Electrical stimulation of the posteromedial thalamus modulates breathing in unanesthetized fetal sheep

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Koos, Brian J., Yoshikazu Kawasaki, Ashwinii Hari, Fanor Bohorquez, Calvin Jan, Jason Roostaeian, Charles L. Wilson, and Lawrence Kruger. Electrical stimulation of the posteromedial thalamus modulates breathing in unanesthetized fetal sheep. J Appl Physiol 96: 115–123, 2004; 10.1152/japplphysiol.00517.2003.—Having previously shown that lesions in the posteromedial group of thalamic nuclei abolish hypoxic inhibition of fetal breathing, we devised this study to identify thalamic loci that depress breathing by focal stimulation of specific sectors of the caudal thalamus and adjacent structures. Multipolar electrode arrays consisting of a series of eight stimulation contacts at 1.25-mm intervals were implanted vertically through guide cannulae into the caudal diencephalon of 12 chronically catheterized fetal sheep (>0.8 term), and central neural tissue was stimulated between adjacent contacts. Each site was stimulated repeatedly with increasing current searching for spatial and stimulus strength parameters for a reliable alteration in respiratory rate. Respiratory period increased when stimulation involved areas of the parafascicular complex (PF), which more than doubled the mean period compared with the baseline of 0.90 ± 0.19 s. The change in respiratory period was due to an increase in expiratory time, whereas inspiratory time and breath amplitude were not significantly affected. Breathing period and expiratory time were also increased when the stimulations involved the intralaminar region surrounding the mediodorsal nucleus, the rostral central gray, zona incerta, and ventral tegmental area. Reductions in respiratory frequency occurred less consistently, with stimulation involving surrounding zones including the sub-PF, ventromedial nucleus, and ventrobasal complex. These findings support the hypothesis that a restricted area of the posteromedial thalamus (principally PF) constitutes part of a neuronal circuitry that modulates respiratory motoneurons.

IN THE MAMMALIAN FETUS, breathing movements (breathing) consist of exercise of respiratory muscles, which results in paradoxical motion of the chest and abdomen with minimal exchange amniotic fluid (10). In sheep (>0.8 term), fetal breathing occurs in episodes coincident with rapid eye movements and low-voltage electrocortical activity. Breathing is not associated with high-voltage electrocortical states, which occur ~30–50% of the time in near-term fetal sheep (10). Hypercapnia and chemical acidemia increase fetal respiratory drive, whereas hypoxia arrests breathing. For example, acutely reducing fetal arterial PO2 by 8–10 Torr (to ~15 Torr) virtually eliminates fetal breathing (10, 26). This respiratory inhibition results from direct effects of low O2 tensions on the fetal brain, although a small component may involve peripheral chemoreflexes (10, 25).

Disruptions of the pons or caudal mesencephalon abolish hypoxic inhibition of breathing (10, 17, 21), but lesions involving the cerebellum, cerebral cortex, or rostral hypothalamus do not (10, 18). We have identified a thalamic sector as being crucial to hypoxic inhibition of fetal breathing (22). In those studies, neuronal lesions of the diencephalon abolished the depressant effects of hypoxia when the disruptions included the posteromedial group of thalamic nuclei (22, 23). The present study was designed to identify the diencephalic region whose activation modulates fetal breathing. Our findings indicate that activation of specific elements within the posteromedial thalamus largely encompassing the parafascicular nuclei constitutes the principal sector involved in lengthening expiratory time and respiratory period.

METHODS

All procedures and experiments were approved by the University of California—Los Angeles Chancellor’s Animal Research Committee and were conducted in accordance with the guidelines of the American Physiological Society.

Surgery. Twelve pregnant ewes (Rambouillet-Columbia cross, ~0.8 term) underwent halothane inhalation anesthesia for in utero fetal surgical procedures that included 1) insertion of polyvinyl catheters in the right brachial artery, the right carotid artery, external jugular vein, and trachea of the fetus; 2) placement of a catheter in the amniotic sac for measuring amniotic fluid pressure; and 3) implantation of bipolar stainless steel electrodes on a medial and lateral orbital ridge of one eye for recording eye movements as well as on dura overlying the left and right parietal cortex for recording electrocortical activity (26). Bilateral stainless steel guide cannulae (2.0 cm shaft length, 1.0 mm outer diameter) were placed 2–4 mm lateral to the midline, inserted into parietal cortex, and stereotaxically directed toward the fetal diencephalon; the cannulae were anchored to the calvaria with stainless steel screws and dental acrylic (22). The guide cannulae, which would subsequently be used as conduits for the diencephalic insertion of stimulating electrodes, were exteriorized through a silastic rubber “window,” which was sutured to uterine and abdominal walls. A Plexiglas cover protected the exteriorized guide cannulae.

