In vivo electrophysiological responses of pedunculopontine neurons to static muscle contraction

Edward D. Plowey, Jeffery M. Kramer, Joseph A. Beatty, and Tony G. Waldrop. In vivo electrophysiological responses of pedunculopontine neurons to static muscle contraction. Am J Physiol Regul Integr Comp Physiol 283: R1008–R1019, 2002. First published July 11, 2002; 10.1152/ajpregu.00075.2002.—The pedunculopontine nucleus (PPN) has garnered attention as a potential regulator of cardiorespiratory drive during exercise as a component of the mesencephalic locomotor region (MLR) (11, 43). The MLR of the rat, located in the mesencephalic tegmentum at the lateral extent of the brachium conjunctivum, is an area from which coordinated locomotion can be evoked via electrical stimulation or chemical disinhibition in a nonanesthetized, decerebrate preparation (16). Garcia-Rill and colleagues (17, 18) demonstrated that the MLR of the rat is highly coexistent with the cholinergic, NADPH-diaphorase positive neurons of the PPN. The PPN is known to be active during locomotion, as has been shown by extracellular neuronal recordings in cats (19) and via examination of c-fos expression in the PPN after treadmill exercise in rats (25).

Activation of the MLR in cats produces feed-forward increases in efferent cardiorespiratory drive that parallel concurrent increases in locomotor drive, yet persist in the absence of elevated muscle feedback during fictive locomotion (11). The capacity of the MLR to produce feed-forward increases in cardiovascular drive has been documented in rats as well (4, 8). Given the apparent importance of the PPN as an anatomic component of the MLR (17, 18), it is reasonable to hypothesize that the PPN may play a role in the regulation of the cardiorespiratory adjustments that accompany exercise through a central command mechanism.

To begin to evaluate the possibility that the PPN contributes a potential modulatory influence to the cardiorespiratory responses evoked by muscle contraction, we determined if neurons of the PPN and the surrounding mesencephalic tegmentum respond to ventrolateral medulla (33), and the dorsal horn of the spinal cord (9). Several authors have hypothesized that orchestration of influences from central command and muscle reflex pathways partly underlies the ability of the central nervous system to evoke alterations in cardiorespiratory drive that are appropriately matched to the metabolic demand of physical activity (32, 33, 35, 43, 44).

The pedunculopontine nucleus (PPN) has previously been implicated in central command regulation of the cardiorespiratory adjustments that accompany exercise. The current study was executed to begin to address the potential role of the PPN in the regulation of cardiorespiratory adjustments evoked by muscle contraction. Extracellular single-unit recording was employed to document the responses of PPN neurons during static muscle contraction. Sixty-four percent (20/31) of neurons sampled from the PPN responded to static muscle contraction with increases in firing rate. Furthermore, muscle contraction-responsive neurons in the PPN were unresponsive to brief periods of hypotension but were markedly activated during chemical disinhibition of the caudal hypothalamus. A separate sample of PPN neurons was found to be moderately activated during systemic hypoxia. Chemical disinhibition of the PPN was found to markedly increase respiratory drive. These findings suggest that the PPN may be involved in modulating respiratory adjustments that accompany muscle contraction and that PPN neurons may have the capacity to synthesize muscle reflex and central command influences.

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evoked static contraction of the hindlimb muscles in anesthetized rats using single-unit extracellular recording. We hypothesized that if the PPN modulates the cardiorespiratory adjustments evoked by muscle contraction, then neurons sampled from the PPN will exhibit alterations in firing rate during static muscle contraction. The data presented suggest that the firing rates of PPN neurons are enhanced during evoked muscle contraction in anesthetized rats and that activation of the PPN may, as observed during muscle contraction, have an impact on respiratory drive.

