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VESTIBULAR AND CEREBELLAR MODULATION OF EXPIRATORY MOTOR ACTIVITIES IN THE CAT

BY QIN HUANG, DAN ZHOU AND WALTER M. ST JOHN
From the Department of Physiology, Dartmouth Medical School, Hanover, NH 03756, USA

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SUMMARY

1. The purpose of our investigation was to evaluate the hypothesis that components of the vestibular and cerebellar systems regulate efferent respiratory-modulated activities of cranial and spinal nerves. The hypothesis was based upon the observation that spinal neural activities during expiration are greatly altered subsequent to a change in posture.

2. In decerebrate and paralysed cats, efferent activities were recorded from the central cut ends of the phrenic nerve, intercostal nerve, branch of the intercostal nerve innervating the triangularis sterni, cranial iliohypogastric (abdominal) nerve and recurrent laryngeal nerve.

3. Animals were artificially ventilated. Those with intact vagi were ventilated by a servo-respirator which produced changes in lung volume in parallel with alterations in integrated activity of the phrenic nerve. Animals with bilateral vagotomy were ventilated with a standard respirator.

4. Aspiration of the entire cerebellar cortex did not produce alterations in levels of neural activities; the respiratory frequency was increased modestly. Following ablation of the ventrolateral portion of corpus medullare and cerebellar peduncles, expiratory activities of spinal nerves were completely eliminated whereas inspiratory activities were not greatly altered. Results were similar in animals having either intact or sectioned vagi.

5. Electrical stimulation or chemical stimulation by glutamate of regions of the ventrolateral cerebellum produced little change in respiratory neural activities except when these stimulations were within the infracerebellar nucleus. Stimulations in this nucleus caused pronounced increases in expiratory activities of spinal nerves. Neither inspiratory activities of spinal nerves nor inspiratory or expiratory activities of the recurrent laryngeal nerve were altered. Studies in animals having intact or sectioned vagi yielded similar results.

6. Bilateral lesions of neurons in the infracerebellar nucleus by injections of kainic acid in animals having intact or sectioned vagi caused an irreversible loss of expiratory activities of spinal nerves with neither inspiratory spinal activities nor inspiratory and expiratory laryngeal activities being altered. Similar findings were obtained following unilateral ablation of the infracerebellar nucleus in vagotomized cats. However, in cats with intact vagi, unilateral ablation of the infracerebellar

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nucleus produced only transient changes in either inspiratory or expiratory neural activities.

7. We conclude that activity descending from the infracerebellar nucleus facilitates expiratory-modulated bulbospinal and/or spinal neuronal activities. These cerebellar efferents establish the 'operating point' of the excitatory neuronal activities.

INTRODUCTION

During quiet breathing, expiration has been considered as a passive phenomenon, resulting from the elastic recoil of the lung and chest wall system. Expiration was only viewed as becoming active under special conditions, such as increased ventilation. The complete absence or minimal expiratory-related discharges of muscles of the chest wall and abdomen in anaesthetized preparations supported this concept of 'passive expiration'. De Troyer & Ninane (1986, 1987) have concluded that this concept requires reconsideration since phasic discharges during expiration can be recorded from muscles of the chest wall and abdomen of unanaesthetized dogs, cats and humans in eupnoea (De Troyer & Ninane, 1986, 1987; Fregosi, Knuth, Ward & Bartlett, 1987; Estenne, Ninane & De Troyer, 1988; De Troyer, Gilmartin & Ninane, 1989; Fregosi & Bartlett, 1989).

Expiratory activities of muscles of the chest wall and abdomen are altered with a change in posture (e.g. Duron & Marlot, 1980; De Troyer & Ninane, 1986, 1987). These changes are considered important to compensate for adverse mechanical effects of postures, such as standing, upon inspiratory muscles of the chest wall and diaphragm (see discussion in Estenne *et al.* 1988). Multiple mechanisms appear to be responsible for these posture-induced changes. Changes in expiratory activities might occur secondarily to changes in lung volume and in the discharge of pulmonary stretch receptors (De Troyer & Ninane, 1987). A change in stretch receptor discharge, such as is induced by positive end-expiratory pressures, causes augmentations of activities of expiratory muscles of the chest wall and abdomen (Bishop, 1967; Oliven & Kelson, 1989).

Changes in pulmonary receptor discharge cannot entirely account for posture-induced changes since such changes in activities of the triangularis sterni are still observed following vagotomy (De Troyer & Ninane, 1987). While posture-induced changes in abdominal muscle activity are eliminated following vagotomy (De Troyer & Ninane, 1987), non-vagal mechanisms still influence these changes. Thus, in animals having intact vagi, changes in abdominal muscle activity in response to a fixed alteration of lung volume differ depending upon the position of the animal (Davies, Sant'Ambrogio & Sant'Ambrogio, 1980).

Since expiratory thoracic and abdominal muscle activities can be altered by small changes in position of the head, a possible role for mechanoreceptors of the upper airway must be considered. Reflexes involving these upper airway receptors are responsible for most of the change in activity of muscles of the upper airway with changes in head position (Bonora, Bartlett & Knuth, 1985). A similar significant role in control of activities of thoracic and abdominal muscles is improbable since posture-induced changes can be recorded in animals in which the upper airway has been bypassed by an endotracheal tube (De Troyer & Ninane, 1987).

The above discussion demonstrates that the expiratory activity of spinal nerves is strongly influenced by a 'postural' component which is independent of mechanoreceptors of upper airways and the lungs. Based on results of the studies described herein, we hypothesize that this 'postural' component is regulated by the vestibulocerebellar system. These studies were undertaken because of the unexpected observation, during other experiments (St John & Zhou, 1989), that expiratory-related activities of the spinal nerves were eliminated when the ventrolateral portion of the cerebellum was removed.

METHODS

General experimental preparation

Seventy-seven adult cats of either sex were used. The surgical preparation has been described previously (e.g. St John & Bledsoe, 1985). Under halothane anaesthesia, the trachea was cannulated, catheters were placed in a femoral artery and vein, and the brain stem was transected at an intercollicular level. Halothane anaesthesia was then discontinued.

By procedures described previously, spinal and cranial nerves were isolated, sectioned and their efferent activities recorded (St John & Bledsoe, 1985; Fregosi *et al.* 1987; St John, Zhou & Fregosi, 1989; St John & Zhou, 1989, 1990). The specific neural activities for which recordings were obtained are as follows: phrenic, recurrent laryngeal, internal intercostal (T8–T9), branch of the intercostal innervating the triangularis sterni (T7–T11), cranial iliohypogastric (abdominal, L1). There were no differences in response of neural activities recorded at the various levels. Not every neural activity was recorded in each animal. Neural activities were integrated by resistance–capacitance circuits.

The vagi were sectioned at a midcervical level in forty-seven cats and within the thorax, caudal to the recurrent laryngeal branches, in nineteen animals. The vagi were intact in the other cats. The animals were paralysed with gallamine triethiodide (5 mg/kg). The vagotomized cats were ventilated by a conventional positive pressure device. Animals with intact vagi were ventilated by a servo-respirator, which produced changes in tracheal pressure, and lung volume, in parallel with changes in integrated activity of the phrenic nerve (St John & Zhou, 1990). As isolation of the intercostal and triangularis sterni nerves had resulted in pneumothoraces, 2 cmH_2O positive end-expiratory pressure was added for animals ventilated with either respirator.

