Characterizations and comparisons of eupnoea and gasping in neonatal rats

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1. Our purpose was to characterize the ventilatory patterns of eupnoea and gasping in the neonatal rat. This study was precipitated by reports, using *in vitro* brainstem spinal cord preparations, that only a single pattern is present in neonatal rats.

2. In anaesthetized or decerebrate rat pups aged less than 13 days, eupnoea was characterized by a sudden onset of inspiratory activity and then a more gradual rise to peak levels. Following vagotomy, frequency fell and peak phrenic activity and tidal volume increased. The rate of rise of inspiratory activity also rose, but peak levels were still achieved during the latter half of inspiration. Vagal efferent activity exhibited bursts during both inspiration and the early expiration. This basic eupnoeic rhythm was not altered after sectioning of the carotid sinus nerves.

3. Upon exposure to hypoxia or anoxia, phrenic activity, tidal volume and frequency initially increased and then declined. In many animals, ventilatory activity then ceased, but later returned with a gasping pattern.

4. Gasping was characterized by a sudden onset of phrenic activity, which reached a peak intensity during the early portion of inspiration. The expiratory burst of vagal activity was eliminated.

5. Reductions of body temperature from 37 to 27 °C resulted in prolongations of inspiration and expiration and decreases of phrenic amplitude; phasic phrenic activity completely disappeared in some animals. Upon exposure to anoxia, gasping was observed, even in animals in which phrenic activity had disappeared in hyperoxia.

6. We conclude that, from the day of birth, rats can exhibit eupnoea and gasping patterns which are very similar to those of adult animals.

7. The rhythmic neural activities of the *in vitro* brainstem–spinal cord preparation, reported by others, differ markedly from eupnoea but are identical with gasping. We therefore conclude that this preparation is not suitable for investigation of the mechanisms that generate eupnoeic breathing.

Since first described in this journal (Suzue, 1984), the *in vitro* preparation of the brainstem and spinal cord of the neonatal rat has received much attention as a model for the genesis of respiratory rhythm. As *in vivo*, this preparation exhibits phasic periods of activity and inactivity of the phrenic nerve, which are synchronized with those of spinal and cranial nerves.

A fundamental question concerns the relationship between the rhythmic neural activities of the *in vitro* preparation and the eupnoeic ventilatory activity of the *in vivo* neonatal rat. Although frequency may change, the pattern of phasic bursts of the *in vitro* preparation is not systematically altered by removal of the pons (Suzue, 1984; Smith, Greer, Liu & Feldman, 1990; Errchidi, Monteau & Hilaire, 1991). Yet, as described by Lumsden (1923), transection of the adult brainstem at the pontomedullary junction results in a fundamental change in the ventilatory pattern from eupnoea to gasping. As in gasping, phrenic activity of the *in vitro* preparation rises rapidly to peak levels; the ramp-like rise characteristic of eupnoea in adult rodents is not seen (e.g. Wang, Fung & St John, 1993; Fung, Wang & St John, 1994*a*, *b*).

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Based upon the similarities of stereotyped neural activities in gasping, as observed in adult animals, and in the *in vitro* preparation, and upon the support of these activities by medullary mechanisms alone, some investigators have raised the possibility that the rhythmic activity of the *in vitro* neonatal rat preparation is gasping (Suzue, 1984; Murakoshi, Suzue & Tamai, 1985). Yet, other investigators have argued that the rhythmic activity of this preparation is similar to eupnoea, as observed in the *in vivo* neonatal rat (Smith *et al.* 1990; Smith, Ellenberger, Ballanyi, Richter & Feldman, 1991; Greer, Smith & Feldman, 1991; Brockhaus, Ballanyi, Smith & Richter, 1993). However, there have been no quantitative comparisons between the rhythmic neural activities of the *in vitro* preparations and either eupnoea and gasping of neonatal rats *in vivo*. Indeed, as noted above, only a single pattern of rhythmic activity has been reported for the *in vitro* preparation.

The absence of detailed information concerning ventilatory patterns of *in vivo* neonatal animals precludes a definitive interpretation of findings for the *in vitro* preparation. The present studies were undertaken to provide such detailed information.

METHODS

General experimental preparation

Pregnant Sprague–Dawley rats were obtained. Pups of either sex were studied from the day of birth (day 0) to 13 days thereafter (Table 1).

Both anaesthetized and decerebrate animals were used. Since the decerebrate neonatal rat is a novel preparation, variables of ventilatory activity were first recorded from anaesthetized animals. These variables enabled comparisons to be made with previous studies using anaesthetized or unanaesthetized animals (Saetta & Mortola, 1987; Fedorko, Kelly & England, 1988; Mortola, 1991).

The anaesthetized group was given 15·0 mg kg^{-1} ketamine and 1·50 mg kg^{-1} xylazine, administered intraperitoneally. A few animals were anaesthetized with 40 mg kg^{-1} pentobarbitone administered intraperitoneally. The level of anaesthesia was assessed frequently by the withdrawal reflex to pinching of the foot. If the reflex was present, supplements of 10% of the anaesthetic dose were administered intraperitoneally. We found that such supplements were not required until a minimum of 60 min after the initial anaesthetic dose. In paralysed animals (see below), experiments were less than 60 min in duration or, if more than 60 min, supplemental doses were administered.

Decerebration was performed under halothane anaesthesia (2–3% in 100% O_2). The skull was opened and the cortex removed by aspiration until the colliculi were visible. We found that, with decerebrations through the level of the superior colliculus, eupnoeic patterns were maintained. With more caudal mesencephalic transections, however, prolongations of the duration of neural inspiration were sometimes seen (see Results). Hence, all except eleven studies involved transections just rostral to the midcollicular level. Following decerebration, all neural tissue rostral to the transection was removed. Halothane anaesthesia was then discontinued.

In both the anaesthetized and decerebrate groups, each rat was placed supine, and the trachea cannulated at a midcervical level. In order to prevent hypoxaemia, animals breathed hyperoxic gas mixtures. The inspired fractional concentration of O_2 was continuously monitored. Measurements of end-tidal fractional concentrations of O_2 and CO_2 were not possible, due to the extremely small tidal volumes of newborn rats (Saetta & Mortola, 1987; Fedorko *et al.* 1988; Mortola, 1991).

Except where noted below, the temperature of the skin of the ventral abdomen was maintained at 36–38 °C using heating pads and lamps. It is important to note that the heating pads surrounded the rats, except for the ventral surface. In four preliminary experiments, we compared the surface temperature with the temperature within the abdominal cavity. Abdominal temperature was 0–2 °C cooler than the surface temperature. Hence, the latter was considered as a reasonable representation of body temperature. Both spontaneously breathing and paralysed animals were studied. The latter group received 40 $\mu g\ g^{-1}$ gallamine triethiodide, administered intraperitoneally. Artificial ventilation with hyperoxic gas was by a positive pressure device. The level of ventilation was adjusted empirically to approximate chest movements of the spontaneously breathing animals. Only twelve anaesthetized rats were studied following administration of gallamine.

Measurements of ventilatory activity

Spontaneously breathing animals. Ventilation was measured with a pneumotachograph. The dead space of this device was 0·05 ml. Signals reflecting inspiratory and expiratory flow were recorded on tape. The area under the curve of inspiratory flow was determined by digital integration, and taken as tidal volume (V_T). Calibrations were established by the movement of known volumes of air, from 0·1 to 1·0 ml, through the pneumotachograph. Curves were constructed comparing these known volumes with areas. Tidal volumes for animals were defined by reference to these calibration curves.

Paralysed and ventilated animals. By a lateral or ventral approach, rootlets of the phrenic nerve were sectioned. These rootlets were drawn up into a suction electrode. A reference electrode was in contact with tissue in a pool of mineral oil adjacent to the pipette of the suction electrode. Activity was amplified, filtered (0·6–6·0 kHz), and recorded on tape. This activity was also integrated by an RC circuit (time constants, 60 ms 'on' and 100 ms 'off'). In some experiments, similar techniques were used to record the activity of the central cut end of the vagus nerve. Activity of this nerve was integrated in the same manner as for phrenic activity.

Experimental alterations

The following were performed: (a) temporary cessation of artificial ventilation, (b) bilateral vagotomy, (c) bilateral sectioning of the carotid sinus nerves, (d) reductions of body temperature, and (e) exposure to hypoxia or anoxia. Exclusive of vagotomy, which was performed in most animals, each intervention was done in a limited number of rats, as noted below.