METHODS

All of the procedures described in this study were executed under animal experimentation protocols that were approved by the Laboratory Animal Care Advisory Committee of the University of Illinois at Urbana-Champaign. These procedures are in compliance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Animal preparation. Male Sprague-Dawley rats (220–350 g, 63 total animals) were anesthetized with intraperitoneal injections of a mixture of α-chloralose (65 mg/kg) and urethane (800 mg/kg) dissolved in Ringer. Adequate depth of anesthesia was maintained via anesthetic supplements that were administered intravenously upon evidence of a positive foot withdrawal response to noxious pinch or of a positive eyelid response to tactile stimulation of the cornea. The trachea was cannulated with PE-205 tubing (Clay Adams, Parsippany, NJ) to maintain a patent upper respiratory tract. A rectal temperature probe, a radiant heat lamp, and a water-perfused heating pad were employed to maintain the animal’s body temperature at 37 °C. An arterial catheter was inserted via the left femoral artery into the abdominal aorta. The right internal carotid artery was dissected from the common carotid and external carotid arteries. A catheter was inserted into the right external jugular vein and left common carotid artery to allow drug administration and measurement of cardiovascular variables, respectively. Pulsatile arterial blood pressure was monitored via a model P23 pressure transducer (Gould, Oxnard, CA) connected to the arterial catheter. Heart rate (HR) was derived from the voltage output of the pressure transducer using a biotachometer (Gould). A rectal temperature probe, a radiant heat lamp, and a water-perfused heating pad were employed to maintain the animal’s body temperature at 37 ± 1°C. The bite bar of the stereotax was then adjusted to the vertical level at the midline. A parietal craniotomy was performed over the mesencephalon in all animals and a water-perfused heating pad was employed to maintain the animal’s body temperature at 37 °C. A craniotomy was then made with high-impedance electrodes (3 MΩ; FHC, Bowdoinham, ME) stereotaxically placed into the PPN. Recording tracts were performed within the following coordinates according to Paxinos and Watson (34): 0.5–1.7 mm rostral, 1.4–2.2 mm lateral, and 5.5–5.9 mm dorsal to interaural zero. Extracellular activity was amplified (100 K; P5 Series AC Preamplifier, Grass Instruments, Quincy, MA) and filtered (300- to 1,000-Hz bandwidth). Single units were isolated with a window discriminator (FHC). Action potentials that fell within the recording window triggered transistor-transistor logic pulses that were sent to a ratemeter (FHC) and to a digital chart recorder (Windows-based PC running PowerLab v.3.4.4, AD Instruments, Grand Junction, CO). Discriminated action potentials were also sent to a storage oscilloscope to check for consistency of the action potential signature to thus ensure a stable recording of a single unit. Extracellular activity was also sent to a speaker to monitor unit activity audibly.

Recording tracts were performed in both the right (ipsilateral) and left (contralateral) sides of the mesencephalon. The responses of isolated mesencephalic units were recorded during a 30-s period of static contraction of the hindlimb muscles. After a 15-min recovery period, response reliability was tested during a subsequent period of muscle contraction. To evaluate the possibility that neuronal responses observed during muscle contraction were evoked by alterations in arterial pressure, the responses of the neurons to brief periods of hypotension or hypertension induced by intravenous injections of sodium nitroprusside (SNP; 5–10 μg; Sigma) or phenylephrine (Phe; 3–5 μg; Sigma), respectively, were documented. To test the possibility that we were directly activating afferents via electrical stimulation to evoke responses in PPN neurons, five muscle contraction-responsive neurons were recorded during electrical stimulation of the tibial nerve at 2 × MT after the nerve was crushed just distal to the stimulation electrode. In all experiments, we made sure that stimulation of the crushed tibial nerve at 2 × MT failed to evoke cardiorespiratory adjustments through direct activation of tibial nerve afferents.

For a subset of six PPN neurons that responded to muscle contraction, the responses to supramesencephalic activation of central command were recorded. Feed-forward increases in cardiorespiratory drive, in the absence of locomotion, were evoked via microinjections of the GABAR receptor antagonist bicuculline (5 mM in Ringer, 60 nl; Sigma) into the CH (11, 42). A microinjection pipette (20- to 30-μm tip aperture) was pulled from a glass capillary tube (1-mm diameter; World Precision Instruments, Sarasota, FL) with a one-stage, up-right pipette puller (Narishige, Tokyo). The pipette was stereotaxically placed in the CH using the following coordinates: rostral +4.8 mm, lateral +0.5 mm, dorsal +1.7 mm relative to interaural zero (34). Microinjections were made with a
PV800 Pneumatic PicoPump (World Precision Instruments) and were measured by monitoring the movement of the mecanus of the injectate through a calibrated microscope reticule (Reichert Scientific Instruments, Buffalo, NY). The firing behavior of PPN neurons was recorded for at least 2 min before the microinjection of bicuculline, throughout the period of CH activation, and for at least 2 min after the return of the cardiorespiratory responses to baseline.

In separate experiments (9 rats), similar animal preparations, except for preparation of the hindlimb for muscle contraction, were used to document the responses of PPN neurons to systemic hypoxia. Hypoxia was induced for 1-min periods by switching the inspired gas from 100% O2 to a gas mixture composed of 10% O2–90% N2. The effects of the hypoxia were either mounted on gelatin-coated slides and stained with neutral red (Sigma) or left unstained to allow determination of the position of the microinjection site. Alternate sections were either stained with neutral red (Sigma) or left unstained to allow determination of the position of the microinjection site.

### Results

**Cardiorespiratory responses to muscle contraction, SNP, and systemic hypoxia.** Cardiorespiratory responses to muscle contraction, intravenous SNP, and systemic hypoxia are summarized in Table 1. Electrical stimulation of the tibial nerve at 2 × MT evoked static contraction of the hindlimb muscles and an increase in tension in the triceps surae muscles and Achilles tendon of 740 ± 50 g. Periods of muscle contraction were associated with decreases in mean arterial pressure (MAP), modest increases in HR, and rapid increases in f (Table 1). In contrast to muscle contraction, intravenous SNP evoked a larger decrease in MAP but no change in f relative to baseline. Hypoxia evoked a similar decrease in MAP compared with muscle contraction, but, in contrast to muscle contraction, mark-