End-tidal fractional concentrations of CO_2 (F_{ET,CO_2}) and O_2 (F_{ET,O_2}) and arterial blood pressure were continually monitored (e.g. St John & Zhou, 1989, 1990). F_{ET,CO_2} and F_{ET,O_2} could be set at desired levels by adjusting the gas mixture delivered to the intake port of the respirators. If required, a mixture of metaraminol and dextran was infused intravenously to maintain mean arterial pressure at a minimum of 80 mmHg. Rectal temperature was continuously monitored and maintained at 37–39 °C by a heating pad.

Stimulation and ablation of cerebellar and vestibular components

A minimum of 1 h following termination of halothane administration, neural activities were recorded in hyperoxia at several levels of F_{ET,CO_2}. Prior to recording, all animals had been positioned prone in a stereotaxic apparatus with the chest and abdomen resting upon the heating pad. For animals having intact vagi, pulmonary inflations were withheld periodically or the lungs were maintained at the end-inspiratory level of inflation for one or more respiratory cycles (St John & Zhou, 1990). A minimum of ten cycles with inflations intervened between these cycles of non-inflation or sustained inflation.

Following these control recordings in five cats, two with intact vagi, the cerebellar cortex, the underlying corpus medullare and cerebellar nuclei and peduncles were gradually ablated by aspiration. Neural activities were periodically recorded.

In other animals, regions of the cerebellum, its peduncles and underlying vestibular nuclei were electrically or chemically stimulated or chemically ablated (Table 1). Note that electrical or chemical stimulations of several regions were performed in some animals. Likewise, chemical ablation usually followed such stimulations. Stereotaxic co-ordinates were used to position a

bipolar electrode, fixed to a needle, into desired regions. The diameter of the electrode was 0·1 mm and that of the needle was 0·5 mm. Electrical stimulation consisted of 2·0 s trains of 0·5 ms pulses delivered at 50 Hz; the current of stimulation was less than 150 μA. This stimulation would activate both cell bodies and fibres. To specifically activate the former (Goodchild, Dampney & Bandler, 1982), 0·5–1·0 μl of a solution of 0·5 M-glutamate was injected. To selectively lesion cell

TABLE 1. Number of experiments in animals with intact and sectioned vagi

Electrical stimulation	Vagi intact	Vagi sectioned
Posterior interposed nucleus	3	8
Anterior interposed nucleus	0	10
Infracerebellar nucleus	12	31
Lateral cerebellar nucleus	0	4
Medial cerebellar nucleus	3	6
Inferior vestibular nucleus	0	3
Lateral vestibular nucleus	0	3
Stimulation with glutamate		
Posterior interposed nucleus	3	9
Anterior interposed nucleus	0	10
Infracerebellar nucleus	7	31
Lateral cerebellar nucleus	0	4
Medial cerebellar nucleus	3	6
Inferior vestibular nucleus	0	3
Lateral vestibular nucleus	0	3
Lesioning with kainic acid		
Posterior interposed nucleus	0	2
Anterior interposed nucleus	0	3
Infracerebellar nucleus	9	29
Lateral cerebellar nucleus	0	0
Medial cerebellar nucleus	0	0
Inferior vestibular nucleus	0	3
Lateral vestibular nucleus	0	3

bodies (see references in Nattie, Mills, Ou & St John, 1988), 0·5–1·0 μl of a 4·69 mM solution of kainic acid was injected into a desired region. Injections were delivered over a period of 1–3 s. After a unilateral injection of kainic acid, an identical volume was injected into the same region contralaterally in most studies. However, in six cats, ventilatory activity was recorded for a number of hours following a single unilateral injection of kainic acid.

Both glutamate and kainic acid were dissolved in mock cerebrospinal fluid (Berger & Cooney, 1982), which also contained Fast Green FCF to localize the site of injection. Glutamate and kainic acid were injected with the cats maintained at various levels of F_{ET,CO_2}; these levels were also altered following injections. Likewise, responses to lung inflation were assessed following injection.

At the end of the experiment, the brain stem and cerebellum were removed for histological verification of regions of injection.

Analysis of data

The time from the onset to the rapid decline of phrenic activity from its peak level defined neural inspiration (T_I). The time to the commencement of the next phrenic burst constituted neural expiration (T_E). Peak integrated neural activities were determined during T_I and/or T_E. These peak activities were normalized as a percentage of control values recorded prior to stimulation or ablation. The control values represented the mean of values for three to five respiratory cycles. Likewise, except during electrical stimulation, values following the various perturbations represented the mean of three to five cycles. Values during electrical stimulation were limited to

that period of stimulation. Statistical evaluations of data were via analysis of variance and a non-parametric Wilcoxon test, adjusted for multiple comparisons. Probabilities less than 0·05 were taken as significant.

RESULTS

Control recordings of neural activities

The respiratory-modulated patterns of discharge of the various spinal and cranial nerves are shown in Figs 1–3 and 5–8. Thus, the recurrent laryngeal nerve typically discharged during both neural inspiration and the initial portion of neural expiration. The discharge of the nerve to the triangularis sterni only commenced during mid- to late expiration, after the recurrent laryngeal discharge had ceased. In some animals, the internal intercostal nerve also only discharged during the late stages of expiration. In other cats, this nerve discharged throughout all of expiration; a separate burst of activity during inspiration was also occasionally observed. Finally, the discharge of the abdominal nerve was characterized by a diminution or complete cessation of activity during inspiration.

Ablation of the cerebellum by aspiration

In five cats, three having bilateral vagotomy, removal of the cerebellar cortex and underlying white matter caused augmentations in both inspiratory and expiratory neural activities and in ventilatory frequency. The changes in neural activities were transient and returned to levels approximating those of control within 10–30 min; the frequency remained elevated (Fig. 1). Removal of the entire medial portion of the cerebellum, including the medial cerebellar nuclei, caused no further alteration of ventilatory activity. However, with ablation of the lateral portion of the cerebellum, expiratory-related activities of spinal nerves were completely and irreversibly eliminated (Fig. 1). This lateral region includes the nucleus interpositus, the lateral nucleus and the infracerebellar nucleus (Berman, 1968). After ablation of this region, elevations of F_{ET,CO_2} to hypercapnic levels did not recruit expiratory spinal neural activities. Results were similar in animals having intact and those with sectioned vagi.

Electrical and chemical stimulation of cerebellar nuclei

Consistent changes in respiratory neural activities were only obtained following electrical or chemical stimulations within the infracerebellar nucleus. This localization and that of other cerebellar regions were confirmed by histology. Stimulations were usually performed in modest hypercapnia ($F_{ET,CO_2} = 0{\cdot}05$–$0{\cdot}06$) since expiratory spinal activities were more clearly defined than at normocapnia.

