Raphe Inhibition of Sympathetic Preganglionic Neurons

Abstract. *Anatomical and electrophysiological studies suggest that the medullary raphe gives rise to a monosynaptic, inhibitory projection on sympathetic preganglionic neurons. Physiological and behavioral data indicate that this sympathoinhibitory pathway participates in the central control of cardiovascular function.*

The projections of the raphe complex to the dorsal horn have received considerable attention as a descending system modulating nociceptive input (*1*). This is not the only significant spinal projection arising from the raphe, however. Evidence is accumulating for a major projection from the medullary raphe to the sympathetic preganglionic neurons (SPN's), thus implicating the raphe complex in the descending regulation of the sympathetic outflow, including that to the cardiovascular system.

The first anatomical evidence that the raphe might project on the SPN's was obtained by Dahlström and Fuxe (*2*). Using histofluorescence techniques these workers concluded that serotonergic terminals located in the sympathetic preganglionic cell column have their cells of origin in the medullary raphe. The presence of serotonin within the column has been confirmed biochemically (*3*), and electron microscopic evidence from the avian spinal cord suggests that presumed serotonergic terminals on SPN's degenerate after either systemic injection of 5,7-dihydroxytryptamine or spinal transection (*4*). There are many data implicating the raphe in the neural control of the cardiovascular system by virtue of its location within the medial medullary "depressor area" (*5, 6*). In this report we provide anatomical, physiological, and behavioral data from the pigeon *(Columba livia)* that show that the medullary raphe has a direct influence on the SPN's and demonstrate the importance of this pathway in the regulation of cardiovascular function.

