MEDIAL LEMNISCAL EVOKED RESPONSES IN THALAMIC ETHMOID NEURONS

by

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ABSTRACT

This thesis describes electrophysiological and pharmacological properties of neurons in the ethmoid nucleus of the rat thalamus. According to the atlas by Paxinos and Watson, the ethmoid nucleus is located dorsal to the medial lemniscus, the major somatosensory input to the thalamus. The ethmoid also lies ventral to the parafascicular nucleus, caudal to the ventrobasal thalamus and rostral to the scaphoid nucleus. The ethmoid is considered a higher order nucleus, which implies that it serves as a link in cortico-thalamo-cortical pathways that process sensory information. The literature on this nucleus is scarce and this thesis represents the first known attempt to study these neurons. Hence, a major objective of this thesis was to determine the passive and active properties of ethmoid neurons.

Recent evidence from this laboratory has shown that stimulation of the medial lemniscus produced glycinergic and GABAergic inhibition in ventrobasal neurons. This inhibition was not sensitive to ionotropic glutamate receptor antagonism by kynurenate. A prediction from these studies was that the ethmoid was an intermediate nucleus in a circuit between the medial lemniscus and neurons of the ventrobasal thalamus. Thus, a key aim of this thesis was to examine the possible involvement of ethmoid neurons in this novel circuit.

This thesis provides evidence that ethmoid neurons have passive and active properties similar to other neurons of the dorsal thalamus. Ethmoid neurons have a mean resting

membrane potential of ~ -53 mV, a mean input resistance of ~670 MΩ and a mean membrane time constant of ~64 ms. Ethmoid neurons also have the ability to generate spikes in the tonic and burst firing modes. The active properties were sensitive to blockade by internal application of QX-314, a quaternary blocker of Na^+ channels and by extracellular application of Ni^{2+}, a Ca^{2+} channel antagonist. These observations are consistent with Na^+ dependent action potentials when a neuron is in the tonic firing mode and low threshold Ca^{2+} spikes when in the burst firing mode.

We showed that stimulation of the medial lemniscus evoked depolarizations in all recorded ethmoid neurons. We categorized the depolarization responses into two groups. Group I were monophasic depolarizations and group II were biphasic depolarizations. The medial lemniscal evoked depolarization of ethmoid neurons persisted in the presence of kynurenate. These observations are consistent with the participation of ethmoid neurons in a circuit that results in glycinergic inhibition in ventrobasal neurons.

This thesis marks the first known attempt to study neurons of the ethmoid nucleus. The observations provide evidence for functional similarities of ethmoid neurons to other thalamic neurons, as well as evidence for novel inputs activated by stimulation of the medial lemniscus.

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Chapter 1: Introduction

1.1 Scope of thesis

This thesis describes electrical and pharmacological properties of the ethmoid, a thalamic nucleus that has received little attention in the literature. The scope of this thesis was to describe some of the passive and active electrical properties of ethmoid neurons. A major objective was to pharmacologically investigate the responses of ethmoid neurons to electrical stimulation of the medial lemniscus.

Recent evidence from this laboratory has shown that stimulation of the medial lemniscus evoked inhibition mediated by glycine and gamma-aminobutyric acid (GABA) receptors in ventrobasal neurons (Ran et al., 2004; Ghavanini et al., 2005). The effects of applying the selective glycine receptor antagonist, strychnine, and $GABA_A$ receptor antagonist, bicuculline, were instrumental in this discovery. Demonstration of glycine receptor mediated inhibition in ventrobasal thalamus was novel, as previous literature suggested that synaptic inhibition in this area was largely mediated by GABA receptors.

The ethmoid nucleus has been implicated in this strychnine-sensitive circuit for three major reasons. Firstly, it has been demonstrated that two or more synapses were involved in medial lemniscal evoked inhibition in ventrobasal neurons (Ghavanini et al., 2005). Secondly, stimulation within the ethmoid nucleus also evokes glycine-receptor mediated inhibition in ventrobasal neurons. Thirdly, inhibition evoked by stimulation of the ethmoid nucleus likely involves only one synapse (Ghavanini, 2006). Hence, the

ethmoid may be an intermediate nucleus in a strychnine-sensitive circuit involving the medial lemniscus and neurons of the ventrobasal thalamus.

This thesis marks the first known effort to characterize the properties of ethmoid neurons. Overall, this approach will enrich the understanding of ethmoid neuron function and will have potential implications for the participation of these neurons in synaptic inhibition.

1.2 Background

1.2.1 The thalamus

The term thalamus is derived from the Greek word thalamos, which refers to an inner room. This is an appropriate name because both thalami are deeply situated in the forebrain. The thalamus consists of a collection of cellular nuclei involved in many different sensory and motor systems.

It is customary to divide the thalamus into three parts: the epithalamus, the ventral thalamus and the dorsal thalamus (Herrick, 1918; Jones, 1985). This division of the thalamus is an indication of the aggregation of cells with common input and output connections to specific areas of the telencephalon (Jones, 1985).

The epithalamus comprises the anterior and posterior paraventricular nuclei and the habenular nuclei. These nuclei do not send fibers to, or receive fibers from, the cerebral cortex. The epithalamus is connected primarily with the hypothalamus, either directly or via the interpeduncular nuclei (Jones, 1985).

The ventral thalamus comprises the reticular nucleus, ventral lateral geniculate, zona incerta, and Fields of Forel nuclei. Neurons of the ventral thalamus receive fibers from the cerebral cortex and possibly from the striatum, but do not send axons to either of these structures (Jones, 1985). The best characterized nucleus of the ventral thalamus is the reticular nucleus. The primary outflow of the reticular nucleus is to the dorsal thalamus and its function is interwoven with that of the dorsal thalamus (Ramón y Cajal, 1904; Jones, 1975; Steriade, 1990).

The dorsal thalamus comprises the majority of thalamic nuclei, including the ventrobasal nuclei, medial group, medial geniculate complex, dorsal lateral geniculate nucleus, anterior complex, intralaminar complex, posterior group (known as the pulvinar in primates; Jones, 1985) and the ethmoid nucleus. Only neurons of the dorsal thalamus send fibers to the cerebral cortex. Hence, the dorsal portion is commonly referred to as the 'relay thalamus' for information flow from the periphery to the cerebral cortex (Jones, 1985).

1.2.2 Thalamic neurons

Investigation of the electrophysiological characteristics of thalamic neurons have revealed three general types: thalamic reticular neurons, intrathalamic interneurons and thalamocortical neurons (Jones, 1985; Steriade et al., 1997).

Thalamic reticular neurons are GABAergic and reside in the reticular nucleus. All known intrathalamic interneurons of the thalamus are also GABAergic, although the percentage

of these neurons is highly variable across nuclei and species (Spreafico et al., 1993). Thalamocortical neurons are present only in the dorsal thalamus and comprise the vast majority of neurons in this region (Steriade et al., 1997). Thalamocortical neurons are characterized by having axons that project to the cerebral cortex or striatum. These neurons also send collaterals to the reticular nucleus of the thalamus but do not send many collaterals to local thalamocortical neurons (Steriade et al., 1990). The decreased number of local collaterals is in strong contrast to other regions of the brain, such as the cerebral cortex or striatum (Miller, 1996). The implication of this feature is that neighbouring thalamocortical neurons are, to a large degree, functionally independent of each another (Miller, 1996). All thalamocortical neurons lack gene expression for glutamate decarboxylase and concomitantly show a lack of immunostaining for this enzyme and its product, GABA (Steriade et al., 1997). For practical reasons, we will focus only on the electrophysiological properties of thalamocortical neurons.

1.2.2.1 Electrophysiological properties of thalamocortical neurons

Neurons are excitable because their membrane potential can be significantly and quickly altered by the opening and closing of ion channels. Depending on the channel type, opening or closing of ion channels can increase or decrease the resting membrane potential, which can serve as a signalling mechanism. Hence, the resting membrane potential of neurons provides a reference against which changes in the membrane potential are expressed.

Action potentials represent the electrical signals by which neurons receive, analyze and convey information. Action potentials are typically generated at potentials positive to the

resting membrane potential. A signal that causes a reduction in membrane potential is termed a depolarization. A depolarization of the neuronal membrane typically enhances the neuron's ability to generate an action potential and is therefore excitatory. Conversely, an increase in membrane potential is called hyperpolarization. Hyperpolarization makes the neuron less likely to generate an action potential and is therefore inhibitory.

Thalamocortical neurons possess two basic mechanisms of action potential generation, burst and tonic (single-spike) mode. The burst firing mode is determined by the inactivation of voltage gated T-type Ca^{2+} channels that generate low-threshold Ca^{2+} spikes (LTSs). This T-type Ca^{2+} current (I_T) activates at membrane potentials positive to approximately -65 mV and inactivates at membrane potentials between -90 mV and -65 mV (Carbone et al., 1984; Nowycky et al., 1985; Huguenard et al., 1992). When a neuron is hyperpolarized beyond -65 mV, there is removal of I_T inactivation (de-inactivation) and upon cessation of a hyperpolarizing current, the membrane repolarizes back towards resting potential, which activates I_T. I_T activates more quickly than it inactivates or de-inactivates (Huguenard et al., 1992). Hence, thalamocortical neurons must be hyperpolarized below approximately -65 mV for several tens of milliseconds in order to remove enough inactivation of I_T to generate an LTS. The de-inactivation of I_T occurs slowly, which results in thalamocortical neurons generating low-threshold Ca^{2+} spikes only at low frequencies (10-12 Hz; McCormick and Feeser, 1990; Huguenard et al., 1992). The LTS is usually accompanied by high-frequency (200-400 Hz) bursts of Na^+ and/or Ca^{2+} action potentials (Huguenard et al., 1992; Tennigkeit et al., 1998).

In contrast, tonic firing occurs when thalamocortical neurons are excited at resting potentials positive to ~ -65 mV. At potentials positive to ~ -65 mV, thalamocortical neurons respond to depolarizations by generating action potential trains, the number of spikes being determined by the intensity and duration of the depolarizing current. Neurons in tonic mode can respond to depolarizing input with the generation of action potential trains at frequencies as high as 100 Hz (Steriade et al., 1997).

The firing mode strongly affects the nature of the signal that is relayed to the cortex (Sherman, 2000). When thalamocortical neurons are in the tonic mode of action potential generation, the threshold for responding to a depolarizing input is lower than if the same neuron is hyperpolarized in the burst mode, i.e. single-spike activity show increased synaptic responsiveness (McCormick and Feeser, 1990). Functionally, this implies that synaptic inputs are conveyed to the cerebral cortex in a more linear and 'faithful' manner when thalamocortical neurons are in the single-spike versus burst mode of action potential generation (Sherman, 1996; Sherman, 2000). Bursting is relatively rare during full wakefulness and more common during periods of inattention, drowsiness and slow-wave sleep. Conversely, single-spike activity occurs during wakefulness and rapid eye movement (REM) sleep (Steriade et al., 1997).

1.2.3 Influence of the brainstem on thalamocortical neurons

The state dependent fluctuations in membrane potential of thalamocortical neurons results from extrinsic synaptic influences. Morphological and electrophysiological data

suggest that cholinergic and glutamatergic neurons of the pedunculopontine and laterodorsal tegmental nuclei (PPT/LDT) play an important role in the transition from burst to tonic firing mode (Steriade et al., 1997). A large part of this transition is achieved by hyperpolarizing thalamic interneurons and/or increasing input resistance of thalamocortical neurons, which increases thalamocortical neuron sensitivity to incoming synaptic volleys (Miller, 1996).

Furthermore, norepinephrine releasing neurons of the locus coeruleus and serotonergic (5-HT) neurons or the dorsal raphe nucleus (Steriade et al., 1997) also contribute to the activity state of thalamocortical neurons. However, these monoamine-containing neurons innervate the thalamus less densely than neurons of the PPT/LDT (Steriade et al., 1997). Another major difference between PPT/LDT neurons and monoamine-containing neurons is the former triggers and maintains thalamic activation. This is achieved by increased spontaneous firing rates of these neurons during both wakefulness and REM sleep. Conversely, monoaminergic neurons slow down their discharge rates during the late stages of resting sleep and are virtually silent during REM sleep (Steriade et al., 1990).

In summary, regulatory systems originating in the brainstem can modulate the functional state of thalamocortical neurons by altering their firing mode for action potential generation. Hence, these neurons can profoundly affect information transfer to the cerebral cortex (Ramcharan, 2005).

