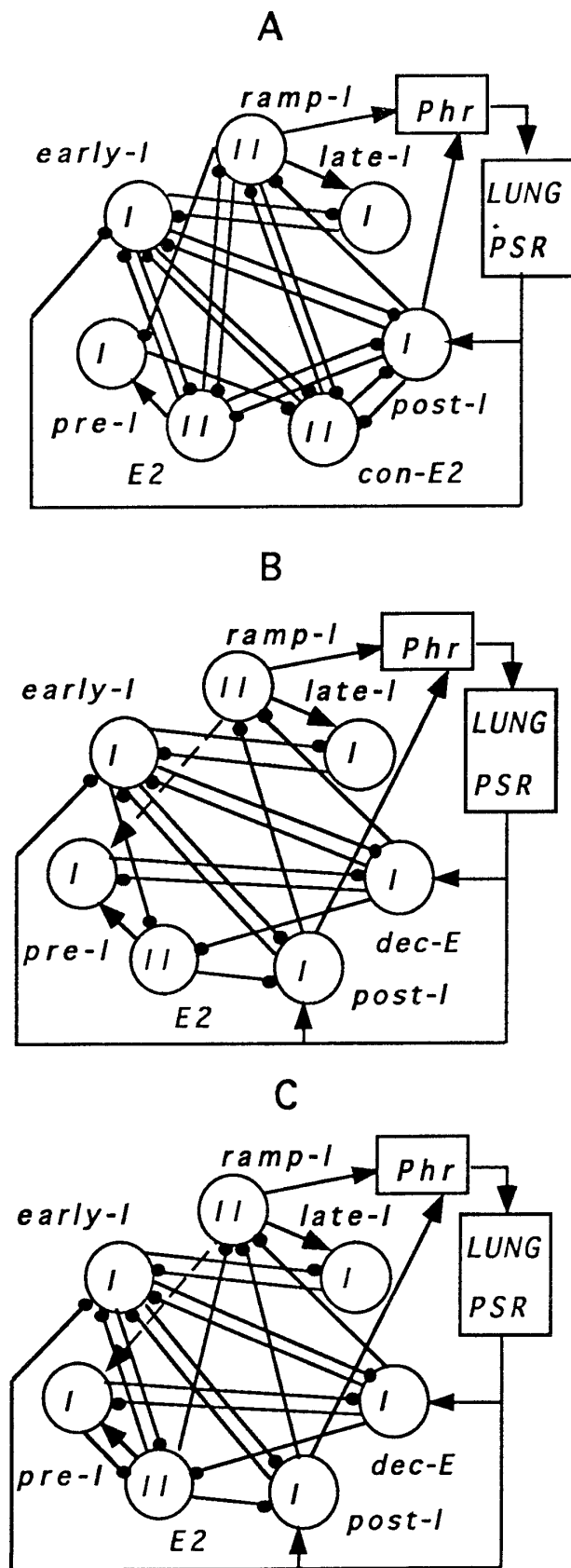


FIG. 4. Schematics of 3-phase CRPG models: model 1 (A), model 2 (B), and model 3 (C). Tonic inputs to neurons are not shown.

from shortening the postinspiratory phase (which probably reflects the loss of the Hering-Breuer expiration-facilitating reflex) (Cohen 1979; Feldman 1986; von Euler 1986).

THREE-PHASE CRPG MODEL 2. Three-phase CRPG model 2 includes early-I, ramp-I, late-I, dec-E, post-I, E2, and pre-I neurons (Fig. 4B³; Table 3B). Model behavior with and without PSR feedback is shown in Fig. 6, A and B.

In this model, the ramp firing pattern of the E2 neuron is produced by disinhibition from the slowly adapting dec-E neuron. The expiratory off-switch mechanism in the three-phase model 2 is similar to that in the three-phase model 1. However, during the late (stage II) expiration, early-I and ramp-I neurons are inhibited by the dec-E neuron (instead of E2 and con-E2 in the three-phase model 1; Fig. 4B; Table 3B). The pre-I neuron provides an inhibitory synaptic input to dec-E neuron but not to con-E2 neuron (Fig. 4B; Table 3B). Termination of dec-E neuron discharge disinhibits early-I and ramp-I neurons. The early-I neuron fires, inhibits all other neurons (except ramp-I and pre-I) and initiates inspiration. Note that the E2 neuron shows rebound spiking in the beginning of the expiratory phase (Fig. 6, A and B) as seen in experimental recordings (Richter et al. 1993).

Similar to the three-phase model 1, disconnection of PSR feedback in model 2 causes an increase in the duration and amplitude of ramp-I neuron and phrenic discharges and a decrease in the duration of the post-I neuron burst and postinspiratory phase (Fig. 6B). However, PSR feedback disconnection produces an increase in the duration of the whole expiratory interval, which is opposite to three phase model 1, but similar to our two-phase models (see above). This may reflect the dependence of expiratory phase duration on the duration of preceding inspiration as reported by Clark and von Euler (1972).

THREE-PHASE CRPG MODEL 3. Three-phase model 3 of CRPG has been developed to combine properties of both the above models and includes the neuron types used in the three-phase model 2 (Fig. 4C; Fig. 7; Table 3C). Similar to the three-phase model 2, the expiratory off-switch in model 3 operates via the pre-I neuron. However, during late (stage II) expiration the early-I and ramp-I neurons are inhibited by both dec-E and E2 neurons (Fig. 4C; Table 3C). Because of this, the pre-I neuron provides inhibitory synaptic inputs to both dec-E and E2 neurons (Fig. 4C; Table 3C). Termination of dec-E and E2 neuronal discharges by the burst of pre-I neuron disinhibits early-I and ramp-I neurons, which initiate inspiration. The respiratory rhythm and membrane trajectories of respiratory neurons in the three-phase model 3 with and without PSR feedback are similar to those in three-phase model 2 (Fig. 7, A and B). However, behavior of models 2 and 3 differ significantly under the conditions of external stimulation (e.g., when short stimuli are applied to PSR feedback at the end of expiration) (see Rybak et al. 1997b).

³ The excitatory synaptic input from the ramp-I neuron to the pre-I neuron in the three-phase models 2 and 3 (shown in Fig. 4, B and C, by the dash lines) does not affect model performance. It was used to simulate depolarization of pre-I neurons at the end of inspiration (see Fig. 1A in Rybak et al. 1997a).