Tetracycline (15 mg/kg im) was administered to the ewes before surgery, and ampicillin (500 mg) was infused into the amniotic sac immediately after the procedure and on the first postoperative day. Buprenorphine HCl (0.006 mg/kg im) was given to the ewes for postsurgical analgesia.

All pressures were measured with calibrated pressure transducers (Argon Medical, Dallas, TX) with tracheal pressure referenced to

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amniotic fluid by subtracting amniotic fluid pressure. Electrooculogram, electrocorticogram, and tracheal pressure were displayed on a Grass polygraph (model 7E).

Experiments. The ewe and fetus were allowed at least four recovery days after surgery before the experimental protocols were begun. An electrode configuration consisting of an eight-contact vertical array of insulated stainless steel wire (145-µm diameter, Rhodes Medical Instruments, Summerland, CA) with 0.3 mm of exposure per contact and 1.25 mm of vertical separation between consecutive contacts was passed through a guide cannula into the diencephalon. In two animals (animals 39 and 244), the electrodes were placed deeper to help ensure that the vertical axis of the electrodes passed through the ventral diencephalon. Silastic rubber wedged between the insulated wire and plastic well of the guide cannula supported the electrode array. An isolated electrical stimulator (Grass Instruments, S48 Stimulator and PSI6 battery-powered isolation unit) was used to deliver current to adjacent pairs of contacts along the microelectrode array while measuring current at each stimulation site. Bipolar stimulation consisting of 3- to 5-s trains of 50-Hz rectangular pulses with a duration of 300 µs was delivered between adjacent contacts, with current intensities ranging from 50 to 1,000 µA.

Fetal breathing, which usually has a frequency of ≥1 Hz, typically occurs in episodes lasting 10–60 min (10). Electrical stimulation regimens began after at least a 5-s period of spontaneous breathing. The two deepest electrode contacts were the first pair tested, with consecutively more proximal pairs tested subsequently (2–3, 3–4, 4–5, etc.); the deeper electrode of each pair was always cathodal, providing consistent polarity of stimulation. The stimulus current, which was begun at 50 µA, was increased in steps of 50–100 µA until a change in breathing frequency was clearly detected or a maximum current of 1.000 µA was reached. The stimulation sequence was repeated in reverse order to establish the reliability of the responses, given the episodic nature of fetal breathing. The recording rate of the polygraph was 5 mm/s during the stimulation experiments.

These brief electrical stimuli were used to search for depressant effects on breathing, exhibited by an increase in expiratory time. This was performed in fetuses with normal arterial PO2, PCO2, and pH, with respective means of 20.8 ± 0.9 Torr, 50.1 ± 1.1 Torr, and 7.31 ± 0.01 before euthanization. A total of 114 electrode contact pairs were stimulated in 12 fetal sheep with the number of stimulations exceeding 2,000, the majority of which failed to significantly alter the rate or amplitude of fetal breathing (Figs. 1 and 2). Electrical stimulation that increased respiratory period principally involved brain sectors within or adjacent to 1) parafascicular nuclear complex (Pf), 2) zona incerta (subthalamus), and 3) the medial zone of the ventral tegmental area of the subthalamus.

Pf. In six fetuses, electrical stimulation of loci within or proximate to the Pf elicited more than a twofold increase in respiratory time and breath period relative to the respective controls of 0.58 ± 0.19 and 0.90 ± 0.19 s (Figs. 1, 3, and 4). Inspiratory time within the first 15 s after electrical stimulation remained within 0.02 s of the baseline mean of 0.38 ± 0.03 s. Mean breath amplitude deviated by <1 mmHg compared with the control of 6.0 ± 0.8 mmHg, which was also a nonsignificant change.

The electrical stimulation elicited particularly strong responses in four fetuses (fetuses 27, 290, 731, and 733), with mean breath periods exceeding 2 s, whereas the stimulations induced smaller increases in mean periods in two other fetuses (fetuses 24 and 296). The respiratory period was not altered consistently when stimulations involved the caudal inferior thalamic respiratory modulation, as described in our laboratory’s previous reports (22, 23).

