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# Electrophysiological properties of cortically projecting histamine neurons of the rat hypothalamus

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The tuberomammillary (TM) nuclei of the hypothalamus appear to be the sole histaminergic cell group in the brain. The extracellular electrophysiological properties of cortically projecting TM neurons were studied in the urethane-anesthetized rat. TM neurons, antidromically activated from either ipsi- or contralateral cerebral cortex, displayed relatively slow conduction velocities, consistent with reports suggesting that TM neurons possess unmyelinated axons. Spontaneous activity was slow and regular, with action potentials of long duration. There was often a noticeable delay between initial segment and somatodendritic portions of spontaneous action potentials, and complete loss of the somatodendritic portion of the second antidromic action potential was commonly seen when double pulse paradigms were employed. These data demonstrate that in addition to anatomical and biochemical similarities, TM neurons share a constellation of physiological properties with other central aminergic neurons.

Mounting evidence suggests that histamine is utilized as a neurotransmitter in the central nervous system (CNS). Thus, histaminergic markers are unevenly distributed in brain [10], synaptosomal fractions contain the requisite cellular machinery for synthesis, storage and release of histamine [12], high affinity receptors are found in brain [21], and their occupation by specific histaminergic agonists and antagonists have well characterized physiological effects [3]. Recent immunohistochemical studies have provided evidence that both histamine [9, 14] and its synthetic enzyme L-histidine decarboxylase (HDC) [11, 23] are localized to neurons. At present, the magnocellular neurons of the tuberomammillary (TM) nuclei of the hypothalamus appear to constitute the sole histaminergic cell group in the brain.

Although a complete and detailed picture of the connectivity of TM neurons is lacking, current evidence suggests that these neurons innervate many regions of the

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CNS [6, 14, 23]. Best studied is the projection to the cerebral cortex; it has been repeatedly demonstrated that TM neurons are the major tuberal source of cortical afferents [6, 15, 17, 20]. The present study has capitalized upon these cortical projections and utilized antidromic activation from the cerebral cortex as a means of identifying presumptive histaminergic TM neurons.

Conventional amplification methods were used to record extracellular action potentials from neurons in the region of the TM nuclei (4 mm caudal to bregma, 1.5–2.0 mm lateral, 7.5–8.5 mm below the cortical surface) using glass micropipettes filled with either 2 M NaCl and 2% Pontamine sky blue or 5% horseradish peroxidase (HRP) in Tris buffer containing 0.25 M KCl in urethane-anesthetized (1.5 g/kg, i.p., supplemented as necessary) rats. A rectal probe connected to a heating pad kept core temperature at 37°C. Constant current anodal stimulation (0.2 ms duration; intensity $= 1.2 \pm 0.5$ mA, mean $\pm$ S.D.) of either frontal, cingulate or parietal cortex was used to identify and study the antidromic properties (criteria: constant latency, high frequency following, and collision [7], Fig. 1A) of cortically projecting TM neurons.

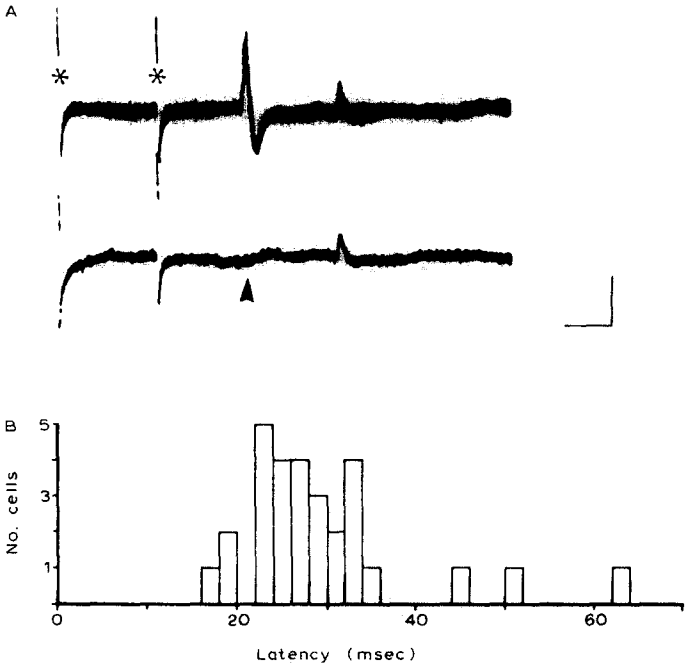


Fig. 1. Antidromic properties of cortically projecting TM neurons. A: the upper trace shows 3 superimposed sweeps of the response of a TM neuron to an externally triggered pair of stimuli (50 $\mu$A, 0.2 ms) delivered at 100 Hz to the frontal cortex (asterisks). The response consisted of two action potentials, both with latencies of 19.5 ms, thereby satisfying the criteria of constant latency and high frequency following. The second antidromic action potential is truncated, probably representing initial segment spikes. The lower trace demonstrates collision of the first antidromic action potential (arrowhead) when the same stimuli were delivered 10 ms following a spontaneous action potential. An antidromic initial segment spike whose timing lies outside of the 'critical window' [7] remains in response to the second stimulus. Calibration bars: 5 ms, 20 $\mu$V. B: histogram of antidromic latencies of cortically projecting TM neurons ($n = 29$).

