

Modulation of Pedunculopontine Tegmental Nucleus and Alterations in Glutamate-Evoked Discharge Rate of Nucleus Ambiguus Cholinoceptive Neurons in Rats

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I. Introduction

The pedunculopontine tegmental nucleus (PPTn) is part of a heterogenous mesopontine complex that provides diffuse ascending projections to diencephalic and forebrain structures as well as descending projections to the brainstem and possibly spinal cord (1). Rye et al. (2) described the PPTn in the rat, based on cytoarchitecture, as including all of the darkly stained magnocellular neurons in the field extending from the substantia nigra and retrorubral field rostral to parabrachial nucleus caudally. The complex is bordered medially by the ascending limb of the superior cerebellar peduncle and laterally by the lateral lemniscus and associated nuclei. The dorsal and ventral extent of the nuclear complex is delimited by the cuneiform nucleus and pontine tegmental field, respectively. At the caudal extreme, the PPTn merges with the laterodorsal tegmental nucleus (LDTn). While admixed with smaller noncholinergic cells, most, if not all, of the large multipolar cells in this area are immunopositive

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for choline acetyltransferase (ChAT). ChAT-positive cells in PPTn and LDTn (PPTn/LDTn) have been designated as the Ch-5 and Ch-6 cholinergic cell groups by Mesulam and co-workers (3)

While attempts to formulate a unitary model for central cholinergic function have been replete with inconsistencies, the hypothesis has been advanced that PPTn/LDTn may be considered a functional unit with an important role in behavioral state regulation (4-7). Ascending cholinergic projections from the PPTn/LDTn may control, in part, shifts in thalamocortical processing associated with different arousal states (reviewed in 8). This conclusion is supported by the finding that PPTn/LDTn (presumably cholinergic) neurons with antidromically identified thalamic projections exhibit "precursor signs" of increased discharge rates during transitions from EEG-synchronized to EEG-desynchronized, or rapid eye movement (REM), sleep state in the chronically implanted cat model (9). Likewise, presumptive experimental evidence supports a role for descending cholinergic projections from PPTn/LDTn to the medulla oblongata in the behavioral manifestations of REM sleep (10). Cholinergic agonists applied to selective sites within the medial pontine reticular formation (MPRF) invoke muscle atonia, EEG desynchronization, pontogeniculo-occipital (PGO) waves, rapid eye movements, and hippocampal theta rhythms. The PPTn/LDTn is considered to be the endogenous source of cholinergic afferents to the MPRF (reviewed in 10). More recently, Lydic and co-workers have reported a series of findings which also argue for the involvement of PPTn/LDTn in state-dependent respiratory depression (6,11-16). While those studies seem to have provided a link between PPTn-related acetylcholine (ACh) release within the MPRF and respiratory depression, it was acknowledged that it is also possible that PPTn/LDTn neurons might provide parallel cholinergic input to pontomedullary respiratory neurons as well as to the MPRF (6).

The evidence is also good that cholinergic mechanisms are involved in cardiovascular and respiratory regulation in the lower brain stem. Earlier studies localized the circulatory and/or respiratory effect of cholinergic substances to chemosensitive zones on the ventral surface of the medulla oblongata (17-19). Subsequent iontophoretic studies have demonstrated that the majority of cardioinhibitory (20,21) and respiratory-related neurons (22) studied in the ventrolateral medulla (nucleus ambiguus area) are also cholinceptive. Additionally, a number of other neuroactive substances are known to be present in the ventrolateral medulla (see 23,24 for review). However, very little is known about their role and synaptic interactions. It is likely that most of these neuroactive substances, including ACh, are modulatory to central chemosensitivity and to the central integration in the medulla of cardiovascular and respiratory reflex afferents.