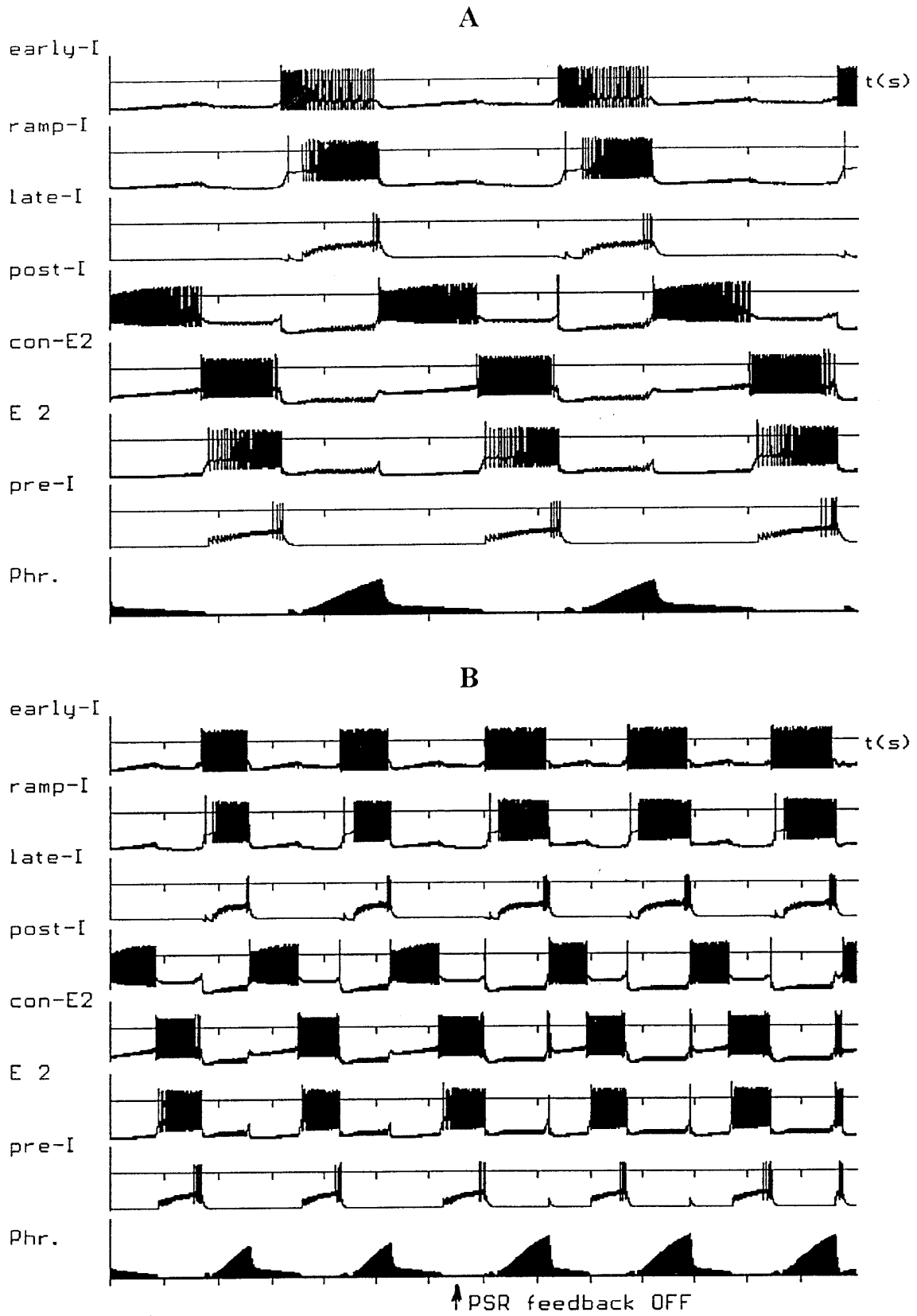


FIG. 5. Performances of the 3-phase model 1 (A and B). In B, PSR feedback was disconnected at time marked (†).

TABLE 3. *Three-phase models: types of neurons and weights of synaptic inputs*

Neuron	Type	Synaptic Weights of Inputs From Other Neurons, W_{ij}							Tonic W_{oi}	PSR W_{PSRi}
		early-I	ramp-I	late-I	post-I	con-E2	E2	pre-I		
<i>A. Three-phase model 1</i>										
early-I	I			-0.15	-0.45	-0.32	-0.15		+1.00	-0.02
ramp-I	II				-2.00	-2.00	-2.00		+1.00	
late-I	I	-0.60	+2.40						-0.50	
post-I	I	-4.20				-0.28	-0.09		+1.00	+0.80
con-E2	II	-2.80	-0.07		-0.40			-0.80	+1.00	
E2	II	-2.80	-0.07		-3.70				+1.00	
pre-I	I		-0.05				+1.93		-0.50	
<i>B. Three-phase model 2</i>										
early-I	I			-0.15	-0.45	-1.80			+1.00	-0.02
ramp-I	II				-3.50	-4.00			+0.90	
late-I	I	-0.60	+2.45						-0.50	
dec-E	I	-4.40						-0.30	+1.25	+0.56
post-I	I	-3.40					-0.70		+1.00	+0.40
E2	II	-3.50				-0.23			+1.00	
pre-I	I		+0.40			-0.25	+2.95		-0.50	
<i>C. Three-phase model 3</i>										
early-I	I			-0.15	-0.45	-0.40	-0.50		+1.00	-0.02
ramp-I	II				-3.20	-4.00	-3.00		+0.90	
late-I	I	-0.60	+2.45						-0.50	
dec-E	I	-3.40						-0.50	+1.25	+0.56
post-I	I	-2.40					-1.00		+1.00	+0.40
E2	II	-2.50				-0.23		-0.45	+1.00	
pre-I	I		+0.42			-0.15	+3.00		-0.50	

Role of intrinsic properties of respiratory neurons in CRPG performance

ROLE OF INTRINSIC PROPERTIES OF RESPIRATORY NEURONS IN CONTROLLING THE DURATIONS OF RESPIRATORY PHASES. We have focused on the dynamics of $[Ca^{2+}]_{in}$ concentration and calcium-dependent potassium conductance $g_{AHP}(Ca)$ in different respiratory neurons and on the role of these dynamics for controlling the respiratory phase durations and phase switching. These intrinsic neuronal processes determine firing patterns of respiratory neurons and rates of spiking frequency changes. This, in turn, influences the durations of respiratory phases.

Figure 8A shows membrane potential trajectories of respiratory neurons with the simultaneous dynamics of $[Ca^{2+}]_{in}$ and $g_{AHP}(Ca)$ in early-I, ramp-I, post-I, and E2 neurons in three-phase model 1. The timing of the inspiratory off-switch and the duration of the inspiratory phase in all our models depend on both the adaptive characteristics of the early-I neuron (which inhibits the late-I neuron) and the ramp firing activity of the ramp-I neuron (which excites the late-I neuron). These characteristics are defined by $[Ca^{2+}]_{in}$ and g_{AHP} dynamics in these neurons. Thus blocking of calcium influx or $[Ca^{2+}]_{in}$ accumulation in the early-I neuron eliminates its adaptive properties and arrests the respiratory cycle in the inspiratory phase (“apneusis”; Fig. 9A). In contrast,

blocking of calcium influx or accumulation in ramp-I neuron causes high-frequency firing patterns instead of slow increases in spike frequency. The duration of inspiration decreases significantly. The resultant breathing pattern does not contain complete inspiratory phases, but only short gasping-like discharges of inspiratory neurons (Fig. 9B).