<table>
<thead>
<tr>
<th>Cardiorespiratory Variable</th>
<th>Baseline</th>
<th>Cardiorespiratory Adjustments</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Muscle contraction</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>106 ± 2</td>
<td>20 ± 2*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>380 ± 5</td>
<td>8 ± 1*</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>73.2 ± 1.1</td>
<td>3.2 ± 0.5*</td>
</tr>
<tr>
<td>Minute /DEMG amplitude,</td>
<td>73.2 ± 1.1</td>
<td>2.0 ± 1.2</td>
</tr>
<tr>
<td>breaths-units·min⁻¹</td>
<td></td>
<td>740 ± 50</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 37 (muscle contraction), 19 (sodium nitroprusside [SNP]), and 9 rats (hypoxia). MAP, mean arterial pressure; HR, heart rate; DEMG, integrated diaphragmatic electromyogram activity; f, respiratory rate. *P < 0.05, significant change compared with baseline; †P < 0.05, significantly different compared with response observed during muscle contraction.

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**Table 1. Cardiorespiratory responses to unilateral, static hindlimb muscle contraction, intravenous injection of SNP, and systemic hypoxia in anesthetized rats**

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**Histology.** After the termination of successful recording tracts or injection experiments in the PPN, animals were prepared for histological analyses of recording and injection sites. The positions of recorded neurons were demarcated with direct current (DC) electrolytic lesions (300 μA, 8–10 s). Injection sites in the PPN and CH were marked with 60-μl microinjections of Chicago sky blue dye (Sigma) in the opposite order of injection. Solutions were separated via thin layers of mineral oil. The microinjection pipettes were stereotaxically placed in the brain using the coordinates employed for extracellular recording. Stable baseline cardiorespiratory variables were recorded for 5 min before injection of bicuculline into the PPN or control sites and then for the duration of observed cardiorespiratory responses. Cardiorespiratory variables were also observed after injection of vehicle and/or Chicago sky blue to ensure that responses to bicuculline were specific.

Cardiorespiratory responses to muscle contraction, intravenous SNP, and systemic hypoxia are summarized in Table 1. Electrical stimulation of the tibial nerve at 2 × MT evoked static contraction of the hindlimb muscles and an increase in tension in the triceps surae muscles and Achilles tendon of 740 ± 50 g. Periods of muscle contraction were associated with decreases in mean arterial pressure (MAP), modest increases in HR, and rapid increases in f (Table 1). In contrast to muscle contraction, intravenous SNP evoked a larger decrease in MAP but no change in f relative to baseline. Hypoxia evoked a similar decrease in MAP compared with muscle contraction, but, in contrast to muscle contraction, mark-

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**Data analyses.** Cardiorespiratory and electrophysiological variables were recorded to the Powerlab digital chart recorder and subsequently analyzed. Cardiorespiratory and electrophysiological responses to muscle contraction were determined by contrasting the means of the variables during the 1-min period immediately before muscle contraction (baseline) with the means of the variables during the entire 30-s period of muscle contraction. Baseline variables were also contrasted to the peak responses observed during muscle contraction. In addition, the SDs of the firing rates from the mean over the entire baseline period were determined. Individual neurons were labeled as responsive if the difference between the mean firing rate before muscle contraction and the mean firing rate during muscle contraction exceeded 1 SD. The means of the cardiorespiratory and electrophysiological variables before and during muscle contraction were contrasted using two-tailed, paired Student’s t-tests, with P < 0.05 deemed significantly different. Similar analyses were employed to independently determine the responses of PPN neurons to SNP-induced hypotension and hypoxia and during the cardiorespiratory responses to inhibition of the CH. Data presented in text and figures are means ± SE.
edly larger increases in respiration and HR were observed during systemic hypoxia.

**PPN responses to static muscle contraction, SNP, and systemic hypoxia.** Figure 1A depicts the response of one neuron sampled from the PPN during static muscle contraction. This PPN neuron exhibited an immediate, dramatic increase in firing rate that concurred with the changes in arterial pressure and respiration during muscle contraction. The same unit failed to respond to a decrease in arterial pressure evoked by intravenous injection of SNP (Fig. 1B). Figure 2A depicts the responses and locations of all mesencephalic recordings in this study. Figure 2B demonstrates that the histological location of the neuron depicted in Fig. 1, inferred from the lesion made by passing DC current through the tip of the recording electrode, was among the NADPH-diaphorase-positive neurons of the PPN. Some PPN units responded to muscle contraction with more gradual increases in firing rate. One such neuron is depicted in Fig. 3, along with a sample of the raw neurogram of the single-unit recording during muscle contraction.