On the other hand, the excitatory amino acid glutamate may play a more key role in the integration of cardiovascular and respiratory reflexes (25). Glutamate is thought to mediate fast synaptic transmission at most excitatory synapses within the central nervous system. Glutamate appears to be released by

central terminals of cardiopulmonary afferent fibers in the nucleus of the solitary tract (NTS) (26,27). Furthermore, central glutamate metabolism and glutamate release from glial elements have been proposed as additional mechanisms of the central respiratory response to hypoxia (25). This latter point is controversial as other investigators have not observed an increase in brain glutamate during hypoxia after peripheral chemodervation (28). However, several studies using cats, dogs, and rats are consistent in demonstrating that excitatory amino acid receptor antagonists alter baro- and chemoreceptor-induced respiratory and cardiovascular responses (29-31). Moreover, immunohistochemical and microinjection studies suggest that glutamate activity is likely to be widespread in medullary cardiorespiratory integration areas (32), including a recently demonstrated NTS to nucleus ambiguus (AMB) pathway (23).

Therefore, in the current chapter, we propose to present evidence for PPTn/LDTn (cholinergic) modulation of glutamate elicited postsynaptic responses in AMB. Our experimental model combined glutamate microdialysis within PPTn with cholinergic and glutamate microiontophoresis in the AMB. The relationship of these findings to the hypothesis that descending mesopontine cholinergic fibers contribute to state-dependent respiratory depression (6) will be discussed.

II. The Rat Pedunculopontine Tegmental Nucleus

A. Functional Considerations

The rat mesopontine tegmentum contains large cholinergic and smaller noncholinergic cells (3). As discussed, cholinergic afferents from PPTn have been implicated in a number of physiological functions, including the production of components of REM sleep, regulation of thalamic activity, as well as stereotypical motor activity (10). Rye and co-workers have attempted to ascribe this latter function to noncholinergic neurons of the "midbrain extrapyramidal area" (1,2); however, this view has recently been challenged (32-34). In *in vivo* extracellular recording studies, although the cholinergic identity of the neuron was questionable, correlations between mesopontine neuronal firing and locomotor behavior (35,36) and sleep-wake states (4,36) have in fact been reported.

Recording from the nucleus parabrachialis lateralis of unanesthetized cats, Saito and co-workers (36) were able to identify three populations of cells based on their activity patterns during the sleep-wake cycle. In a population of 84 single units, 31% exhibited a marked reduction or cessation of firing during REM or paradoxical sleep. These were termed PS-off cells. A majority (55%) of the units isolated increased their firing during REM sleep and were termed PS-on cells. Six of the PS-on cells were phase-locked with PGO waves and fired in bursts of 3-5 action potentials 5-25 ms before the PGO wave was recorded in the thalamus. PS-on cells, therefore, might participate in the generation of thalamocortical PGO waves. The question then arises as to whether the firing patterns of mesopontine neurons are reflections of synaptic input or arise from intrinsic membrane properties. In other words, are PPTn/LDTn

neurons the "pattern generator" for thalamocortical oscillations? This question has been addressed, in part, by *in vitro* studies.

Based on the electrophysiological properties of PPTn/LDTn neurons in brain slice preparations from guinea pigs, three types of neurons also have been described (37). Type I neurons make up approximately 10% of the populations and are characterized by low-threshold spikes (LTS). These LTS are manifested as rebound bursts after hyperpolarizing current pulses. A voltage-dependent calcium current underlies the LTS. The conductance was inactivated at the resting membrane potential and de-inactivated at hyperpolarized membrane potentials. It is unlikely that type I neurons are cholinergic due to their small size and the apparent lack of projections outside the tegmentum. Type II neurons fired spontaneously and exhibited a prominent late after-hyperpolarization (AHP) in contrast to the rebound excitation of type I neurons. Several conductances contributed to the firing behavior of type II neurons. Action potentials were preceded by two-component (fast and sustained) tetrodotoxin (TTX)-sensitive depolarizing prepotentials. Transient outward A-currents were apparently responsible for the delay in returning to the baseline potential following an action potential. In addition, high-threshold cadmium-sensitive (calcium) spikes were uncovered after TTX blockade of fast action potentials. Morphologically, type II and type III neurons were described as large fusiform multidendritic putatively cholinergic cells. Type III cells were also spontaneously active and exhibited A-currents as well as LTS. It was noted that these two currents may provide a sensitive mechanism for controlling rebound excitability in light of their opposing actions and susceptibility to neurochemical modulation. It was also acknowledged that type II and type III neurons might represent two functional states of the same neuron. Similar results were reported from rat brain slices by Kang and Kitai (38).