Adaptive properties of dec-E neurons are very important for the expiratory off-switch and rhythmogenesis in all our models except the three-phase model 1. In both two-phase models, the existence and timing of the expiratory off-switch depend directly on the adaptive properties of dec-E neuron. In three-phase models 2 and 3, adaptation of the dec-E neuron defines the ramp firing patterns of the E2 neuron, which excites the pre-I neuron to terminate expiration. Because the adaptive properties of the dec-E neuron are defined by $[Ca^{2+}]_{in}$ and g_{AHP} dynamics, blocking of calcium influx or accumulation in this neuron prevents adaptation of firing and stops the respiratory oscillations. In two-phase model 2 and three-phase models 2 and 3 the final state can be considered as a “postinspiratory apnea” because dec-E and post-I neurons became active permanently whereas all other neurons are inhibited. In two-phase model 1 the final state can be considered as an “expiratory apnea,” because dec-E and E2 are active permanently after cessation of the rhythm.

Three phase model 1 is the only model in which the intrinsic properties of post-I and E2 neurons independently control

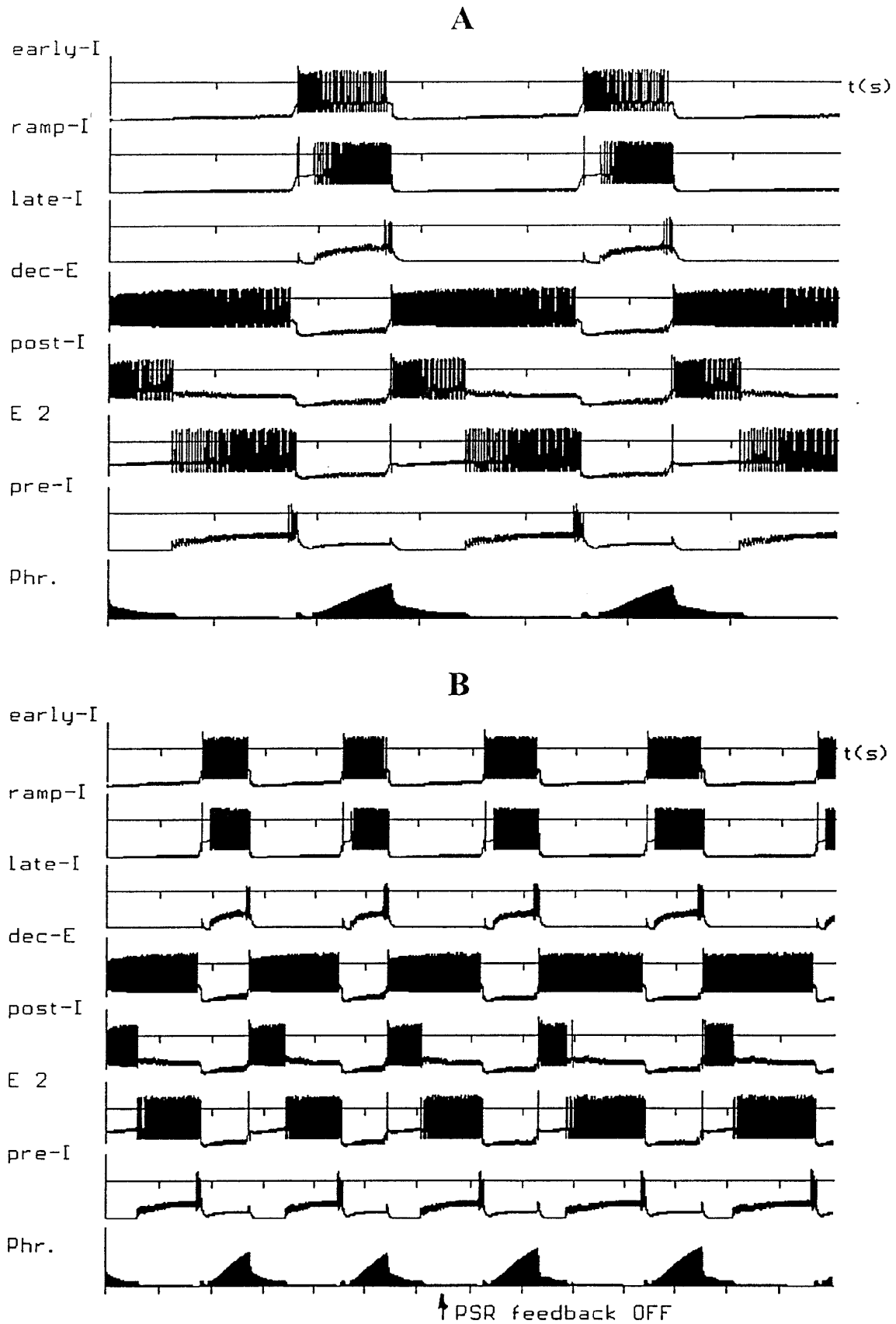


FIG. 6. Performances of the 3-phase model 2 (A and B). In B, PSR feedback was disconnected at time marked (†).