1.2.4 Influence of the cerebral cortex on thalamocortical neurons

Connectivity between the thalamus and the cerebral cortex is bi-directional. The topography of corticothalamic connectivity is as precise as that of thalamocortical connectivity (Jones, 1985). Corticothalamic axons project to the nuclei from which they receive thalamic input and also send collaterals to the reticular nucleus and to intrathalamic interneurons (Jones, 1975). Corticothalamic axons arise from pyramidal cells in layers V and VI of the cerebral cortex and synapse on distal dendrites of thalamocortical neurons (Jones, 1985). The strength of the corticothalamic synapse is equally as important in modulating the state of thalamocortical neurons as synapses from ascending brainstem afferents. In fact, the major development in the evolution of the brain of higher primates is the enrichment of the corticothalamic system (Llinás and Paré, 1991).

1.2.5 First order and higher order relay neurons

Thalamic relay nuclei of the dorsal thalamus can be categorized as first order or higher order. Thalamic nuclei that receive many afferents from neurons in cortical layer VI are considered first order. (Guillery and Sherman, 1998; Sherman, 2000). Neurons of first order nuclei also receive input from subcortical sites and relay that information to the cortex for the first time. In contrast, higher order nuclei are characterized by neurons that receive afferents from cells in cortical layers V and VI (Guillery and Sherman, 1998; Sherman, 2000). Higher order neurons also relay information from one cortical area to another cortical area (Sherman, 2005). It is believed that higher order relay nuclei have a more elaborate role in processing sensory information.

There are at least two main differences in the innervation patterns of first and higher order thalamic relay nuclei. Firstly, cholinergic inputs from the midbrain parabrachial region cause hyperpolarization of thalamocortical neurons more frequently in higher order than in first order nuclei (Mooney et al., 2004). Secondly, a GABAergic input from the zona incerta selectively targets higher order thalamic relays (Boker et al., 2005). This difference in innervation pattern suggests greater hyperpolarization of higher order neurons and hence, more frequent bursting among higher order neurons (Ramcharan, 2005).

1.2.6 Somatosensory thalamus

The somatosensory system is simply the sensory system of somatic sensation. The medial lemniscal tract (also known as the dorsal column tract) is the major somatosensory input to the thalamus. It transmits information on fine touch, vibration, two-point discrimination and proprioception (position sense) from the skin and joints to the central nervous system (CNS; reviewed by Fitzgerald, 2005).

The medial lemniscus consists of the fasiculus gracilis and fasiculus cuneatus. The fasiculus gracilis conveys information from the lower half of the body and the fasiculus cuneatus conveys inputs from the upper half of the body (Fitzgerald, 2005). Fibers from both the gracilis and cuneate fasiculi ascend in the dorsal white column of the spinal cord and synapse with second order neurons in the lower brainstem. These second order fibers decussate, giving rise to the medial lemniscus (Fitzgerald, 2005). Many medial lemniscal fibers make excitatory glutamatergic synapses with third order neurons in the ventrobasal

thalamus (cf. Castro-Alamancos, 2002). Third order ventrobasal axons ascend to the somatosensory cortex, via the internal capsule, and exhibit somatotopic organization (Steriade et al., 1997). The ventrobasal thalamus, comprised of the ventral posterior lateral and ventral posterior medial nuclei, constitutes the principal thalamic relay and processing centre for somatosensory information (Jones, 1991).

1.2.6.1 GABAergic and glycinergic inhibitory transmission in ventrobasal nuclei

Synaptic inhibition of ventrobasal thalamocortical neurons can alter the firing mode for action potential generation. Hence, inhibition can profoundly affect information transfer to the cerebral cortex (cf. Ramcharan, 2005).

GABA is the major inhibitory neurotransmitter in parts of the CNS rostral to the brainstem. GABA mediates inhibition via three receptor types: ionotropic $GABA_A$ and $GABA_C$ receptors and metabotropic $GABA_B$ receptors (Johnston, 1996; Bettler et al., 2004; Chebib, 2004). Nineteen isoforms have been identified for $GABA_A$ receptor subunits: α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, π, ρ_{1-3} and θ (Sieghart and Sperk, 2002; Simon et al., 2004; Khom et al., 2005). The subunit composition is variable and greatly affects the pharmacology of $GABA_A$ receptors (Wingrove et al., 1997).

Glycine receptors have an established role in synaptic inhibition in the spinal cord and brainstem (Werman et al., 1968). Glycine receptors are composed of homomeric α subunits or a combination of α and β subunits (reviewed by Lynch, 2004). The α subunit, of which there are four isoforms (α_{1-4}), bears the ligand binding site and is required for

functional glycine receptors. The α_2 and α_4 isoforms are present during embryonic periods and α_1 and α_3 isoforms are expressed in the adult rat (Singer et al., 1998). In some brain regions, however, α_2 persists into adulthood (Piechotta et al., 2001). Glycine receptors have three known endogenous agonists: glycine and the glycine like amino acids, β-alanine and taurine (Curtis et al., 1968). The selective glycine receptor antagonist, strychnine, blocks the action of all three endogenous glycine receptor agonists.

Both glycine and ionotropic GABA receptors are pentameric ligand-gated ion channels. Activation of either type of receptor results in Cl^- influx, which changes the membrane potential towards the Cl^- equilibrium (E_{Cl}). Since E_{Cl} is usually more negative than the neuron's resting potential in rodents, activation of these receptors causes hyperpolarization of the postsynaptic membrane, which drives the membrane potential away from action potential threshold. However, in some nerve terminals and dendrites and in the embryonic phase, neurons have an elevated concentration of intracellular Cl^-. In these cases, activation of chloride channels results in a depolarization, generating excitation (Kuner and Augustine, 2000). Glycine and ionotropic GABA receptors also cause inhibition by increasing membrane conductance. This causes inhibition by short-circuiting excitatory postsynaptic potentials (EPSPs), a phenomenon termed "shunting" (cf. Ries and Puil, 1999). Shunting is far more effective in blocking action potentials than hyperpolarization (cf. Ries and Puil, 1999).

Until recently, synaptic inhibition in ventrobasal thalamus was thought to be mediated almost exclusively by GABA receptors. This paradigm is changing as functional glycine

receptors in ventrobasal thalamus have been demonstrated. Immunocytochemical studies show that there is low to moderate staining for both α_1 and α_2 glycine receptor subunits in ventrobasal thalamus (Rampon et al., 1996; Ghavanini et al., 2005). The expression of both subunits was punctate and diffuse, suggesting synaptic and extrasynaptic glycine receptors, respectively (Ghavanini et al, 2005). The functional nature of the α subunits has been verified by applying glycine receptor agonists to ventrobasal neurons. The agonists (glycine, β-alanine, taurine) caused a shift in the membrane potential towards E_{Cl} and decreased neuron input resistance. These effects were due to an increased Cl^- conductance, as shown by their reversal at E_{Cl} (Ghavanini et al., 2005).

Furthermore, it has been demonstrated that stimulation of the medial lemniscus evokes glycine receptor- and $GABA_A$ receptor-mediated inhibitory postsynaptic potentials (IPSPs) or inhibitory postsynaptic currents (IPSCs) in ventrobasal neurons (Ghavanini et al., 2005; Ghavanini 2006). This was shown by blockade with specific glycine receptor antagonist, strychnine, and $GABA_A$ receptor antagonist, bicuculline. However, since strychnine antagonizes receptors for glycine, β-alanine and taurine, it is unknown whether the observed strychnine-sensitive inhibition was due to mediation by glycine, β-alanine, taurine or combinations of the three.

The location of the neurons containing glycine or glycine-like amino acids is currently unknown. Studies using latency fluctuation and chronaxie and rheobase analysis implicate two or more synapses in medial lemniscal evoked inhibition of ventrobasal neurons (Ghavanini et al., 2005). Local circuit interneurons are scarce in the rat

ventrobasal thalamus (Harris and Hendrickson, 1987; Ohara and Lieberman, 1993). Hence, local circuitry is unlikely involved in this inhibition. The picture that has emerged is that stimulation of the medial lemniscus excites neurons that reside outside of the ventrobasal nuclei, which in turn, cause strychnine-sensitive IPSPs or IPSCs in ventrobasal neurons. A likely intermediate nucleus involved in this polysynaptic circuit is the ethmoid. It has recently been demonstrated that stimulation within the ethmoid nucleus evokes strychnine-sensitive IPSPs or IPSCs in ventrobasal neurons (Ghavanini, 2006). Furthermore, it appears that ethmoid evoked inhibition in ventrobasal neurons likely involves only one synapse (Ghavanini, 2006)

Interestingly, the medial lemniscal and ethmoid evoked inhibition in ventrobasal neurons occurred in the presence of the non-specific N-methyl-D-aspartic acid (NMDA), α-amino-5-hydroxy-3-methyl-4-isoxazole propionic acid (AMPA) and kainate receptor antagonist, kynurenate, as well as in the presence of broad spectrum metabotropic glutamate receptor antagonist, alpha-methyl(4-carboxy-phenyl) glycine (MCPG; Ghavanini et al., 2005; Ghavanini, 2006). This is surprising because, as noted above, known excitatory medial lemniscal synapses are glutamatergic (Salt and Eaton, 1996; Castro-Alamancos, 2002).

1.2.7 The ethmoid nucleus

The ethmoid nucleus is located caudal to the ventrobasal thalamus, rostral to the scaphoid nucleus, dorsal to the medial lemniscus and ventral to the parafascicular nucleus (Paxinos and Watson, 1986; Fig. 2.2B). A striking anatomical feature of the ethmoid nucleus is

the perforations by vertically directed fibers, seemingly coming from the medial lemniscal region (cf. Paxinos and Watson, 1986). As a result of this anatomical feature, Paxinos and Watson (1986) named this nucleus ethmoid, which is Greek for 'sieve-like'. The literature on this nucleus is confusing as many authors have erroneously referred to the ethmoid as the medial part of the medial geniculate body (Lund and Webster, 1967a; Lund and Webster, 1967b; cf. Paxinos and Watson, 1986). Based on the time course of neuroepithelial generation, it is believed that the ethmoid nucleus is part of the posterior thalamic nucleus (Altman and Bayer, 1979a; Altman and Bayer, 1979b).

The ethmoid is considered to be a higher order relay nucleus, suggesting an important role in complex information processing (Boker et al., 2005). Interestingly, the ethmoid nucleus appeared to stain positively for glycinergic cell bodies in the mouse (cf. Zeilhofer et al., 2005). It is also known that ethmoid neurons receive input from the anterior pretectal nucleus (Bokor et al., 2005). However, the functional implications of this connection are unknown. Furthermore, the active and passive properties of ethmoid neurons, as well as other input and output connections, are currently unknown.

The scarce literature on the ethmoid nucleus has resulted in difficulty determining which receptors may be expressed by neurons of this nucleus. However, receptor expression in the posterior nuclear group and other nuclei surrounding the ethmoid may provide insight on possible receptor types expressed by ethmoid neurons.

1.2.7.1 Ionotropic glutamate receptors

Three groups of ionotropic glutamate receptors have been identified, NMDA, AMPA and kainate. Functional NMDA receptors are widely distributed throughout the thalamus (Salt and Eaton, 1996). mRNA expression for AMPA receptor subunits is generally low in the thalamus, although not absent (Keinaenen et al., 1990). Furthermore, the dorsal thalamus shows low and diffuse immunoreactive staining for several kainate receptor subunits (Petralia et al., 1994). Thus, NMDA, kainate and possibly AMPA receptors may be expressed by ethmoid neurons.

1.2.7.2 α-adrenergic receptors

Currently, nine distinct α-adrenergic receptor subtypes have been identified, all of which are G-protein-coupled receptors (Gocayne et al., 1987). Regions surrounding the ethmoid nucleus, including the parafascicular nucleus and the posterior nuclear group all stain positively for a variety of α- adrenergic mRNA subtypes (Day et al., 1997).

1.2.7.3 Acetylcholine receptors

Neuronal acetylcholine receptors are categorized into two families, nicotinic and muscarinic. Nicotinic receptors are ionotropic receptors formed by five protein subunits (Lindstrom, 1997; Changeux et al., 1998). There are two general nicotinic subunits: α and β. Different combinations of these subunits can generate a variety of nicotinic receptor types (Schimerlik, 1989). Immunocytochemical studies using specific antibodies show that cells in almost all thalamic nuclei stain positive for several of these subunits (Deutch et al., 1987; Swanson et al., 1987).

Muscarinic receptors belong to the superfamily of G-protein-coupled receptors (Schimerlik, 1989). Five subtypes have been identified, M1 – M5 (Caulfield, 1993). Expression of several muscarinic receptor subtypes has been shown throughout all dorsal thalamic nuclei (Hersch et al., 1994).

1.2.7.4 5-HT receptors

5-HT receptors are grouped into seven families (5-HT_{1-7}) comprising a total of 14 structurally and pharmacologically distinct mammalian subtypes (reviewed by Barnes and Sharp, 1999). Electrophysiological evidence for 5-HT_{1A} and 5-HT_7 receptors has been demonstrated in the parafascicular nucleus (Harte et al., 2005).