(a) Temporary cessation of artificial ventilation. In forty-seven paralysed animals, artificial ventilation was discontinued at end-

expiration in order to remove the influence of the phasic discharge of pulmonary stretch receptors.

(b) Vagotomy. The vago-sympathetic trunks were identified lateral to the trachea. In fifty-seven spontaneously breathing animals, ventilatory activity was recorded before and after these vago-sympathetic trunks were sectioned. The vagi were sectioned in sixty-nine paralysed and ventilated animals.

(c) Sectioning of the carotid sinus nerves. In eleven rats, the carotid arteries were stripped of all adherent tissue from the midcervical level to as far rostral as possible. Verification of sectioning of both carotid sinus nerves was obtained by exposing the animals to mild hypoxia. Prior to carotid sinus nerve sections, newborn animals exhibit a 'biphasic' response to hypoxia (e.g. Mortola, 1991). Hence, respiratory frequency, peak integrated phrenic activity and tidal volumes initially increase and then decline to levels below those of hyperoxia (see Results). Following carotid sinus nerve sections, only the depressive component of the 'biphasic' response is observed.

(d) Exposure to hypoxia or anoxia. The inspired gas mixture was switched from hyperoxia to various levels of oxygen in nitrogen, to 100% N_2 or to 0·5–1·0% carbon monoxide in air. After variable periods of exposure, ventilation with hyperoxic mixtures was recommenced. In eight animals, anoxia was produced by a cessation of artificial ventilation.

(e) Reductions in body temperature. In nine rats, the skin temperature was allowed to fall from 38–36 °C to 26 °C by termination of heating; in some cases, ice was also placed around the body. Rewarming of the animals to 37 °C was performed.

Evaluation of data

For spontaneously breathing animals, T_I was defined as the period between the beginning of inspiratory airflow to the beginning of expiratory flow. T_E was the time between the commencement of expiratory flow to the beginning of the next respiratory cycle. Hence, T_E included expiratory flow and end-expiratory pauses. The respiratory frequency (f) and tidal volume (V_T) were also determined. The rate of rise of inspiratory activity was assessed by determining the time at which peak inspiratory flow was reached and normalized as a percentage of the duration of neural inspiration (time to peak as % T_I). Hence, an augmented rate of rise of inspiratory activity was evidenced by a reduction in the time to peak.

Integrated phrenic nerve activity was analysed to determine the following variables: durations of neural inspiration (T_I), expiration (T_E), respiratory frequency (f) and peak height. Again, as an index of the rate of rise of phrenic activity, the percentage of neural inspiration was defined at which integrated phrenic activity reached its peak value (time to peak as % T_I). Neural inspiration was defined as the period of the phrenic burst, from the onset to rapid decline. The time to the commencement of the next burst constituted neural expiration. Peak heights of integrated activity of the vagus nerve during neural inspiration and expiration were also determined.

Data from a minimum of ten ventilatory cycles under each condition in each animal were averaged, except for studies involving temporary cessations of ventilation. For these studies, the first five cycles without ventilation were averaged. Statistical evaluations of data were by the unpaired Mann–Whitney U test and the Wilcoxon matched pairs test. Probabilities less than 0·05 (two-tailed) were considered significant.

Table 1. Total number of rats of various ages from which data were obtained

Age (days)	Number of animals
0	14
1	29
2	29
3	24
4	35
5	44
6	30
7	11
8	10
9	7
10	2
11	3
12	6
13	3

RESULTS

Experimental groups

Data were collected from a total of 247 neonatal rats (Table 1). This represents approximately 70% of the total experiments attempted. Hence, mortality was high, especially in initial studies.

Experimental difficulties

As noted above, animals in the initial experiments had a high mortality, even before measurements were made. When difficulties with anaesthesia or tracheal cannulation occurred, animals quickly became apnoeic and/or altered their breathing pattern to 'gasp-like' efforts. Most of these animals did not survive for a sufficient period for measurements to be made. However, some animals which did survive, were found to have patterns of ventilatory activity that differed from those of the great majority of other animals examined. Such 'abnormal' patterns were rarely observed as experience with the preparation increased. Specific comments concerning patterns of ventilatory activity are presented below.

A potential confounding factor was the inability to measure end-tidal fractional concentrations of CO_2 or O_2 in these small animals. While adequate levels of oxygenation were assured by exposure to hyperoxic mixtures, levels of end-tidal CO_2 without doubt varied between animals. This variability might underlie the range of values for components of ventilatory activity, as described below.

Characterizations of eupnoea

Spontaneously breathing animals

The eupnoeic ventilatory patterns of an anaesthetized and a decerebrate animal, having intact vagi, are shown in Fig. 1. Mean values of variables of ventilatory activity for all twenty-nine anaesthetized animals and forty-three decerebrate rats are given in Fig. 2. Animals were divided into age groups of 0–4 and 5–13 days. This grouping was done since some investigators report an age-dependent difference in the pattern of ventilatory activity (Smith *et al.* 1990).

Data for eleven decerebrate rats are not included in Fig. 2. Prior to vagotomy, these animals had values of inspiratory duration which were greater than those of the other decerebrate or anaesthetized animals. Following bilateral vagotomy, these animals exhibited pronounced pauses in the inspiratory position, with T_I in excess of 1800 ms. Thus, these rats exhibited apneusis.

These animals represented some of the earliest experiments, in which the decerebrations were at the midcollicular level. In subsequent experiments, the level of decerebration was moved slightly more rostral, as noted in Methods, and the apneustic pattern was not seen. Data for these eleven apneustic animals are not considered further.

Pre- and post-vagotomy. Prior to vagotomy, older rats had higher frequencies and larger tidal volumes than the 0–4 day animals (Fig. 2). The latter is obviously expected since the older animals were larger.

Following vagotomy, the durations of inspiration and expiration and tidal volume increased, and frequency decreased (Figs 1 and 2). Such changes were only significant for the decerebrate animals (Fig. 2). The rate of rise of inspiratory activity also increased following vagotomy with peak flow being achieved at a mean of 54–49% of T_I in the various groups; comparable means prior to vagotomy varied from 65 to 55% of T_I (Fig. 2).

Responses to hypoxia and anoxia. Upon exposure of either the anaesthetized or decerebrate vagotomized animals to hypoxia or anoxia, the respiratory frequency and tidal volume initially increased and then fell below

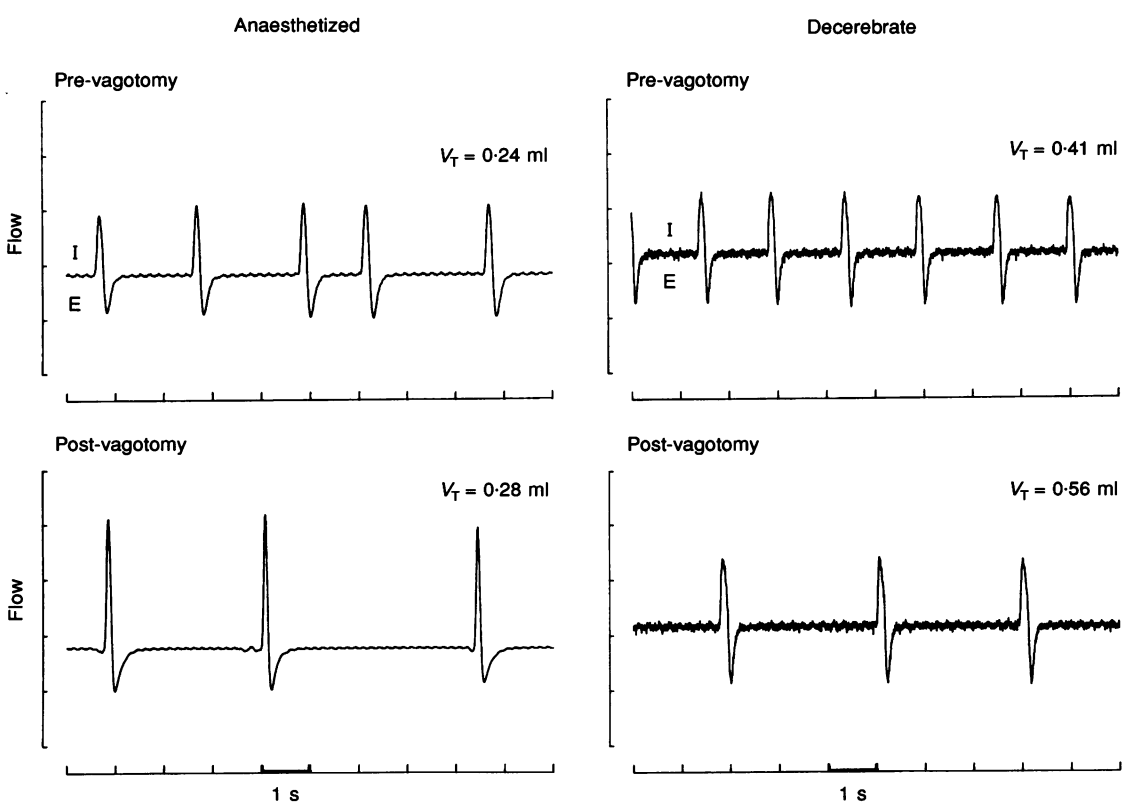

Figure 1. Ventilatory activity of spontaneously breathing neonatal anaesthetized and decerebrate rats before and after vagotomy

Left panel shows pneumotachograph records from an anaesthetized animal. I and E designate directions of inspiratory and expiratory flows, respectively; mean values of tidal volume (V_T) are given for the breaths shown. Right panel shows comparable recordings for a decerebrate animal.