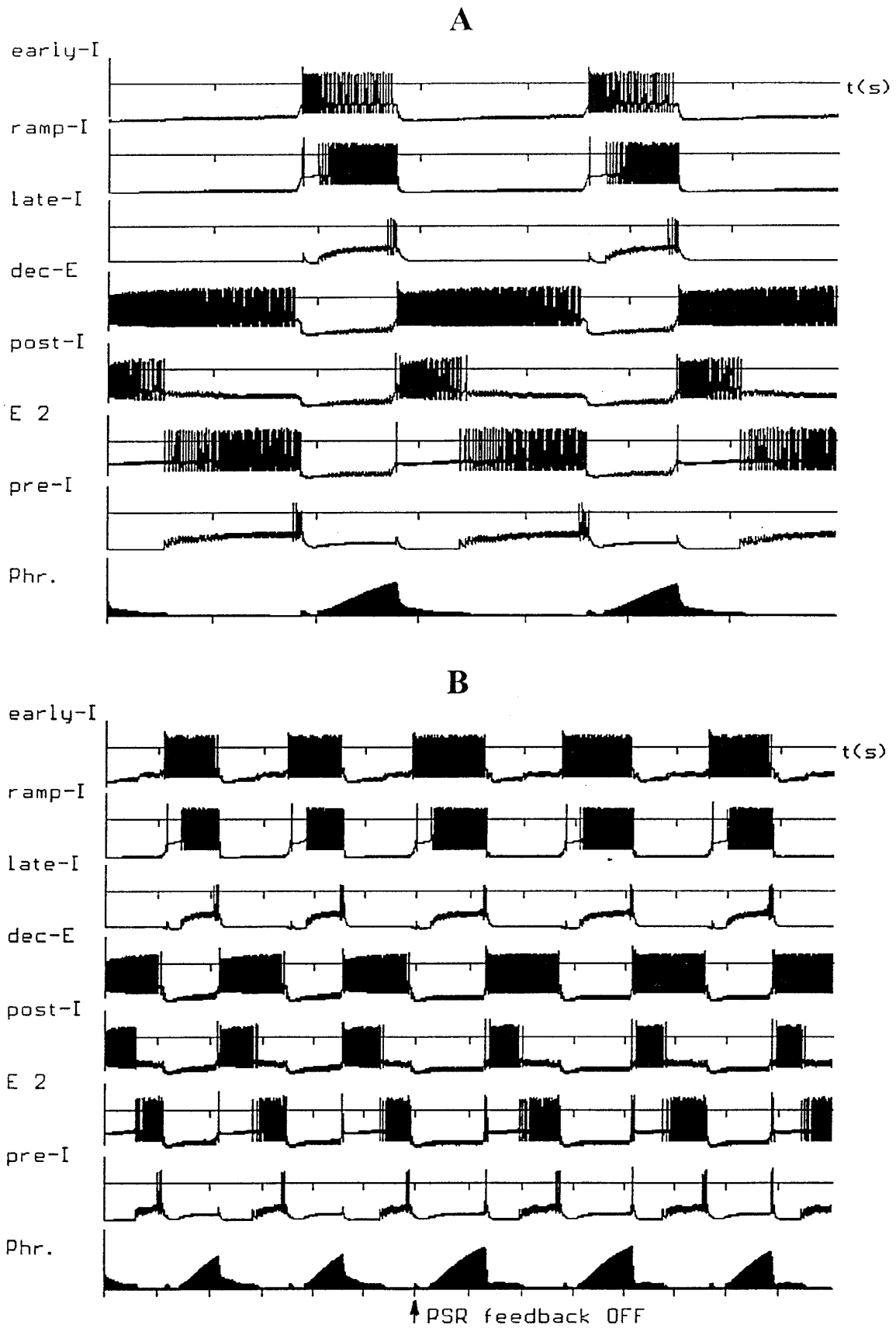


FIG. 7. Performances of the 3-phase model 3 (A and B). In B, PSR feedback was disconnected at time marked (\uparrow).

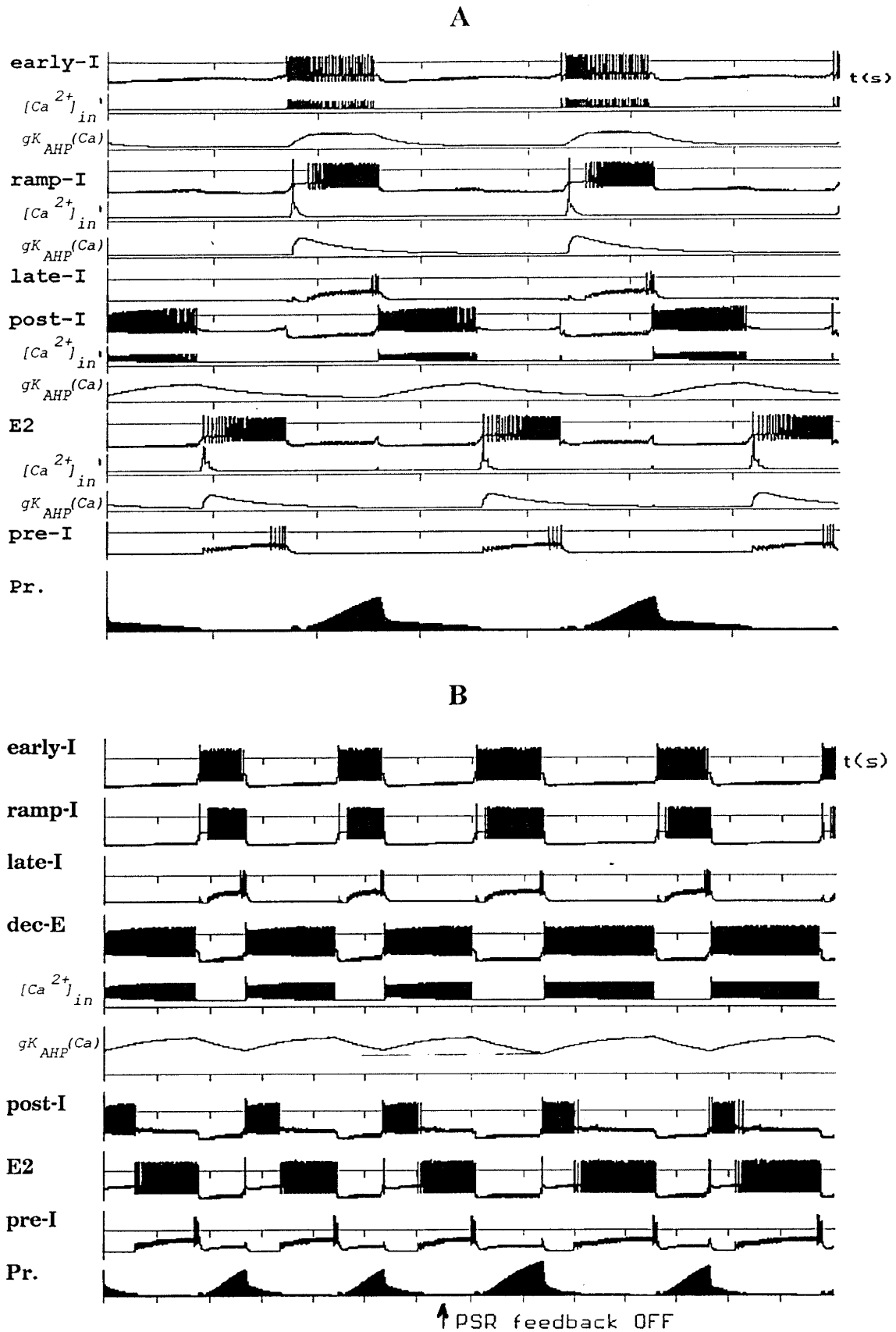


FIG. 8. Dynamics of $[Ca^{2+}]_{in}$ and g_{AHP} during CRPG performance. *A*: 3-phase model 1 (trajectory of membrane potential of stage II constant firing expiratory neuron is not shown). *B*: 3-phase model 2. Trajectories of $[Ca^{2+}]_{in}$ and g_{AHP} in early inspiratory (early-I), ramp inspiratory (ramp-I), postinspiratory (post-I), and stage II expiratory (E2) neurons in *A* and in decrementing expiratory (dec-E) neuron in *B* are shown *below* corresponding membrane potential trajectories.