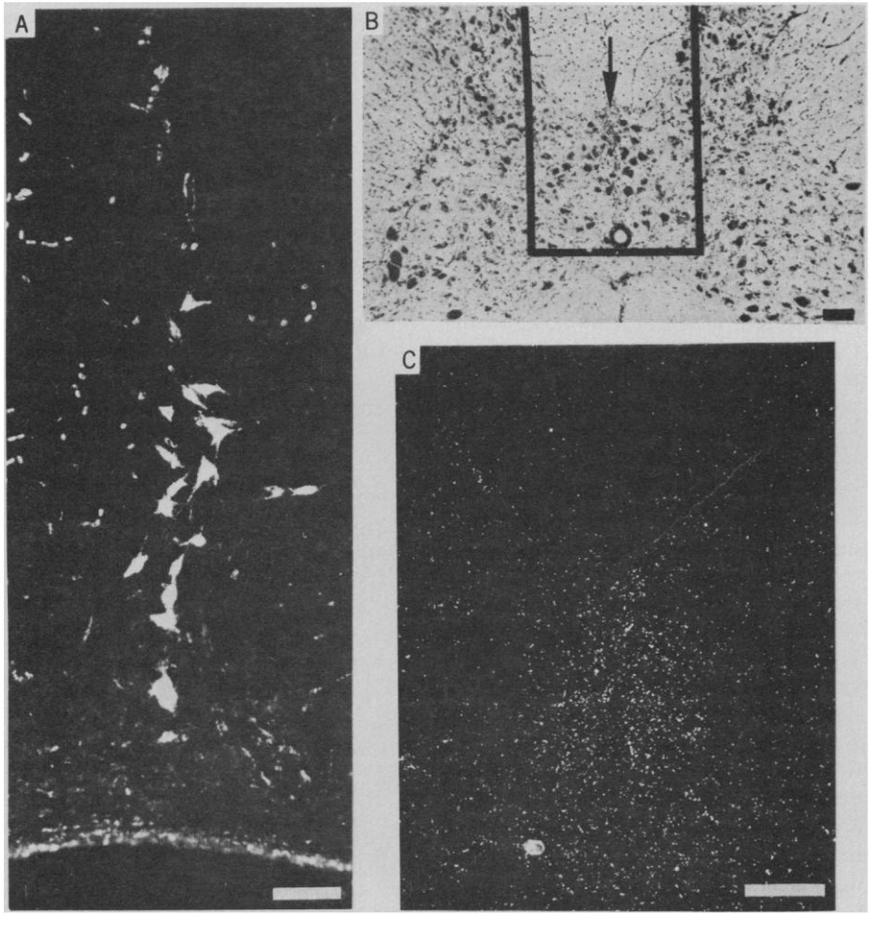

Fig. 1. Spinal cord projections of the medullary raphe nuclei. (A) Darkfield photomicrograph showing dense labeling of raphe neurons after an injection of HRP in the right lateral funiculus of the brachial cord. (B) Transverse, Nissl-stained section of high thoracic cord in the pigeon illustrating the location of SPN's (arrow). The outline on the photomicrograph indicates the field of view for (C). (C) Darkfield autoradiograph showing presence of silver grains over SPN's after an injection of [^{3}H]proline in the raphe. Background count was 1.6 grains per 1600 μm^2. Scale bars are 100 μm.

We first conducted anatomical experiments (i) to establish the presence of an avian raphe-spinal projection by means of retrograde transport of horseradish peroxidase (HRP) and (ii) to determine, by means of anterograde transport and degeneration studies, if such a projection includes terminations on the SPN's. For the retrograde transport studies, single injections of 1 to 2 μl of HRP (33 to 50 percent solution) were made in the right lateral funiculus of the brachial ($N = 5$), thoracic ($N = 2$), and lumbar ($N = 1$) spinal cord. This injection site was chosen because descending serotonergic fibers have been located in this area (*2, 6, 7*). After 48 hours, brains and spinal cords were removed and processed according to a conventional technique (*8*). For the anterograde transport studies two pigeons were injected with [^{3}H]proline (30 μCi/μl, 0.1 to 0.25 μl) in the medullary raphe, and after 3 or 5 days they were killed and the tissue processed for autoradiography (*9*). For anterograde degeneration experiments electrolytic lesions were made in the medullary raphe of three pigeons; after 5 to 10 days the pigeons were killed and the brains and spinal cords processed by the Fink-Heimer methods (*10*). Both the lesions and injections involved more than the raphe, encroaching on the adjacent nucleus paramedianus and the ventromedial aspects of the nuclei reticularis gigantocellularis and pontis caudalis (*11*).

Figure 1A shows the extensive retrograde labeling of neurons in both the dorsal and ventral divisions of the raphe (*12*) after injection of the lateral funiculus. Such labeling was evident throughout the medullary extent of the raphe, with many fewer labeled cells rostral to the pontomedullary junction. Although this established a spinal projection of the avian raphe similar to that observed in mammals (*13*), anterograde experiments were necessary to determine whether this projection includes terminations on the SPN's. Figure 1B shows a transverse section of high thoracic spinal cord in the pigeon, with the SPN's located in a well-defined cell column just dorsal to the central canal (*14, 15*). The autoradiograph in Fig. 1C shows a concentration of silver grains over the sympathetic preganglionic column in a pigeon that received an injection of [^{3}H]proline in the medullary raphe. The average grain density within the column is approximately eight times background. Supporting observations were made in pigeons that re-

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ceived raphe lesions, but the silver degeneration results were not as striking as the autoradiographic findings. Thus, the combined retrograde and anterograde data provide evidence for a direct projection from the medial medulla to the SPN's, and we conclude that this projection probably arises in large part from the medullary raphe. A similar projection has been described in the cat (*16*).

We then conducted electrophysiological experiments. Eight pigeons were stimulated in the ventral division of the rostral medullary raphe, recordings being made from antidromically identified SPN's with maintained activity (*17*). The left panel of Fig. 2A shows control histograms where the maintained activity of several SPN's has been pooled. The right panel shows poststimulus time histograms illustrating the effects on the SPN's of raphe stimulation. Two major effects are evident. Raphe stimulation strongly inhibited SPN discharge; and increasing the stimulus duration lengthened the period of decreased SPN activity, indicating temporal summation. Closer examination of the initial 50 msec (Fig. 2B) after stimulus onset reveals that the inhibitory effect began after 10 msec and was at a maximum by 30 msec. On the basis of this observation and an approximated conduction distance of 12 cm, the conduction velocity of the raphe-spinal inhibitory pathway is estimated at 4.0 to 12.0 m/sec.