Data analysis. Fetal breathing was detected by characteristic negative changes in intrathoracic pressure as reflected in the tracheal pressure recordings. Breath period or cycle was measured from the start of each breath to onset of the subsequent breath. Breath amplitude was the absolute change in tracheal pressure associated with a respiratory effort. The record for analysis included the 5 s immediately before stimulation (baseline) and the subsequent 15 s, which comprised the stimulation and recovery periods. Mean inspiratory time, expiratory time, period, and amplitude were calculated for each 5-s epoch, and significant changes were identified within separate brain sectors for each animal. Because relatively large brain sectors did not exhibit uniform breathing responses to stimulation, analysis was confined to multiple measurements (2–4 measurements) for each current intensity from pairs of contacts that elicited respiratory responses within specific sectors. Mean inspiratory time, expiratory time, period, and amplitude changes from baseline were compared by time and brain sector via a repeated-measures analysis of variance model (SAS Procedure MIXED; SAS, Cary, NC), controlling for random comparison among animals. Specific comparisons were made by using the post hoc t-test method and the Tukey-Fisher criterion under the repeated-measures model. Mean differences were considered statistically significant if P < 0.05. Data are presented as means ± SE.

RESULTS

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Fig. 2. Outline drawings of histological sections showing electrical stimulations sites distant from Pf. AV, anteroventral nuclei; cl, central lateral nucleus; mt, mammillothalamic tract; oc, optic chiasm; ot, optic tract; Pa, anterior paraventricular nucleus; pc, posterior commissure; PIT, pituitary; Rh, rhomboid nucleus.

☐ no response

- period >2s

★ period

+ period

period >2s, amplitude

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border of Pf (fetuses 372 and 39) or the zone along the medial edge of Pf (fetus 170).

Statistically significant increases in respiratory period and expiratory duration occurred 5–10 s after the onset of stimulation. The mean response latency calculated in four fetuses (with breath periods of >3 s) was 5.5 ± 0.7 s after the onset of stimulation and 2.5 ± 0.7 s after the end of stimulation.

Fig. 3. Fetal breathing response to electrical stimulation (400 μA) of the most dorsal sector of the brain of fetus 27 (see Fig. 1). Fetal breathing is depicted by the negative deflections in tracheal pressure. Stimulation time is shown by the horizontal bar.

Fig. 4. Changes in mean respiratory period (cycle time) after the start of electrical stimulation relative to baseline (control). Pf in 6 fetuses; ZI in 3 fetuses; VTAm, ventral tegmental area, medial zone (in 3 fetuses). *P < 0.05, **P < 0.005, ***P < 0.0001 compared with control.
The electrocorticogram was recorded in one fetus (fetus 27), whereas the electrocologram was recorded in three fetuses (fetuses 27, 290, and 733). Electrical stimulation had no consistent effect on electrocortical activity or rapid eye movements (Fig. 3).

Zona incerta. In three fetuses (fetuses 22, 296, and 58), electrical stimulation within the external medullary lamina, most strikingly in the medial expamse constituting the zona incerta, also increased expiratory time and respiratory period compared with the respective controls of 0.39 ± 0.23 s and 0.73 ± 0.24 s (Figs. 2 and 4). Mean inspiratory time remained within 0.04 s of the control of 0.35 ± 0.04 s, a nonsignificant change.

The stimuli significantly increased ($P < 0.01$) mean amplitude by 2.4 ± 0.9, 4.6 ± 0.9, and 3.2 ± 0.9 mmHg for epochs 0–5, 5–10, and 10–15 s, respectively, after the start of stimulation, compared with the control of 10.8 ± 0.8 mmHg. In a fourth fetus (fetus 731), stimulation within an area medially adjacent to zona incerta increased both respiratory rate and breath amplitude.

Ventral tegmental area. Stimulation near the medial border of ventral tegmental area (VTA) decreased respiratory frequency in three fetuses (fetuses 24, 290, 296) without altering mean inspiratory time or breath amplitude (Figs. 1, 2, and 4). In these fetuses, baseline measurements revealed a mean inspiratory time of 0.18 ± 0.04 s, expiratory time of 0.37 ± 0.28 s, period of 0.56 ± 0.29 s, and amplitude of 5.4 ± 1.3 mmHg. In a fourth, fetus 372, electrical stimulation of the lateral margin of VTA did not alter respiratory period or amplitude.