1.2.7.5 Dopamine receptors

Dopamine receptors are grouped into two categories, D_1-like and D_2-like (reviewed by Neve et al., 2004). D_1-like receptors include D_1 and D_5 subtypes and D_2-like receptors include D_2, D_3, and D_4 subtypes. Anatomical studies show that many neurons in the dorsal thalamus express D_2 mRNA (Wamsley et al., 1989).

In summary, a variety of receptor types may be expressed by ethmoid neurons. These candidate receptors may contribute to the activity of ethmoid neurons.

1.3 Experimental rationale, objectives and hypothesis

While this thesis is essentially descriptive in content, there is sufficient rationale for a supposition that the ethmoid nucleus participates in a circuit involving the medial lemniscus and neurons of the ventrobasal thalamus. Electrical stimulation of the medial lemniscus evoked strychnine-sensitive inhibition in ventrobasal neurons. It was likely that this evoked inhibition involved more than one synapse. Electrical stimulation within the ethmoid nucleus also evokes strychnine-sensitive IPSPs or IPSCs in ventrobasal neurons (Ghavanini, 2006). Interestingly, ethmoid evoked inhibition likely involves only one synapse. Furthermore, the inhibition evoked by stimulation of the medial lemniscus and the ethmoid nucleus is not sensitive to blockade by kynurenate.

The potential link between the medial lemniscus and neurons of the ethmoid nucleus has not received attention in the literature. In this thesis, I will use whole-cell patch clamp and pharmacological techniques to investigate the electrical properties of ethmoid neurons and investigate responses to electrical stimulation of the medial lemniscus. These data will be used to examine the link between the medial lemniscus and neurons that reside in the ethmoid nucleus.

I hypothesise that stimulation of the medial lemniscus causes a depolarization of ethmoid neurons that persists during blockade of ionotropic glutamate transmission by kynurenate application. Investigating this hypothesis is an important step in further understanding the complexity of synaptic inhibition in the dorsal thalamus and will provide baseline information about the capabilities of ethmoid neurons.

Chapter 2: Materials and Methods

2.1 Imaging

Bright field microscopy was performed on haematoxylin and eosin stained parasagittal brain sections with ×2.5 dry (N.A. 0.075) and ×63 oil-immersion (N.A. 1.4) objective lenses on a Zeiss AxioImager Model Z1 (Carl Zeiss, Germany). Images were processed using Adobe Photoshop software (Adobe, San Jose, U.S.A.).

2.2 Whole-cell patch clamp recording

2.2.1 Tissue slice preparation

The experimental procedures received approval by the Animal Care Committee of The University of British Columbia. Sprague-Dawley rats (10-15 days old) were anesthetized with halothane and decapitated after approximately 1 minute under deep anesthesia. The brain was then removed and quickly submerged in an ice-cold (4 °C) sucrose solution containing (in mM): 26 $NaHCO_3$; 1.25 NaH_2PO4; 2.5 KCl; 2 $MgCl_2$; 2 $CaCl_2$; 25 dextrose; 250 sucrose. This solution was saturated with carbogen (95% O_2: 5% CO_2) and had a pH of 7.3-7.4 and mean osmolarity of 315 mOsmol. Osmolarity was measured by a freezing point osmometer (Advanced Instruments, Norwood, USA; courtesy of Dr. Edwin Moore). Brains were dissected along the interhemispheric fissure into two identical tissue blocks. A Vibroslicer (Campden Instruments, London, UK) was employed to section tissue blocks into 200-250 μm-thick parasagittal slices. Slices showing landmarks of the scaphoid nucleus and medial lemniscus (Paxinos and Watson, 1986, plate 81 and 81a) were kept at room temperature (23–25°C) for a minimum of 1 h and maximum of 5 h in artificial cerebrospinal fluid (aCSF) containing (in mM): 124

NaCl; 26 $NaHCO_3$; 1.25 NaH_2PO_4; 4 KCl; 2 $MgCl_2$; 2 $CaCl_2$; 10 dextrose. The aCSF solution was saturated with carbogen (95% O_2:5% CO_2) and had a pH of 7.3–7.4 and osmolarity of 305 mOsmol.

2.2.2 Electrophysiological recording

Slices were placed in a Perspex recording chamber (~2 ml volume) and immobilized with a polypropylene mesh. Slices were perfused with oxygenated aCSF at room temperature, at a rate of 1.5–2 ml/min. A Narishige puller (World Precision Instruments, Sarasota, U.S.A.) was used to make recording microelectrodes (resistance ranged from 4 to 9 MΩ) from thin-wall borosilicate glass tubing. Electrodes were filled with an intracellular solution containing (in mM): 140 K-gluconate; 5 KCl; 4 NaCl; 3 $MgCl_2$; 1 $CaCl_2$; 10 EGTA; 10 HEPES; 3 MgATP; 0.3 Na_2GTP. The pH of the intracellular solution was adjusted to 7.3-7.4 with 50% gluconic acid and 1M KOH. Ethmoid neurons were identified under a differential interference contrast microscope at a magnification of 400x (Axioskop 2, Carl Zeiss, Oberkochen, Germany).

In all experiments, the control aCSF contained 1 mM kynurenate and 20 μM bicuculline, with the exception of two cases. In section 3.4.1, aCSF did not contain kynurenate or bicuculline. In section 3.4.2, aCSF only contained kynurenate. In all experiments, E_{Cl} was ~ –53 mV.

Whole-cell patch clamp recordings were obtained using an Axoclamp-2A (Axon Instruments, U.S.A.) or a List EPC-7 amplifier (HEKA, Germany) in the current clamp

mode. Signals were filtered at 3 kHz, digitized at 10 kHz with a 16-bit data acquisition system (Axon Instruments) and stored using pClamp software (Axon Instruments).

Tonic and burst firing was evoked by depolarizing and hyperpolarizing current pulses (–300 pA to 300 pA, 400 ms).

2.2.3 Electrical stimulation

Anatomical locations of the medial lemniscus and thalamic ethmoid nucleus, (site of electrophysiological recording and electrical stimulation, respectively) are illustrated in Figure 2.2. Stimulation of the medial lemniscus was performed with a bipolar tungsten electrode (~5 MΩ resistance, World Precision Instruments). The distance between the two poles of the electrode was ~0.3 mm. The distance between the stimulating electrode and the recording electrode was ~1 mm. An isolated stimulator (Digitimer, Hertfordshire, UK) was connected to the bipolar electrode and controlled by pClamp software. Stimulation was delivered at ≤0.5 Hz and the average duration and amplitude of each stimulus was 50 μs and 60 V, respectively.

2.3 Drug application

Drugs were applied by bath perfusion at 1-2 ml/min. Stock drug solutions were prepared in distilled water or dimethylsulfoxide (DMSO) and diluted in aCSF just prior to use. All drug solutions were bubbled with carbogen (95% O_2:5% CO_2) before application. Kynurenate, bicuculline, strychnine, carbenoxalone, suramin, methylsergide, mecamylamine and QX-314 were purchased from Sigma Chemical Co. (St. Louis,

U.S.A.). Pancuronium bromide (pancuronium) was purchased from Baxter Corporation (Ontario, Canada), phentolamine from Novartis Pharmaceuticals (Dorval, Quebec), nickel (Ni^{2+}) from J.T. Baker Chemical Co. (Phillipsburg, N.J.) and atropine from Glaxo-Gllenburgs.

2.4 Data analysis

2.4.1 Passive membrane properties

Electrophysiological data was analyzed using pClamp software (Axon Instruments). Input resistance was measured from <10 mV voltage responses to hyperpolarizing current pulses. The time constant of each neuron was calculated by fitting a single exponential to the decay of a <10 mV response to a hyperpolarizing current pulse. The capacitance of the neuron was calculated using the following equation:

$$C_i = \tau_m / R_i$$

where τ_m was the time constant, R_i was the input resistance and C_i was the input capacitance of the neuron. Voltage-current relationships were measured from voltage responses to depolarizing and hyperpolarizing current pulses (-100 pA to 100 pA, 400 ms).

2.4.2 Spikes evoked by current pulse injection

The amplitude of spikes evoked by current pulse injection was calculated by the voltage difference between onset of the spike, defined as the point at which the depolarization of the spike visibly exceeded the noise level, and the peak of the spike (difference between arrow 2 and arrow 1 in Fig. 2.1). The duration of the spike was calculated from the time

of onset to the time where the response crossed the baseline (difference between arrow 3 and arrow 1 in Fig. 2.1) dV/dt values were calculated by the change in potential from the onset of the spike to the peak divided by time to peak (change in potential between arrow 2 and arrow 1 divided by change in time between arrow 2 and arrow 1 in Fig. 2.1). Amplitude of afterhyperpolarization (AHP) was calculated by the change in potential between the termination of the spike and the peak of the hyperpolarization phase (change in amplitude between arrow 4 and arrow 3 in Fig. 2.1).

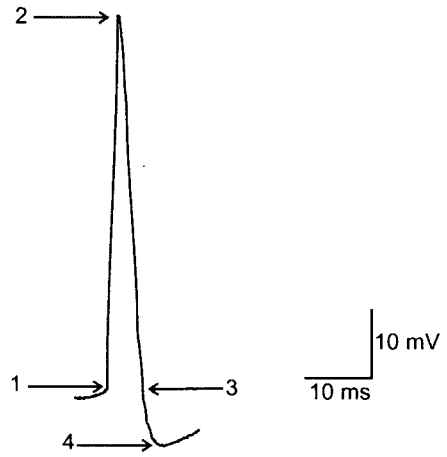

Figure 2.1 Example of a spike evoked by current pulse injection. The amplitude of the spike potential was calculated from the change in potential between arrow 2 and arrow 1. The duration of the spike was calculated from the change in time between arrow 3 and arrow 1. The average dV/dt was calculated from the change in potential between arrow 2 and arrow 1 divided by the change in time between arrow 2 and arrow 1. Amplitude of the afterhyperpolarization was calculated by the change in potential between arrow 4 and arrow 3.

Mean amplitude, duration and dV/dt values for spikes other than the first spike generated in a train, were calculated by taking the amplitude, duration and dV/dt of the second, middle and last spike in a train and averaging the values. This was done for each neuron.

2.4.3 Medial lemniscal evoked depolarizations

The amplitude of medial lemniscal evoked depolarizations was calculated by the change in potential from baseline to the peak of the depolarization. Negligible sensitivity to an antagonist was defined as <10% reduction in the amplitude of the depolarization. An antagonist was said to eliminate a response when >90% reduction in the amplitude of the response occurred on it's bath application. Partial blockade was defined as a 10-90% reduction in the amplitude of the depolarization. The mean latency of medial lemniscal evoked depolarizations was calculated for 5-10 depolarizations of each neuron. Latency of the response was measured as the time interval from the start of the stimulus to the onset of the response, defined as the point at which the depolarization visibly exceeded the noise level.

2.4.4 Statistical analysis

All data were expressed as mean ± SEM and n denoted the number of neurons tested. Data were statistically analyzed using Microsoft Excel software. The Student's *t*-test was used for comparing two groups. Significance was defined as $P < 0.05$.

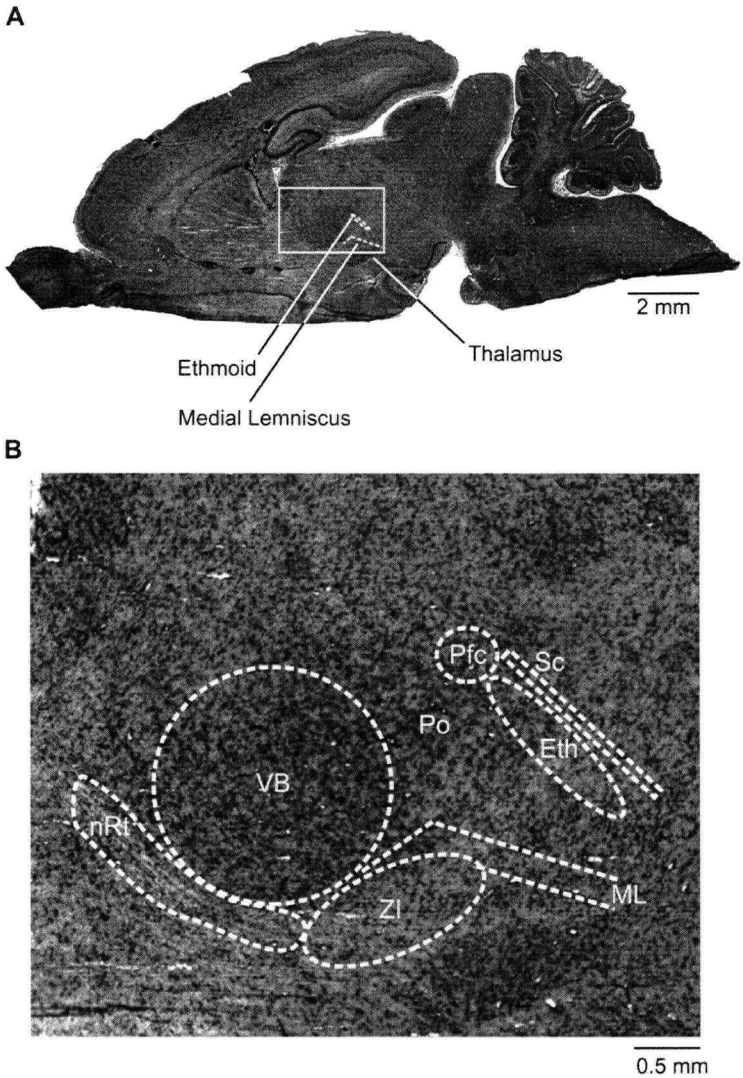

Figure 2.2 Bright field microscopy of parasagittal section of juvenile rat brain. *A*, Low-power magnification (×2.5 objective) of slice (10 μm-thick), stained with haematoxylin and eosin. Note the location of the medial lemniscus and ethmoid nucleus, sites of electrical stimulation and recording, respectively. *B,* Enlargement of inset in A, showing ethmoid nucleus (Eth), medial lemniscus (ML) and surrounding nuclei, including zona incerta (ZI), reticular nucleus (nRt), ventrobasal complex (VB), posterior group (Po), parafascicular nucleus (Pfc) and the scaffoid nucleus (Sc).