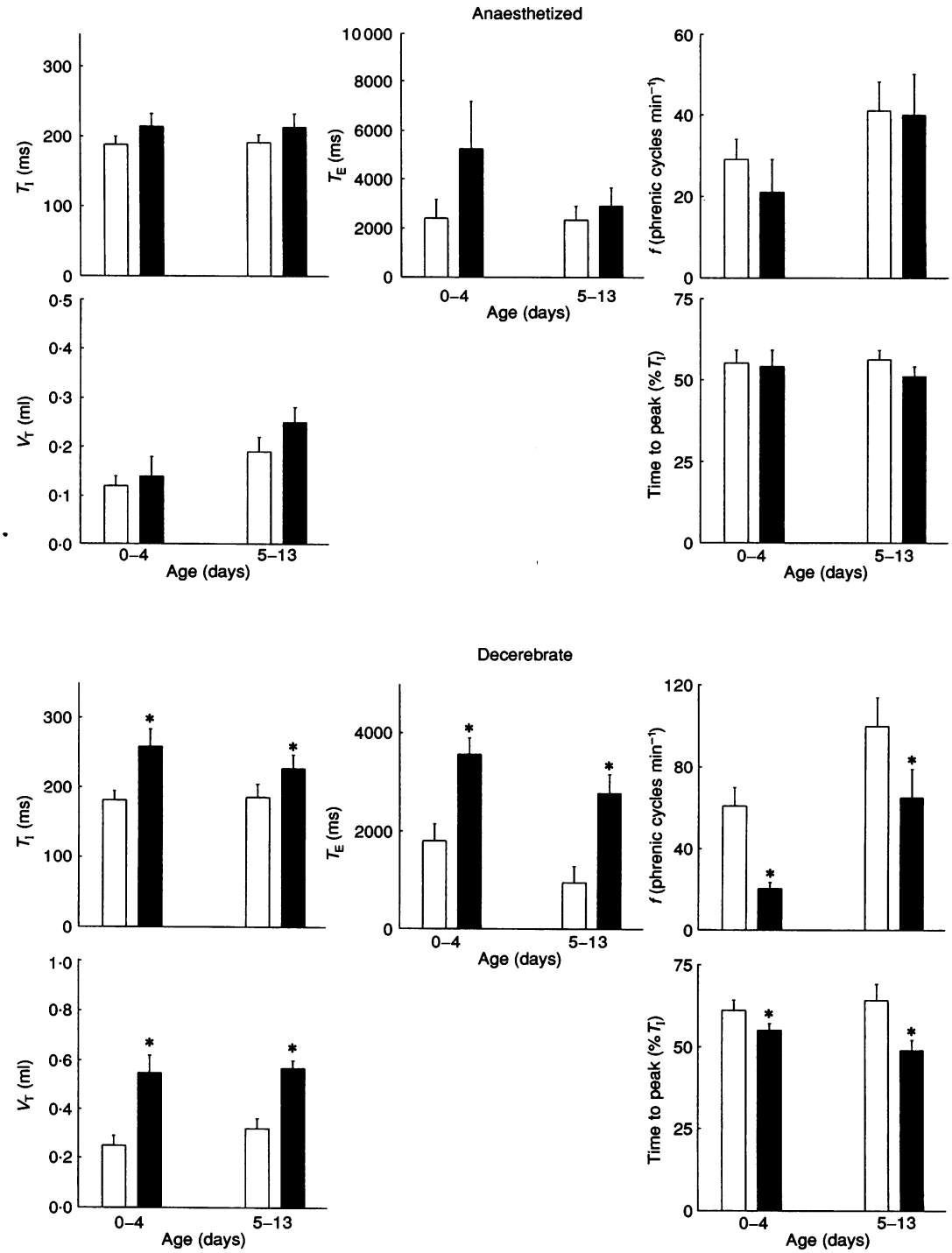

Figure 2. Variables of ventilatory activity for spontaneously breathing neonatal anaesthetized and decerebrate rats before and after vagotomy

Values are means (+ S.E.M.) for animals of ages 0–4 and 5–13 days. □, pre- and ■, post-vagotomy. * Significant difference between conditions, $P < 0{\cdot}05$. Values are durations of neural inspiration (T_I), neural expiration (T_E), frequency (f), V_T, and the time to reach peak inspiratory flow (Time to peak), expressed as a percentage of T_I.

levels observed during hyperoxia. Complete apnoea resulted in approximately half of the animals exposed to hypoxia and all exposed to anoxia (Fig. 3). This apnoeic period was interrupted, in some animals, by a pattern in which inspiratory flow rose more quickly to a peak value than in eupnoea; this pattern appeared to be one of gasping. However, since most animals either died without exhibiting gasping or exhibited only a few gasps, we did not attempt to study this gasping pattern systematically in spontaneously breathing animals.

Ventilated animals

Pre-vagotomy: cessation of ventilation. Twelve anaesthetized and thirty-six decerebrate animals were examined. In either preparation having intact vagi, phrenic activity was exceedingly variable during artificial ventilation. Thus, peak phrenic height and the duration of expiration varied greatly (Fig. 4). In some animals, no phrenic activity could be discerned. This variability undoubtedly reflected varying degrees of entrainment by the activation of pulmonary stretch receptors.

When ventilation was terminated, the phasic patterns of phrenic activity were clearly manifested (Fig. 4). Variables from the first five cycles without lung inflation were measured and averaged. While arterial partial pressures of CO_2 and O_2 must have changed during this period without ventilation, note in Fig. 4 that no systematic increases in either peak phrenic activity or respiratory frequency were evident.

Phrenic activity reached a peak value, on average, in the latter half of neural inspiration (Figs 4 and 5). Whereas there was scatter of data, T_I averaged less than 250 ms, T_E was below 2000 ms, and frequency was above 75 phrenic cycles min^{-1} for both anaesthetized and decerebrate animals (Fig. 5) Note that Fig. 5 represents mean data for rats of all ages. Examination of data for individual animals revealed no systematic change in variables with age.

Post-vagotomy. For both anaesthetized and decerebrate animals, T_E was significantly longer, and respiratory frequency was significantly lower following vagotomy, compared with temporary cessations of ventilation prior to vagotomy (Figs 4 and 5). Neither T_I nor the rate of rise was significantly altered (Fig. 5).

Concerning the rate of rise, note in Figs 4, 6 and 7 that, after onset, phrenic activity exhibited a ramp-like rise to reach a peak value in the latter half of neural inspiration. Rats younger than 4 days (Figs 4, 6 and 7) did not have rates of rise of phrenic activity which differed from those of older rats.

Efferent activity of the vagus nerve was recorded in four animals (Fig. 8). This activity was characterized by a burst during the inspiratory period and a burst of greater amplitude during the initial portion of neural expiration.

Sectioning of the carotid sinus nerves. For all ten decerebrate rats having both bilateral vagotomy and carotid sinus nerve sections, values of the durations of neural inspiration and expiration and the time to reach peak phrenic activity were similar to those of animals having vagotomy alone (Fig. 9). Note specifically in Fig. 9 that peak phrenic height was reached in the latter half of inspiration for all but one rat. Although frequencies overlapped with those of vagotomized animals, two animals did have frequencies in excess of 200 phrenic cycles min^{-1}.

Characterizations of gasping

Figures 4, 6, 7 and 8 show records of phrenic activity for rats from the day of birth to 7 days thereafter. Recordings were obtained following vagotomy with animals being ventilated with 100% O_2 (hyperoxia) and during anoxia.