Electrical stimulations of the infracerebellar nucleus caused pronounced increases in expiratory intercostal, triangularis sterni and abdominal discharges; the concomitant augmentations in peak expiratory laryngeal discharge were modest and not significant (Fig. 2, Table 2). Peak inspiratory laryngeal discharge did increase significantly following stimulation; however, such augmentations were not obtained following microinjections of glutamate. These glutamate injections resulted in significant augmentations of expiratory spinal activities but not expiratory laryngeal discharge (Table 2, Fig. 3). The respiratory frequency was significantly elevated following injections of glutamate, being $115 \pm 4{\cdot}9\%$ of control values.

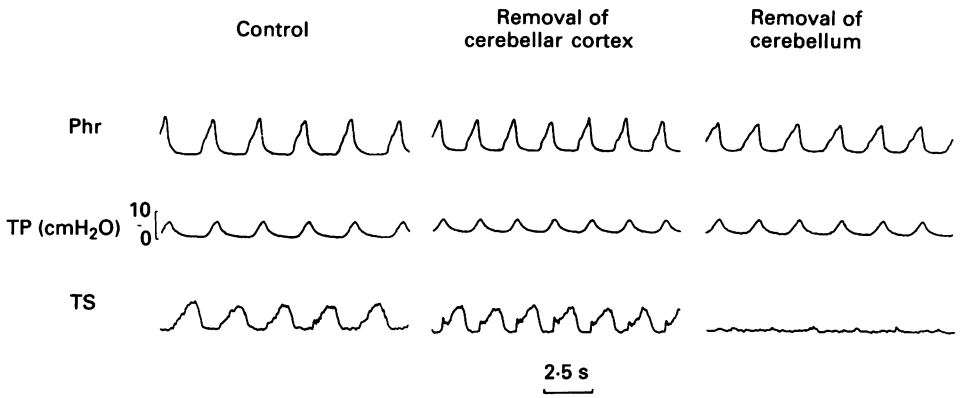

Fig. 1. Changes in inspiratory and expiratory spinal neural activities following ablation of the cerebellum. Note the minimal changes in phrenic (Phr) and triangularis sterni (TS) activities following ablation of the entire cerebellar cortex and the selective elimination of expiratory activity following removal of the corpus medullare and cerebellar peduncles. TP, tracheal pressure. Cat had intact vagi.

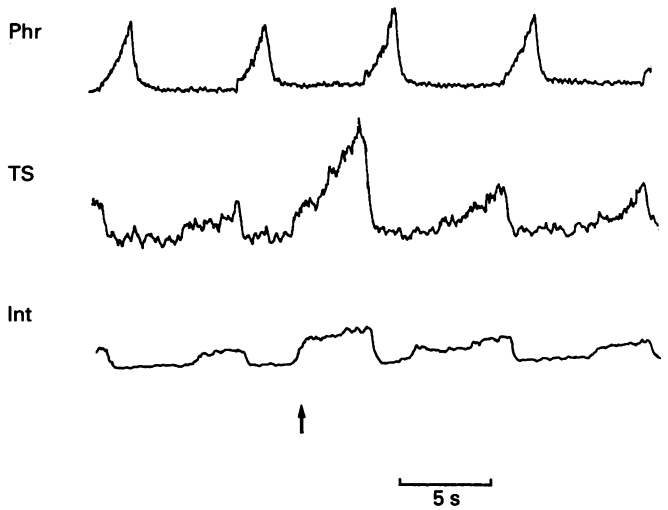

Fig. 2. Alterations of inspiratory and expiratory neural activities following electrical stimulation of the infracerebellar nucleus. Note augmentations of expiratory activities of triangularis sterni (TS) and intercostal (Int) nerves following stimulation. Arrow designates start of a train of 2 s duration. Data are from a cat with sectioned vagi.

The latency for maximal changes to be achieved following the glutamate injections was variable in different trials. Thus, in the left panel of Fig. 3, note that whereas the peak activity of the triangularis sterni was elevated in the respiratory cycle following glutamate injection, the maximal elevation was not achieved until the third cycle. In contrast, the maximal elevations of peak expiratory spinal neural activities were attained immediately after the glutamate injection for the trial shown in the right panel of Fig. 3. This variability in latency might represent slight differences in the

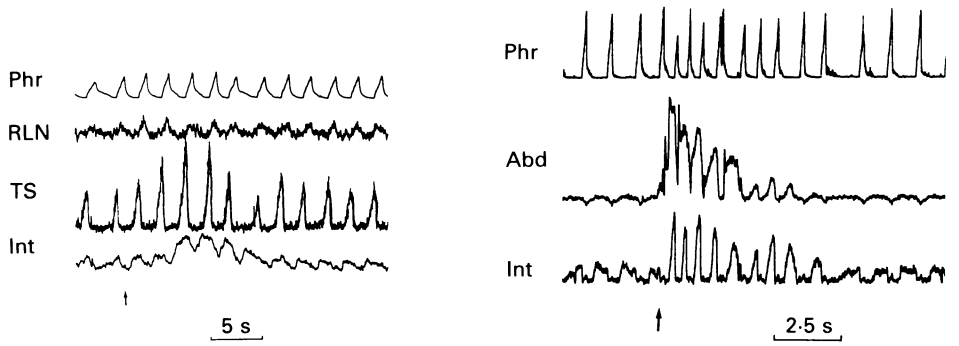

Fig. 3. Alterations in inspiratory and expiratory neural activities following injections of glutamate into the infracerebellar nucleus. Data are from two cats, both having intact vagi. Records in the left panel show responses to injection of 0·5 μl of 0·5 M-glutamate. Note absence of changes in activities of the recurrent laryngeal nerve (RLN) and phrenic nerve (Phr). TS, triangularis sterni; Int, intercostal. Records of right panel show alterations in activities of the phrenic nerve (Phr), abdominal nerve (Abd), and intercostal nerve (Int) following injection of 1·0 μl of 0·5 M-glutamate.

TABLE 2. Alterations of inspiratory and expiratory neural activities following electrical and chemical stimulations of the infracerebellar nucleus

	Electrical stimulation	Stimulation with glutamate
Phrenic	103 ± 3·4	97 ± 1·4
RLN_{insp}	114 ± 4·3*	104 ± 6·5
RLN_{exp}	114 ± 11·8	91 ± 7·0
Intercostal	165 ± 18·0*	135 ± 9·00*
TS	128 ± 10·0*	132 ± 6·3*
Abdominal	—	188 ± 25·3*

Values are means ± S.E. of the mean and are expressed as percentage of control cycles, without stimulations. * = $P < 0·05$ compared to control values. RLN_{insp}, inspiratory activity of recurrent laryngeal nerve; RLN_{exp}, expiratory activity of recurrent laryngeal nerve; intercostal, expiratory activity of intercostal nerve; TS, expiratory activity of nerve to the triangularis sterni; abdominal, expiratory activity of abdominal nerve. Data are both for animals having intact and sectioned vagi. Number of observations for electrical stimulation are: phrenic, 31; RLN_{insp} and RLN_{exp}, 10; intercostal, 15; TS, 16; abdominal, 0. Numbers of observations for glutamate stimulation are: phrenic, 31; RLN_{insp} and RLN_{exp}, 14; intercostal, 26; TS, 27; abdominal, 6.

region of injection. Also, the glutamate injections which were used most probably caused both augmentations and, slightly later, diminutions of neuronal activities in the region (Lipski, Bellingham, West & Pilowsky, 1988). This mixed response might have contributed to the latency for maximal excitation.