Other structures. Respiratory slowing was revealed by stimulation involving the lateral margin of the central (periaque ductal) gray in the mesencephalon (fetus 372), the internal medullary lamina of the thalamus (fetus 731), and the substantia nigra (fetus 244). Electrical stimulation increased respiratory frequency in three fetuses (fetuses 170, 296, and 290), each involving a separate locus (Figs. 1 and 2). Stimulation of other nearby locations, including the subparafascicular nucleus, ventromedial and ventrobasal nuclei, lateral posterior nucleus, posterior group, or medial mammillary nucleus, did not produce consistent changes in breath period or amplitude.

DISCUSSION

Electrical stimulation of sectors within or near the Pf decreased respiratory frequency, indicating that Pf and perhaps adjacent elements modulate fetal breathing. This area has been more precisely delimited by observing the absence of respiratory responses with electrical stimulation of adjacent sites. The relatively long latency preceding respiratory slowing suggests that, during stimulation, respiratory drive may be reduced through polysynaptic or possibly reverberating connections. Thus these results with electrical stimuli, along with our previous work with neuronal lesions of the fetal thalamus, support a posteromedial thalamic origin of a pathway inhibitory to respiratory motoneurons.

Electrical stimulation of several zones connected to Pf (e.g., periaque ductal gray, zona incerta, ventral tegmental area, substantia nigra) also prolonged cycle time. These depressant effects might have been mediated through Pf, which would further bolster the notion that Pf is involved in respiratory inhibition.

The diencephalic regions in which alterations in respiratory rate and/or amplitude are distinctively restrictive in that the major sensory and association nuclei of the thalamus, constituting the vast majority of thalamic volume, are essentially unresponsive to electrical stimuli relative to breathing alterations. Minor exceptions for small changes were noted on stimulating the ventromedial nucleus, recipient of visceral and gustatory input, only in a limited sample. One needle track through the caudal mediodorsal nucleus (fetuses 731–55) revealed clear apneic responses, but this region is rostrally juxtaposed to the Pf. It should be emphasized, however, that the responsive region does not accurately approximate the spatial limits of Pf and that there are clearly unresponsive sectors within this structure.

Electrical stimulation of the mediodorsal nucleus of the thalamus as well as substantia nigra increases respiratory rate in conscious cats (3), and stimulation of central gray increases the amplitude and rate of respiration in anesthetized cats (20). Electrical stimulation of structures including the habenula or habenulo-interpeduncular tract (fasciculus retroflexus) decreases respiratory amplitude and frequency in anesthetized cats (20), which suggests that cells surrounding (para) the fasciculus triggered the response because the fiber tract itself is an unlikely source. In conscious rats, electrical stimulation of the lateral parafascicular nucleus, which corresponds to the nucleus centrum medianum in primates, inhibits spontaneous and stimulation-induced movements without noticeably affecting respiration (32). The present observations of electrical stimulation of the thalamus in unanesthetized fetal sheep are unique in that breath period increased without changing amplitude when the stimulations involved areas within or adjacent to the Pf, ventral tegmental area, rostral central gray, and substantia nigra.

Connections of the parafascicular nucleus. In adult mammals, afferents to Pf include fibers from nuclei regulating sleep (e.g., hypothalamus, parabrachial nucleus, locus ceruleus, penduncular n., lateral dorsal tegmental nucleus, brain stem reticular formation), motor function (i.e., frontal cortex, substantia nigra, zona incerta, superior colliculus, periaque ductal gray, ventral tegmental area), autonomic function (i.e., hypothalamus, rostral ventrolateral medulla, solitary tract nucleus), and somatic and gustatory sensation (i.e., parabrachial nucleus, trigeminal complex, solitary tract nucleus). In turn, Pf projects to sectors involved in motor activity (i.e., striatum, frontal cortex, zona incerta, fields of Forel, substantia nigra, central periaque ductal gray, red nucleus), sleep (i.e., cerebral cortex, hypothalamus, reticular formation), sensation, and respiration (4, 16, 27, 31, 39, 40).