With exposure to anoxia, the biphasic response noted above began. Hence, peak integrated phrenic activity and the respiratory frequency initially increased and then declined to apnoea. This apnoeic period was succeeded by phrenic bursts which rose quickly to a peak value immediately after onset and then declined during the remainder of neural inspiration (Figs 4, 6 and 7). This pattern is similar to gasping in adult animals. When gasping commenced, reventilation with oxygen could result

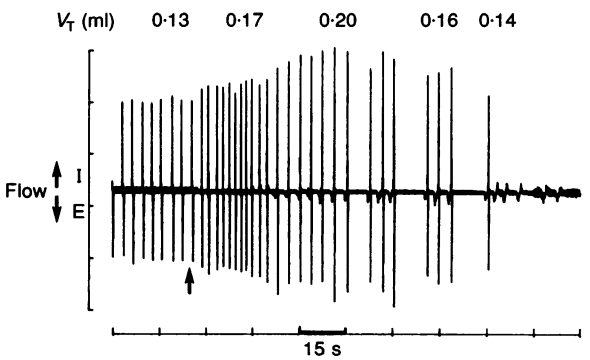

Figure 3. Biphasic response to hypoxia in spontaneously breathing decerebrate neonatal rat

Pneumotachograph records are shown during hyperoxia and after introduction of hypoxia (arrow). Hypoxic mixture was 12 % O_2 in nitrogen. Values of V_T are given above breaths. Note complete cessation of breathing less than 2 min after onset of hypoxia.

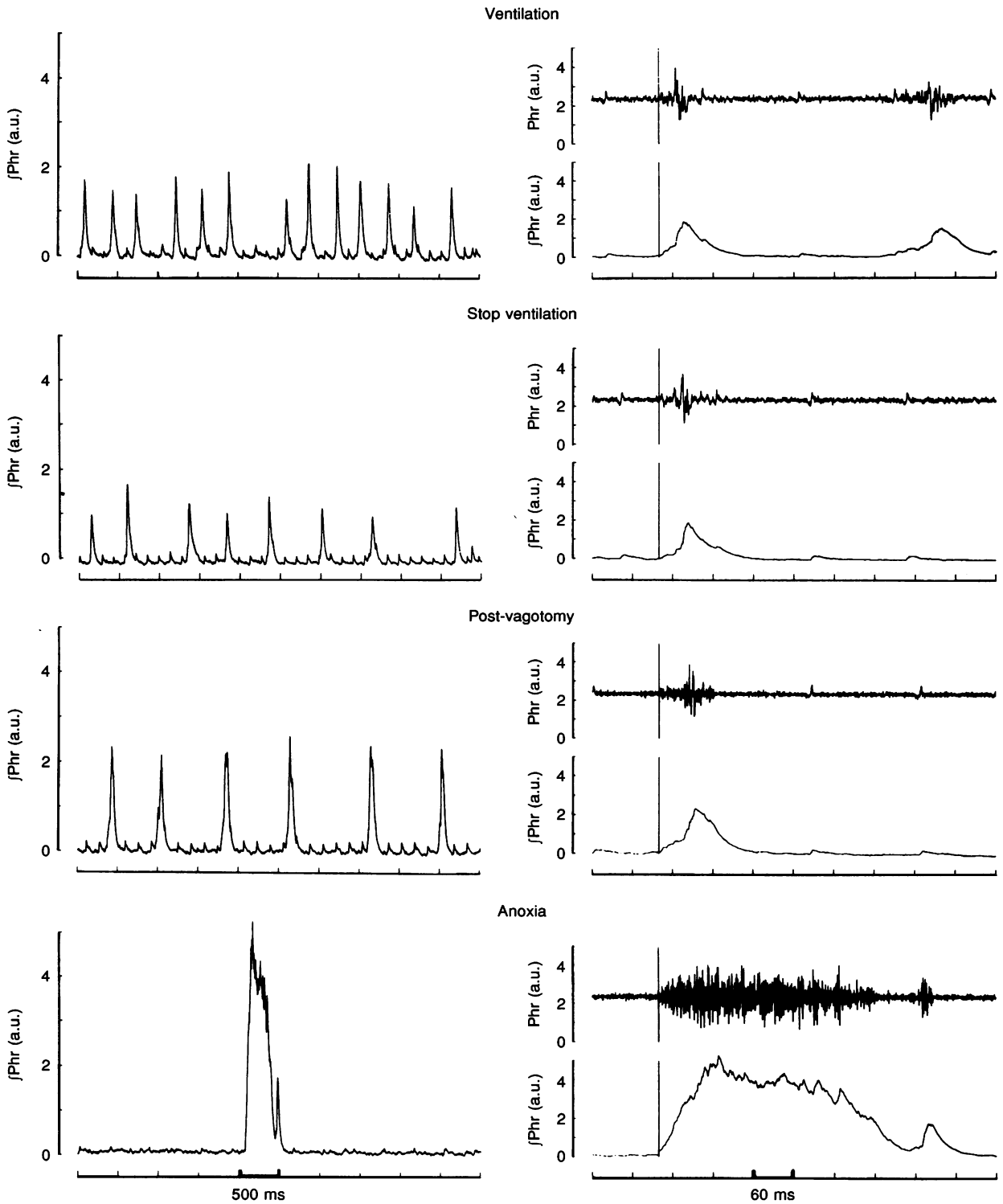

Figure 4. Phrenic activity before and after bilateral vagotomy

Left panels show patterns of integrated phrenic activity. In the right panels, phrenic activity (Phr) and its integral (∫Phr) are shown on an extended time base. Units of activity are arbitrary (a.u.). The vertical line identifies the start of the burst. Patterns are for the period of artificial ventilation, during temporary cessation of ventilation, following bilateral vagotomy and during anoxia. Note the differences between the pattern in anoxia-induced gasping and in eupnoea. Data are for a 3-day-old rat.