Table 2 summarizes the responses of PPN neurons to static muscle contraction. Of the entire sample of PPN neurons recorded \((n = 31)\), 20 (64%) responded to muscle contraction with increases in firing rate, 3 (10%) responded with decreases in firing rate, and 8 (26%) PPN neurons were unresponsive to muscle contraction. Of the 20 PPN neurons that responded to muscle contraction with increases in firing rate, 11 of these neurons exhibited immediate increases in firing rate (peak response within 5 s of muscle contraction); the remainder (9 PPN neurons) responded with gradual increases in firing rate (peak response after 5 s of muscle contraction). The basal firing rates of all PPN neurons sampled were contrasted with their firing rates observed during muscle contraction (Fig. 4). The entire sample of PPN neurons \((n = 31)\) exhibited significant increases in mean firing rate and peak firing rate during muscle contraction. In contrast, the preponderance of neurons sampled from the cuneiform nucleus (CnF) and the inferior colliculus (IC) exhibited no changes in firing rate or decreases in firing rate during muscle contraction (Table 2). Neuron samples from the CnF and IC failed to exhibit significant alterations in sample firing rate (peak response and average response) during muscle contraction (Fig. 4).

To test the possibility that the PPN responses observed during muscle contraction were due to deactivation of baroreceptor afferents, 14 of the 20 muscle contraction-responsive PPN neurons were observed during brief bouts of hypotension evoked by intravenous injections of SNP (as demonstrated in Fig. 1B). Only 1 of the 14 PPN neurons tested responded with an increase in firing rate during SNP-evoked hypotension. The sample firing rate of muscle contraction-responsive PPN neurons was unaltered during SNP-induced hypotension \((7.4 \pm 0.9 \text{ Hz at baseline vs. } 7.5 \pm 0.8 \text{ Hz during transient hypotension}; P > 0.05)\). In addition, consistent with the lack of responses to SNP-evoked changes in blood pressure, three PPN neurons in this sample did not exhibit alterations in firing rate when blood pressure was transiently elevated via intravenous injection of Phe.

We were also interested in testing whether the cardiorespiratory and neuronal responses we observed were due to activation of muscle afferents by muscle contraction or due to direct activation of sensory afferents in the tibial nerve by our stimulation electrode. In all experiments, stimulation of the tibial nerve at 2× MT after the nerve was crushed distal to the stimulation electrode failed to evoke changes in arterial pressure, HR, respiration, and muscle tension. In addition, five muscle reflex-responsive PPN neurons were recorded during electrical stimulation of the tibial nerve after the nerve was crushed distal to the stimulation site (Fig. 5). In all five cases, stimulation of the crushed tibial nerve at 2× MT failed to reproduce the neuronal

![Figure 1](http://ajpregu.physiology.org/)

**Figure 1.** Response of one pedunculopontine nucleus (PPN) neuron to static muscle contraction. A: electrical stimulation of the tibial nerve at 2× motor threshold (MT) evoked static contraction of the hindlimb muscles. The PPN unit depicted in this trace exhibited an immediate, robust increase in firing rate that concurred with muscle contraction and the associated cardiorespiratory adjustments. B: same PPN neuron failed to respond to a decrease in arterial pressure evoked by intravenous injection of 5 μg sodium nitroprusside (SNP). DEMG, diaphragmatic electromyogram activity; \(f\), respiratory rate; TTL, transistor-transistor logic.
responses observed during muscle contraction, while stimulation at higher voltages did result in activation of PPN neurons.

The responses of 13 PPN neurons during systemic hypoxia, induced by spontaneous ventilation of 10% O₂, were documented in separate animals. Figure 6 depicts the response of one PPN unit during 1 min of exposure to the hypoxic gas mixture. Note that the neuron exhibited an increase in firing rate that coincided with the decrease in arterial pressure and increases in HR and respiration. Eight of the 13 PPN units tested responded with increases in firing rate, two responded with decreases in firing rate, and three failed to respond during 1 min of systemic hypoxia. The basal firing rate of this sample of PPN units was 7.9 ± 1.2 Hz. The firing rate of the entire sample increased to 12.4 ± 1.9 Hz (P < 0.05) at the peak of the response to hypoxia and averaged 10.1 ± 1.6 Hz (P < 0.05) during the period of hypoxic ventilation.

**PPN responses to disinhibition of the CH.** A subset of the muscle contraction-responsive neurons of the mesencephalic tegmentum was also recorded during activation of cardiorespiratory drive evoked via disinhibition of the CH. Figure 7A depicts the response of one muscle reflex-responsive PPN neuron (see Fig. 3 for response to muscle contraction) to microinjection of 60 nl of 5 mM bicuculline into the CH. This treatment resulted in a modest increase in arterial pressure as well as robust increases in HR and respiration. The PPN neuron exhibited a marked, relatively sustained elevation in firing rate during the period of enhanced cardiorespiratory drive induced by disinhibition of the CH. Similar responses to disinhibition of the CH were observed in three other PPN neurons that responded to muscle contraction, all observed in separate animals. Two additional muscle contraction-responsive PPN neurons exhibited triphasic responses consisting of peak increases in firing rates during the onset and
decline of the cardiorespiratory responses to disinhibition of the CH. The peaks in firing rate surrounded periods of increased firing rate variability and general decreases in firing rate from the peaks of the responses, although at all times the firing rates of these neurons remained elevated relative to baseline. Overall, disinhibition of the CH was associated with a marked increase in the firing rate of muscle reflex-responsive PPN neurons from $6.7 \pm 2.1 \text{ Hz at baseline}$ to $20.4 \pm 5.2 \text{ Hz (} P < 0.05; \ n = 6 \)$. Microinjections of identical volumes of vehicle (Ringer) and Chicago blue into the CH failed to evoke similar responses in PPN neurons.