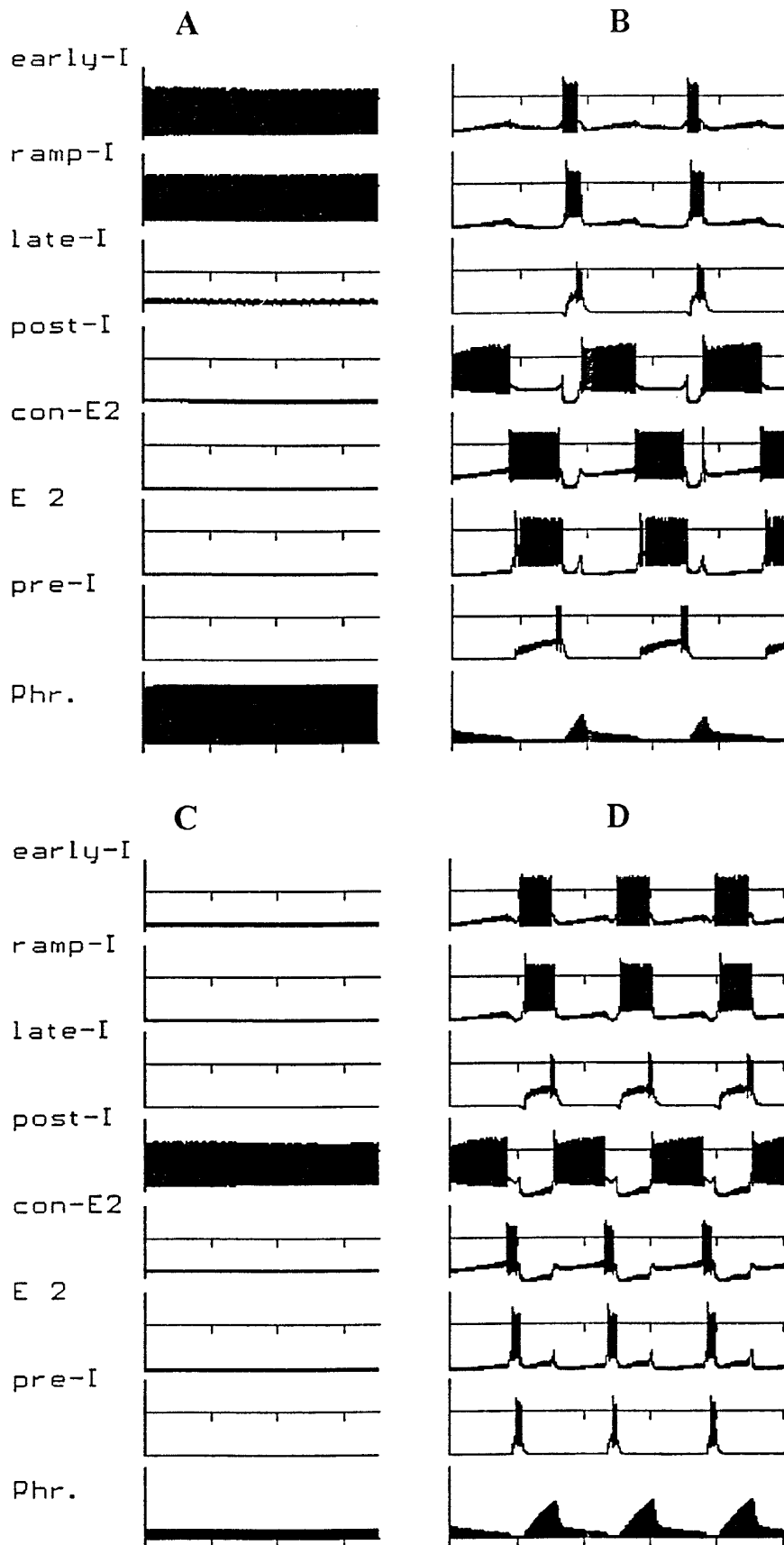


FIG. 9. Performances of the 3-phase model 1 when calcium accumulation was blocked ($[Ca^{2+}]_{in} = [Ca^{2+}]_{in0} = \text{const}$) in different respiratory neurons: early-I neuron (A); ramp-I neuron (B); post-I neuron (C); E2 neuron (D).

the duration and timing of the postinspiratory and late expiratory phases, respectively. The switching between postinspiration and late expiration in this model depends on the adaptation in firing of the post-I neuron. Blockage of calcium influx or accumulation in the post-I neuron abolishes its adaptive properties and produces a “postinspiratory apnea” (Fig. 9C). The expiratory off-switch in this model depends on ramp firing characteristics of the E2 neuron, which are defined by $[Ca^{2+}]_{in}$ and g_{AHP} dynamics in this neuron. Blocking of calcium influx or accumulation in the E2 neuron causes high-frequency firing patterns instead of a slow increase of spiking frequency. Consequently, the duration of the late expiratory phase decreases significantly. The resultant breathing pattern contains only two complete phases: inspiration and postinspiration, and short bursts of the E2 neuron (Fig. 10D).

In summary, 1) the duration of inspiration and the timing of the inspiratory off-switch are controlled by $[Ca^{2+}]_{in}$ and g_{AHP} dynamics in the early-I and ramp-I neurons; 2) the duration of expiration and the timing of the expiratory off-switch are controlled by $[Ca^{2+}]_{in}$ and g_{AHP} dynamics in the dec-E neuron in all our models, except three-phase model 1; and 3) in three-phase model 1, the duration of the postinspiratory phase and timing of the switch between postinspiration and late expiration are controlled by $[Ca^{2+}]_{in}$ and g_{AHP} dynamics in the post-I neuron, whereas the duration of the postinspiratory phase and timing of the expiratory off-switch are controlled by $[Ca^{2+}]_{in}$ and g_{AHP} dynamics in the E2 neuron.

INTRINSIC PROPERTIES OF RESPIRATORY NEURONS CAN MAINTAIN THE RELATIONSHIP BETWEEN THE DURATIONS OF INSPIRATORY AND EXPIRATORY PHASES. Clark and von Euler (1972) showed in both cat and man that expiratory duration (T_E) was dependent on inspiratory duration (T_I), usually with an approximately linear relationship, so that when T_I was shortened or lengthened (e.g., by perturbations applied to the lung), the succeeding T_E was reduced or increased, respectively⁴ (Cohen 1979). It is known that vagotomy decreases breathing frequency as a result of the increase in duration and deepness of both inspiration and active expiration (Cohen 1979). However, it is difficult to find an explanation for the T_E dependence on T_I in a pure “network paradigm” because all inspiratory neurons are silent during expiration and cannot produce such long-term aftereffects and PSR feedback is almost ineffective in late expiration or even does not operate after vagotomy.