Chapter 3: Results

Intracellular recordings were obtained from a total of 48 neurons. The locations of all recorded neurons were within the boundaries of the ethmoid nucleus, delineated by Paxinos and Watson (1986; cf. Fig. 2.2).

3.1 Membrane properties

The mean resting membrane potential of ethmoid neurons was -53 ± 8 mV (n = 6). From potentials of ~ -60 mV, current pulses were injected to investigate passive and active membrane properties of ethmoid neurons. The mean input resistance of ethmoid neurons was 670 ± 75 MΩ with a range of 83 to 1102 MΩ. The mean τ_m was 64 ± 19 ms with a range of 24 to 102 ms. The mean input capacitance was 105 ± 24 pF, with a range of 60 to 218 pF (n = 6).

The voltage-current relationship of ethmoid neurons was determined from ~ -60 mV. The voltage-current relationship exhibited an apparent s-shaped relationship (n = 15; Fig. 3.1).

3.2 Spike generation

Depolarizing current pulses (10 to 300 pA, 400 ms) from a holding potential of ~ -60 mV generated tonic firing in ethmoid neurons. Single spikes evoked by just threshold current pulses were not observed. Therefore, the amplitude, duration and dV/dt of the first spike evoked in a train was calculated (cf. Fig. 2.1, Table 3.1).

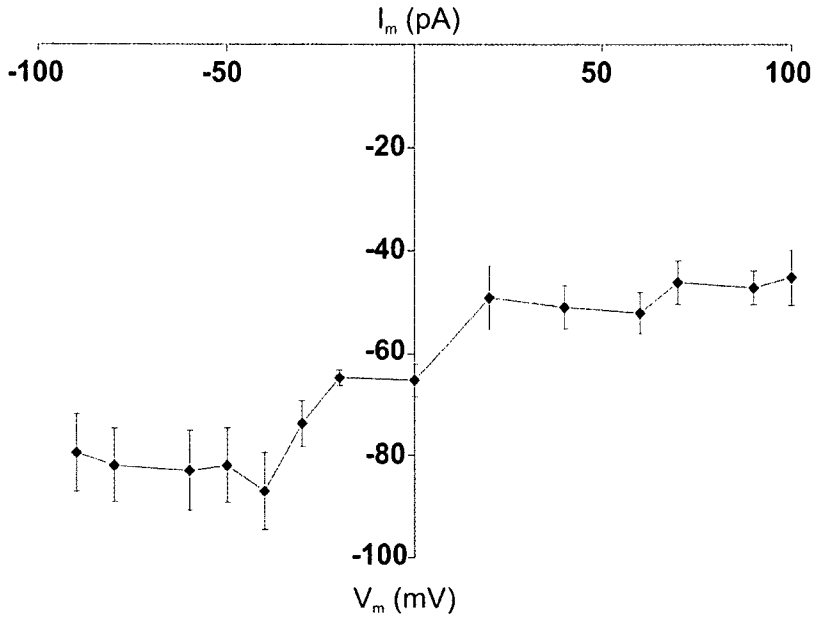

Figure 3.1 Voltage-current relationship of ethmoid neurons. Note the apparent s-shaped relationship. Each point represents an average from 5-15 ethmoid neurons.

The first spike was evoked at potentials ranging from -42 to -49 mV by a mean current of 71 ± 1 pA. Increasing the magnitude of the current pulse increased the frequency of evoked spikes, where a maximum number of 12 spikes were observed during the 400 ms depolarizing current pulse. The mean amplitude, duration and dV/dt values of the first spike in a train were 60 ± 2 mV, 6 ± 1 ms and 33 ± 3 mV/ms, respectively (n = 14; Table 3.1). A fast AHP was part of these spikes and had a mean amplitude of -8 ± 1 mV (Fig. 3.2Ac, black arrow). Following this hyperpolarization, a biphasic repolarization occurred (Fig. 3.2Ac).

Table 3.1 Characteristics of the first spike evoked in a train.

	Threshold Range (mV)	Mean Spike Amplitude (mV)	Mean Spike Duration (ms)	Mean dV/dt (mV/ms)	Mean AHP Amplitude (mV)
First spike (n=14)	-42 to -49	60 ± 2	6 ± 1	33 ± 3	-8 ± 1

Values are ± SEM

Interestingly, spikes within a train gradually developed shorter amplitudes, longer durations and slower dV/dt values compared to the first spike. For example, the mean amplitude, duration and dV/dt of subsequent spikes in a train were 33 ± 4 mV, 23 ± 3 ms and 5 ± 1 mV/ms, respectively (n = 14). The changes in amplitude, duration and dV/dt were more pronounced in some neurons than in others (Fig. 3.2 vs. Fig. 3.3).

From membrane potentials of ~ -60 mV, hyperpolarizing current pulses (-10 to -300 pA, 400 ms) were followed by a rebound spike at the offset of the current pulse in 78% of recorded neurons. Application of 600 μM Ni^{2+} (6 minutes) abolished the rebound spike, confirming that it was an LTS (Hernandez-Cruz and Papé, 1989; Fig. 3.2Bb). One or two depolarizing spike potentials were evoked at the apex of the LTS. In all cases, the first spike was larger in amplitude and shorter in duration than the subsequent spike. For example, the mean amplitude, duration and dV/dt of the first spike in a burst were 66 ± 4 mV, 4 ± 1 ms and 46 ± 4 mV/ms, respectively (n = 9; Fig. 3.2Bc). The mean amplitude, duration and dV/dt of the second evoked spike in a burst were 35 ± 6 mV, 5 ± 1 ms and 31 ± 3 mV/ms, respectively (n = 9; Fig. 3.2Bc). It should be noted that three spikes were observed in two neurons. In these cases, the amplitude, duration and dV/dt of the third spike were similar to the characteristics of the second spike (data not shown).

Under current clamp conditions, activation of the hyperpolarization-activated cation current (I_h) is characterized by a voltage- and time-dependent depolarizing "sag", which typically occurs in thalamic neurons displaying an LTS (McCormick and Papé, 1990). A prominent voltage sag in ethmoid neurons was not detected (n = 48).

3.2.1 Effects of QX-314 on passive and active properties

We sought to investigate the effects of QX-314 on the firing properties of ethmoid neurons. The local anaesthetic, QX-314, is an analog of lidocaine and is potent in blocking neuronal Na^+ channels from the intracellular side of the plasma membrane (reviewed by Butterworth and Strichartz, 1990). Since QX-314 was applied intracellularly, the control membrane properties or spike characteristics before application of the drug could not be determined. However, comparing the passive and active properties during intracellular application of QX-314 to the passive and active properties of neurons in the absence of QX-314 application was helpful in determining the effects of QX-314.

During intracellular application of QX-314 (3 mM, ~10 minutes), the average resting membrane potential was -59 mV. Comparing this value to the average resting membrane potential of ethmoid neurons in the absence of QX-314, revealed a significant decrease, i.e. hyperpolarization ($n = 4$, $P < 0.05$, unpaired *t*-test).

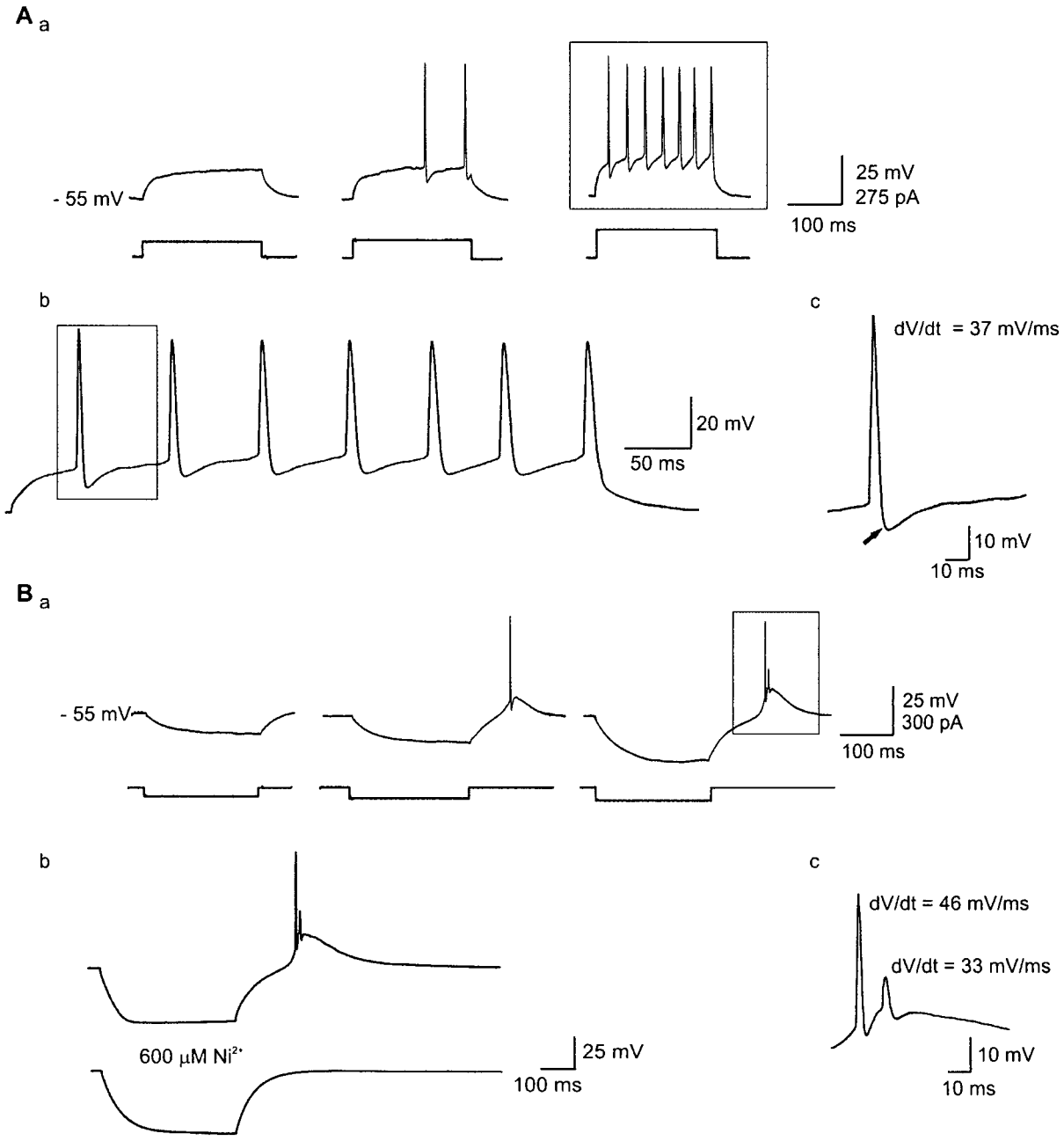

Figure 3.2 Tonic and burst firing in ethmoid neurons. *Aa*, Current pulses from -55 mV generated tonic firing. Note that increasing current injection increased the number of observed spikes. *Ab,* Enlargement of inset in Aa. Note the decrease in amplitude and increased duration of spikes within the train. *Ac,* Example of a typical first spike in a train (enlargement of inset in Ab). Note the large amplitude, short duration, fast dV/dt and AHP (black arrow) followed by a bi-phasic repolarization. *Ba*, Hyperpolarizing current pulses from -55 mV from the same neuron generated a rebound LTS with one or two spikes. *Bb,* In a different neuron, application of Ni^{2+} (600 μM, 6 minutes) abolished the LTS. *Bc,* Enlargement of inset in Ba. Note the difference in amplitude, duration and dV/dt between the first and second spike.