Microinjections of bicuculline into the PPN evoked increases in cardiorespiratory drive. To document the physiological effects associated with activation of PPN neurons, microinjections of 5 mM bicuculline (60 nl, $n = 22$) were executed among the NADPH-diaphorase-positive PPN neurons in anesthetized rats. Figure 8 and Table 3 demonstrate that microinjection of bicuculline into the PPN results in marked, sustained increases in $f$ and minute JDEMG amplitude, as well as moderate increases in MAP and HR. Injections of vehicle (Ringer) and Chicago blue into the PPN failed to reproduce these responses.

**DISCUSSION**

**Responses of PPN neurons to muscle contraction.** The principal aim of this study was to document the responses of neurons in the PPN to unilateral evoked static contraction of the hindlimb muscles. A substantial majority of PPN units exhibited increases in firing rate during muscle contraction, and the sample of PPN units as a whole exhibited statistically significant increases in $f$ and minute JDEMG amplitude, as well as moderate increases in MAP and HR. Injections of vehicle (Ringer) and Chicago blue into the PPN failed to reproduce these responses.

**Table 2. Response profiles of neuronal samples from the PPN, CnF, and IC during static muscle contraction**

<table>
<thead>
<tr>
<th>Region</th>
<th>Increase (%)</th>
<th>Decrease (%)</th>
<th>No response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPN ($n = 31$)</td>
<td>20(64)</td>
<td>3(10)</td>
<td>8(26)</td>
</tr>
<tr>
<td>CnF ($n = 24$)</td>
<td>5(21)</td>
<td>7(29)</td>
<td>12(50)</td>
</tr>
<tr>
<td>IC ($n = 24$)</td>
<td>7(29)</td>
<td>5(24)</td>
<td>12(50)</td>
</tr>
</tbody>
</table>

Response profile values are no. of neurons; nos. in parentheses are percentage of total neurons ($n$) sampled in each region. PPN, pedunculopontine nucleus; CnF, cuneiform nucleus; IC, inferior colliculus.

**Fig. 3. Single-unit extracellular recording of a PPN unit that responded to muscle contraction with a gradual increase in firing rate. A: unit rate response to muscle contraction. B: side-by-side comparison of extracellular action potential signature from the storage oscilloscope. The action potential at left was observed before muscle contraction; the action potential at right was observed during muscle contraction. C: neurogram activity from bracketed period in A depicting the unit response to muscle contraction.**

**Fig. 4. Effects of static muscle contraction on the firing rates of neuron samples from the PPN, CnF, and IC. PPN neurons exhibited a statistically significant increase in sample firing rate above baseline during muscle contraction ($*P < 0.01; \ n = 31$). Samples of neurons from the CnF ($n = 24$) and IC ($n = 24$), in contrast, did not exhibit alterations in sample firing rate during muscle contraction.**
creases in mean firing rate and peak firing rate during muscle contraction. The majority of responsive PPN units exhibited an immediate increase in firing rate, although many were observed to gradually increase their firing rates during the period of muscle contraction. As demonstrated by local bicuculline injections, PPN activation resulted in significant increases in f and minute f/DEMG amplitude. On the basis of these results, we conclude that muscle contraction can activate PPN neurons, leading to increases in cardiac output and respiratory drive.

Fig. 5. Direct electrical stimulation of the crushed tibial nerve did not evoke responses in PPN neurons that were excited during muscle contraction. Left: response of a PPN neuron to static muscle contraction. Middle: stimulation of the crushed tibial nerve at 2× MT failed to evoke muscle contraction and a similar increase in firing rate in this unit. Right: stimulation of the crushed tibial nerve at 8× MT evoked a neuronal response presumably due to direct stimulation of tibial afferents.

Fig. 6. Response of one PPN neuron to 1 min of acute hypoxia (10% O2). The PPN unit depicted in this trace responded to hypoxia with an increase in firing rate.
results, we conclude that the PPN is activated during evoked static muscle contraction in the anesthetized rat and further hypothesize that the PPN may play a role in the regulation of the respiratory adjustments that accompany muscle contraction in the rat. Further experiments are necessary to conclusively test this hypothesis.

In this study, we conducted two important control procedures to evaluate potential confounding variables of the observed PPN responses during muscle contraction. First, we demonstrated that the PPN responses observed during muscle contraction were not due to the depressor response that accompanies muscle contraction in chloralose-urethane-anesthetized rats. This conclusion is supported by the observation that brief periods of hypotension induced via intravenous injections of SNP were ineffective at altering the firing rates of PPN neurons. Second, we showed that observed responses were not due to direct activation of afferent neurons in the tibial nerve as stimulation of the proximal end of the crushed tibial nerve at 2× MT failed to evoke responses in PPN neurons. Increases in the intensity of the stimulation of the crushed tibial nerve resulted in reproduction of the responses previously observed during muscle contraction, probably due to direct stimulation of tibial afferents. The results of these two control experiments suggest that the increases in PPN activity we observed during muscle contraction were evoked by activation of reflex pathways specific to muscle contraction.