The changes in neural activities were similar for injections of glutamate ipsilateral and contralateral to the neural recording site. Likewise, changes were similar in animals having intact or sectioned vagi. Neither mean arterial blood pressure nor heart rate was consistently altered following either electrical or chemical stimulation of the infracerebellar nucleus.

Augmentations of spinal expiratory activities following stimulations of the infracerebellar nucleus were completely eliminated if the stimulus was delivered as

little as 1·0 mm from this nucleus. Hence, the augmentation of expiratory activities following stimulation was used as a means of identifying the location of the infracerebellar nucleus for injections of kainic acid.

Neither the respiratory frequency nor any variable of spinal or cranial neural activities was altered by electrical stimulation of the anterior or posterior divisions

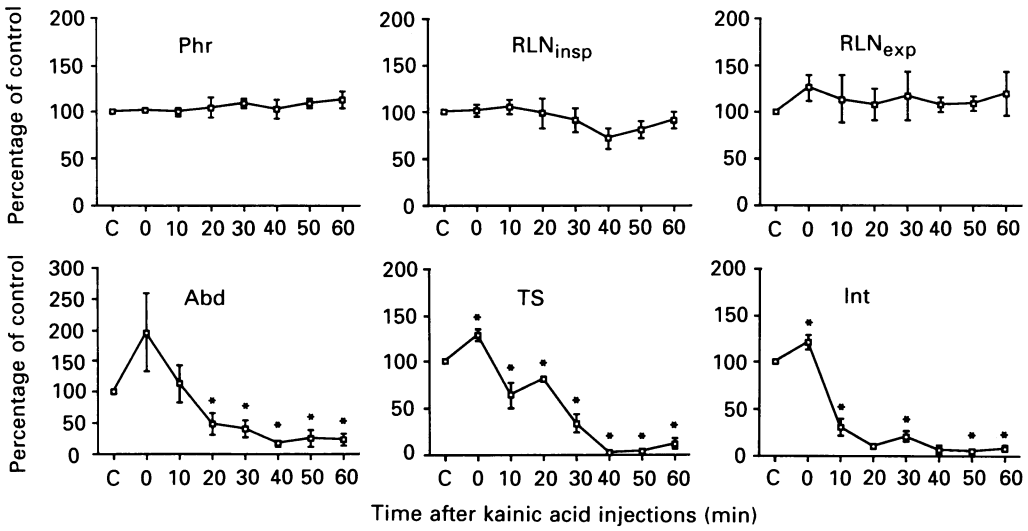

Fig. 4. Time course of changes in inspiratory and expiratory neural activities following bilateral injections of kainic acid into the infracerebellar nucleus. Values of peak neural activities prior to injection are expressed as 100%. Note that ordinate scale differs in various panels. Number (n) of animals for each variable were: phrenic (Phr), $n = 31$; inspiratory and expiratory activities of recurrent laryngeal nerve (RLN_{insp}, RLN_{exp}), $n = 6$; triangularis sterni (TS), $n = 29$; internal intercostal (Int), $n = 26$; abdominal (Abd), $n = 6$. * = $P < 0{\cdot}05$ compared to control value.

of the nucleus interpositus or injections of glutamate into this region. Similar negative results were obtained from perturbations of the medial and lateral nuclei with the exception that some electrical stimulations of the lateral nucleus caused reductions in peak phrenic activity and reductions in peak triangularis sterni activity followed stimulation of the medial nucleus. Since injections of glutamate caused no comparable changes, responses to electrical stimulation may have been due to activation of fibres passing through the nuclei. Likewise confirming previous findings (Bradley, Pascoe, Paton & Spyer, 1987), mean arterial blood pressure increased following electrical, but not glutamate-induced stimulation of the medial cerebellar nucleus.

Ablation of cerebellar neurones by kainic acid

Infracerebellar nucleus

In thirty-one experiments, kainic acid was injected into the infracerebellar nucleus bilaterally with less than 5 min separating the two injections. Changes in neural activities were similar in cats having intact ($n = 5$) or sectioned vagi ($n = 26$).

Immediately following injections, the respiratory frequency was elevated as were peak expiratory activities of the triangularis sterni, intercostal and abdominal nerves. Neither peak phrenic activity nor inspiratory or expiratory activities of the recurrent laryngeal nerve were altered. Exclusive of the augmentation of frequency,

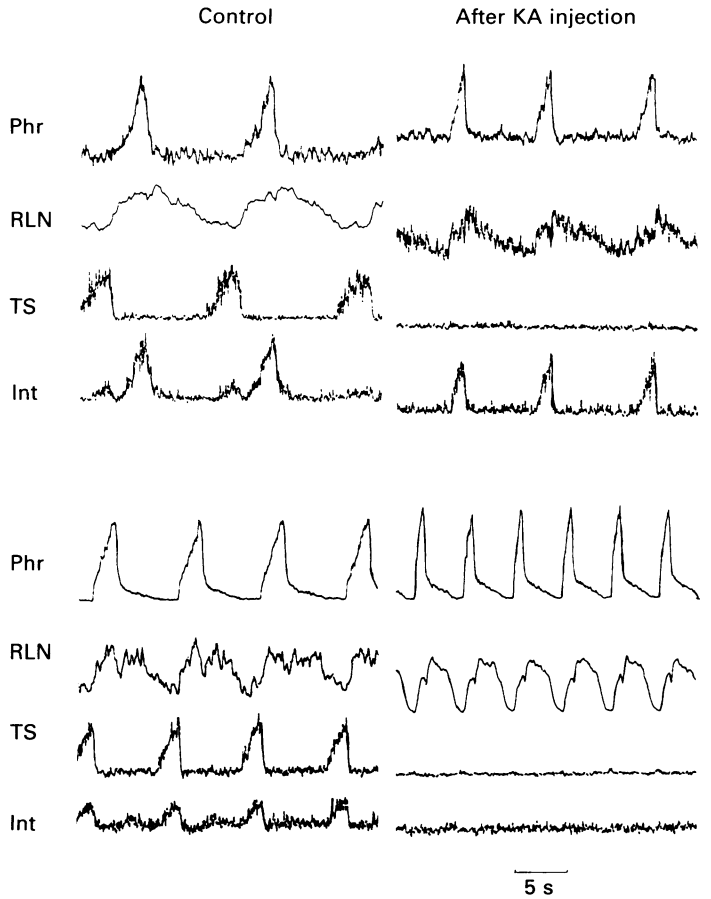

Fig. 5. Changes in neural activities following bilateral injections of kainic acid (KA) into the infracerebellar nucleus. Recordings of left panel were obtained prior to injections; right panel tracings were obtained 1 h following injections. Records were obtained from two vagotomized cats. Phr, phrenic; RLN, recurrent laryngeal nerve; TS, triangularis sterni; Int, intercostal nerve. Note elimination of expiratory, but not inspiratory spinal discharges following kainic acid.

the other augmentations were transient with expiratory neural activities declining progressively and sharply (Figs 4 and 5). One hour after the injections, peak expiratory spinal activities had significantly declined, being completely eliminated in many experiments (16 of 29 expiratory intercostal, 16 of 30 triangularis sterni, 2 of 6 abdominal). In contrast, neither the expiratory nor inspiratory activities of the

recurrent laryngeal nerve, peak phrenic activity or inspiratory intercostal activity was significantly altered (Fig. 4). The respiratory frequency was greatly elevated, being $186 \pm 11{\cdot}8\,\%$ of control. This increase was due to diminutions in both T_I and T_E. Neither blood pressure nor heart rate was systematically altered.