Our analysis showed that all our models except the three-phase model 1 demonstrate the above functional relationship between T_E and T_I . This allows a hypothetical explanation for the above dependence drawn from our simulations. The explanation is based on a combination of intrinsic neuronal and network properties. Figure 8B shows the trajectories of membrane potentials of all neurons and dynamics of $[Ca^{2+}]_{in}$ and g_{AHP} in dec-E neuron in the three-phase model 2. Disconnection of PSR feedback causes a prolongation of both the inspiratory phase and entire expiratory period. The mechanism for changing the duration of expiration (T_E) after a change in the duration of the preceding inspiratory phase (T_I) is the following. Figure 8B shows that the conductance

of $K_{AHP}(Ca)$ channels (g_{AHP}) in dec-E neuron decreases during inspiration and increases during expiration. When the duration of the inspiratory phase (T_I) increases (e.g., because of vagotomy), the value of g_{AHP} in dec-E neuron decreases for a longer period of time and reaches a lower level. Therefore, the shunting effect of $K_{AHP}(Ca)$ current on the activity of the dec-E neuron is diminished. This increases the spike frequency of dec-E, thereby increasing the inhibition of E2 by dec-E during the following expiration. Consequently, the spike frequency of E2 neuron increases longer, which delays both the firing of the pre-I neuron and the expiratory off-switch so prolonging the duration of expiration (T_E). We believe, that this intrinsic neuronal mechanism can operate in the respiratory system to maintain the relationship between the inspiratory and expiratory durations.

Three-phase models of CRPG based on biphasic switching neurons

Analyzing recordings of pre-I neurons obtained in different laboratories, we found that some of these neurons generated two bursts per respiratory cycle: the first during the transition from expiration to inspiration and the second during the transition from inspiration to expiration (Onimaru and Homma 1987; Paton 1996; Schwarzacher et al. 1995; K. Morris, personal communication). Using three-phase models 1–3 as bases, we have developed three additional CRPG models, which have been called “three-phase BS” (biphasic switcher) models 1, 2, and 3, respectively. These CRPG models use a biphasic switching neuron, called a late/pre-I neuron, which combines the functions of both late-I and pre-I neurons and generates two bursts per respiratory cycle providing both inspiratory and expiratory off-switching. Figure 10 shows the schematic of one of these models: the three-phase BS model 1. The weights of synaptic connections used in this model are presented in Table 4 and its behavior is shown in Fig. 11.

Simulations showed that performance of each three-phase BS model is similar to the corresponding basic three-phase model. This indicates the possibility that CRPG generates the respiratory rhythm using biphasic switching neurons which provide both off-switching mechanisms (inspiratory and expiratory).

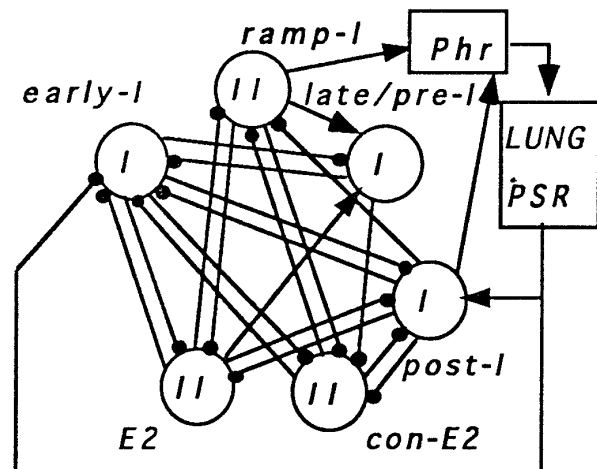


FIG. 10. Schematics of the 3-phase BS model 1. Tonic inputs to neurons are not shown.

⁴ Several studies have shown that the coupling of T_E and T_I in vivo is restricted to a limited set of circumstances (e.g., Mitchell et al. 1982).

DISCUSSION

This study represents the first attempt to develop CRPG models based on realistic Hodgkin-Huxley type models of single respiratory neurons. Using these single neuron models, we have developed a variety of CRPG models. The results of our simulations must be considered with the account of limitations of the modeling approach used. At the cellular level, these limitations result from employing single compartment models of neurons without considering dendritic integration and presynaptic mechanisms. Then, because of the insufficiency of biophysical data from respiratory neurons, we have taken some data from unidentified brain stem and thalamic neurons and used arbitrary numbers for channel density. At the network level, the limitations concern arbitrary numbers of synaptic weights. In addition, the CRPG models presented here do not contain populations of each neuronal type that would be more realistic. At the systems level, the limitations relate to very simple models of lung and PSR and simplified configuration of PSR feedback to the respiratory network. We have not considered pontine structures and their descending influence, which may play a role in rhythm generation (see, for example, Feldman 1986; von Euler 1986) although our models do not exclude a role for the pons in rhythm generation.

However, all our CRPG models generate respiratory rhythm and firing patterns consistent with experimental data. Our simulations clearly show that the intrinsic properties of respiratory neurons are integrated with the network properties of CRPG at three hierarchical levels: at the cellular level, to provide the specific firing patterns of respiratory neurons (e.g., ramp firing pattern), at the network level, to provide switching between respiratory phases, and at the systems level, to control the duration of inspiration and expiration under different conditions (e.g., lack of PSR feedback).

Ramp firing patterns and performance of CRPG models

One of the main findings in our previous paper (Rybak et al. 1997a) was that the ramp firing patterns of respiratory neurons may result from a combination of preceding synaptic inhibition and intrinsic neuronal properties [$K_{AHP}(Ca)$ and Ca_T membrane channels]. In the network CRPG models described in the present paper, we have used successfully this mechanism to reproduce the ramp firing patterns of respiratory neurons instead of recurrent excitation that was employed in previous models (Botros and Bruce 1990; Gottschalk et al. 1994; Ogilvie et al. 1992). It should be emphasized that our network findings (architectures of different CRPG models) have independent significance because all our network models operated well when reciprocal excita-

tion was used as a mechanisms for the ramp firing pattern of ramp-I neurons. However, in this case, it was not possible to obtain the specific membrane trajectory of ramp-I neurons such as rebound spike followed by delayed ramp firing (see Fig. 1, *A* and *B*, in Rybak et al. 1997a).

Inspiratory off-switch mechanism: a contribution of PSR feedback

The mechanism for the inspiratory off-switch used in all our models was described previously by Cohen and Feldman (Cohen 1979; Cohen and Feldman 1977; Feldman 1986) and incorporated into some earlier models (Botros and Bruce 1990; Gottschalk et al. 1994; Ogilvie et al. 1992). This mechanism operates via the off-switching late-I neuron. The late-I neuron depolarizes during inspiration due to both increasing excitation from the ramp-I neuron and decreasing inhibition from the adapting early-I neuron. When membrane potential reaches firing threshold, the late-I neuron fires and inhibits the early-I neuron; the latter, in turn, disinhibits the post-I (or dec-E) neuron, which activates and initiates expiration.

In our models, PSR feedback inhibits early-I and excites post-I (and dec-E) neurons. The inhibitory effect of PSR feedback on early-I neuron reduces inhibition to the late-I neuron, which fires earlier and shortens inspiration. This is the mechanism that provides the Hering-Breuer inspiration-inhibiting reflex (Cohen 1979; Feldman 1986; von Euler 1986) in our models. In each model, the trajectory of phrenic activity with PSR feedback reproduce that without feedback until the inspiratory phase is terminated. Thus PSR feedback does not influence phrenic activity before the inspiratory termination, and the inspiratory off-switch mechanism used is of the ‘‘all-or-none type’’ (Cohen 1979). In contrast, Younes et al. (1978) showed that PSR feedback influenced phrenic neurogram during some period of time before the inspiratory off-switching and hence provides a ‘‘gradual’’ process of inspiratory termination. The network models presented here, composed of single neurons of each type, fail to reproduce this phenomenon. However, we found that such gradual process of inspiratory termination can be demonstrated with our populational CRPG models (comprising populations of 25 respiratory neurons of each type with randomly distributed weights of connections) (unpublished data).