Early mapping studies of the somatic afferent projections to the cat thalamus revealed a large, nontopographic input to the parafascicular complex (which includes the nucleus centrum medianum in most descriptions of the cat thalamus) when activated by strong nerve volleys (29) and shown in single-neuron recordings to be activated by noxious pinprick and C fiber activation (2), stimuli that elicit pain in awake animals and an invariant abrupt, concomitant interruption of respiratory rhythmicity. Input to the Pf from the pontine respiratory group, known as the “pneumotoxic center” [largely comprised of the parabrachial nuclear complex, particularly the Kölliker-Fuse nucleus, and “intertrigeminal” and “peri trigeminal” portions of the sensory trigeminal nuclei (14)], constitutes an entity of
heterogeneous and ambiguously defined structures, and is principally polysynaptic. The pontine respiratory group is recognized as projecting to the ventral respiratory medullary group (7, 15) and contains neurons whose stimulation by glutamate elicits a marked respiratory slowing (7, 9, 30), putatively N-methyl-D-aspartate receptor mediated (13). Interestingly, stimulation of the region of the Kölliker-Fuse nucleus in humans has been employed in the relief of chronic pain (43).

The topographically organized somatic, visceral, and gustatory projections to the thalamus terminate in the ventral tier of nuclei, principally the ventrobasal and ventromedial nuclei. The “peritrigeminal” neurons by contrast lack somatotopic activation patterns (28) and are activated only by high-threshold, putatively noxious stimuli, thus resembling the characteristics of parafascicular neurons (2). The relation between excitation of peritrigeminal and parafascicular neurons by an abrupt noxious stimulus or strong nerve volley and the invariant concomitant inspiratory respiratory arrest elicited by such stimuli remains obscure, but there is little doubt that nociceptive reflexes profoundly modulate respiratory and cardiac rhythmicity.

Relation to hypoxic inhibition. Hypoxia-induced expression of the protooncogene c-fos has been used to identify polysynaptically activated neurons in the brain of fetal sheep (6, 34, 35). For example, hypoxia increased expression of the nuclear protein Fos encoded by c-fos in neurons of the central periaqueductal gray (6), which has particular relevance to the present work because 1) electrical stimulation involving this sector strongly inhibited breathing and 2) the periaqueductal gray is connectioned to Pf. Fos was also expressed in the substantia nigra, a locus ventrolateral to red nucleus, and the habenula. No specific mention was made of whether Fos immunoreactivity was present in the parafascicular nucleus (34), although the few thalamic neurons labeled for control and hypoxia-exposed levels appear to include the region we would interpret as medial Pf in O and P (Fig. 1 of Ref. 34) for both 100- to 105- and 130- to 133-gestational day examples. These Fos results must be interpreted cautiously, however, because 1) not all activated neurons express Fos, 2) Fos expression can occur independently of neuronal activity, and 3) inhibited neurons do not express Fos (34).

Limitations. Interconnections and complex geometry of the brain limit the extent to which predictions can be made regarding the elements excited by electrical stimulation (36, 37). Neurons and unmyelinated and myelinated axons, as well as glia and blood vessels, all have different resistivities that affect the pattern and extent of current flow in neuropil (36). The size of cell bodies and fibers, nodal length, number and shape of dendrites, cell membrane capacitance, and orientation to current flow also determine the expression of electrical stimulation (36, 37). Electrical currents can block as well as elicit action potentials, thus accounting for the selectivity with which elements are excited. For example, only a “shell” of axons surrounding the bipolar electrode may be excited, whereas axons close to the electrode may not be stimulated. However, smaller diameter axons might be stimulated near the electrode under the same conditions. Cell bodies are generally less easily depolarized than myelinated axons because of their larger capacitance (36, 37). The heterogeneous composition and orientation of neuropil limit our ability to determine the type and distribution of excited elements that surround the bipolar electrode.

Current spread from monopolar electrodes has been estimated based on measurements in cats (36). From these data, current spread in our experiments might have extended up to 1–2 mm from electrode pairs at the maximum stimulation current. But information from cats cannot be directly applied to our work because the electrodes in those studies were monopolar rather than bipolar with different dimensions and composition, stimuli consisted of single pulses, and experiments were performed in brains of adult mammals, which probably would have had greater myelination than the brains of fetal sheep. Presumably, our estimate of current spread is conservatively large because monopolar stimulation generally results in significantly greater current spread than between closely spaced electrode pairs.

Information on the spatial limits of specific thalamic nuclei or the elements (fibers, neurons) involved in electrical stimu-
lution cannot be precisely derived from these studies (Fig. 5). Nevertheless, reasonable conclusions can be drawn about general sectors involved in respiratory depression based on inter-animal consistency of responses, likely anatomic connections, and the findings of prior experiments with selective destruction of thalamic neurons (22). Overall, the results are congruent with our previous observations with neuronal lesions implicating the Pf as a critical component of the neuronal circuitry involved in hypoxic inhibition of breathing.