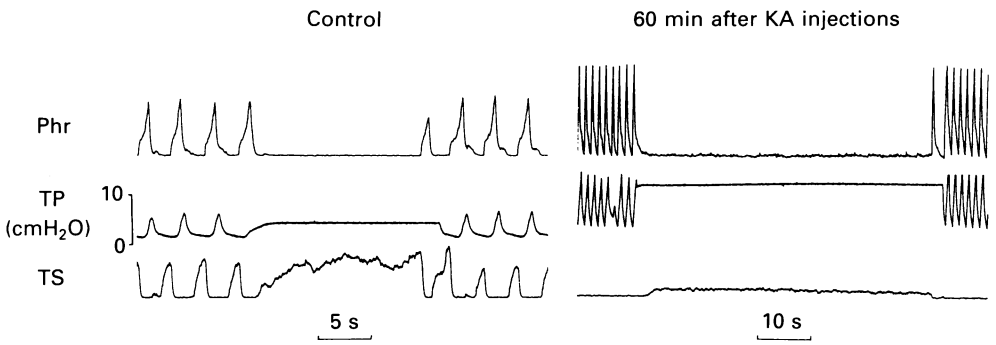

Fig. 6. Influence of changes in pulmonary stretch receptor discharge upon phrenic (Phr) and triangularis sterni (TS) activities before and after bilateral ablation of the infracerebellar nucleus by injection of kainic acid (KA). In the left panel, note that pulmonary inflations eliminated phrenic activity and augmented triangularis sterni discharge. Following injection of kainic acid (KA; right panel), phasic triangularis sterni discharges were not discernible during cycles with periodic lung inflations and augmentations during sustained lung inflations were much reduced, compared to pre-injection changes. TP, tracheal pressure.

In those animals in which spinal expiratory activities had been eliminated, extreme hypercapnia or asphyxia did not elicit discernible phasic discharges in 83% of the animals. In the other cats, low level phasic discharges were recruited. A similar recruitment was also obtained by sustained lung inflation in cats with intact vagi.

As exemplified by recordings of Fig. 6, prior to bilateral ablation of the infracerebellar nucleus, lung inflation caused a prolonged interruption of phrenic activity and a concomitant augmentation of expiratory spinal discharges. Withholding lung inflation caused augmentation of phrenic discharge and diminutions of the expiratory neural activities. One hour following bilateral injections of kainic acid, the respiratory frequency was substantially elevated. In this animal, peak phrenic activity was also elevated; such elevations were not a consistent finding in cats with intact vagi. These increases in peak phrenic activity resulted in increases in the volume of ventilation and, hence, the inspired fraction of CO_2 was increased to maintain isocapnia. Withholding lung inflation still caused further augmentations of peak phrenic activity and a reduction or elimination of any minimal spinal expiratory activities. Likewise, sustained lung inflation still greatly prolonged neural expiration with the time before a 'break-through' of phrenic activity being typically in excess of that prior to lesioning of the infracerebellar nucleus. During this sustained expiratory period, expiratory spinal neural activities were recruited, but at extremely low levels. All of these responses to changes in lung volume were eliminated by bilateral vagotomy. Thus, augmentations of the discharge of vagal pulmonary receptors were capable of modifying changes in expiratory spinal neural

activities following bilateral ablation of the infracerebellar nucleus. Such modification by vagal mechanisms was more clearly demonstrated following unilateral ablation of this nucleus.

In six cats, kainic acid was injected unilaterally into the infracerebellar nucleus. Some neural activities were recorded ipsilateral and others contralateral to the site

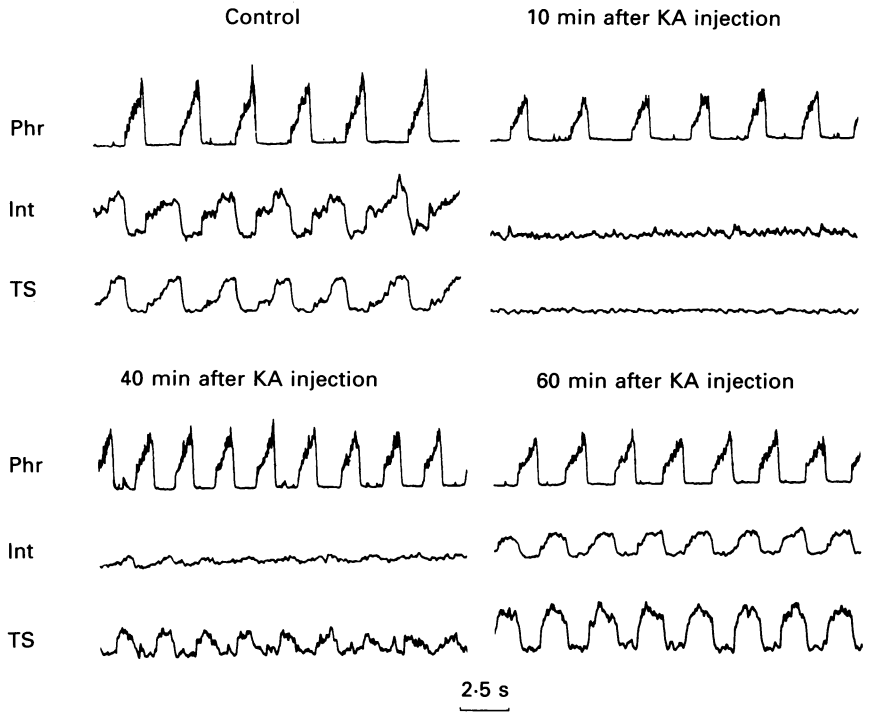

Fig. 7. Recovery of expiratory neural activities following unilateral injection of kainic acid (KA) into the infracerebellar nucleus of an animal having intact vagi. Note elimination of expiratory triangularis sterni (TS) and intercostal (Int) discharges 10 min following injection and recovery of these neural activities over the next 50 min. Phr, phrenic discharge.

of injection. There were no differences between the changes in neural activities which followed the ipsilateral or contralateral injections. For the two animals having sectioned vagi, changes in neural activities were very similar to those following bilateral injections with expiratory spinal activities being completely eliminated. In contrast, for all four cats having intact vagi, expiratory spinal activities were only transiently eliminated with such activities being only modestly below control levels 1–2 h following the unilateral injection (Fig. 7). The respiratory frequency was slightly elevated. Changes in phrenic and expiratory spinal activities in response to withholding or sustained lung inflation were likewise not substantially altered (Fig. 7).

Other cerebellar nuclei

Injections of kainic acid into the anterior (three cats) or posterior (two cats) divisions of the nucleus interpositus caused no alterations of either inspiratory or expiratory spinal neural activities or of the respiratory frequency. Changes in these neural activities were obtained by perturbations of the vestibular system.