In summary, electrophysiological data are consistent with state-dependent phasic and tonic discharge patterns of mesopontine neurons. While correlations between state-dependent *in vivo* discharge behavior and the *in vitro* findings relative to the intrinsic membrane conductances are not straightforward, it is apparent that these neurons exhibit properties compatible with regulation of behavioral states, including the sleep-wake cycle.

B. Transmitter-Specific Afferents

Ultimately, the discharge patterns of mesopontine neurons must reflect the interplay of intrinsic membrane conductances and afferent inputs. However, the neurochemicals employed by afferent pathways to PPTn/LDTn have not been thoroughly delineated. Reportedly, the rat PPTn/LDTn receive multiple inputs from limbic structures, basal forebrain, oculomotor nuclei, ascending sensory systems, substantia nigra and the brainstem reticular formation (2,5,38). Afferents from the limbic system include massive inputs from the lateral-hypothalamus-zona incerta area and the midbrain central gray, with lesser inputs

from the medial prefrontal cortex. The afferents from the lateral hypothalamic area, a region associated with regulating autonomic functions, appear to contain α -melanocyte-stimulating hormone (5). Little is known about projections from the midbrain central gray, although they originate in areas found to be rich with neuropeptides, including somatostatin, substance P, vasoactive intestinal peptide, and opioid peptides (39). It has therefore been proposed that neuropeptides are involved in the long-term modulation of state control (40). On the other hand, the synaptic input from the prefrontal cortex is thought to be via excitatory amino acid non-NMDA receptors (41). Reportedly, glutamate is also contained within an efferent subpopulation of PPTn/LDTn neurons (42). Considering the interconnections between ipsi- and contralateral mesopontine neurons (5), it is reasonable to assume that cholinergic mesopontine neurons might also receive an excitatory synaptic input from intrinsic mesopontine glutamatergic neurons. Moreover, studies utilizing excitatory neurotoxins suggest that this region contains a high density of NMDA receptors (43). Inhibitory GABAergic inputs appear to arise, in part, from the substantia innominata of the basal forebrain (44) as well as the substantia nigra pars reticulata (38,45). Other neurotransmitters inhibitory to PPTn/LDTn neurons include serotonin from the dorsal and median raphe nuclei (5,46) and noradrenaline from the locus ceruleus (5,47). Moreover, carbachol has been demonstrated to reliably suppress the firing of at least one type of mesopontine neuron, presumably via a muscarinic autoreceptor (4,48).

Hence, PPTn/LDTn receive inputs from areas known to be involved in behavioral and autonomic regulation. Additionally, the postsynaptic effects of the afferent neurochemicals are likely to be consistent with known state-dependent pharmacology and firing behavior of PPTn/LDTn neurons (36,40). For example, the increased tonic firing of some putative cholinergic PPTn/LDTn neurons during arousal and REM sleep appears to be enabled by a decrease in monoaminergic (noradrenaline and serotonin) afferent tone. The state-dependent excitatory drive to tonic PPTn/LDTn neurons likely comes from peptides and glutamate. Phasic or burst firing PPTn/LDTn neuronal behavior might reflect rebound excitation in type I and possibly type II neurons subsequent to GABAergic-mediated hyperpolarization.

C. Efferent Projections of PPTn/LDTn

Widely distributed diffuse cholinergic and noncholinergic projections ascend and descend from the mesopontine tegmentum (1-3,8,34,39). While this chapter focuses on descending effects on PPTn/LDTn modulation, ascending projections in the rat are reportedly distributed to the thalamus, basal forebrain, thalamus, limbic cortex, and perhaps the basal ganglia (reviewed in 2). Descending projections from PPTn/LDTn have been described in the cat (49,50) and rat (1,51-57). Descending projections were found mainly distributed to pontomedullary reticular nuclei. In the rat, Woolf and Butcher (57) utilized retro-