Three-phase versus two-phase CRPG modes and post-I versus dec-E neurons

The number of independently controlled respiratory phases has been extensively tested in our CRPG simulations.

TABLE 4. *Three-phase BS model 1: types of neurons and weights of synaptic inputs*

Neuron	Type	Synaptic Weights of Inputs From Other Neurons, W_{ij}						Tonic W_{oi}	PSR W_{PSRi}
		early-I	ramp-I	late/pre-I	post-I	con-E2	E2		
early-I	I			-0.15	-0.45	-0.32	-0.15	+1.00	-0.02
ramp-I	II				-2.00	-2.00	-2.00	+1.00	
late/pre-I	I	-0.60	+2.40				+1.93	-0.50	
post-I	I	-4.20					-0.15	+1.00	+0.80
con-E2	II	-2.80	-0.07	-0.80	-0.40			+1.00	
E2	II	-2.80	-0.07		-3.70			+1.00	

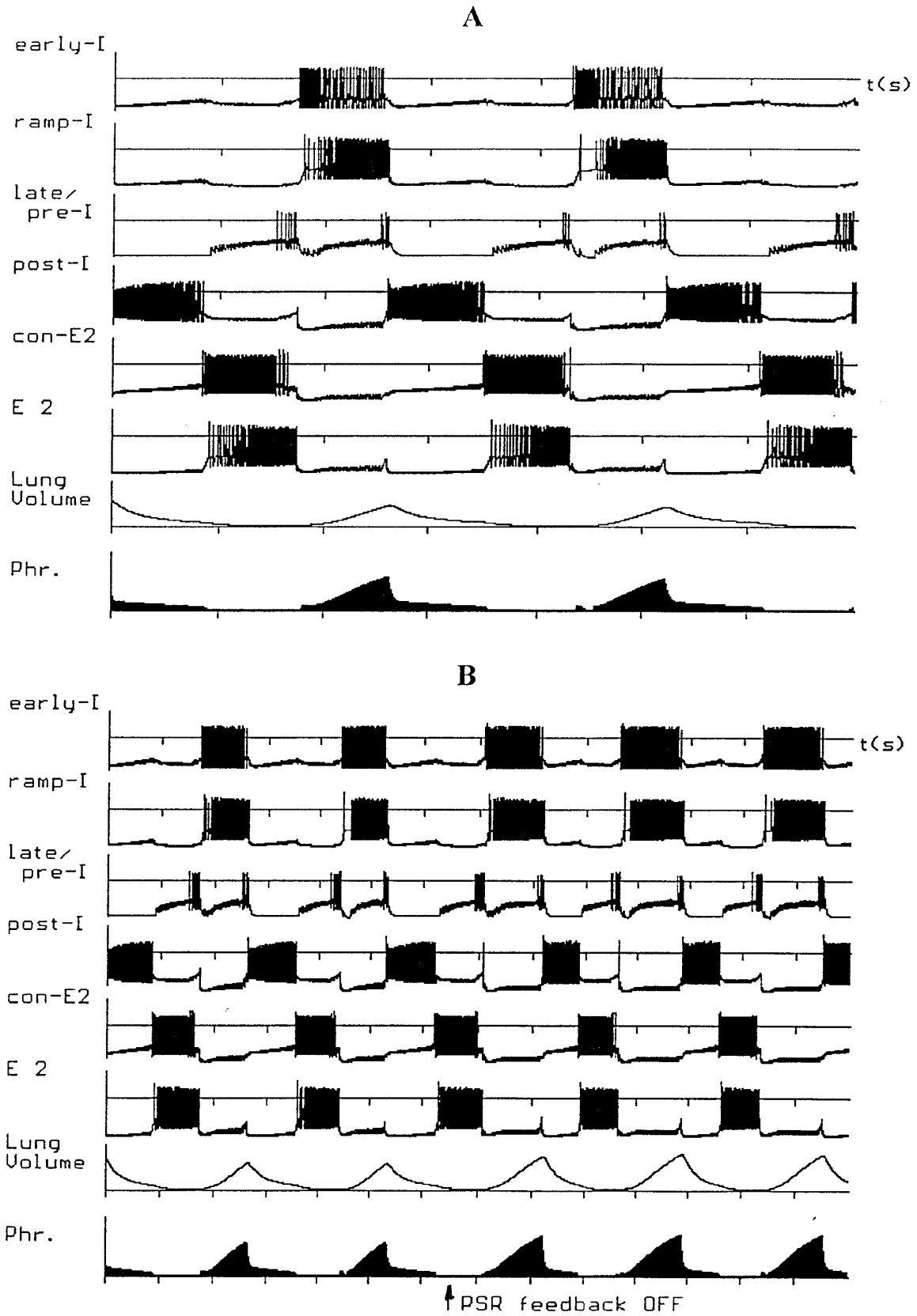


FIG. 11. Performances of the 3-phase BS model 1 (A and B). In B, PSR feedback is disconnected at time marked (↑).

We have considered two two-phase models (1 and 2) and six three-phase models (1; 2; 3; BS 1; BS 2; BS 3). All models contain post-I and E2 neurons. However, in both the two-phase models, post-I and E2 neurons are not necessary for respiratory rhythm generation or for controlling expiratory duration. In these models, the dec-E neuron is essential. The adaptive properties of the dec-E neuron and interactions between the dec-E and the early-I neurons control both the expiratory off-switch and the duration of the entire expiratory interval. These models are consistent with the concept of Duffin et al. (1995) but contradict the evidence supporting independently regulated postinspiratory and late expiratory (stage II) phases (Klages et al. 1993; Remmers et al. 1986; Richter 1996; Richter and Ballantyne 1983; Richter et al. 1986a, 1987; Schwarzacher et al. 1991).

The dec-E neuron also plays an important role in the three-phase models 2 and 3 (and BS 2 and BS 3). Adaptive properties of this neuron and its inhibitory synaptic input to the E2 neuron control the duration of both postinspiratory and late expiratory phases. Thus the post-I neuron is much less important in these models than dec-E neuron. This contradicts the three phase theory of Richter that considers the post-I neuron to be a key element of CRPG (Gottschalk et al. 1994; Ogilvie et al. 1992; Richter 1996; Richter and Ballantyne 1983; Richter et al. 1986a).

Three-phase model 1 and the BS model 1 are the only models in which the post-I neuron plays an essential role in controlling the postinspiratory phase and switching between the postinspiratory and late expiratory phases, which is consistent with the three-phase theory (Richter 1996; Richter and Ballantyne 1983; Richter et al. 1986a, 1987).