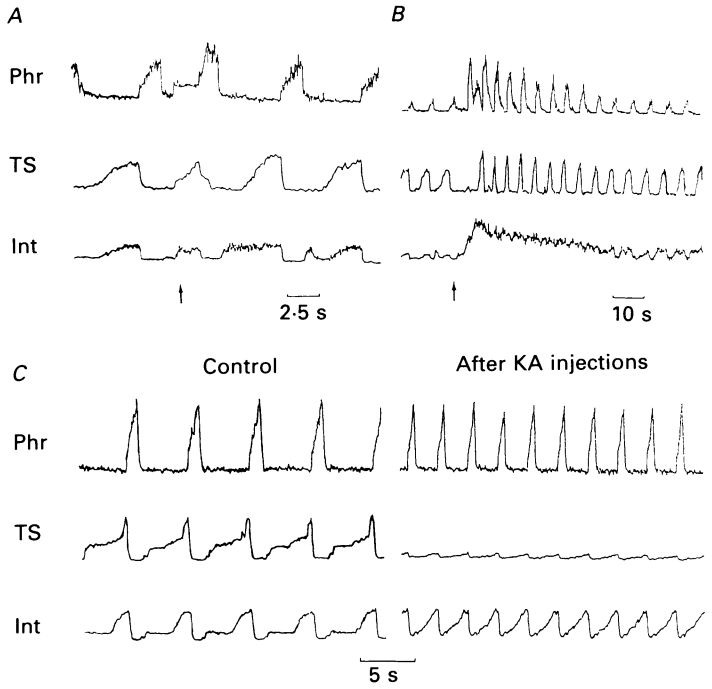

Fig. 8. Changes in inspiratory and expiratory neural activities following electrical and chemical stimulation of the vestibular nuclei and lesioning of vestibular neurons by kainic acid. Panels *A* and *B* show changes following electrical stimulation and injections of glutamate (1·0 μl, 0·5 M) into the inferior vestibular nucleus of a vagotomized cat. *C* shows changes before and 30 min after bilateral injection of kainic acid (KA) into the lateral vestibular nucleus. Phr, phrenic nerve; TS, triangularis sterni; Int, intercostal nerve.

Perturbations of the vestibular system

An augmentation of both inspiratory and expiratory spinal neural activities was obtained following stimulation of the inferior or lateral vestibular nuclei or injections of glutamate into these nuclei in three cats (Fig. 8). Microinjections of kainic acid into these same nuclei resulted in an augmentation of peak phrenic activity and declines in expiratory spinal neural activities. In none of the six animals was the expiratory spinal neural activity completely eliminated following kainic acid (Fig. 8).

Histological evaluations

Figure 9 is an example of an original histological section which was used to document the region of injection of kainic acid. The end of the physical lesion caused by the electrode and needle (arrow on left side) and the centre of the distribution of

Fast Green FCF defined the centre of this region. Note that this region was in the infracerebellar nucleus. Also note, on the right side, the lesion tracts made by the electrode and needle (arrow); again the end of the physical lesion was in the region of the infracerebellar nucleus.

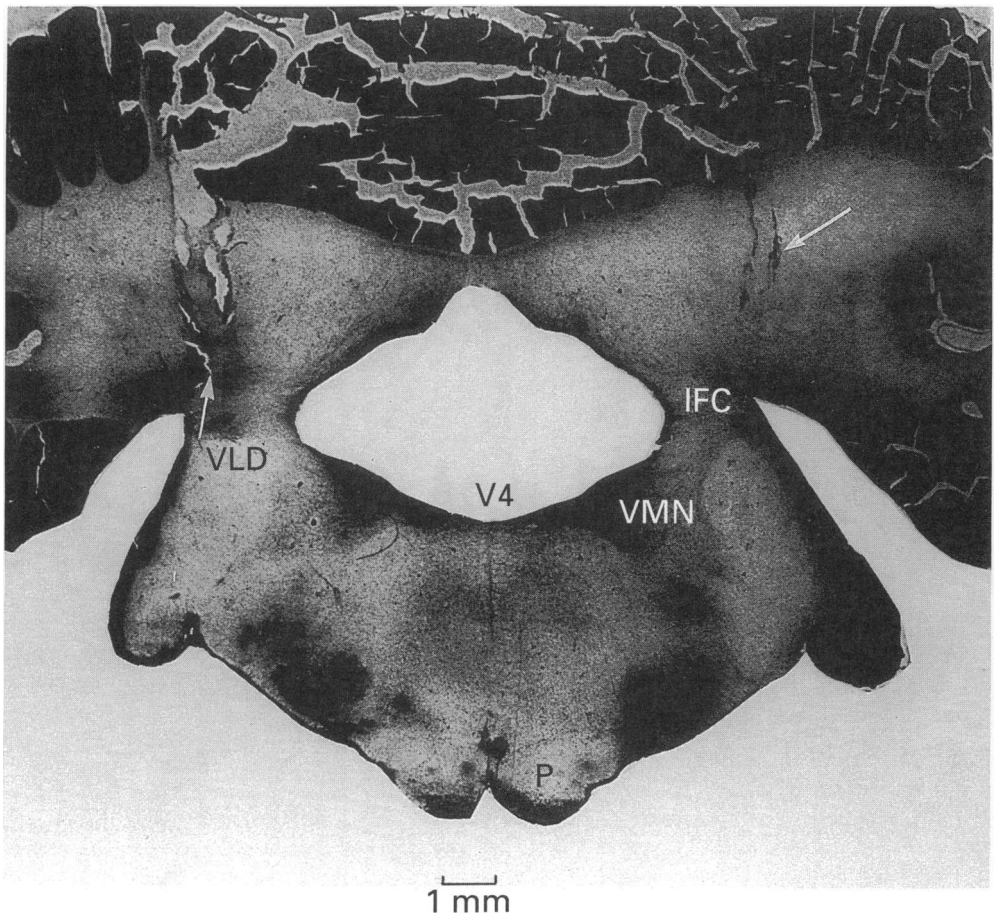

Fig. 9. Example of location of region of injection of kainic acid. Section is taken through the brain stem and cerebellum at the level of the infracerebellar nucleus. Note on the left side that the termination of tract made by the electrode and needle (arrow) was in the infracerebellar nucleus. Tract approaching this nucleus is also shown on the right side (arrow). See text for further details. IFC, infracerebellar nucleus; P, pyramidal tract; V4, fourth ventricle; VLD, lateral vestibular nucleus, dorsal division; VMN, medial vestibular nucleus.

Concerning the volume of distribution of Fast Green and, hence, glutamate and kainic acid, this was difficult to estimate. This difficulty was largely due to the spread of dye dorsally, along the tract made by the needle and electrode. However, Fast Green was distributed in a sphere approximately 0·4–0·8 mm from the centre of the injection.