grade tracer with ChAT histochemistry and described mesopontine projections to reticular spinal nuclei, which included the raphe magnus nucleus, lateral reticular nucleus, medullary reticular nucleus, and the oral and caudal pontine reticular nuclei. These latter projections, along with specific efferents to the gigantocellular reticular nucleus, were confirmed by *Phaseolus vulgaris*-leukoagglutinin anterograde tracer studies (51). These projections appear to be involved in REM sleep induction and the muscle atonia of REM sleep (58). Additionally, a ChAT immunoreactive pathway has been described from PPTn to the rostral ventrolateral medulla (RVLM), which has implications for cardiovascular regulation. Rye and co-workers (1) also described pathways from the mesopontine tegmentum, which innervated autonomic control areas in the medulla. The NTS and caudal VLM (CVLM), including AMB and adjacent cardiorespiratory control neurons, appear to be innervated by the ventrolateral branch of Probst's tract. It was believed that most of these fibers originated in the predominantly noncholinergic parabrachial nucleus. However, it would appear that the CVLM is innervated by some cholinergic PPTn fibers (see Table 2, case 1 in Ref. 1).

As mentioned, it is likely that some of the noncholinergic cells that contribute to the efferent output of PPTn/LDTn are glutamatergic (42). Furthermore, PPTn/LDTn neurons exhibit immunoreactivity for a number of peptides including substance P, corticotropin releasing factor, bombesin, and atriopeptin, at least in the rat (59,60,61). The terminal field neuronal effects of PPTn/LDTn stimulation are not well understood. Stimulation of PPTn and LDTn, under the appropriate conditions, elicited a short latency excitation of thalamic neurons followed by a long latency and prolonged depolarization superimposed by a barrage of action potentials (see Ref. 9). While the early depolarization is thought to be mediated by nicotinic receptors (NAChR), the slow depolarization may be via muscarinic receptor (MAChR) mechanisms and/or peptide modulation.

III. Nucleus Ambiguus

A. Functional Considerations

In the rat, the AMB and associated ventroambigial (periambigual) region appear as aggregations of small to large, mainly multipolar neurons located ventral to the parvocellular reticular nucleus and dorsal to the lateral reticular nucleus (62). The cell column extends from the rostral "retrofacial," or Botzinger, complex to the caudal "retroambigial" nucleus. Presumably, this cell column represents a developmental ventral descent of the visceral efferent column of the medulla. In most mammals, these motor and premotor neurons are integral to cardiorespiratory control including the control of laryngeal and bronchial musculature.

Both electrophysiological (63) and morphological (64,65) studies confirm that cardioinhibitory fibers originate from the dorsal motor nucleus of the vagus (DMV) and AMB—mainly from the AMB. Standish and co-workers (64) have proposed functional differences between them, in that DMV neurons have primarily unmyelinated axons that mediate slow vagal effects, whereas AMB neurons are myelinated and mediate fast effects. This latter study also demonstrated the ventricular innervation of the vagus. The AMB neurons are also in close proximity to the caudal ventrolateral group of cardiovascular neurons (CVLM), which are premotor to sympathetic preganglionic neurons (66).

The ventral respiratory group (VRG) is also associated with the AMB cell column. The caudal AMB-retroambigial complex exhibits primarily expiratory activity and most of these neurons project to the thoracic spinal cord, where they innervate internal intercostal motoneurons (66,67). Others appear to be bulbar output neurons innervating laryngeal, bronchial, and/or esophageal musculature.

B. Neurochemical Modulation of Ambiguous Neurons

Precise information regarding the localization, postsynaptic effect, and physiological impact of neurochemicals on autonomic reflexes within the ventrolateral medulla is not available (see 68,69). Nevertheless, it appears likely that reflex excitatory synaptic interactions in AMB are mediated by excitatory amino acids, and these synaptic effects are in turn modulated by classical neurotransmitters and neuropeptides.