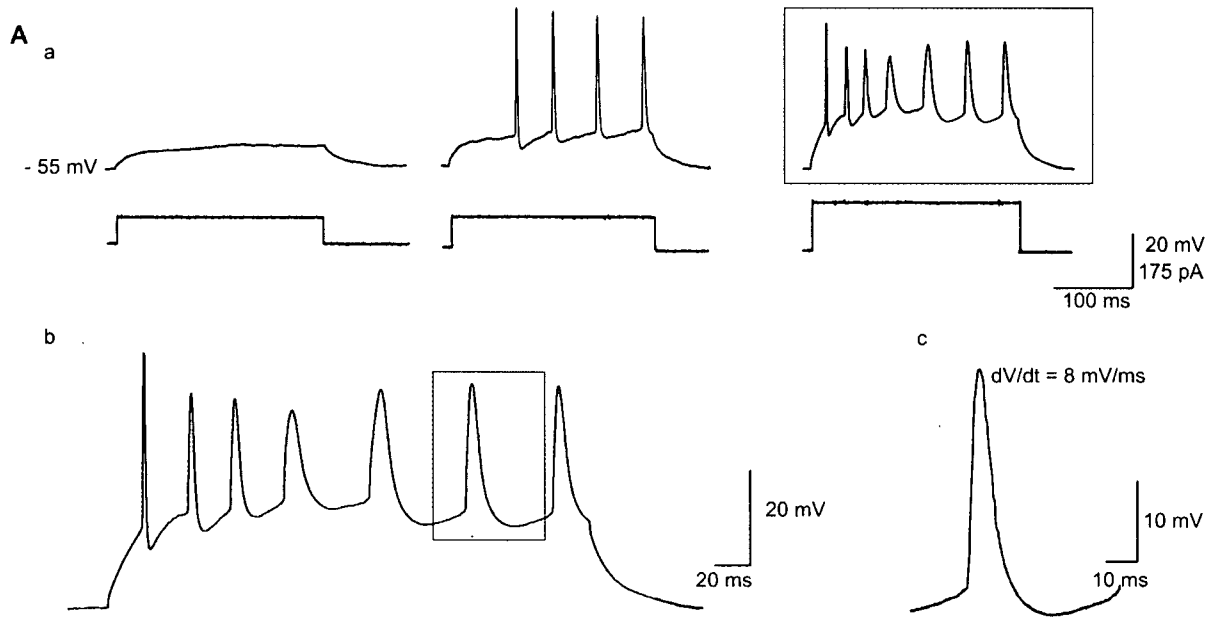

Figure 3.3 Tonic firing showing spikes with smaller amplitude and longer duration in a single ethmoid neuron. *Aa*, From a membrane potential of -55 mV, increasing the current pulse generated spikes with reduced amplitude, prolonged duration and slower dV/dt values. *Ab*, Note the apparent change in spike characteristics within the train (enlargement of inset in Aa). *Ac*, Enlargement of a typical low amplitude, long duration, slow dV/dt spike (from inset in Ab).

The mean input resistance during QX-314 application was 1300 ± 481 MΩ (n = 4). Using the same approach to compare changes in input resistance revealed a significant increase (~270%) in input resistance during intracellular application of QX-314 ($P < 0.05$, unpaired *t*-test). The mean τ_m during application of QX-314 was 97 ± 9 ms, which was also a significant increase (~180%) compared to control ($n = 4$, $P < 0.05$, unpaired *t*-test).

The most striking effects of QX-314 were on tonic firing (Fig. 3.4). The mean amplitude, duration and dV/dt values of observed spikes in the presence of QX-314 were 28 ± 3 mV, 124 ± 12 ms and 0.6 ± 0.3 mV/ms, respectively (n = 3). In these neurons,

hyperpolarizing current pulse injections did not evoke an LTS. However, in another neuron, we observed burst firing in the presence of QX-314 but did not observe tonic firing. In this neuron, only one spike was generated at the peak of the LTS (data not shown).

In summary, the apparent partial blockade of spikes by QX-314 suggested that Na^+ currents are important for spike generation. The remaining resistance of spikes to QX-314 application implied that Na^+ currents may not be the only currents involved in generating depolarizing spikes during tonic firing.

3.2.2 Effects of Ni^{2+} on passive and active properties

We tested the effects of the T– and R– type Ca^{2+} channel antagonist Ni^{2+}, to investigate if these channels contributed to spike generation evoked by current injection. At membrane potentials of ~ -60 mV, application of Ni^{2+} (500 μM, 3-6 minutes) changed input resistance but had no effects on other passive membrane properties. In three out of six neurons, Ni^{2+} decreased input resistance by a mean value of 36 ± 14%. In the remaining three neurons, Ni^{2+} increased input resistance by 20 ± 5%.

When burst firing was present, application of Ni^{2+} abolished the LTS (n = 3). Increasing the concentration of applied Ni^{2+} to 600 μM (6 minutes) abolished all tonic and burst firing (n = 1; Fig. 3.5B).

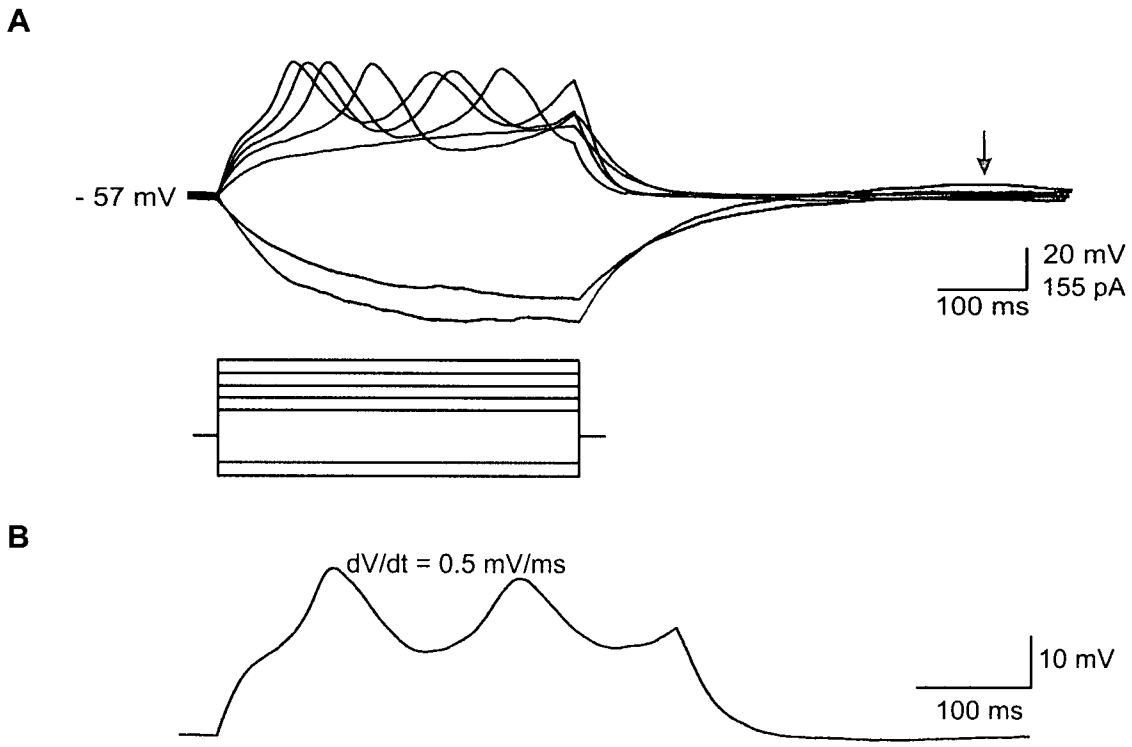

Figure 3.4 Effects of intracellular application of QX-314 in a single ethmoid neuron. *A,* During intracellular application of QX-314 (3 mM, ~10 minutes), direct depolarizing current pulse injections from -57 mV evoked spikes with small amplitude, long duration and slow dV/dt values. Hyperpolarizing current pulses from -57 mV did not evoke a pronounced rebound LTS at the termination of the pulse, although a small rebound LTS may have been generated (gray arrow). *B,* Enlargement of tonic firing from A (current pulse was 255 pA, not shown). Note the apparent reduced amplitude, prolonged duration, and reduced dV/dt of evoked spikes.

During tonic firing, application of Ni^{2+} (500 μM, 4-6 minutes) did not affect the first spike evoked in a train but decreased the number of subsequent spikes observed in a train by 61% (n = 5; Fig. 3.5A). The mean amplitude, duration and dV/dt of the first evoked spike in a train in the control were 62 ± 1 mV, 5 ± 0.1 ms and 39 ± 1 mV/ms. The mean amplitude, duration and dV/dt of the first evoked spike in a train during application of Ni^{2+} were 64 ± 1 mV, 6 ± 0.1 ms and 33 ± 1 mV/ms. The effects of Ni^{2+} on the

characteristics of the first spike were not significant (n = 5, P > 0.05, paired *t*-test). The effects of Ni^{2+} in all recordings were not reversible during a 60 minute washout of drug (n = 3).

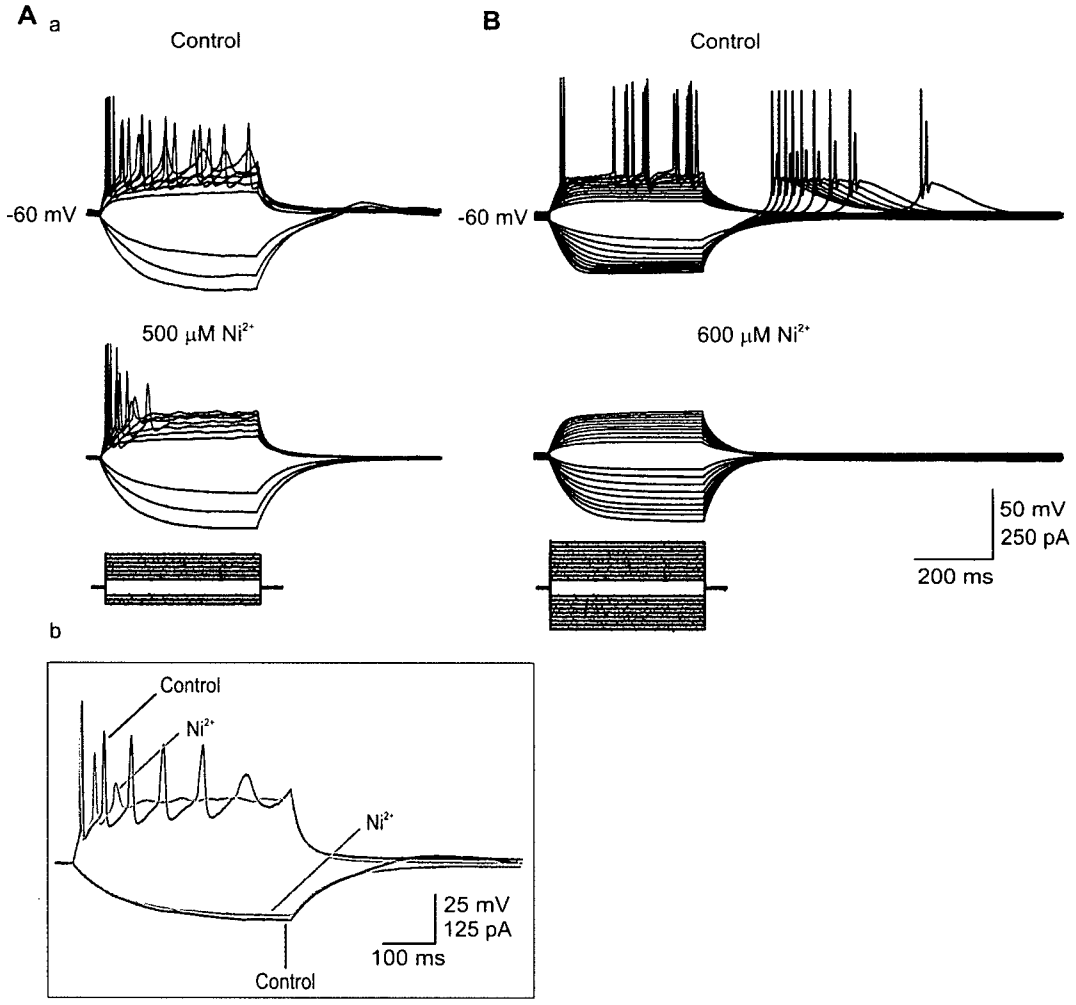

Figure 3.5 Effects of Ni^{2+} on firing properties in two ethmoid neurons. *Aa,* From a membrane potential of -60 mV, application of Ni^{2+} (500 μM, 4 minutes) did not affect the first evoked spike in a train but decreased the frequency of subsequent spikes. *Ab,* Overlay of voltage responses during control and Ni^{2+} application from Aa (pulse amplitude was 100 pA, not shown). *B,* From a membrane potential of -60 mV, application of Ni^{2+} (600 μM, 6 minutes) abolished all tonic and burst firing. These effects were not reversible on washout of drug (~60 minutes).