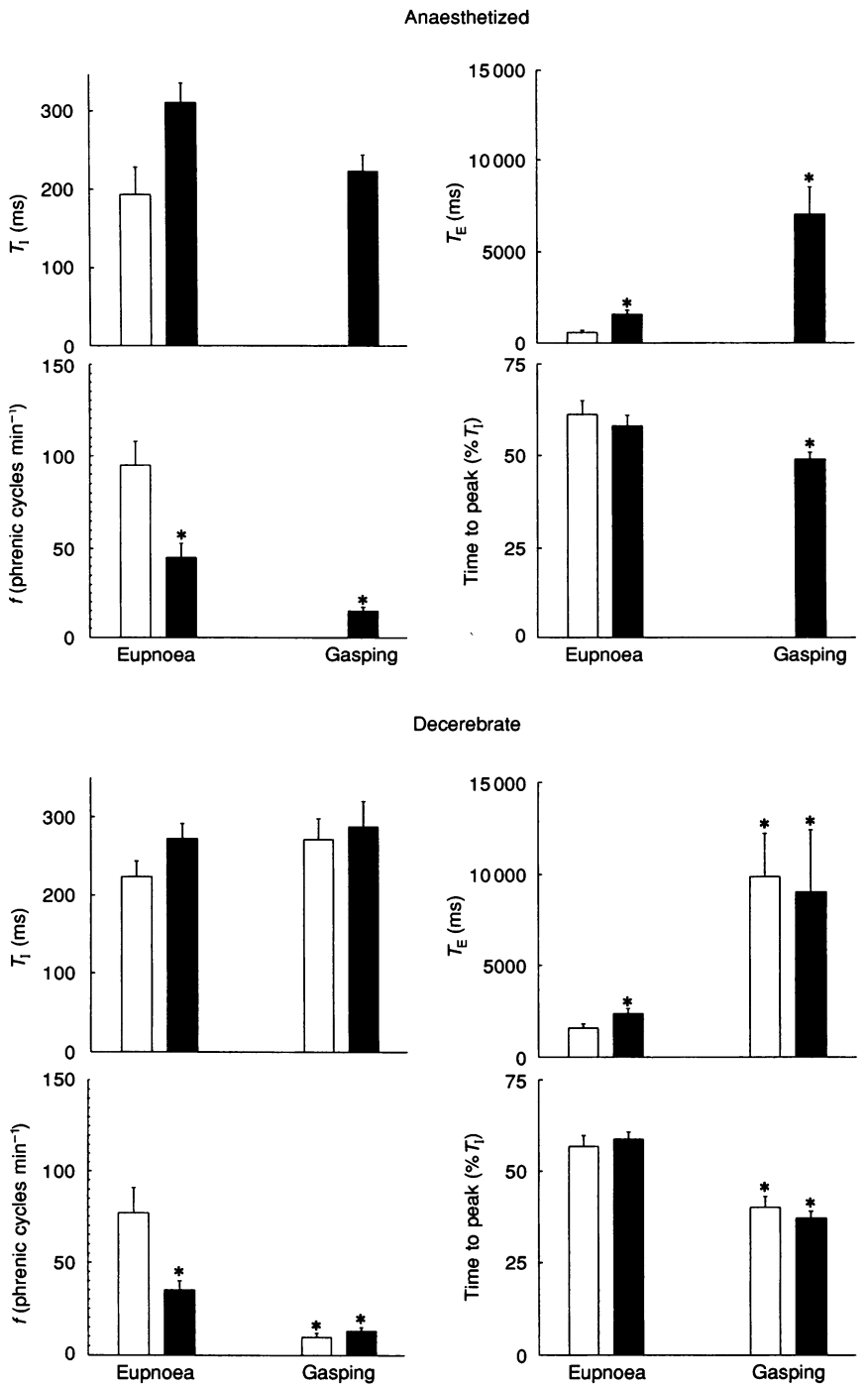

Figure 5. Variables of phrenic activity in anaesthetized and decerebrate neonatal rats during eupnoea and gasping

Values reported are means (+ S.E.M.). □, temporary cessations of ventilation before vagotomy; ■, values following vagotomy. * above bars in eupnoea designates significant difference between pre- and post-vagotomy values. * above bars in gasping designates significant difference compared with post-vagotomy values in eupnoea.

in the re-establishment of eupnoea. However, even after hypoxic exposures of less than 10 min, some animals continued gasping for the remainder of the experiment, even during ventilation with oxygen.

Phrenic activity in gasping was very stereotyped between animals. For both anaesthetized and decerebrate neonatal rats (Fig. 5), the duration of the phrenic burst in gasping was not significantly different from that of animals in eupnoea. However, T_E was significantly longer and frequency was significantly lower in gasping. Peak phrenic activity was achieved significantly earlier in neural inspiration in gasping compared with eupnoea. Hence, the rate of rise of phrenic activity was significantly increased in gasping. Exactly the same differences emerge when gasping, resulting from a termination of artificial ventilation in decerebrate animals, was compared with

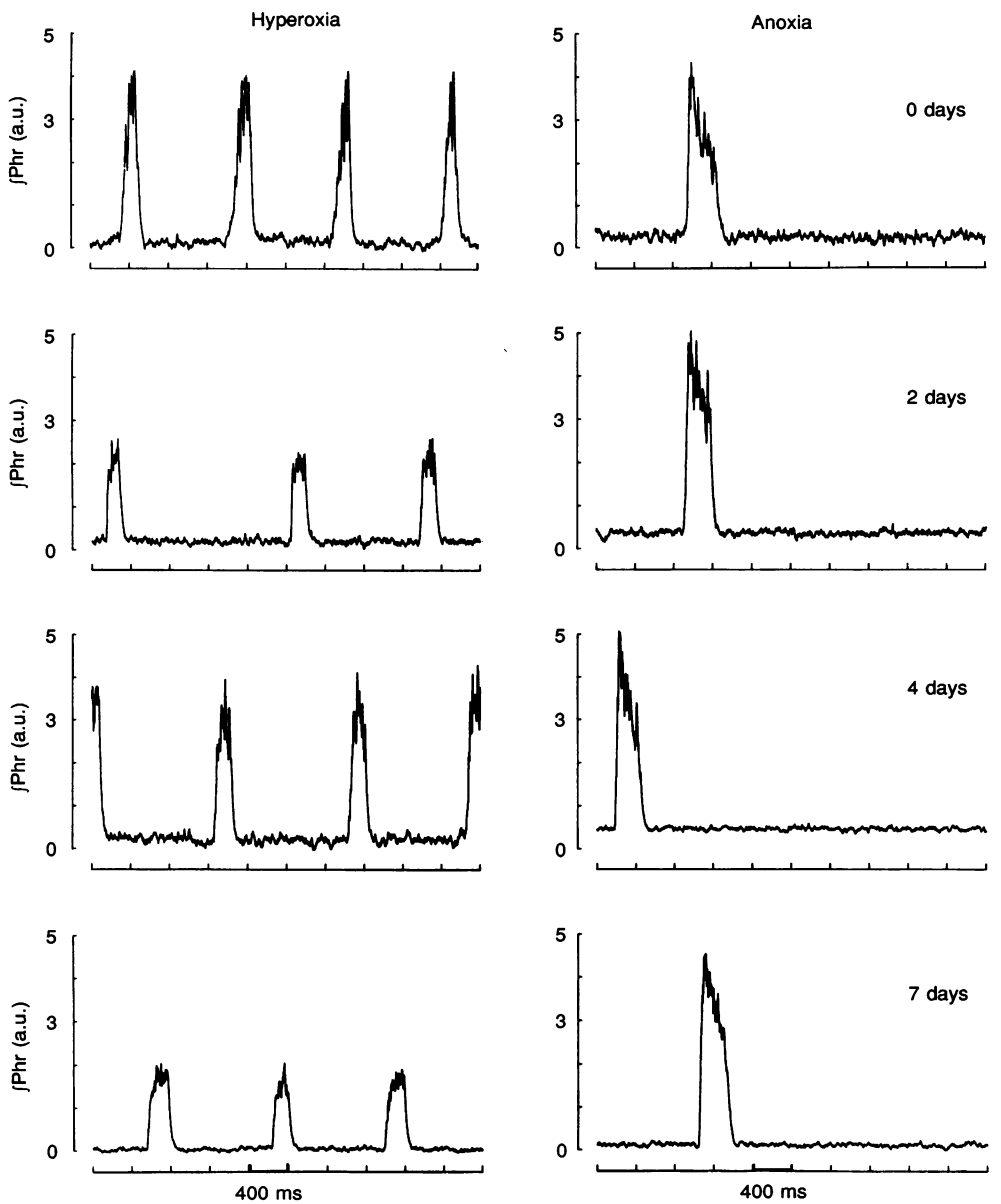

Figure 6. Examples of phrenic activity in eupnoea and gasping in four neonatal rats

Records of integrated activity in eupnoea were recorded in hyperoxia; records in gasping were in anoxia. Note that the durations of neural expiration were so extended in gasping that only a single phrenic burst is shown. Number of days indicates days after birth.

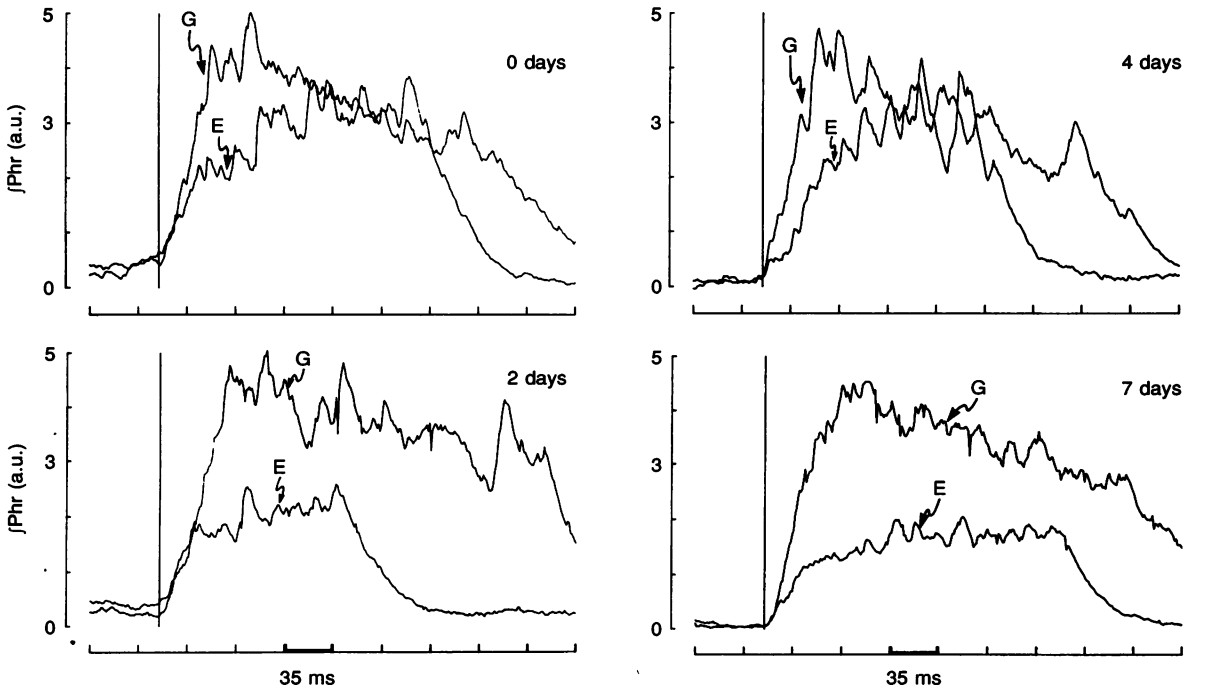

Figure 7. Comparison of rates of rise of phrenic activity in eupnoea and gasping for four neonatal rats

Note that peak values in gasping (G) are achieved much earlier in neural inspiration than in eupnoea (E). Number of days indicates days after birth. More extended records for these same animals are in Fig. 6.