The most direct evidence comes from exogenous iontophoretic application or microinjection of putative neurotransmitter agonists or antagonists to observe their cellular and/or autonomic effects. Excitatory amino acids have been routinely used to locate cardioinhibitory neurons in the AMB by their ability to induce bradycardia (20,21). In addition, recent evidence suggests that NMDA receptors might participate in solitario-ambigial synaptic transmission (70). Somatostatin may be colocalized with glutamate within this pathway (71), and somatostatin enhances glutamate-evoked excitation of AMB motoneurons. It is also likely that the AMB region receives glutamatergic efferents from the mesopontine tegmentum (42). Reportedly cardioinhibitory AMB neurons are also excited by dopamine, enkephalins, substance P, and acetylcholine (21,72), whereas they appear to be inhibited by glycine (73) and possibly serotonin and noradrenaline.

Although more variable, pharmacological studies of the VRG mirror the responses of cardioinhibitory AMB neurons (63). Bradley and Lucy (22) reported that the iontophoretic administration of glutamate resulted in excitation of inspiratory, expiratory, phase-spanning, and nonrespiratory units in the region of the AMB. Glutamate often converted phasic activity to tonic activity. In the same study, acetylcholine administration caused excitation in 80% of the neurons studied; no inhibition was reported. However, two different time

courses of the response to ACh were noted: a short latency onset and offset, and a slow onset with persistent excitation after the iontophoretic current was turned off. This latter finding is particularly significant as it seems to suggest that ACh might modulate or enable response plasticity in cardiorespiratory reflex circuitry. However, the underlying mechanisms are not straightforward. In the forebrain, the M1 muscarinic receptor is primarily associated with response plasticity (74,75). In the case of AMB, the actions of cholinergic antagonists seem to suggest the presence of both muscarinic and nicotinic receptors. Zhang et al. (76) and Wang et al. (77) have provided further evidence for an AMB cholinergic nicotinic synapse, mediating information transfer in brainstem vagal motoneurons. Moreover, M2 seems to be the predominant muscarinic type in the rat brainstem, presumably subserving an autoreceptor role (78). The source of cholinergic afferents in these studies is left unclear.

Therefore, we designed a series of experiments to test the hypothesis that activity within the PPTn is capable of modulating glutamate-evoked neuronal activity in AMB, the underlying assumption being that glutamate microiontophoresis mimics the effect of visceral reflex interneurons on AMB neurons. The specific aim of these experiments was (a) to test the effect of pharmacological modulation of PPTn on AMB neurons, (b) to assess the effect of muscarinic antagonist (i.e. atropine), and (c) to look for persistent effects of PPTn stimulation.

Studies were performed on urethane-anesthetized (1.5 g/Kg, IP) male Sprague-Dawley rats. Neuronal activity was recorded via 3 M NaCl-filled center barrels of 7 barreled glass multipipettes. For microiontophoresis, the multipipettes also contained acetylcholine chloride (0.5 M) atropine sulfate (0.025 M), sodium glutamate (0.2 M), and artificial cerebrospinal fluid (aCSF). Single-unit AMB activity was discriminated and collected via a F. Haer counter that reset each 5 s. Data were converted to a rate per second display in software and analyzed as peak firing rates. The remaining barrels were filled with the retrograde tracers, rhodamine, and/or fluorescein latex microspheres. The rhodamine-labeled latex microspheres were deposited at the end of the recording session; fluorescein was used as a nonspecific marker and was placed in the medial reticular formation. The AMB was approached using stereotaxic coordinates, and recording sites were selected based on the ability of high-current (~100 nA) delivery of glutamate to effect changes in heart rate. Microdialysis of glutamate (10 mM), GABA (10 mM), or aCSF was through a Carnegie Medicin CMA/12 microdialysis probe (1-mm membrane tip) directed at the caudal PPTn. Perfusion rate was 2 μ L/min with in vitro transfer factor ranging from 36% to 54%.