Figure 10 is a drawing of a section through the brain stem at the level of the infracerebellar nucleus. This figure illustrates the centre of regions in which kainic acid was found to have been injected, as documented from histology, such as that of Fig. 9. Note that injections into the lateral vestibular nucleus, very close to the

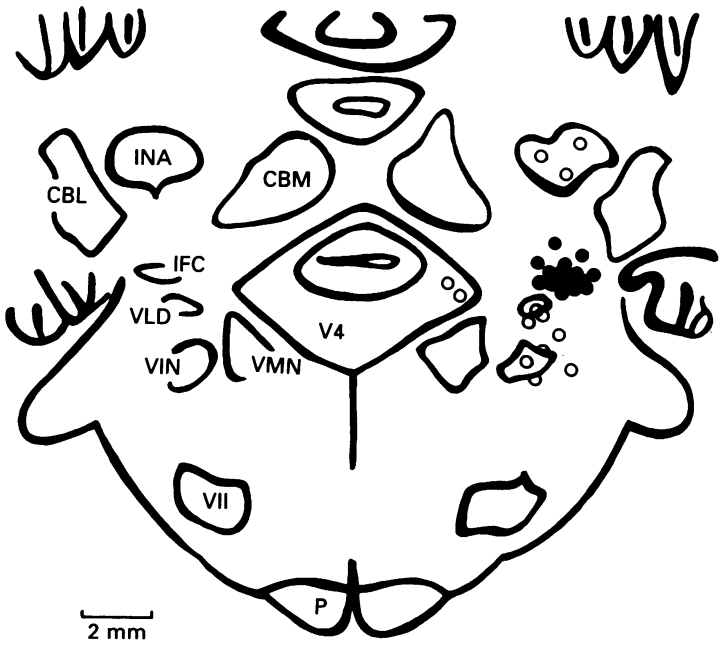

Fig. 10. Localization of sites of injection of kainic acid. Right half of section shows histological localization of injections. For bilateral injections, only those on one side are illustrated. Likewise, for injection sites in the infracerebellar nucleus (IFC), there were many sites which overlapped. ●, sites taken to be within the infracerebellar nucleus which resulted in eliminations of expiratory neural activities. ○, sites which resulted in no or modest changes in expiratory neural activities. CBL, lateral cerebellar nucleus; CBM, medial cerebellar nucleus; INA, anterior interposed nucleus; IFC, infracerebellar nucleus; P, pyramidal tract; VII, facial nucleus; V4, fourth ventricle; VIN, inferior vestibular nucleus; VLD, lateral vestibular nucleus, dorsal division; VMN, medial vestibular nucleus.

infracerebellar nucleus, never resulted in elimination of expiratory spinal neural activities. Likewise, injections into the fourth ventricle produced no changes in neural activities.

DISCUSSION

The major conclusion of this study is that activity descending from the infracerebellar nucleus of the cerebellum provides an input which serves to facilitate expiratory-modulated spinal neural activities. Indeed, a given level of such facilitation appears necessary for this spinal expiratory activity to be manifested. If the other major facilitatory system involving the vagus is intact, the infracerebellar nucleus on one side appears capable of providing this critical level of facilitation. To

use the terminology of Sears (1966), these cerebellar efferents establish the 'operating point' of the expiratory spinal neurons and/or expiratory bulbospinal neurons. Inherent to our conclusion is the concept that these cerebellar efferents must not be distributed ubiquitously to all medullary respiratory neurons, but rather terminate discretely upon the expiratory bulbospinal population. We also hypothesize that some or all of these efferent cerebellar projections impinge upon spinal neurons by a pathway which bypasses the medullary respiratory nuclei.

A striking finding was that stimulations or ablations of the infracerebellar nucleus caused specific alterations in expiratory spinal neural activities with neither inspiratory spinal activities nor inspiratory or expiratory laryngeal activities being consistently altered. Within the medullary respiratory nuclei, there is some degree of separation of laryngeal, inspiratory and expiratory bulbospinal neuronal activities within the ventral nucleus (Euler, 1986; Feldman, 1986). Hence, it is possible that efferent influences from the infracerebellar nucleus selectively terminate upon expiratory bulbospinal cells. Parenthetically, it would appear necessary that some such efferent influences, or those from a surrounding region, must reach the pontile or medullary brain stem since respiratory frequency was altered following injections of glutamate or kainic acid into the infracerebellar nucleus.

Concerning actions upon the brain stem, the possibility arises that efferents from the infracerebellar nucleus alter spinal expiratory neural activities exclusively by perturbations of neuronal activities which control the ventilation cycle. These spinal activities typically reach peak discharge during the late stage of expiration. This late state has been designated as phase II. Phase I is the period between the peak of phrenic discharge and the commencement or rapid rise of many spinal neural activities, for example, that of the triangularis sterni. This phase I is also the period of laryngeal adduction (see Richter, 1982; Richter, Ballantyne & Remmers, 1986). In the context of the present study, augmentations or diminutions of spinal expiratory neural activities could result from alterations of those neural circuits which are theorized to control phase I and phase II (Richter, 1982; Richter *et al.* 1986). However, some of our results are inconsistent with this interpretation.

In two previous reports in this journal, we presented evidence of a reciprocal relationship between both the magnitude and duration of activities during phase I and phase II (St John & Zhou, 1989, 1990). As an index of phase I, we recorded activity of the branch of the recurrent laryngeal nerve innervating the thyroarytenoid muscle of the larynx, a primary adductor. Experimental manoeuvres which increased or decreased thyroarytenoid activity caused opposite alterations of activity of the triangularis sterni. While not as consistent as changes in thyroarytenoid discharge, spinal nerves which were active during phase I did exhibit alterations which were opposite of those during phase II.

As opposed to these earlier studies, alterations of spinal neural activities during phase II following changes in activity within the infracerebellar nucleus were not accompanied by reciprocal changes during phase I. Thus, for example, activity of the recurrent laryngeal nerve during early expiration was little altered during large increases or decreases of triangularis sterni discharge. Following elimination of triangularis sterni activity, a gap, corresponding to phase II, was still evident between the termination of recurrent laryngeal activity and the onset of phrenic

discharge. Finally, for spinal nerves which were active during both phase I and phase II, these activities were altered in parallel (e.g. Fig. 7). We thus consider that influences from the infracerebellar nucleus are directed primarily upon spinal expiratory neurons, or their premotor bulbospinal neurons, rather than upon intrinsic brain stem neuronal circuits. Given the known neuroanatomical connections of the infracerebellar nucleus, an influence upon expiratory spinal neurons would seem possible.

There have only been very limited examinations of the infracerebellar nucleus, which is also referred to as the 'y' nucleus of Brodal & Pompeiano (1957). This nucleus is usually divided into dorsal and ventral divisions which differ in afferent and efferent projections. Neurons of the dorsal division receive afferents from the flocculus whereas vestibular afferents project upon neurons of the ventral division (Brodal & Hoivik, 1964; Gacek, 1977; Sato & Kawasaki, 1987). Efferents from the nucleus include projections to the flocculus, the lateral vestibular nucleus and the superior, medial and descending vestibular nuclei (Gacek, 1977; Carleton & Carpenter, 1983; Epema, Getties & Voogd, 1988). There are also interconnections between the dorsal and ventral divisions (Gacek, 1977). In the present studies, we did not attempt to define if changes in expiratory spinal neural activities reflected alterations within the dorsal or ventral division. However, as discussed below, the dependence of these expiratory activities upon vestibular processes implies that neurons of the ventral division are of greatest importance.