In summary, these data suggested that L- and R-type Ca^{2+} currents are likely not important in the generation of the first spike but may be involved in the generation of subsequent spikes evoked in a train.

3.3 Stimulation of the medial lemniscus

Stimulation of the medial lemniscus evoked depolarizations in all recorded neurons (n = 44). The evoked depolarizations were divided into two groups based on the number of apparent peaks observed (Table 3.2). Group I responses were monophasic depolarizations with a mean amplitude and duration of 6 ± 1 mV and 16 ± 20 ms, respectively (n = 26; Fig. 3.6). The mean latency of group I depolarizations was 2 ± 0.4 ms (n = 26). Group II depolarizations were characterized by two peaks. The mean amplitude of the first and second peak were 27 ± 15 mV and 15 ± 5 mV, respectively (n = 18; Fig. 3.6). The mean duration and latency of group II depolarizations were 134 ± 23 ms and 1 ± 0.1 ms, respectively (n = 18). The latency of group I and group II depolarizations were significantly different ($P < 0.05$, unpaired *t*-test).

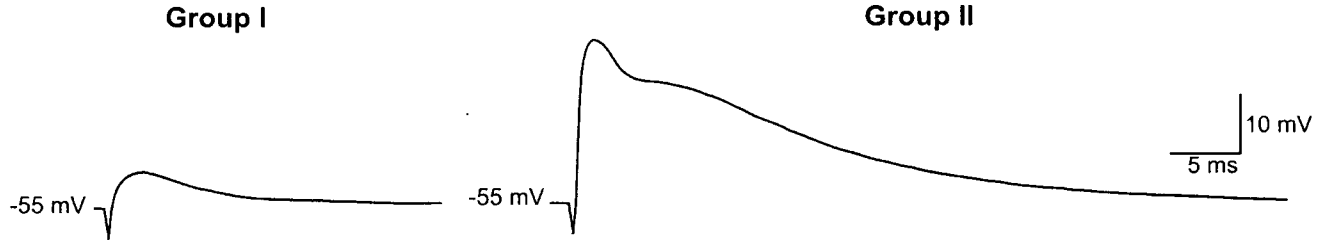

Figure 3.6 Example of group I and group II depolarizations in two ethmoid neurons. From a membrane potential of -55 mV, electrical stimulation of the medial lemniscus generated a depolarization with one apparent peak (group I) or a depolarization with two apparent peaks (group II). Traces of group I and group II responses are averages of 10 recordings.

We did not investigate significance of the amplitude or duration between group I and group II because the onset of the apparent second peak of group II could not be determined. Hence, a test of significance would not have been meaningful.

Table 3.2 Characteristics of group I and group II depolarizations evoked by stimulation of the medial lemniscus.

Depolarization Group	Number of Peaks	Latency (ms)	Amplitude (mV)		Duration (ms)
			Peak 1	Peak 2	
I (n = 26)	1	2 ± 0.4*	6 ± 1	N/A	16 ± 20
II (n = 18)	2	1 ± 0.1*	27 ± 15	15 ± 5	134 ± 23

Values are ± SEM. N/A Not Applicable. * $P < 0.05$, unpaired *t*-test.

3.4 Pharmacology

By applying various receptor antagonists, we sought to investigate the pharmacological nature of the medial lemniscal evoked depolarizations. Unless otherwise stated, ethmoid neurons were held at a membrane potential of ~ -60 mV.

3.4.1 Effects of kynurenate

As stated in the Introduction, all known excitatory medial lemniscal synapses are glutamatergic (Salt and Eaton, 1996; Castro-Alamancos, 2002). Hence, we first examined the effects of the non-specific ionotropic glutamate receptor antagonist, kynurenate (1 mM, 10 minutes). Application of kynurenate had no apparent effects on current evoked firing (Fig. 3.7A). The effects of kynurenate were not tested on group I

depolarizations, however when applied to group II depolarizations, kynurenate decreased the amplitude of the first peak by 22 ± 9% and decreased the second peak by 40 ± 2% (n = 3; Fig. 3.7B).

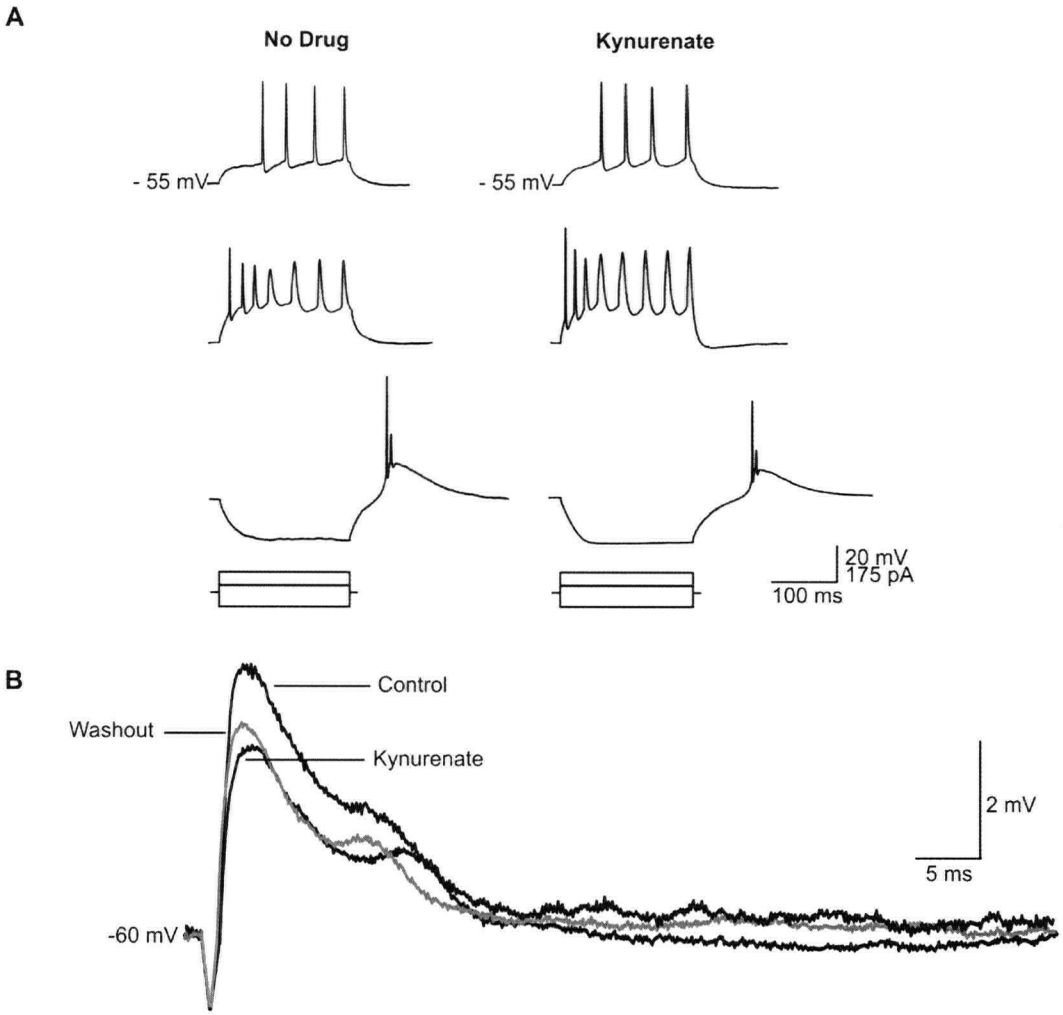

Figure 3.7 Effects of kynurenate in a single ethmoid neuron. *A*, From a membrane potential of -55 mV, application of kynurenate (1 mM, 10 minutes) did not affect the occurrence of tonic or burst firing. *B,* From a membrane potential of -60 mV, application of kynurenate to a group II depolarization decreased the first and second peak. These effects were partly reversible on washout of drug (~45 minutes). Responses in B are averages of 10 recordings.

In summary, it appeared that depolarizations evoked by medial lemniscal stimulation are only partly sensitive to kynurenate, suggesting that ethmoid neurons receive other depolarizing inputs in addition to glutamatergic inputs. Since medial lemniscal evoked inhibition in ventrobasal neurons has been shown to be insensitive to kynurenate (Ghavanini et al., 2005), 1 mM kynurenate was included in the control aCSF to isolate the kynurenate-insensitive depolarization.

3.4.2 Effects of bicuculline

A complex interplay of excitatory and inhibitory synaptic mechanisms take place on neurons of the dorsal thalamus (Sherman and Guillery, 1996). Since recordings were typically performed at potentials more negative than E_{Cl} (~ -53 mV), activation of Cl^- channels would depolarize ethmoid neurons. Hence, we investigated the effects of the $GABA_A$ receptor antagonist, bicuculline.

When neurons were held at -45 mV, a potential more positive than E_{Cl}, application of bicuculline increased the amplitude of group I depolarizations by ~18% (n = 2; Fig. 3.8A). When neurons were held at -65 mV, a potential more negative than E_{Cl}, application of bicuculline decreased the amplitude by ~20% (n = 2; data not shown).

From membrane potentials near -65 mV, application of bicuculline to group II depolarizations had no effects on the first peak and decreased the second peak by ~23% (n = 2; Fig. 3.8B).

These results suggested that $GABA_A$ergic inhibition mediates part of the response evoked by medial lemniscal stimulation. Since we were interested in identifying receptors that could cause excitation, we included bicuculline in the control aCSF.

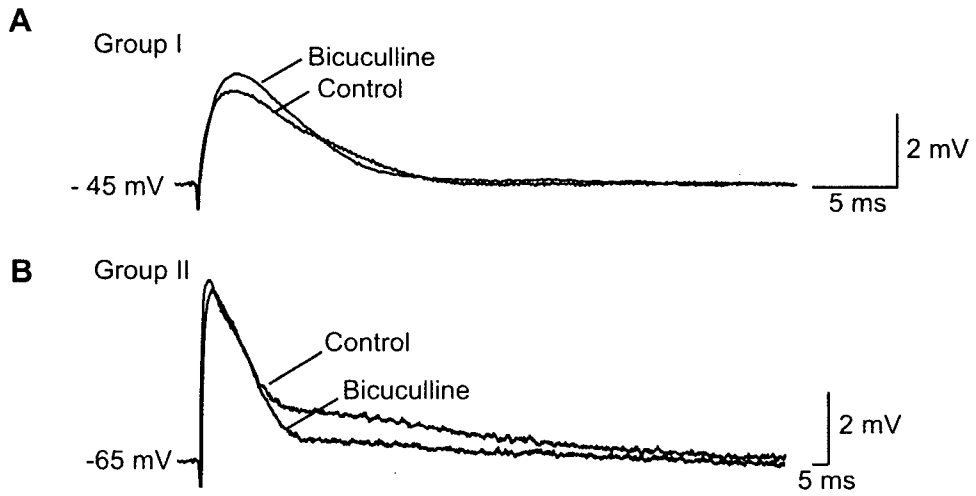

Figure 3.8 Effects of bicuculline in two ethmoid neurons. *A,* Bicuculline (20 μM, 10 minutes) increased the amplitude of a group I depolarization when an ethmoid neuron was held at a membrane potential more positive than E_{Cl} (~ -53 mV). *B,* Bicuculline decreased the amplitude of the second peak of a group II depolarization when an ethmoid neuron was held at a membrane potential more negative than E_{Cl}. Responses are averages of 10 recordings.

Table 3.3 Effects of kynurenate and bicuculline on passive membrane properties of ethmoid neurons

Property	Control (n = 6)	Kynurenate (1 mM) + Bicuculline (20 μM) (n = 21)
Resting membrane potential (mV)	-53 ± 8	-54 ± 4
R_i (MΩ)	670 ± 75	503 ± 25
τ_m (ms)	64 ± 19	53 ± 7

Values are mean ± SEM.

The effects of kynurenate and bicuculline on ethmoid membrane properties are summarized in Table 3.3. Kynurenate and bicuculline did not have significant effects on the membrane properties of ethmoid neurons ($P > 0.05$, unpaired *t*-test).