Results are summarized for 26 neurons that responded with excitation to direct application of ACh with an atropine-sensitive change in firing rate. During glutamate microdialysis into PPTn, 42% (n = 11) of the AMB neurons exhibited an increase in net peak evoked response compared to the glutamate-evoked response during aCSF microdialysis into PPTn. 54% (n = 14) of the

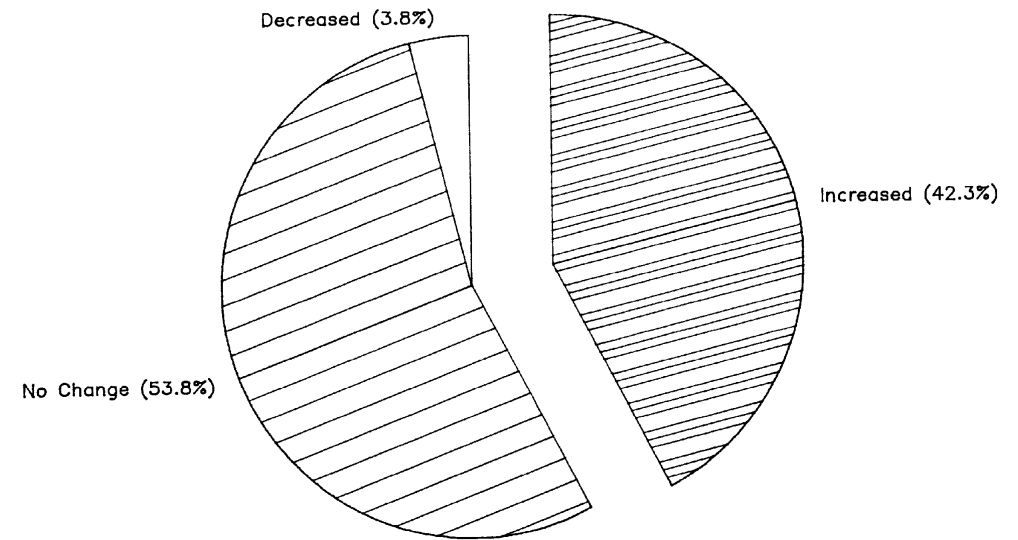


Figure 1 Differential effect of glutamate-evoked activity in nucleus ambiguus neurons (n = 26) during glutamate microdialysis into PPTn as compared to microdialysis of aCSF. Exploded slice represents 42.3% of the neurons (n = 11), which exhibited a net increase in peak glutamate-evoked activity.

neurons demonstrated no change in net evoked response, and only 4% (n = 1) showed a decrease in net evoked response (Fig. 1). A typical experiment is depicted in Figures 2A and 2B. In Figure 2, panel A, the increase in glutamate-evoked discharge in AMB during glutamate microdialysis is illustrated, along with the progressive attenuation by simultaneous application of atropine. Atropine attenuated the response in each of the 11 neurons exhibiting PPTn facilitation. Atropine was equally effective in blocking the excitation produced by ACh in all 26 neurons tested (data not shown). Panel B of Figure 2 is a continuation of panel A (lapsed time between graphs is 30 min). It demonstrates the recovery after atropine antagonism and the progressive increase in background and evoked discharge with continuous glutamate microdialysis into PPTn. It should be noted that both remained elevated for up to 30 min after glutamate microdialysis was terminated in three neurons. The data are summarized for the 11 facilitated neurons in Figure 3. During PPTn aCSF microdialysis, the glutamate-evoked response tended to be more robust as compared to ACh. GABA microdialysis into PPTn was ineffective in altering glutamate-evoked activity in AMB.

IV. Discussion

The literature reviewed and data presented in this chapter strongly suggest that descending projections from the PPTn/LDTn complex might be involved in