More direct evidence that raphe-spinal axons conduct in this range was obtained in two pigeons with a stimulating electrode in the sympathetic preganglionic column and a recording electrode in the rostral medullary raphe (*18*). Figure 2C shows the conduction velocities of eight antidromically activated raphe neurons which are plotted as a function of antidromic activation latency. This largely substantiates the conduction velocity range estimated from the raphe stimulation experiments (see Fig. 2, B and C); all eight of these neurons were located in the ventral division of the complex.

Although these studies indicate a direct inhibitory raphe projection on the SPN's, they provide no information about the cardiovascular effects of activating this system. To investigate this we stimulated the ventral division of the rostral medullary raphe in unanesthetized pigeons ($N = 3$) and monitored blood pressure and heart rate (*19*). In all these pigeons raphe stimulation resulted in significant depressor responses, with systolic and diastolic pressures decreasing 20.5 ± 0.85 mm-Hg (mean ± standard error) and 28.1 ± 1.19 mm-Hg, respectively; response latency was 0.86 ± 0.07 second. The decreases in systolic and diastolic pressures differed significantly ($P < .001$), but there were no significant differences caused by stimulus frequency or duration. Bradycardia was noted in two of the experiments (*20*).

We next assessed the behavioral effects of raphe lesions in a classical conditioning paradigm that reliably produces conditioned cardioacceleration: we paired whole-field retinal illumination as a conditioned stimulus with foot-shock as an unconditioned stimulus (*21*). Compared with controls, pigeons with such lesions showed a marked enhancement of the conditioned cardioacceleratory response ($P < .005$) (Fig. 3). We interpret this as a change in performance rather than learning, since it also occurs in a sensitization paradigm where lights and shocks are unpaired. The results are thus consistent with the medullary raphe's providing an inhibitory input to the SPN's, and interruption of this input apparently compromises an important system for limiting the magnitude of cardioacceleration to exteroceptive stimulation.

Our physiological and behavioral data implicate the medullary raphe as having a major sympathoinhibitory role in the pigeon, and thus extend the inhibitory action of the descending raphe projections to yet another spinal system and another species (*1*). Whether these different spinal effects are exerted by different raphe cell groups remains to be determined. The anatomical and elec-