The depressor response observed in chloralose-urethane-anesthetized rats during evoked static muscle contraction in this study and by others from our laboratory (28) contrasts with the pressor responses observed in anesthetized preparations of several species, including the cat (20, 30), the dog (13), the mouse (27), and the chicken (37), as well as the pressor response observed in conscious humans (14). Depressor responses (36) and inconsistent, diminutive pressor responses (38) to static muscle contraction have been reported in separate studies of halothane-anesthetized rats. Rats anesthetized with α-chloralose only exhibit no change in blood pressure during static contraction (40). Smith et al. (36) recently demonstrated that de-
cerebration and withdrawal of anesthetic reverts the depressor response evoked by muscle contraction in halothane-anesthetized rats to an increase in blood pressure. Attenuated pressor responses were observed in decerebrate rats after restoration of halothane anesthesia. Their results suggest that anesthetic alters central neural regulation of the cardiovascular responses that accompany evoked muscle contraction. Because of the integrative nature of cardiovascular and respiratory neural control systems, we acknowledge the possibility that the PPN responses we observed during muscle contraction are not fully representative of those that occur in the absence of anesthetic. Future inquiries into the responses of PPN neurons during evoked muscle contraction in decerebrate, nonanesthetized rats may elucidate this issue.

It is not surprising that the PPN, given its apparent role in regulating coordinated muscular output patterns (15), also receives feedback from contracting muscles. However, at this point we cannot definitively identify the pathway through which muscle reflex activation influences PPN activity. Muscle feedback influences may perhaps be mediated via spinomesencephalic secondary afferent projections known to connect lamina I of the dorsal horn of the spinal cord with the PPN (31). Alternatively, PPN neurons may be influenced through projections from other sites/pathways implicated in the regulation of muscle reflex responses, including the posterior hypothalamus (44, 45) and the ventrolateral medulla (1, 2, 33), the latter of which appears to receive secondary afferent projections that are possibly activated selectively by ergoreceptors (46). Further research is necessary to elucidate these details.

Table 3. Cardiorespiratory responses associated with injections of 5 mM bicuculline into the PPN

<table>
<thead>
<tr>
<th>Cardiorespiratory Variable</th>
<th>Baseline</th>
<th>Control mesencephalic injections</th>
<th>PPN injections</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>125 ± 2</td>
<td>3 ± 3</td>
<td>5 ± 2*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>385 ± 5</td>
<td>2 ± 6</td>
<td>17 ± 2*</td>
</tr>
<tr>
<td>(f_r), breaths/min</td>
<td>70.1 ± 1.1</td>
<td>2.4 ± 1.9</td>
<td>15.7 ± 2.5†</td>
</tr>
<tr>
<td>DEMG amplitude, units</td>
<td>1.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Minute / DEMG amplitude, breaths·units·min(^{-1})</td>
<td>70.1 ± 1.1</td>
<td>1.5 ± 3.3</td>
<td>20.4 ± 4.3†</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE; n = 8 (control mesencephalic injections) and 22 (PPN injections). *P < 0.05 significantly greater increase compared with baseline; †P < 0.05 significantly greater increase compared with response to control injections.
the anatomic reconstruction of our extracellular recording sites. The results demonstrated that neurons sampled from the NADPH-diaphorase-positive PPN were largely excited by muscle contraction, whereas the preponderance of neurons sampled from the CnF and the IC were unresponsive or inhibited by muscle contraction. The anatomic specificity of the neuronal responses to muscle contraction we observed in the PPN parallels the uniqueness of the PPN as a mediator of locomotor drive from the mesencephalic tegmentum, or MLR, as described by Garcia-Rill and colleagues (17, 18).

**PPN responses to systemic hypoxia.** Given previous reports of potential involvement of the PPN/peribrachial mesencephalic tegmentum in modulation of respiratory drive (7, 10, 11, 24, 29), we were interested to test the responses of PPN neurons to systemic hypoxia induced by spontaneous ventilation of 10% O2, a stimulus that evokes robust increases in respiration in the anesthetized rat (23). We found that a large percentage of PPN neurons exhibited increases in firing rate during the hypoxic stimulus. The same PPN neurons failed to respond to decreases in arterial pressure evoked by intravenous injections of SNP and thus were not likely affected by the decrease in arterial pressure that accompanies hypoxia in anesthetized rats. These data suggest that the PPN might contribute a modulatory influence to respiratory adjustments evoked by hypoxia and may perhaps be indicative of a more general role for the PPN in the regulation of respiratory adjustments that accompany physiological stressors and behavioral adjustments. Although such roles for the PPN have yet to be thoroughly investigated, it is interesting to note that the PPN has been hypothesized to contribute to the pathologies of obstructive sleep apnea (5) and sudden infant death syndrome (6, 15).