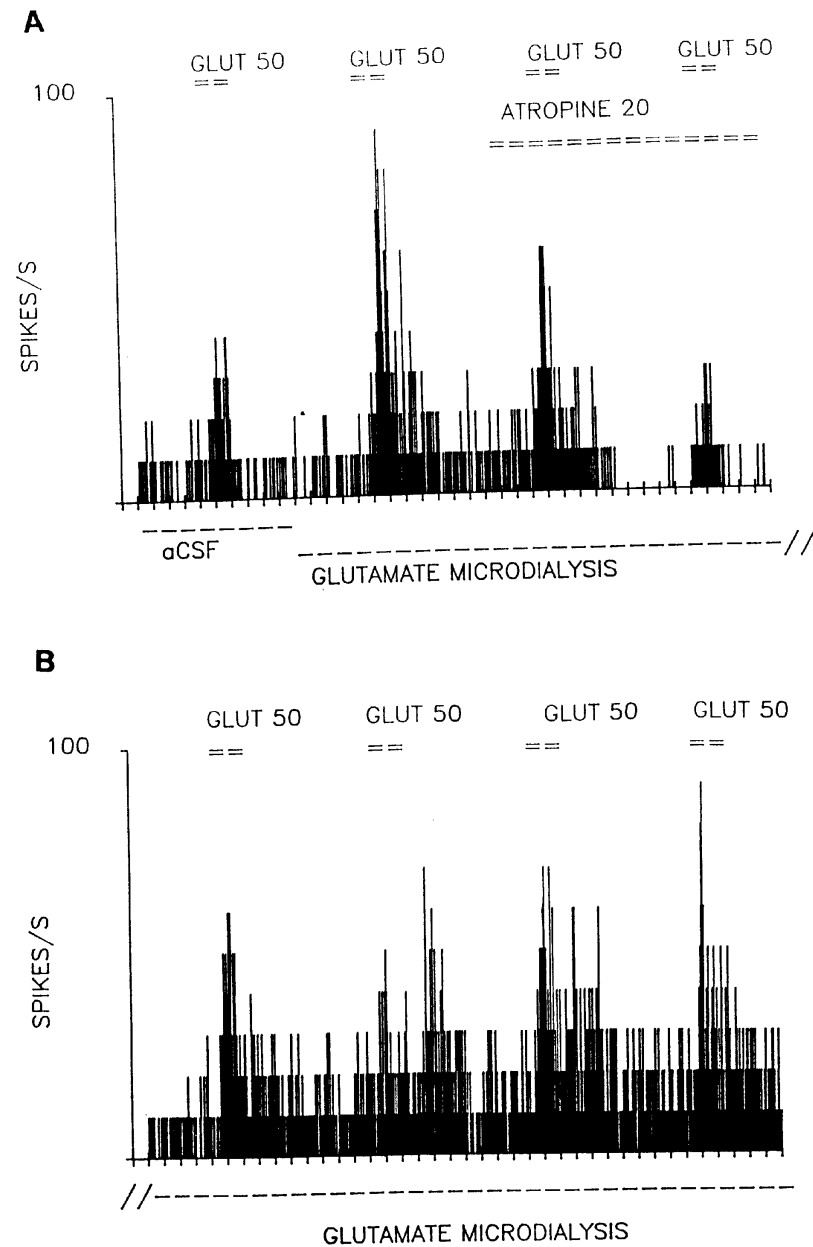


Figure 2 Discharge behavior of a typical facilitated unit during experimental protocol. (A) From left to right, discharge behavior of a single unit to two 50-s test pulses of 50-nA glutamate in the presence of aCSF, 50-nA glutamate after 200 s of PPTn microdialysis of 10-mM glutamate, and the progressive attenuation of glutamate-evoked activity by simultaneous application of 20-nA atropine sulfate. (B) Continuation of panel A after 30 min lapsed time. Figure illustrates progressive buildup of glutamate-evoked discharge and background firing with continuous PPTn glutamate microdialysis. Time between tick marks on x-axis equal 50 s, bin equal 5 s.