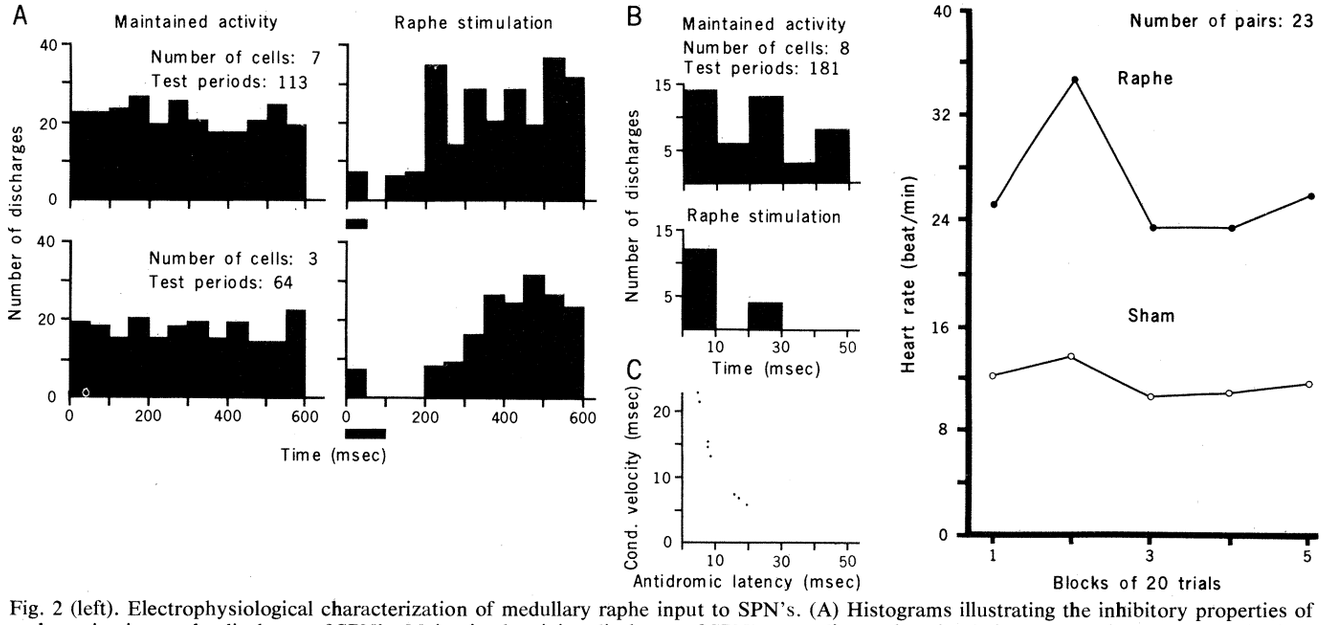

Fig. 2 (left). Electrophysiological characterization of medullary raphe input to SPN's. (A) Histograms illustrating the inhibitory properties of raphe activation on the discharge of SPN's. Maintained activity: discharge of SPN's preceding raphe stimulation. Test periods: number of paired observations of the discharge of SPN's in the maintained activity and raphe stimulation periods. Raphe stimulation: discharge of SPN's subsequent to electrical stimulation of the raphe. Dark bars beneath raphe stimulation histograms indicate stimulus duration. Upper right panel: 50-msec stimulus period. Lower right panel: 100-msec stimulus period. Stimulus frequency: 100 Hz. Bin width: 50 msec. (B) Histograms showing onset of the inhibitory effects of raphe activation. Bin width: 10 msec. (C) Conduction velocity of raphe-spinal axons plotted as a function of the latency of antidromic activation of their cells of origin within the medullary raphe. Fig. 3 (right). Conditioning performances of pigeons with medullary raphe lesions relative to the performances of their controls (*Sham*). All curves represent mean heart rate changes between the 6-second conditioned stimulus and preceding control periods. Each point is a group mean for a block of 20 training trials.

trophysiological results suggest that this effect may be mediated by way of a direct pathway from the raphe to the SPN's. Although other mechanisms such as disfacilitation or disynaptic inhibition involving a spinal interneuron cannot be definitively excluded, the weights of the combined evidence suggest that the medullary raphe inhibits the SPN's by a direct pathway.

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17. Stimuli were 0.5-msec, cathodal pulses delivered by a constant-current stimulator through No. 00 stainless steel insect pins insulated to give tip exposures of ≃ 100 μm. Electrode localization was verified histologically from 50-μm celloidin sections stained with cresyl echt violet. Threshold was at stimulus intensities of 30 to 60 μA with supramaximal responses at 50 to 100 μA. The SPN's were identified on the basis of collision [I. Darian-Smith, G. Phillips, R. D. Ryan, *J. Physiol. (London)* **168,** 129 (1963)] of a spontaneous discharge with an antidromic discharge elicited by bipolar stimulation of the preganglionic axons in sympathetic ganglion 14 [J. B. Cabot and D. H. Cohen, *Brain Res.* **131,** 73 (1977)]. Recording electrodes were 4*M* NaCl micropipettes with tip resistances of 6 to 10 megohms.
18. The collision test was applied in all cases, and raphe recording sites were verified histologically.
19. Blood pressure and heart rate were recorded by conventional methods (*14*). Stimulating electrodes were located in the raphe while the animals were anesthetized with ether; the animals were allowed to recover for 2 to 2.5 hours before stimulation. Stimulus trains were delivered for 3 or 5 seconds at frequencies of 25, 50, or 100 Hz. Threshold was at intensities of 25 to 50 μA with supramaximal responses at 30 to 100 μA.
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22. We thank D. Hannum, D. Goff, and N. Richardson for technical assistance. This research was supported by NSF grant BNS-75-20537 to D.H.C. J.B.C. and J.M.W. were supported by fellowships from the Alfred P. Sloan Foundation to the Neuroscience Program at the University of Virginia.