3.4.3 Effects of strychnine

In light of the recent demonstration of strychnine-sensitive inhibition in ventrobasal thalamus (cf. Introduction), we sought to investigate the effects of glycine receptor antagonist, strychnine. Application of strychnine (2 μM, 10 minutes) did not affect the amplitude of medial lemniscal evoked depolarizations (n = 3; Fig 3.9). These results suggested that glycine receptors were not a prominent factor in medial lemniscal evoked responses in ethmoid neurons.

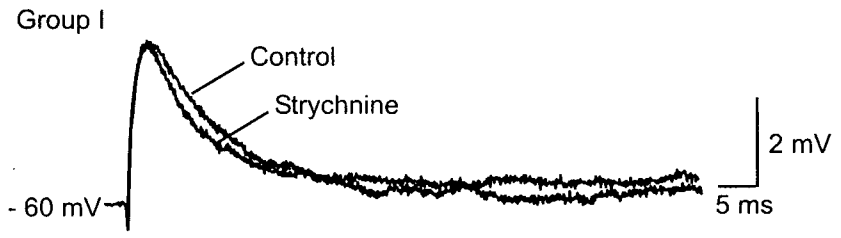

Figure 3.9 Effects of strychnine in a single ethmoid neuron. From a membrane potential of -60 mV, application of strychnine (2 μM, 10 minutes) did not affect the amplitude of a group I depolarization. Responses are averages of 10 recordings.

3.4.4 Effects of Ni^{2+}

To examine whether Ca^{2+} currents contributed to the depolarization evoked by electrical stimulation of the medial lemniscus, we applied the T– and R– type Ca^{2+} channel antagonist, Ni^{2+}. From a resting membrane potential of -45 mV, application of Ni^{2+} (500

μM, 4-6 minutes) decreased the mean amplitude of group I depolarizations by 83% in one neuron and by 30% in a second neuron (Fig. 3.10). Application of Ni^{2+} to group II depolarizations did not affect the first peak but abolished the second peak (n = 1; Fig. 3.10). Application of Ni^{2+} to a second group II depolarization, did not affect the first peak and decreased the second peak by 32%. In summary, these data suggested that L- and R-type Ca^{2+} currents may be involved in generating the depolarization evoked by stimulation of the medial lemniscus.

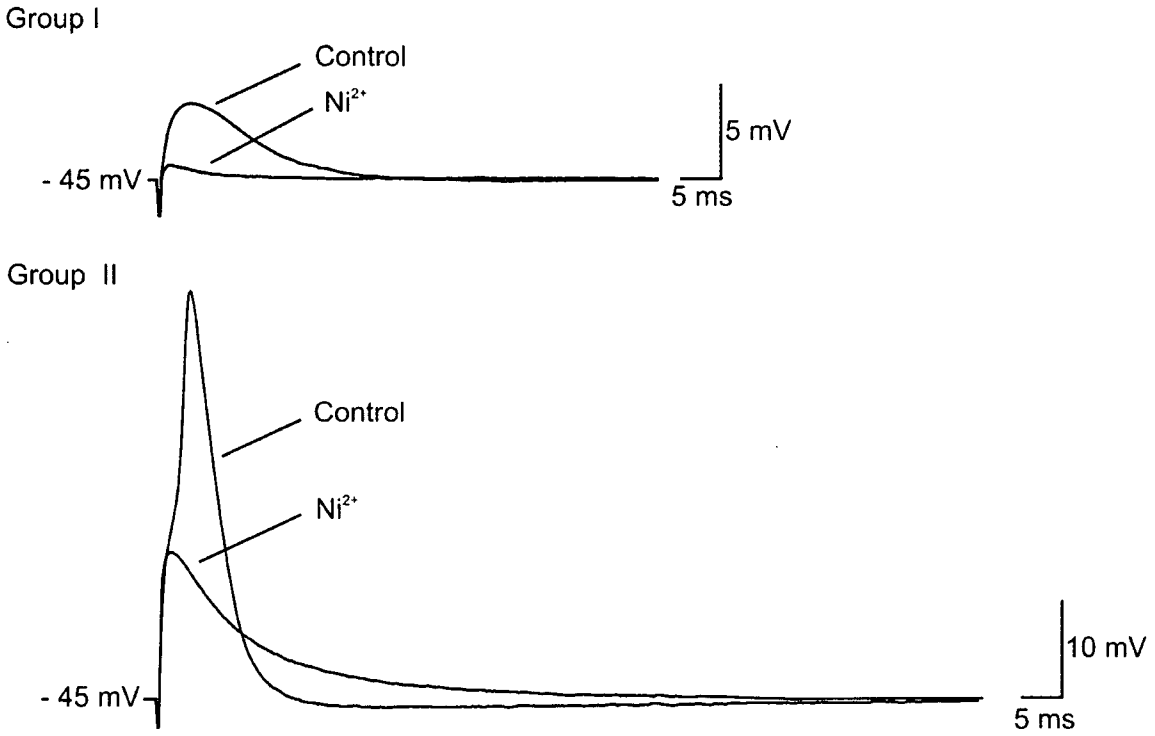

Figure 3.10 Effects of Ni^{2+} in two ethmoid neurons. From a membrane potential of -45 mV, application of Ni^{2+} (500 μM, ~4 minutes) decreased the amplitude of a group I depolarization and abolished the second peak of a group II depolarization. These effects were not reversible on washout of drug (~60 minutes). Responses are averages of 10 recordings.

3.4.5 Effects of phentolamine

Since α-adrenergic receptors are expressed in regions near the ethmoid (cf. Introduction), we investigated the effects of non-specific α-adrenergic antagonist, phentolamine, on the amplitude of medial lemniscal evoked depolarizations.

Phentolamine at 5 μM, 20 μM, 50 μM and 100 μM did not reveal apparent concentration dependent effects on the amplitude of group I or group II depolarizations (n = 3-9). Thus, we pooled the effects of phentolamine.

When applied to nine group I depolarizations, phentolamine (10-12 minutes) decreased the amplitude of one neuron by 10% and decreased the amplitude of a second neuron by 50%. Phentolamine also increased the amplitude of group I depolarizations by 20 ± 9% (n = 5). Phentolamine had no effects on the remaining group I depolarizations (n = 2; Fig. 3.11).

When applied to group II depolarizations, phentolamine had no effects on the first peak and partly decreased the amplitude of second peak by ~29% (n = 2). In three out of six group II depolarizations, phentolamine decreased both the first and second peak by a mean value of 42 ± 6% and 85 ± 7%, respectively. These effects were partly reversible on washout of drug. For example, in one neuron, the first peak recovered to 96% and the second peak recovered to 69% of control (Fig. 3.11C). Phentolamine had no significant effect on the amplitude of the remaining neuron.

In summary, group I and group II depolarizations were partly sensitive to application of phentolamine. Thus, it is possible that channels sensitive to phentolamine may participate in the medial lemniscal evoked depolarization of ethmoid neurons.

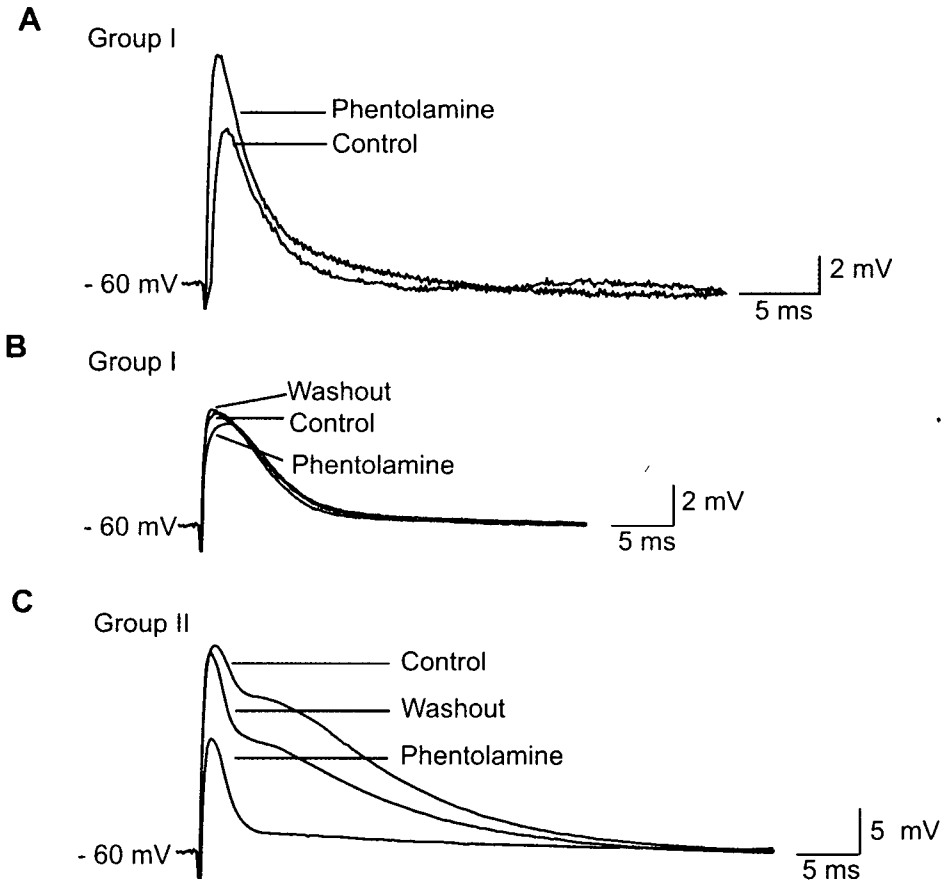

Figure 3.11 Effects of phentolamine in three ethmoid neurons. From a membrane potential of -60 mV, application of phentolamine (20 μM, 12 minutes) increased the amplitude of a group I depolarization in one neuron *(A)* and decreased the amplitude of a group I depolarization in another neuron *(B)*. Effects in B were reversible on washout (~45 minutes). *C*, Phentolamine (20 μM, 10 minutes) decreased the amplitude of the first and second peak of a group II depolarization. These effects were reversible on washout of drug, the first peak recovered to 96% and the second peak recovered to 69% of control. All responses are averages of 10 recordings.

3.4.6 Effects of suramin

It is known that many glutamatergic synaptic vesicles contain adenosine triphosphate (ATP; Harvey et al., 2000). Since medial lemniscal evoked depolarization of ethmoid neurons appeared to be partly mediated by glutamate, we hypothesized that ATP may be co-released with glutamate. The non-selective purinergic-2 (P2) receptor antagonist, suramin (100 μM, 10 minutes) did not affect the amplitude of group I or group II depolarizations (n = 3; Fig. 3.12). These lack of effects of suramin on medial lemniscal evoked depolarizations suggested that P2 receptor mediation of the response was unlikely.

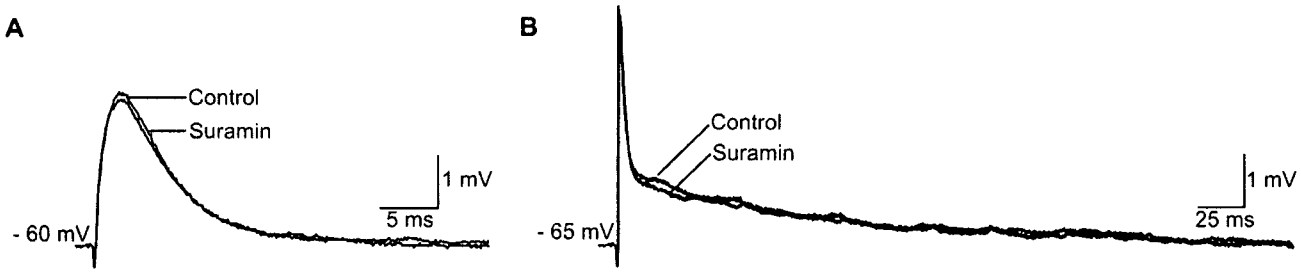

Figure 3.12 Effects of suramin in two ethmoid neurons. *A,* From a membrane potential of -60 mV, application of suramin (100 μM, 10 minutes) did not affect the amplitude of a group I depolarization. *(B)* From a membrane potential of -65 mV, application of suramin (100 μM, 10 minutes) did not affect the amplitude of a group II depolarization. Traces are averages of 10 recordings.

3.4.7 Effects of mecamylamine and pancuronium

Since there is a widespread expression of various nicotinic receptor types in the dorsal thalamus (cf. Introduction), we considered the possibility that nicotinic receptors may mediate the medial lemniscal evoked depolarization. Since there are many subtypes of neuronal nicotinic receptors, we applied non-specific nicotinic receptor antagonists, mecamylamine and pancuronium. During medial lemniscal stimulation, mecamylamine

(10-30 μM, 10 minutes) increased the amplitude by 28% in one group I depolarization but did not have effects on four other tested group I depolarizations (Fig. 3.13A). Application of mecamylamine did not affect the amplitude of group II depolarizations (n = 1). Application of pancuronium (40 μM, 10 minutes), did not have effects on the amplitude of group I depolarizations (n = 2; data not shown). In summary, these results suggested that nicotinic receptors likely do not contibute to the medial lemniscal evoked depolarization of ethmoid neurons.