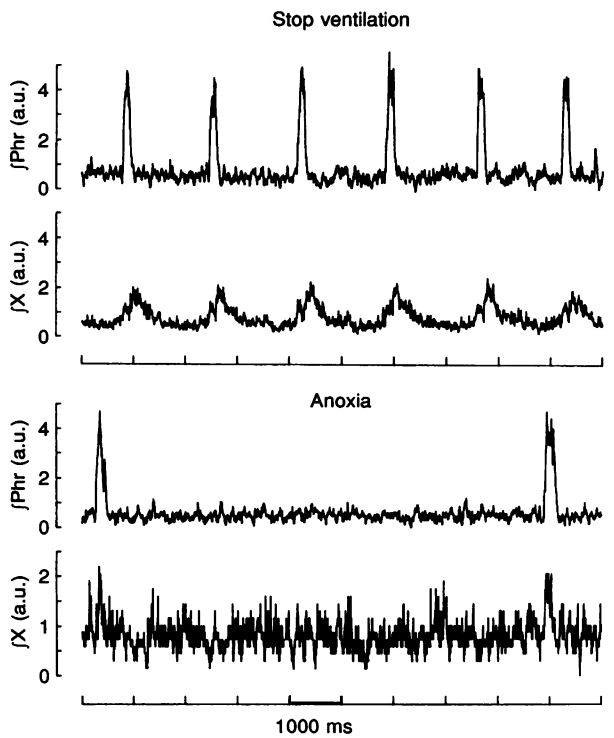

Figure 8. Examples of phrenic and efferent vagal activities in eupnoea and gasping

Activities were recorded in a vagotomized animal. Records of integrated vagal activity (∫X) became clearer when ventilation was terminated. Upper panels show phrenic and vagal activities immediately after the cessation of ventilation; lower panels are for gasping. Note the early expiratory burst of vagal activity in eupnoea and its disappearance in gasping. The gain of the vagal recording is doubled in gasping as compared with eupnoea.

phrenic activity during brief periods of non-inflation (compare open bars in Fig. 5). Similarly, the same differences were also apparent when animals were divided into groups, depending upon age. In particular, from the day of birth, rates of rise of inspiratory activity were greater in gasping than eupnoea.

These significant differences among variables of eupnoea and gasping, reported above and shown in Fig. 5, are the result of paired comparisons with data for the same animal in eupnoea and gasping. However, an unpaired comparison of data for all animals in eupnoea and gasping, using a Mann–Whitney U test, yielded very similar findings.

In addition to these evaluations of phrenic activity, the changes in vagal activity revealed marked differences between eupnoea and gasping. Hence, as in adult animals, the early expiratory bursts were greatly reduced or totally eliminated during gasping and the discharges during neural inspiration remained (Fig. 8).

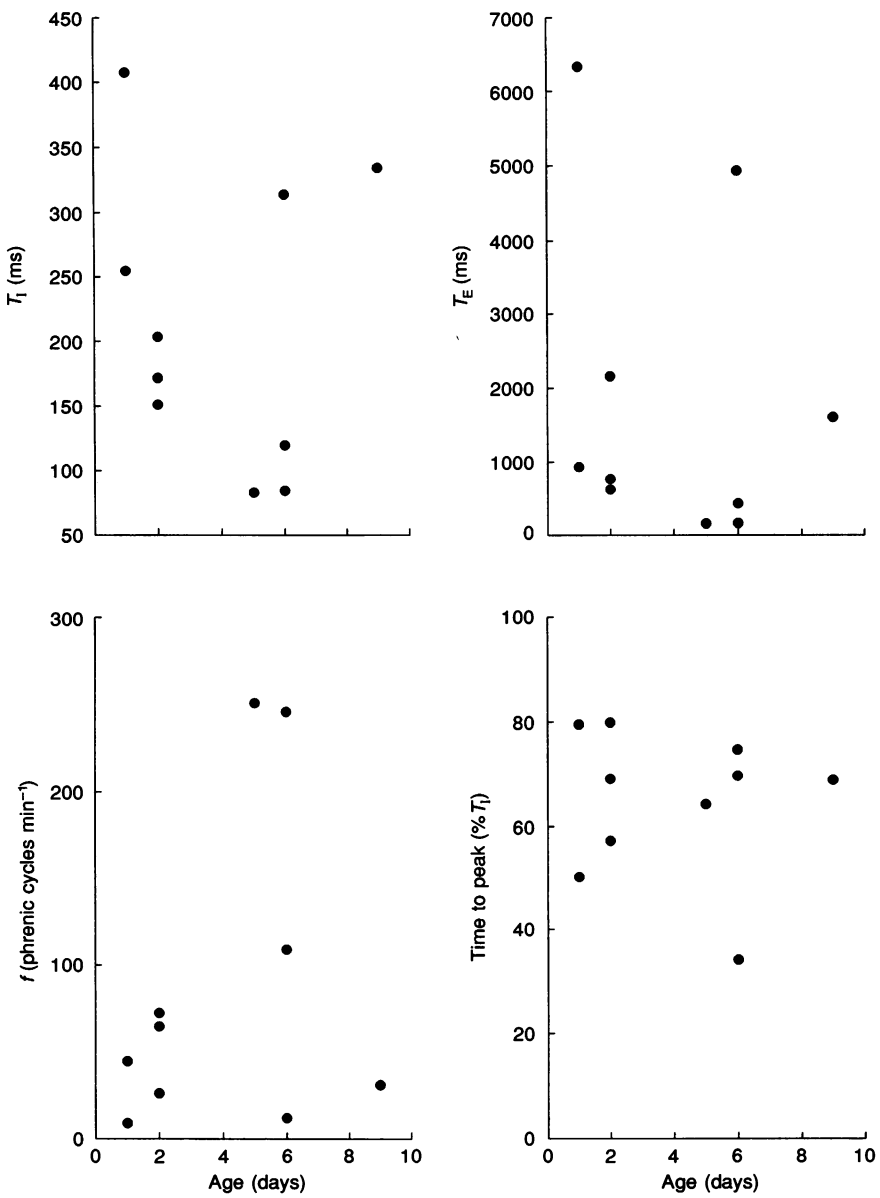

Figure 9. Variables of integrated phrenic activity for decerebrate paralysed and ventilated neonatal rats following vagotomy and bilateral section of the carotid sinus nerves

Each point represents data for individual animals examined at the ages designated (days).

Alterations of ventilatory activity with reduction in body temperature

The eupnoeic pattern was markedly altered with reductions of body temperature from control levels of 38–37 °C to 27–26 °C (Figs 10 and 11). In twelve of thirteen animals, regardless of age, both T_I and T_E increased and peak phrenic activity decreased. Despite these changes, the rate of rise of phrenic activity approximated the control value. This generalization requires qualification in that five rats exhibited apnoea at or before the temperature reached 27–26 °C (Fig. 10). These rats varied in age from 3 to 7 days.

Upon exposure of eight animals to anoxia, an apnoeic period, followed by gasping, was observed in those animals having phasic phrenic activity at 27–26 °C. For animals that were apnoeic, gasping was also elicited. These gasps were similar to those elicited at 38–37 °C with peak phrenic activity and its rate of rise increasing greatly; however, values of T_I were longer in hypothermia (Figs 10 and 11).

DISCUSSION

The major conclusion of this study is that, from the day of birth, the neonatal rat can exhibit eupnoea and gasping. These patterns are clearly distinguishable, based primarily upon the greater rate of rise of inspiratory activity in the gasp.