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Zea diploperennis (Gramineae): A New Teosinte from Mexico

Abstract. *A perennial teosinte or "wild maize" endemic to the Cerro de San Miguel, Sierra de Manantlan, Jalisco, Mexico differs from* Zea perennis *by dimorphic rhizomes, robust habit, and a larger number of longer, laxer tassel branches. The fact that it is a diploid (2*n *= 20) has taxonomic and agronomic significance. The seeds are used locally for food.*

Earlier this year, Guzmán (*1, 2*) reported his remarkable rediscovery of perennial teosinte, thought extinct in the wild since 1921 (*3*), at two sites in southern Jalisco, Mexico. Subsequently, both sites were visited by three of us (H.H.I., J.F.D., and R.G.M.), and specimens, seeds, and rhizomes were collected and initial analyses were made. This report confirms Guzmán's conclusion regarding the Ciudad Guzmán population—that it is, indeed, conspecific with the tetraploid (2*n* = 40) *Zea perennis* (Hitchcock) Reeves and Mangelsdorf, originally discovered in this area by Hitchcock in 1910. However, the plants from the second location, Cerro de San Miguel, though similar in many ways, are a clearly distinct diploid taxon, here described for the first time:

Zea **diploperennis** Iltis, Doebley & Guzmán. **sp. nov.**

Similis a *Zea perennis* sed robustior, culmis 1-2 cm diam., rhizomatibus perennibus dimorphis (gracilioris non nisi 5-15 cm × 5-10 mm, brevioris crassis, tuberosis 1-4 cm × 9-15 mm), uterque cum internodiis brevibus 2-6 mm longis, foliis multo majoribus (40-80 × 4-5 cm), inflorescentiis ♂ cum 3-13 ramis, robustioribus et 6-15 cm longis. Typus: Iltis, Doebley & Guzmán 450.

Robust, erect, maizelike, loosely clump-forming perennial, with five to ten, or more, primary culms from one rhizome system; rhizomes of two intergrading sorts, (i) *cordlike long shoots*, 5 to 15 cm long, 5 to 10 mm in diameter, these with many dense short (2 to 6 mm) internodes, scaleless when mature, usually vertical or strongly ascending and changing abruptly into the much thicker culms, or less often horizontal and producing one to several culms from short lateral shoots, or (ii) *thick and tuberous, ovoid to obovoid short shoots* 1 to 4 cm long, 9 to 15 mm in diameter, each of these produced horizontally from the lowest two or three nodes of the primary culms, clothed when young with triangular, strongly convergent-veined, overlapping, connivent scales, at times growing upward (into a long shoot?) and producing a culm, or sometimes remaining dormant to eventually produce one to four lateral short or long shoots (or both).

Primary culms 10 to 25 dm tall, 1 to 2 cm in diameter, unbranched (or with one to three inconspicuous lateral branches), the nodes, internodes, and leaf sheaths glabrous throughout except for a more or less dense fringe of long hairs on upper sheath margin and auricles of the upper leaves; ligule a thin membrane 1 to 2 mm long, the collar prominent; leaf blades linear-lanceolate, *the major central or lower ones 40 to 80 cm long, 4 to 5 cm wide*, subcordate, glabrous, or subglabrous, except for a few marginal long hairs near base.

Male inflorescences with (2 to) 3 to 13 ± divergent to nodding branches; these 6 to 15 cm long, 12 to 20 mm wide, the central one barely exceeding the others; branching axis 1 to 4 cm long; spikelets in sessile or pedicellate pairs (pedicels 1.5 to 3 mm long), crowded and overlapping (for example, 14 spikelet pairs in 4 cm); the branch internodes short (2 to 6 mm); the branch rachis about 1 mm wide, in cross-section triangular with ciliate edges; spikelets 8.5 to 11.5 mm long, about 3 mm wide; outer glumes very thin and translucent, often purple-

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