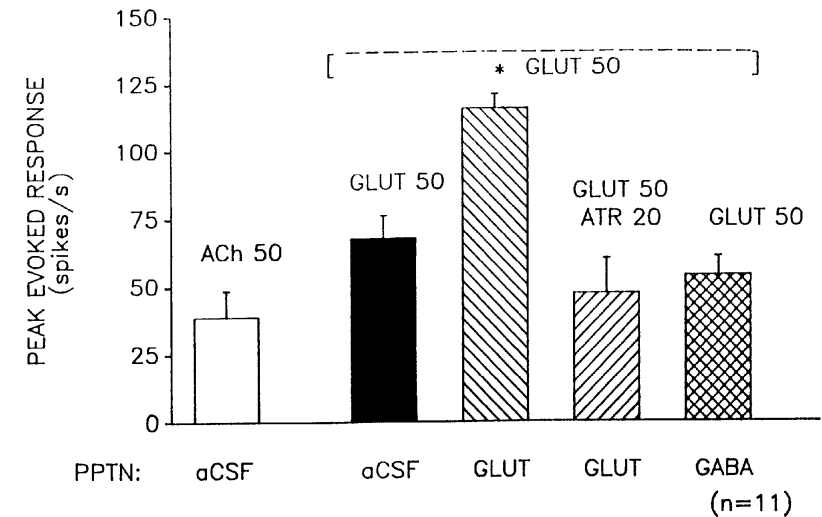


Figure 3 Summary of average net peak evoked activity for facilitated neurons ($n = 11$). Bottom legend provides substance microdialyzed into PPTn while top legend indicates substance(s) applied by microiontophoresis into the AMB. Average peak evoked activity was as follows: 50-nA ACh in presence of aCSF, 38.8 ± 9.7 spike/s (mean \pm SEM); 50-nA glutamate with aCSF, 67.8 ± 8.3 spike/s; 50-nA glutamate in presence of 10-mM glutamate microdialysis, 115.6 ± 5.3 spike/s; 50-nA glutamate with 20-nA atropine in presence of glutamate microdialysis, 47.2 ± 13.0 spike/s; and 50-nA glutamate in presence of 10-mM GABA microdialysis, 53.8 ± 7.0 . Treatments were randomized. * $P < 0.05$ (ANOVA with Bonferoni-corrected post hoc comparisons).

state-dependent regulation of visceral sensory-motor integration in the CVLM. Presumptive cholinergic and noncholinergic cells in this complex exhibit electrophysiological characteristics consistent with a role in behavioral state modulation of neuronal activity up and down the neuraxis. State control seems to reflect an interplay between cholinergic activity and the other neurochemicals, specifically noradrenaline and serotonin, both within the PPTn/LDTn and at target sites. Neurons within and around the AMB, premotor, and motor efferents to visceral effectors are integral to cardiorespiratory reflex integration and are overwhelmingly cholinergic. Cholinergic mechanisms have been implicated in state-dependent modulation of neuronal activity throughout the central nervous system. Finally, our studies reveal that the discharge rate of a significant number of AMB neurons can be facilitated by putative pharmacological (glutamate microdialysis) upregulation of PPTn firing.

However, the current study is not without limitations. Because the retrograde tracing studies failed to provide a conclusive link between mesopontine cholinergic neurons and AMB (Rucker et al., unpublished), the origin of cholinergic afferents to AMB remain unclear. It remains to be demonstrated directly that PPTn glutamate microdialysis increases ACh release in the cortex. In addi-

tion, detailed pharmacological studies are needed to clarify the receptor type(s) involved in the observed responses.

Nevertheless, if we assume that descending cholinergic mechanisms were, at least in part, responsible, then several points can be addressed. During the transition from waking to slow-wave sleep (SWS) to REM sleep, subpopulations of PPTn neurons exhibit tonic firing during waking, become silent during SWS, and display bursting activity during REM sleep (58). Therefore our glutamate microdialysis should have induced PPTn firing behavior consistent with waking and/or REM sleep, and GABA microdialysis should have mimicked PPTn behavior during SWS. We saw no effects with PPTn GABA microdialysis. However, the increase in AMB neuron firing during PPTn glutamate microdialysis is consistent with the increased cardioinhibitory tone during waking and REM sleep. Furthermore, the decreased respiratory tone of REM sleep is no doubt linked to the atonia-related hyperpolarization of respiratory motor neurons in the lower brainstem, which has been linked to cholinergic mechanisms (11–16). This phenomenon may involve cholinergic activation of inhibitory interneurons, and is therefore also consistent with our current findings. Further speculation at establishing correlations between PPTn behavior and cardiorespiratory regulation in the lower brainstem is clearly not warranted at the present time; however, further study in this area is indicated.

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