Respiratory responses after stimulation of sectors involving Pf were unlikely to result from direct stimulation of the red nucleus (42), which lies >5 mm inferior and caudal to Pf at this gestational age. Furthermore, stimulations in closer proximity to the red nucleus did not slow respiration. Current spread from stimulation of Pf in our studies would also be unlikely to extend to the parabrachial nuclear complex of the pons, which lies ~10 mm caudal to Pf in the brain of fetal sheep (~0.8 term).

More accurate anatomic localization could be obtained along the axial plane of stimulation because the location of the electrode tips could be identified in tissue by visualizing the Prussian blue reaction. This marker enabled the analysis to factor in tissue shrinkage after fixation. The accuracy of anatomic analysis that could be achieved by this method is illustrated in fetus 244, in which stimulation of a small sector bounded virtually by the nuclear limits of substantia nigra elicited marked respiratory slowing (Fig. 2).

Stimulation of neurons rather than fibers of passage within the posteromedial thalamus likely elicited the fall in respiratory rate because selective neuronal lesions of this sector interrupt a crucial portion of the neurocircuitry mediating hypoxic depression of breathing (22). Further studies with chemical stimulation of this locus would help confirm that prolongation of expiratory time resulted from neuronal excitation. Although stimulation studies cannot exclude the possibility that fasciculus retroflexus or other fiber tracts contribute to an increased respiratory period and expiratory time, the absence of distinct respiratory responses when the target habenular and interpeduncular nuclei are stimulated suggests that Pf neuron excitation is crucial.

Targeting increased expiratory duration rather than prolonged apnea, as occurs with fetal hypoxemia (change in arterial PO₂ ≥ −8 Torr), is an unavoidable limitation of this study. Although electrical activation of neuronal elements within the posteromedial thalamus increased respiratory period and expiratory time, the brief periods of electrical stimulation did not elicit prolonged apnea. Thus uncertainty exists whether the reduction in breathing rate is due to activation of the neurocircuitry involved in hypoxic inhibition of breathing.

A distinct advantage of electrical stimulation is the ability to accurately measure the response latency, although latency is more difficult to determine when the breathing rate is irregular, as in the fetus. The response latency for stimulations involving Pf was ~5.5 s relative to the start of the stimulus and ~2.5 s relative to the cessation of current. These results suggest that respiratory modulation was mediated by a polysynaptic pathway, whether the increased respiratory period was due to the onset or termination of the current. Besides activation of neurons in the posteromedial thalamus, respiratory slowing might have been elicited through a stimulus-induced rise in local blood flow and/or metabolism locally or in connected brain sectors (33). Adenosine, generated through degradation of ATP released by electrical stimulation, might also have modulated respiration through activation of adenosine A₂ receptors with a time lag of 2.5–5.5 s (24).

Although electrical stimulation of the thalamus did not produce detectable changes in electrocortical activity or rapid eye movements, prolongation of expiratory time might have resulted from an interruption of the phasic respiratory drive of rapid eye movement sleep (19), which has been implicated in hypoxia-induced apnea (22). Besides disfacilitation, reduced respiratory rate might have occurred via direct suppression of respiratory motoneurons.

Repeated-measures ANOVA would ideally take into account current as well as time and brain sector if it were practical to extensively analyze multiple electrode tracks in a large number of fetuses. Because the stimulus sites in each fetus were never in exactly the same locus in each sector, current could not be distinguished as a separate variable from the position of the electrode pairs within a brain sector. However, because a maximum current was used to evoke a response in all cases, a constant current criterion was used.

In summary, electrical stimulation of sectors within, proximate, or connected to the Pf increases respiratory period in fetal sheep. These findings, along with previous studies revealing abolition of hypoxic inhibition by neuronal lesions of this thalamic locus (22), support the hypothesis that neurons within Pf and/or adjacent structures contribute a crucial part of the neuronal circuitry involved in the hypoxic arrest of fetal breathing. Representing the rostral convergence of fibers involved in somatic sensation, sleep, and motor function, the Pf appears to be a critical structure linking O₂-sensitive neurons to disfacilitation/inhibition of respiratory motoneurons. Whether this previously unrecognized locus involved in fetal breathing also modulates respiration postnatally remains to be determined.

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