It is not clear whether dec-E and post-I neurons belong to the same or different populations of respiratory neurons because the difference between their firing patterns is not significant (Ezure 1990; Feldman 1986; von Euler 1986). However, we have focused on the possible functional consequences of this difference. Our simulations demonstrate that dec-E and post-I neurons can play principally different roles for the key mechanisms of respiratory pattern generation.

Expiratory off-switch mechanism

Because the expiratory off-switch mechanism is understood poorly, we explored two plausible mechanisms. The first mechanism was based on the adapting pattern of the dec-E neuron and mutual inhibitory connections between the early-I and the dec-E neurons (two-phase model 1 and 2). The second mechanism employed phase switching neurons (the pre-I neuron in three-phase models 1, 2, and 3, and the late/pre-I neuron in three-phase BS models 1, 2, and 3). Both these mechanisms allow the ramp firing pattern of E2 neurons and the expiratory off-switch simultaneously. However, the second mechanism is more plausible if a three-phase model of CRPG is accepted. The best experimental support for this off-switch mechanism would be the finding of an excitatory input from an E2 neuron to a phase switching pre-I neuron.

It cannot be ruled out that the pre-I neurons, which contribute to the expiratory off-switch, have unique bursting or pacemaker properties as previously reported in neonatal rats (Smith et al. 1991). However, these properties were not explored in the present study because it remains unknown

whether pacemaker properties are important for eupneic breathing or even present in pre-I neurons of the adult respiratory network.

Some post-I neurons display a second burst at the end of expiration as recorded in vivo (Klages et al. 1993; Remmers et al. 1986; Richter et al. 1987; Richter 1996). It was supposed that this second burst contributed to the expiratory off-switch mechanism (Richter et al. 1987; Richter 1996). In some of our models, post-I neurons also demonstrate a second bursts at the end of expiration (2-phase model 2 in Fig. 3; 3-phase model 1 in Fig. 5; 3-phase BS model 1 in Fig. 11B). However, the second burst of post-I neuron in these models is a consequence and not the source of the expiratory off-switch. Indeed, we have been unable to find a network configuration in which the second burst in post-I neurons was necessary for the expiratory off-switch mechanism.

Mechanisms for the ramp firing patterns of E2 neurons

Two different mechanisms for the ramp firing pattern of the E2 neuron were explored in our CRPG simulations. The first mechanism (used in 2-phase model 1, 3-phase model 1 and BS model 1) based on the intrinsic properties of E2 neurons (neurons type II) (see Rybak et al. 1997a) and on a rapid release from inhibition. This mechanism requires the presence of the additional con-E2 neuron, which receives a weaker inhibition from the post-I neuron than the E2 neuron, fires earlier and abruptly inhibits the post-I neuron to provide rapid disinhibition of the E2 neuron. Like the E2 neuron, the con-E2 neuron is active in the late expiratory phase but does not show a ramp firing pattern. The existence of this kind of neuron awaits experimental support. Interestingly, preliminary analysis of our populational CRPG models (comprising populations of respiratory neurons with randomly distributed weights of connections) showed that a subpopulation of con-E2-like neurons always was present in the population of E2 neurons (unpublished data). The described mechanism for the ramp firing pattern of E2 neurons can be tested experimentally. If the idea about this mechanism is correct, blocking the calcium influx or accumulation in E2 neurons should cause high-frequency patterns instead of a slow increase in spiking frequency.

The second mechanism for ramp frequency E2 discharges used in the two-phase model 2, three-phase models 2 and 3, and BS models 2 and 3 was based on disinhibition from slowly adapting dec-E neurons. This mechanism requires the presence of dec-E neurons, since the post-I neuron is not active during the late expiratory phase.

The three-phase models described here differ from the mechanism used for the ramp firing of E2 neurons and by the mechanism for termination of expiration. Although these details look insignificant for CRPG performances under normal conditions, behavior of the models become significantly different under phasic afferent stimulation applied during expiration. A descriptions of such computational experiments, model comparison, and comparable evaluation of the models are presented in the following paper (Rybak et al. 1997b).

Three-phase BS models and biphasic switching neurons

Our three-phase models were also successful when a biphasically firing neuron was incorporated in CRPG models

replacing late-I and pre-I neurons (BS models 1–3). Because biphasically firing respiratory neurons of similar type were found in different research laboratories (Onimaru and Homma 1987; Paton 1996; Schwarzacher et al. 1995; K. Morris, unpublished data), our results permit consideration of this type of neuron as a candidate for a dual (inspiratory and expiratory) off-switching neuron. The question is whether this neuronal type belongs to a specific group of neurons or may be included into a neuronal group considered earlier (e.g., pre-I or late-I neuronal groups). It is interesting that preliminary data from our populational model demonstrate that late-I, pre-I, and late/pre-I neurons can belong to the same neural population but show either purely late-I or purely pre-I or both type of discharge patterns depending on randomized weights of connections (unpublished results).

Role of intrinsic properties of respiratory neurons in CRPG performance

The dynamics of calcium and calcium-dependent potassium conductances are known to be involved in the control of respiratory phase durations and in phase switching mechanisms by controlling the degree of adaptation in some respiratory neurons (Champagnat and Richter 1994; Champagnat et al. 1986; Pierrefiche et al. 1995; Richter et al. 1983, 1986b, 1993). It was emphasized that the early-I and post-I neurons were the most relevant neurons in which intrinsic properties contribute to respiratory rhythmogenesis. Our analysis showed that the role of intrinsic properties of different respiratory neurons depends strongly on the accepted mechanisms for phase switching and for shaping of the specific firing patterns of respiratory neurons. In our models, the dynamics of calcium and calcium-dependent potassium conductances in early-I and ramp-I neurons regulate the inspiratory duration and timing of the inspiratory off-switch. In contrast, intrinsic properties of post-I neurons were unimportant in all our models, except three-phase model 1 and BS model 1, in which they controlled the duration of the postinspiratory phase. In the latter models, the intrinsic properties of E2 neurons contributed to controlling the duration of the late expiratory phase. In contrast to post-I neurons, the dynamics of calcium and calcium-dependent potassium conductances in dec-E neurons are essential for controlling the entire expiratory duration and timing of the expiratory off-switch in all our models except the three-phase model 1 and BS model 1. Finally, our theoretical explanation for the dependence between the durations of expiration and preceding inspiration (based on the dynamics of calcium-dependent potassium conductances in dec-E neuron) allows the supposition that intrinsic neuronal properties can contribute to CRPG performance not only at the network level but at the system level as well.

In conclusion, our simulations have provided unique insights into the possible architectures of CRPG, which are based on different mechanisms for phase switching. We also show the significance of intrinsic membrane properties of different respiratory neurons for neural mechanisms underlying CRPG performance and phase switching. The data presented here prompted numerous hypotheses, which now await experimental investigation.

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