Consideration of PPN responses to systemic hypoxia has also led us to believe that the PPN responses we observed during muscle contraction were not due to changes in pulmonary affenter activity secondary to increases in respiration associated with muscle contraction. Eldridge and Chen (10) reported that bilateral vagotomy enhanced the excitatory effect of systemic hypoxia on neurons in the peribrachial mesencephalic tegmentum, an indication that vagal afferents have an inhibitory effect on this neuronal population. It is thus improbable that increases in vagal reflex activation secondary to increases in respiration during muscle contraction are responsible for the excitatory effects of muscle contraction on neurons in the PPN. In addition, if there were a direct, causal relationship between vagal afferent activity and PPN activation, it would stand to reason that a much larger degree of PPN activation would be observed during systemic hypoxia than during muscle contraction since the former stressor induces a much larger increase in respiratory drive than muscle contraction. The data indicate that unilateral static muscle contraction and systemic hypoxia have comparable effects on neuronal firing rate in the PPN. These observations suggest that it is unlikely that pulmonary afferent activation during static muscle contraction is responsible for the PPN responses we observed.

**Responses of muscle contraction-responsive PPN neurons to disinhibition of the CH.** Several authors have suggested that integration of central command and muscle reflex drives may be an important mechanism whereby the central nervous system is able to appropriately match cardiorespiratory adjustments with the intensity of the locomotor task (32, 33, 35, 43, 44). Previous studies have demonstrated the potential to synthesize muscle reflex and central command influences in the CH (11, 12, 45), the ventrolateral medulla (33), and the dorsal horn of the lumbar spinal cord (9). In the current study, we describe muscle reflex-responsive neurons in the PPN that are robustly activated by central command activation via chemical disinhibition of the CH. Feed-forward activation of the PPN from the CH is also supported by anatomic data in which direct, reciprocal projections have been demonstrated between the two nuclei (3). The ability of single PPN neurons to respond to both evoked muscle contraction and disinhibition of the CH suggests that the PPN may have the capacity to synthesize muscle reflex and central command influences on respiratory drive.

**Cardiorespiratory responses to disinhibition of the PPN.** To obtain an indication of the potential physiological consequences of increased activity in the PPN, as was qualitatively observed in response to muscle contraction, disinhibition of the CH, and hypoxia, we injected the GABA<sub>A</sub> receptor antagonist bicuculline into the NADPH-diaphorase-positive PPN. Disinhibition of the PPN evoked robust increases in f and minute f/DEMG amplitude, as well as modest increases in MAP and HR that were not significantly different from those observed in response to injections outside the PPN. These responses were qualitatively similar to those previously documented with electrical stimulation (11, 21, 22, 24). This period of accelerated cardiorespiratory drive eventually returned to baseline in 20–30 min. The observation that activation of the PPN results in an acceleration of cardiorespiratory drive is support for the hypothesis that PPN activation during muscle contraction may play a role in the modulation of the cardiorespiratory adjustments that accompany muscle contraction. The fact that disinhibition of the PPN evoked larger respiratory adjustments in contrast to cardiorespiratory adjustments could be indicative of a more important role for the PPN in the regulation of respiratory drive. Further experimentation is necessary to fully test these hypotheses.