Eupnoea and gasping in neonatal rats *in vivo*

As in adults, inspiratory activity of eupnoea was characterized by a sudden onset and then more gradual rise to a peak level in the mid- to late portions of neural inspiration (St John & Knuth, 1981; St John, Bledsoe & Sokol, 1984; St John, Bledsoe & Tenney, 1985; Wang *et al.* 1993; Fung *et al.* 1994*a*,*b*). This pattern was similar in anaesthetized and decerebrate animals, and was also similar to data obtained from spontaneously breathing animals. Specifically, tidal volumes and inspiratory durations that we recorded in decerebrate or anaesthetized animals were within ranges reported for spontaneously breathing, unanaesthetized neonatal rats (Saetta & Mortola, 1987; Fedorko *et al.* 1988; Mortola, 1991). In

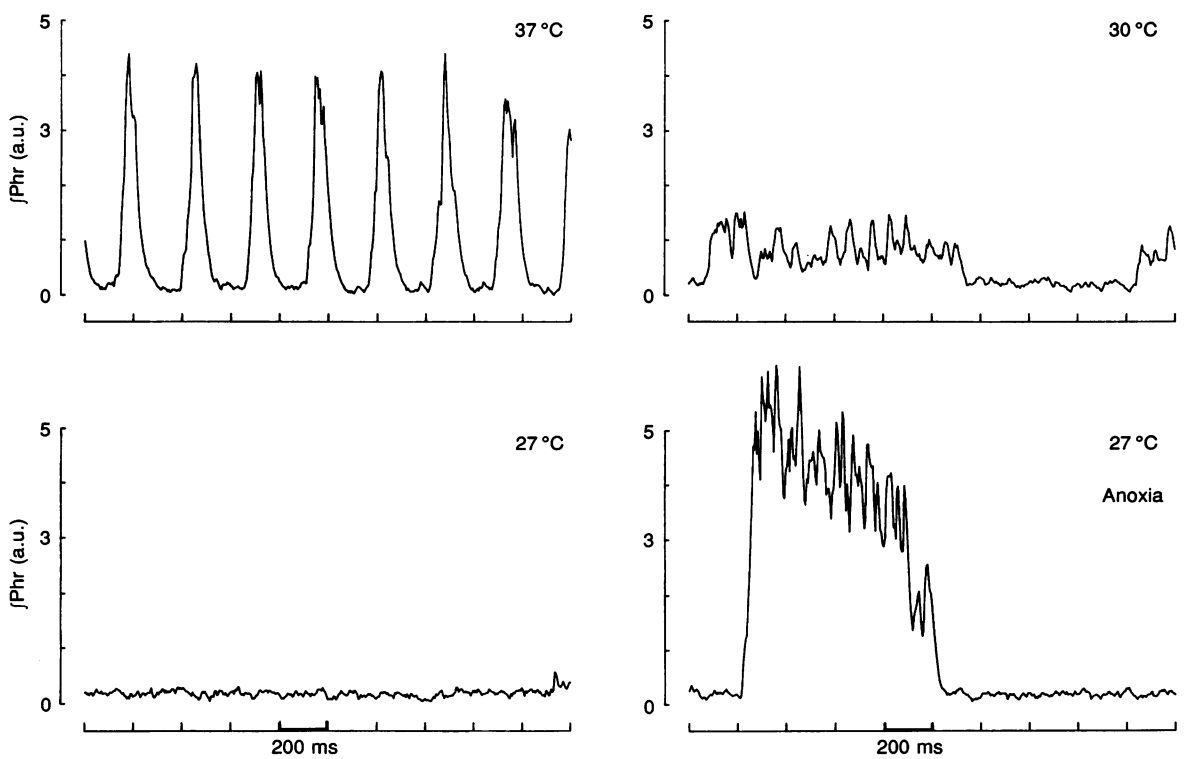

Figure 10. Influence of reductions in body temperature upon phrenic activity in a decerebrate and vagotomized neonatal rat

Recordings were all obtained in hyperoxia except for recording in anoxia in lower right panel.

addition, the pattern of vagal activity in eupnoea was similar to that described previously for the recurrent laryngeal nerve in adult animals (e.g. St John, Bartlett, Knuth & Hwang, 1981).

The eupnoeic pattern remained in both spontaneously breathing and paralysed ventilated animals following bilateral sectioning of the vagi and carotid sinus nerves. Some spontaneously breathing animals did exhibit profound reductions of frequency and minute volume following vagotomy; it is likely that these animals were hypercapnic and acidotic. Such reductions in minute ventilation could account for the high mortality following vagotomy (see Discussion in Fedorko *et al.* 1988). In this context, Smith *et al.* (1990) have reported that diaphragmatic electromyographic discharges are markedly different following vagotomy in rats younger than 4 days

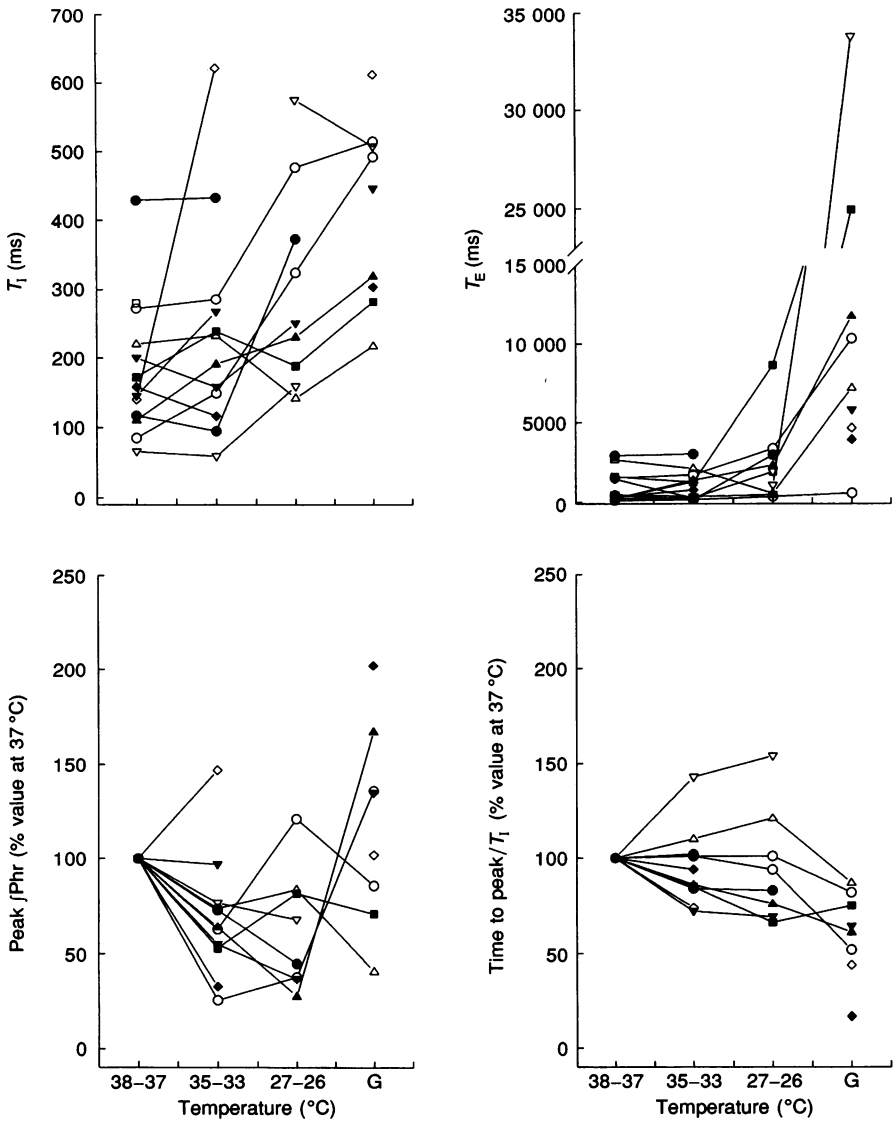

Figure 11. Variables of integrated phrenic activity for decerebrate paralysed and ventilated neonatal rats following reductions of body temperature

Each point represents data for individual animals examined at designated temperatures. Animals of ages 1–9 days were examined. Values during gasping (G) were recorded at 27 °C in anoxia. Breaks in lines indicate apnoea at next lowest temperature. Examination of some animals only commenced at reduced body temperatures. Peak ∫Phr and time to peak are expressed as a percentage of values recorded at 38–37 °C.

compared with older animals, with gasping seemingly elicited by vagotomy alone (Smith *et al.* 1990). We noted a similar pattern following vagotomy only when rats, regardless of age, had been previously exposed to anoxia or had experienced apnoeic episodes during surgery. Hence, we find no support for the conclusion that vagotomy fundamentally transforms the pattern of spinal neural activities in the neonate (Smith *et al.* 1990). Rather, such transformations occurred only during anoxia-induced gasping.