It is obvious from our studies involving ablations of cerebellar regions that connections of the infracerebellar nucleus with intracellular circuits are not required for the steady-state regulation of expiratory activity. Cerebellar regions may be involved in regulating transient responses and, indeed, expiratory spinal neural activities are diminished following stimulation of the medial group of cerebellar nuclei in opossums (Faber, 1987). We observed similar changes following electrical stimulations of these medial nuclei but stimulations with glutamate produced no alterations. Concerning steady-state responses, Euler (1986) has concluded in his review that results from many ablation experiments demonstrate, at best, a modest role for cerebellar mechanism in the regulation of ventilation *per se*. In this context, it must be emphasized that, given its location, the infracerebellar nucleus would be spared during most cerebellectomies. Also, to our knowledge, activities of nerves having expiratory-related discharges have not been recorded during these ablations.

Given the close relationship of the infracerebellar nucleus with the vestibular system, it is possible that some of the changes following injections of glutamate or kainic acid into the infracerebellar nucleus were due to alterations of activities within these nearby vestibular nuclei. Indeed changes following stimulations or ablations of the vestibular and infracerebellar nuclei were similar in some aspects. Thus, for both nuclei, injections of glutamate caused augmentations of expiratory spinal neural activities and the respiratory frequency. Peak phrenic activity also consistently increased following stimulation of the vestibular nuclei; such increases were only occasionally observed for stimulations of the infracerebellar nucleus. Reductions of expiratory spinal neural activities were much greater following injections of kainic acid into the infracerebellar nucleus than the reductions following injections into the vestibular nuclei. Nevertheless, these qualitatively similar alterations have two

implications. First, it is possible that changes which were only occasionally observed following perturbations of the infracerebellar nucleus, such as increases in phrenic activity, reflected concomitant changes in activity within the vestibular nuclei. Second, it is possible that all of the changes resulting from stimulation or ablation of the infracerebellar nucleus could be reproduced if more extensive, or different, regions of the vestibular nuclei were subjected to similar procedures. This question would seem most acute for the lateral vestibular nucleus, which is immediately adjacent to the infracerebellar nucleus in both the ventral and rostral planes (see Fig. 9 and Berman, 1968). We hypothesize that such extensive ablations of the vestibular nuclei would, in fact, reproduce changes following lesions of the infracerebellar nucleus. This hypothesis is based upon the extensive neuroanatomical interconnections, considered above. Indeed, such interconnections would appear necessary for the infracerebellar nucleus to exert any function in the control of ventilatory activity.

The connection of the infracerebellar nucleus with the vestibular system provides a mechanism, via the vestibulospinal tract, by which expiratory activities of spinal motoneurons might be altered independent of the expiratory bulbospinal neuronal activities. Projections have been described from the medial and lateral vestibular nuclei of cats to the thoracic and lumbar spinal cord (Wilson, Yoshida & Schor, 1970; Miller, Tan & Lakos, 1989). This possibility of an independent spinal activation is also supported by the finding that stimulation of laryngeal afferents (Jodkowski & Berger, 1988) or induction of emesis (Miller, Tan & Suzuki, 1987) can augment expiratory spinal neural activities separately from the activity of expiratory bulbospinal neurons. Yet, as Miller and his colleagues have demonstrated, induction of emesis also causes changes in activities of medullary inspiratory and expiratory neurons (Miller *et al.* 1987; Miller, Nonaka, Lakos & Tan, 1990). Thus, we believe it most probable that efferent influences from the infracerebellar nucleus project both to the medulla and, independently, to the spinal cord.

Since vestibular afferent activity is altered with a change in posture, the interrelationship with the vestibular system provides the mechanisms whereby activity within the infracerebellar nucleus could cause alterations in expiratory spinal neural activities, independent of vagal mechanisms. Such independence does not imply an unimportant role of vagal afferents in the definition of expiratory activities for, as noted in the Introduction and as demonstrated in Results, elevations of pulmonary stretch receptor discharge can augment or, in anaesthetized animals, elicit phasic expiratory-modulated spinal activities.

The absence of phasic expiratory spinal activities, exclusive of the intercostal and triangularis sterni, in anaesthetized animals has been paradoxical (see discussions in Fregosi *et al.* 1987; Fregosi & Bartlett, 1989). While anaesthesia causes a similar diminution or elimination of respiratory-modulated activities of cranial nerves (Eyzaguirre & Taylor, 1963; Hwang, St John & Bartlett, 1983; St John & Bledsoe, 1985), the mechanisms for anaesthesia-induced depression would appear to differ. Thus, while respiratory-modulated activities of pontile and medullary motoneurons must be diminished to cause eliminations of their neural activities, both inspiratory and expiratory bulbospinal neuronal activities are numerous even in extremely deeply anaesthetized preparations (Merrill, 1974). It therefore appears that

anaesthesia had eliminated some facilitatory input upon the spinal motoneurons. The elimination of expiratory spinal activities by ablation of the infracerebellar nucleus, the hypothesized dependence of this nucleus upon vestibular afferents for its tonic discharge and the suppression of activities in the vestibulo-cerebellar circuity by anaesthesia (Goldberg & Fernandez, 1984), all support the possibility that the depression of expiratory activities of spinal nerves in anaesthesia is, in fact, reflective of depression of the activity within the infracerebellar nucleus.

Two points remain to be discussed. The first concerns the recovery of expiratory spinal neural activities following a unilateral injection of kainic acid into the infracerebellar nucleus of cats with intact vagi. We have concluded that such recovery demonstrates that facilitatory influences from a single infracerebellar nucleus are sufficient, if the vagal facilitatory system is intact, to support expiratory spinal neural activity. However, it is also possible that the injection of kainic acid did not destroy the neurons of the infracerebellar nucleus but, rather, produced a long-lasting, but ultimately reversible, depolarization block (Lipski *et al.* 1988). We consider the latter explanation as improbable since recovery of spinal expiratory neural activities was never observed in any of the other preparations, including the vagotomized cats which received unilateral injections of kainic acid.

The final point concerns the well-recognized role of the vestibulo-cerebellar system in the emesis of motion sickness (e.g. Reason & Brand, 1975). The possibility that our findings are related only to emesis and not to ventilatory activity *per se* appears improbable. Thus, during emesis, there is co-activation of diaphragmatic and abdominal muscles (Miller *et al.* 1987); such co-activation was never observed following stimulations of the infracerebellar nucleus.

In extension of the concept of Duron & Marlot (1980), we propose that the expiratory discharge of spinal motoneurons is defined by the interaction of 'postural' components, primarily of vestibulo-cerebellar origin, with 'respiratory' components resulting from the discharge of bulbospinal neurons. While the input of the latter is clearly phasic and respiratory-modulated, we propose that the input of the former is tonic. The bulbospinal component can be adjusted by afferents from the peripheral and central chemoreceptor mechanisms and from the discharge of slowly adapting pulmonary stretch receptors. The input from the vestibulo-cerebellar system can be adjusted by changes in the position of the animal. This system for the control of expiratory spinal activities, would appear capable of providing an explanation for a number of heretofore unanswered physiological questions.

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