**Conclusion.** In summary, we sampled the responses of neurons of the NADPH-diaphorase-positive PPN during several stimuli that evoked cardiorespiratory adjustments, including evoked static muscle contraction. PPN neurons were found to respond specifically to muscle reflex activation with increases in firing rate. We also observed robust increases in respiratory drive on chemical disinhibition of the NADPH-diaphorase-positive PPN. Taken together, these observations suggest that the PPN might contribute a modulatory influence to the respiratory adjustments that accompany
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activation of cardiorespiratory drive from the CH was 
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REFERENCES
Eldridge FL and Chen Z. 
behavioral contexts that involve respiratory adjust-
en exercise-related stimuli and physiological stressors/
the PPN warrants future attention regarding potential 
roles in the modulation of respiratory drive during 
exercise-related stimuli and physiological stressors/behavioral contexts that involve respiratory adjust-
ments.
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1. Bauer RM, Iwamoto GA, and Waldrop TG. Ventrolateral 
medullary pressor neurons modulate pressor reflex to 
muscular contraction. Am J Physiol Regul Integr Comp Physiol 257: 
2. Bauer RM, Iwamoto GA, and Waldrop TG. Discharge pat-
terns of ventrolateral medullary neurons during muscular con-
traction. Am J Physiol Regul Integr Comp Physiol 259: R606– 
3. Bayev KV, Beresovskii VK, Kebkalo TG, and Savoskina 
LA. Afferent and efferent connections of brainstem locomotor 
regions: study by means of horseradish peroxidase transport 
exercise: the decerebrate rat locomotor preparation. J Appl 
5. Bellingham MC and Funk GD. Cholinergic modulation of 
respiratory brain-stem neurons and its function in sleep-wake 
2000.
6. Boop FA, Garcia-Rill E, Dykman, and Skinner RD. The P1; 
insights into attention and arousal. Pediatr Neurosci 20: 57– 
7. Chen Z, Eldridge FL, and Wagner PG. Respiratory-associ-
ated rhythmic firing of midbrain neurons in cats: relation to 
8. Chong RK and Bedford TG. Heart rate, blood pressure, and 
running speed responses to mesencephalic locomotor region 
stimulation in anesthetized rats. Pfliegers Arch 434: 280–284, 
1997.
inhbits the discharge of dorsal horn neurons responsive to 
10. Eldridge FL and Chen Z. Respiratory-associated rhythmic 
firing of midbrain neurons is modulated by vagal input. Respir 
11. Eldridge FL, Millhorn DE, Kiley JP, and Waldrop TG. 
Stimulation by central command of locomotion, respiration and 
12. Eldridge FL, Millhorn DE, and Waldrop TG. Exercise hyper-
pernea and locomotion: parallel activation from the hypothal-
13. Fisher ML and Nutter DO. Cardiovascular reflex adjustments 
to static muscular contractions in the canine hindlimb. Am J 
14. Friedman DB, Peal C, and Mitchell JH. Cardiovascular re-
sponses to voluntary and nonvoluntary static exercise in 
15. Garcia-Rill E. The pedunculopontine nucleus. Prog Neurobiol 
16. Garcia-Rill E. The basal ganglia and the locomotor regions. 
17. Garcia-Rill E, Houser CR, Skinner RD, Smith W, and 
Woodward DJ. Locomotion-inducing sites in the vicinity of the 
18. Garcia-Rill E, Kinjo N, Atsuta Y, Ishikawa Y, Webber M, 
and Skinner RD. Posterior midbrain-induced locomotion. 
19. Garcia-Rill E, Skinner RD, and Fitzgerald JA. Activity in 
the mesencephalic locomotor region during locomotion. Exp Neu-
20. Hayashi N, Hayes SG, and Kaufman MP. Comparison of the 
exercise pressor reflex between forelimb and hindlimb muscles 
in cats. Am J Physiol Regul Integr Comp Physiol 281: R1127– 
21. Hilton SM. The defence-arousal system and its relevance for 
circulatory and respiratory control. J Exp Biol 100: 159–174, 
1982.
22. Hilton SM and Redfern WS. A search for brain stem cell 
groups integrating the defence reaction in the rat. J Physiol 378: 
23. Horn EM and Waldrop TG. Modulation of the respiratory 
responses to hypoxia and hypercapnia by synaptic input onto 
24. Hugelin A and Cohen ML. The reticular activating system and 
respiratory regulation in the cat. Ann NY Acad Sci: 586– 
603, 1963.
25. Iwamoto GA, Wappel SM, Fox GM, Buettow KA, and 
Waldrop TG. Identification of diencephalic and brainstem cardio-
spiratory areas activated during exercise. Brain Res 726: 109– 
122, 1996.
26. Kaufman MP and Forster HV. Reflexes controlling circula-
tory, ventilatory and airway responses to exercise. In: Handbook 
of Physiology. Exercise: Regulation and Integration of Multiple 
t, p. 431–447.
27. Kramer JM, Aragones A, and Waldrop TG. Reflex cardiovas-
cular responses originating in exercising muscles of mice. J Appl 
28. Kramer JM and Waldrop TG. Spontaneously hypertensive 
rats exhibit altered cardiovascular and neuronal responses to 
29. Lydic R and Bagdoyan HA. Pedunculopontine stimulation 
alters respiration and increases ACh release in the pontine 
reticular formation. Am J Physiol Regul Integr Comp Physiol 
30. McCloskey DI and Mitchell JH. Reflex cardiovascular and 
respiratory responses originating in exercising muscle. J Physiol 
41D, 877, 1980.
31. Menetrey D, Chaouch A, and Besson JM. Location and 
properties of dorsal horn neurons at origin of spinoreticular tract 
in lumbar enlargement of the rat. J Neurophysiol 44: 862–877, 
1980.
32. Mitchell JH. Cardiovascular control during exercise: central 
and reflex neural mechanisms. Am J Cardiol 55: 34D–41D, 
1985.
33. Nolan PC and Waldrop TG. Integrative role of medullary 
nurons of the cat during exercise. Exp Physiol 82: 547–558, 
1997.
34. Paxinos G and Watson C. The Rat Brain in Stereotaxic Coor-
35. Rybicki KJ, Streemal RW, Iwamoto GA, Mitchell JH, and 
Kaufman MP. Occlusion of pressor responses to posterior dience-
phalic stimulation and muscular contraction. Brain Res Bull 
36. Smith SA, Mitchell JH, and Garry MG. Electrically induced 
static exercise elicits a pressor response in the decerebrate rat. 
37. Solomon IC and Adamson TP. Static muscular contraction 
elicits a pressor reflex in the chicken. Am J Physiol Regul Integr 
38. Toney GM and Mifflin SW. Mediators of contraction-evoked 
skeletal muscle depressor response in anesthetized rats. J Appl 