Neuronal firing rates were determined by a rate meter from a continuous 60-s sample of spontaneous activity. When HRP-filled electrodes were used, TM neurons were characterized extracellularly, penetrated, and HRP iontophoresed intracellularly in order to study their histochemical and morphological properties (Reiner et al., in preparation). Confirmation of the recording site was accomplished by one or more of the following: stereotaxic localization, Pontamine sky blue dye spot (iontophoresed at the end of a track), or recovery of intracellularly filled HRP-positive neurons.

Thirty cortically projecting TM neurons were antidromically driven from ipsilateral ($n=26$) or contralateral ($n=4$) cerebral cortex. Antidromic latencies were notably long, with a mean of $29.1 \pm 9.5$ ms (Fig. 1B). Mean absolute refractory period of the axonal-somatic ensemble was $3.4 \pm 1.6$ ms. Seventy-five percent of cortically projecting TM neurons exhibited loss of the somatodendritic portion of the antidromic action potential with high frequency stimulation (Fig. 1A); in rare instances, only initial segment potentials could be evoked by antidromic stimuli.

Ultrastructural studies have provided evidence for the existence of both myelinated and unmyelinated HDC immunoreactive axons [4, 16]. Assuming a conduction pathway of 10–15 mm, the mean conduction velocity of cortically projecting TM axons encountered in the present study falls within the range of 0.3–0.5 m/s, suggesting that their axons are probably unmyelinated [5].

All cortically projecting TM neurons reported in the present study were spontaneously active. TM neurons typically fired slowly ($2.0 \pm 1.5$ Hz, Fig. 2D), with a rela-

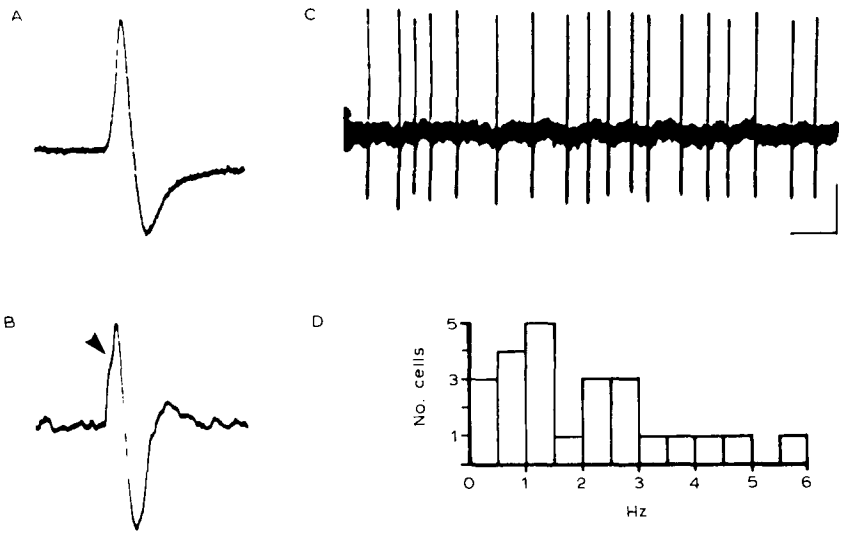


Fig. 2. Spontaneous activity of cortically projecting TM neurons. A and B demonstrate two different action potentials, both of long duration (6 and 4 ms, respectively). The neuron in A has a biphasic action potential, while that shown in B is triphasic. Additionally, the neuron in B has a prominent notch in the ascending limb (arrowhead), representing electrotonic delay between the initial segment and somatodendritic components of the action potential. C: spontaneous activity of an antidromically identified TM neuron. Calibration: A, 2.5 ms, 75 $\mu$V; B, 2 ms, 50 $\mu$V; C, 0.5 s, 20 $\mu$V. D: histogram of spontaneous firing rates of cortically projecting TM neurons ($n=24$).

tively regular pattern (Fig. 2C). Action potentials (Figs. 2A and B) were bi- or triphasic, of long duration (2–6 ms), and there was frequently an inflection on the ascending limb of the action potential (Fig. 2B), probably representing electrotonic delay between spontaneously generated initial segment and somatodendritic spikes.

That the cortically projecting TM neurons described herein are histaminergic derives from several lines of evidence. HDC immunoreactive neurons in the TM nuclei have been doubly labelled following HRP injections into the cerebral cortex [17]. Similarly, TM neurons staining positively for several other transmitter related enzymes (glutamate decarboxylase [20], adenosine deaminase [8], monoamine oxidase [15]) and for Met-enkephalin heptapeptide [6], some of which have been co-localized with HDC [13], project to the cerebral cortex. Finally, virtually all TM neurons are HDC positive [23], and the relatively homogeneous physiological properties displayed by cortically projecting TM neurons were also exhibited by many neighboring neurons which could not be antidromically driven from the cerebral cortex. Taken together, these data suggest that cortically projecting TM neurons are histaminergic.

The constellation of physiological properties displayed by cortically projecting TM neurons is strikingly similar to that of other aminergic systems with respect to action potential morphology, conduction velocity and spontaneous activity [1, 2, 22]. Furthermore, it has been reported that neurons in the region of the TM nuclei exhibit stereotypical changes in firing rate across behavioral states in unanesthetized cats [19], in a fashion similar to central noradrenergic [1] and serotonergic [18] neurons. Parallels between central histaminergic neurons and other aminergic cell groups have been previously drawn on the basis of biochemical [12] and anatomical [23] data. The results of the present study suggest that this homology may be valid with respect to physiology as well.

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