In anoxia, the ramp-like rise of phrenic activity of eupnoea was replaced by a pattern in which peak activity was achieved very soon after the onset of the phrenic burst. Integrated phrenic activity then declined slightly from this peak level. This pattern is identical to that reported for gasping produced by anoxia or by brainstem transections at the pontomedullary junction (e.g. Lumsden, 1923, 1924; St John & Knuth, 1981; St John *et al.* 1981, 1984, 1985; St John, Zhou & Fregosi, 1989; St John, 1990; Fung *et al.* 1994*b*). Moreover, the elimination of the early expiratory burst of vagal efferent activity and retention of the inspiratory discharge during anoxia is similarly identical to that reported during gasping (St John *et al.* 1981, 1989).

Comparison of *in vivo* and *in vitro* neonatal preparations

A number of laboratories have studied the *in vitro* preparation of the neonatal rat (Suzue, 1984; Monteau, Errchidi, Gauthier, Hilaire & Rega, 1989; Smith *et al.* 1990; Brockhaus *et al.* 1993; McLean & Remmers, 1994). Recordings of cervical neural activities from these various laboratories show a stereotyped 'rapidly peaking-slowly decrementing...I-phase discharge' (Smith *et al.* 1990, p. 1155). When these recordings are compared with the figures in this paper, especially Figs 4, 6 and 7, the marked differences from eupnoea and similarity to gasping are clear.

A reduction in expiratory activities of cranial and spinal nerves is a common feature of gasping (St John *et al.* 1981, 1989; Zhou, Wasicko, Hu & St John, 1991). In a compilation of recordings from a number of *in vitro* preparations, Smith *et al.* (1990) show both early and late expiratory discharges of cranial and spinal nerves. Compared with the recordings from *in vivo* neonatal rats reported here, and adult animals, such expiratory activities are much reduced; indeed, all tracings are dominated by bursts of inspiratory activity. Moreover, such expiratory neural activities were apparently not consistently recorded in the *in vitro* preparation, with no expiratory activities of the vagus being discernible in some figures of Smith *et al.* (1990) or Brockhaus *et al.* (1993). Thus, to summarize, patterns of neural activities *in vitro* are identical to those of gasping.

The methods for producing the *in vitro* preparation appear to make it unlikely that any pattern except gasping can be exhibited. The animal must be apnoeic for some period during the removal of the brainstem and its placement in culture. Moreover, in a culture medium maintained at 27 °C and saturated with 100% O_2, all of the preparation, except a periphery of 450–600 μm, is completely anoxic (Brockhaus *et al.* 1993; Okada, Muckenhoff, Holtermann, Acker & Scheid, 1993). Again, as described by Lumsden (1924), such anoxia is sufficient to induce gasping in intact animals. The finding that the *in vitro* preparation can be maintained under such anoxic conditions may reflect the greater capacity of the neonate than the adult to maintain medullary functions by anaerobic metabolism (Ballanyi, Kuwana, Volker, Morawietz & Richter, 1992).

A final indication that the *in vitro* preparation is exhibiting gasping results from the hypothermia at which the preparation is maintained. We found prolongations of the duration of neural inspiration and diminutions of respiratory frequency with progressive reductions of body temperature from 38–37 °C to 27 °C in the vagotomized neonatal rat, with the addition that five rats exhibited apnoea at or above 27 °C. When anoxia was introduced in hypothermia, gasping was recorded in all animals.

The pattern of neural activities of the *in vitro* preparation is not altered with changes from 35 to 27 °C; the duration of the burst and the period between bursts are prolonged (Smith *et al.* 1990). Again, this absence of a change in pattern is only compatible with the *in vitro* preparation exhibiting gasping at all temperatures. In this context, a hallmark of the *in vitro* preparation is the low frequency of its rhythmic bursts. While hypothermia might contribute to such low frequencies, even at 35 °C, frequencies are less than half of those following vagotomy, *in vivo* (Smith *et al.* 1990). These low frequencies *in vitro* are very close to those of gasping. Responses to hypoxia in the *in vitro* preparation also point to the conclusion that the rhythmic activities are gasps.

As shown here, neonatal animals exhibit a 'biphasic' response to hypoxia with frequency, peak phrenic activity and tidal volume increasing and then declining. Following carotid sinus nerve sections, only decreases in both frequency and depth of breathing are recorded in hypoxia. For *in vitro* preparations, anoxia causes a transient rise in the frequency and then a gradual decline to a complete cessation of activity (Brockhaus *et al.* 1993). This pattern is identical to the hypoxia-induced alterations in gasping of animals having either intact or sectioned carotid sinus nerves (St John & Knuth, 1981). It must be emphasized that the pattern of neural activities of the *in vitro* preparation is never altered in anoxia, except to a complete cessation of activity. If this pattern is not gasping, why is

the pattern not altered? In fact, anatomical considerations also point to an identity of this pattern with gasping.

We have previously identified a medullary 'region for gasping' in adult cats and rats. Ablation of this region irreversibly eliminates gasping, but causes no alteration of the eupnoeic rhythm (St John *et al.* 1984, 1985; Fung *et al.* 1994*b*). Two adjacent regions have been defined as critical for the generation of the rhythmic activities of the *in vitro* neonatal preparation: a 'pre-Botzinger' complex and a 'pre-I' region (Onimaru, Arata & Homma, 1988, 1989, 1990; Smith *et al.* 1990, 1991; Errchidi *et al.* 1991; Di Pasquale, Monteau & Hilaire, 1994; Johnson, Smith, Funk & Feldman, 1994). While the pre-I region has only been identified *in vitro*, a pre-Botzinger complex has been described in adult cats (Connelly, Dobbins & Feldman, 1992; Schwarzacher, Smith & Richter, 1995) and rats (Dobbins & Feldman, 1994), *in vivo*. This description is based upon neuronal discharge patterns and the tracing of interconnections among brainstem respiratory neurons.

At its medial border, the pre-Botzinger complex, defined *in vitro*, overlaps slightly with the region for gasping, defined in adult rats, *in vivo* (Smith *et al.* 1990, 1991; Johnson *et al.* 1994; Fung *et al.* 1994*b*). More substantial overlap is apparent when histology from adult *in vivo* animals is compared (Connelly, Dobbins & Feldman, 1992; Dobbins & Feldman, 1994; St John *et al.* 1984, 1985; Fung *et al.* 1994*b*; Schwarzacher *et al.* 1995). Most conclusively, in cats, the pre-Botzinger complex is now shown extending from dorsomedial to ventrolateral to the nucleus ambiguus. Neurons at this dorsomedial region appear to lie within the region for gasping. Moreover, the dendrites of these neurons and those in more ventral regions, having respiratory-modulated discharge patterns, are extensively distributed in the region for gasping (Pilowsky, Jiang & Lipski, 1990; Schwarzacher *et al.* 1995).

These morphological considerations make it evident that, rather than different regions, the region for gasping, the pre-Botzinger complex and the pre-I region may represent a single continuous column of neurons, with extensive and intermingled dendritic processes. The question then arises as to whether neuronal mechanisms in these regions, which generate the gasp, are responsible for the neurogenesis of eupnoea. Stated differently, are such neuronal activities a 'kernel' for the neurogenesis of all automatic ventilatory patterns.

Since the studies of Lumsden (1923, 1924), the question has been considered as to whether eupnoea, apneusis and gasping represent variants of a single rhythm or if multiple mechanisms underlie the various rhythms (see discussion in St John, 1990). One strong piece of evidence in support of the latter is the finding that one region of the medulla is indispensable for the neurogenesis of gasping but not of eupnoea. We have no information as to the role, if any, that neuronal activities in this region play in the neurogenesis of eupnoea. A similar statement is accurate for the regions of medulla which underlie rhythmic activities of *in vitro* preparation of neonatal rats. Indeed, it is undefined whether neuronal activities of the pre-Botzinger complex, recorded *in vivo*, are synonymous with those underlying the neurogenesis of rhythmic activities *in vitro*. Only synonymous are the rhythmic activities of the *in vitro* brainstem and spinal cord preparation of the neonatal rat with gasping of *in vivo* neonatal and adult animals.

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