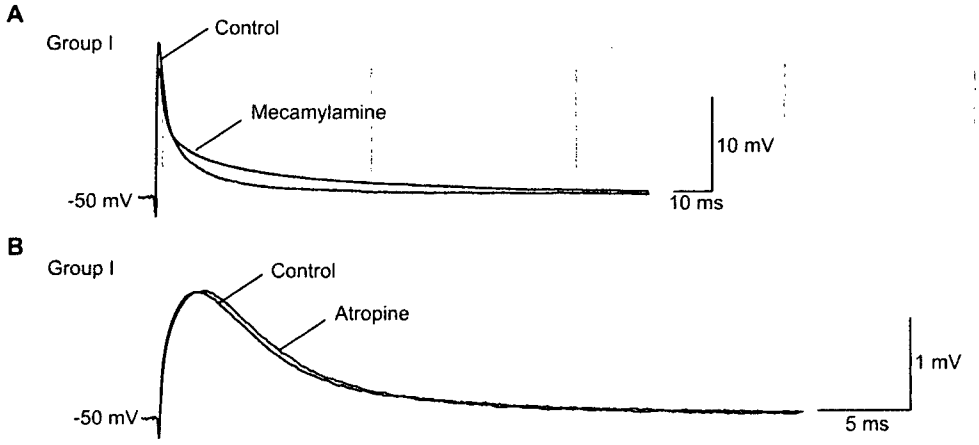

Figure 3.13 Effects of acetylcholine receptor antagonists in two ethmoid neurons. *A*, From a membrane potential of -50 mV, application of mecamylamine (20 μM, 10 minutes) decreased the amplitude of a group I depolarization. *B*, From a membrane potential of -50 mV, application of atropine (50 μM, 10 minutes) did not affect the amplitude of a group I depolarization. Responses are averages of 10 recordings.

3.4.8 Effects of atropine

Since muscarinic receptor subtypes are expressed throughout all dorsal nuclei (cf. Introduction), we considered the contribution of these receptors to the medial lemniscal evoked depolarization of ethmoid neurons. From a membrane potential of -50 mV, application of muscarinic receptor antagonist, atropine (50 μM, 10 minutes), did not

affect the amplitude of group I or group II depolarizations (n = 3; Fig. 3.13B). Thus, it seemed unlikely that muscarinic receptors are involved in the medial lemniscal evoked depolarization.

3.4.9 Effects of methylsergide

Functional 5-HT_{1A} and 5-HT_7 receptors have been demonstrated electrophysiologically in regions near the ethmoid nucleus (cf. Introduction). Hence, we considered a 5-HT receptor contribution to the medial lemniscal evoked depolarization. Application of the non-selective 5-HT_1, 5-HT_2, 5-HT_7 receptor antagonist, methylsergide (10 μM - 20 μM, 10 minutes) did not affect the amplitude of group I depolarizations (n = 3). When applied to group II depolarizations, methylsergide had no effects on the amplitude in one neuron and decreased the amplitude of the first and second peak by 25% in a second neuron (Fig. 3.14A). This evidence is not conclusive for 5-HT_1, 5-HT_2, 5-HT_7 receptor involvement in medial lemniscal evoked depolarization.

3.4.10 Effects of haloperidol

Anatomical studies show high expression of D_2 receptors in dorsal nuclei (cf. Introduction). Hence, we investigated the D_2 receptor antagonist, haloperidol (300 μM, 10 minutes). Application of haloperidol did not affect the amplitude of medial lemniscal evoked depolarizations (n = 3; Fig. 3.14B). Thus, it is unlikely that D_2 receptors mediate the medial lemniscal evoked depolarization.

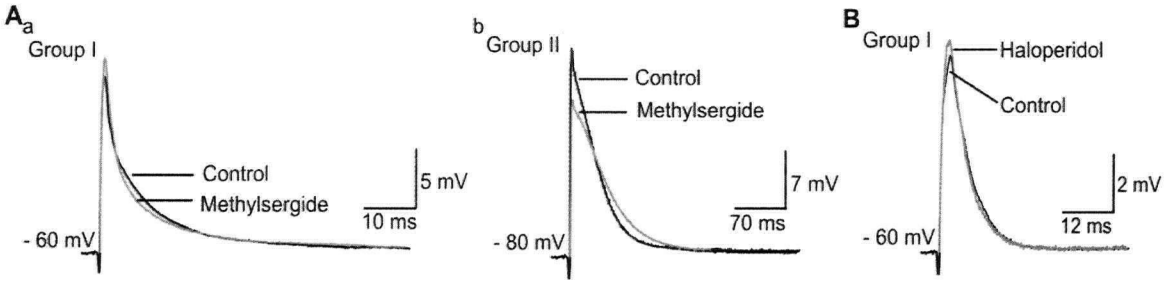

Figure 3.14. Effects of methylsergide and haloperidol in three ethmoid neurons. *Aa,* From a membrane potential of -60 mV, application of methylsergide (20 μM, 10 minutes) did not significantly affect the amplitude of a group I depolarization. *Ab*, From a membrane potential of -80 mV, application of methylsergide (20 μM, 10 minutes) decreased the amplitude of the first and second peak. *B,* From a membrane potential of -60 mV, application of haloperidol (300 μM, 10 minutes) did not affect the amplitude of a group I depolarization. Responses are averages of 10 recordings.

3.5 Effects of carbenoxalone

Many neurons in the CNS communicate through electrical synapses, defined as gap junction-mediated connections. Gap junctions are reciprocal pathways for ionic current and small organic molecules (reviewed by Connors and Michael, 2004). Hence, we sought to investigate if gap junctions were a feature of ethmoid neurons.

Application of gap junction/hemichannel antagonist, carbenoxolone (200 μM, 10-12 minutes; D'Antuono et al., 2005) had effects on membrane input resistance (Fig. 3.15A). In two out of four neurons, carbenoxalone increased the input resistance by ~30%, which was associated with an increase in τ_m by ~10%. In a third neuron, carbenoxalone decreased input resistance by 31% and decreased τ_m by 27%. Carbenoxalone had no effects on the passive properties of the remaining neuron. Furthermore, application of carbenoxalone did not have apparent effects on spikes generated in tonic and burst firing mode (n = 4).

Next, we determined the effects of carbenoxalone on group I and group II depolarizations. Application of carbenoxalone (200 μM, 12-15 minutes) increased the amplitude of a group I depolarization by 383% in one neuron and increased the amplitude by 15% in a second neuron.

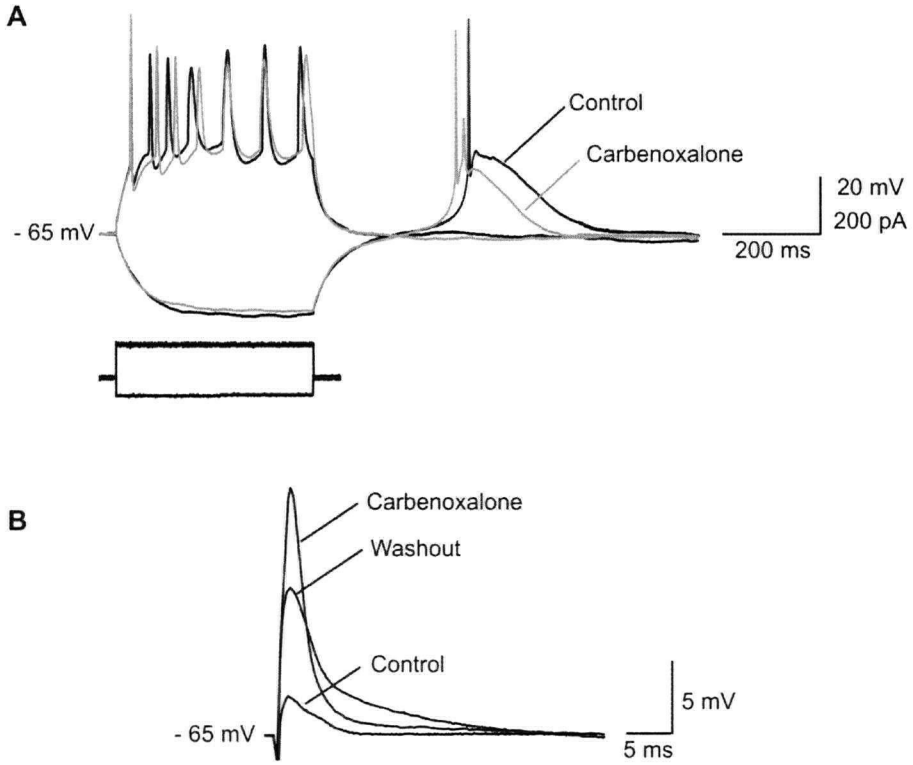

Figure 3.15 Effects of carbenoxalone in a single ethmoid neuron. *A,* From a membrane potential of -60 mV, application of carbenoxalone (200 μM, 15 minutes) did not have significant effects on spikes evoked during tonic and burst firing. *B*, From a membrane potential of -60 mV, application of carbenoxalone (200 μM, 15 minutes) increased the amplitude of a group I depolarization. Responses in B are averages of 10 recordings.

These effects were partly reversible during a 60 minute washout of carbenoxalone (Fig. 3.15B). Carbenoxalone did not have significant effects on the amplitude of group II

depolarizations (n = 2). There was an association between carbenoxalone effects on membrane properties and its effects on the amplitude of group I depolarizations. In both cases, when carbenoxalone increased input resistance it also caused an increase in amplitude. In summary, these data are not conclusive for the presence of gap junctions in ethmoid neurons.

Chapter 4: Discussion

4.1 Summary of the results

This thesis studied passive and electrical properties of thalamic ethmoid neurons. A major objective was to assess the possibility that thalamic ethmoid neurons provide a feasible link between medial lemniscal stimulation and ventrobasal inhibition. The hypothesis was that electrical stimulation of the medial lemniscus depolarized ethmoid neurons in the presence of kynurenate.

Investigation of the passive membrane properties revealed a resting membrane potential, input resistance and τ_m values of ~ -53 mV, 670 MΩ and 64 ms, respectively. Characterization of the firing properties revealed that ethmoid neurons can generate spikes in the tonic and burst firing mode. An interesting feature of tonic firing was the presence of spikes with various amplitudes, durations and dV/dt values. Application of Na^+ channel antagonist, QX-314, and R- and T-type Ca^{2+} channel antagonist, Ni^{2+}, suggested that both Na^+ and Ca^{2+} currents were important for the generation of spikes in the tonic mode.

We also successfully demonstrated that stimulation of the medial lemniscus depolarized ethmoid neurons. Stimulation of the medial lemniscus generated two general types of depolarizations in ethmoid neurons. Depolarizations exhibiting a single peak were termed group I and depolarizations with two peaks were termed group II. A significant finding was that the non-specific ionotropic glutamate receptor antagonist, kynurenate, only partly blocked these depolarizations. This was significant for two major reasons.

First, most known excitatory medial lemniscal synapses within the dorsal thalamus are glutamatergic (Salt and Eaton, 1996; Castro-Alamancos, 2002). Second, medial lemniscal evoked inhibition in ventrobasal neurons also persisted in the presence of kynurenate.

In addition to kynurenate, ten receptor antagonists were used as pharmacological tools to determine the identity of the evoked responses in ethmoid neurons. Negligible sensitivity on the amplitude of group I and/or group II depolarizations were observed during application of strychnine, suramin, mecamylamine, pancuronium, atropine, methylsergide and haloperidol. We demonstrated partial blockade of the depolarizations during application of bicuculline, phentolamine and Ni^{2+}.

4.2 Passive properties

The passive properties of ethmoid neurons were similar to reported values for other dorsal neurons (Turner et al., 1997). The mean resting membrane potential of ethmoid neurons (~ -53 mV) was more positive than the resting membrane potential for other neurons of the dorsal thalamus, which range from -60 to -80 mV (Turner et al, 1997, Tennigkeit et al., 1998; Ghavanini, 2006). This suggested that ethmoid neurons could have a reduced number of K^+ channels or an increased number of Na^+ channels open at resting potential. However, we also cannot exclude the possibility that the increased resting membrane potential was due to cell damage.

The input resistance of ethmoid neurons was in line with input resistance values reported for other neurons of the dorsal thalamus. For example, input resistances for thalamocortical neurons of the lateral geniculate nucleus and ventrobasal nuclei are ~322 MΩ and ~400 MΩ, respectively. (Turner et al., 1997; Govindaiah and Cox, 2006). However, we did observe a wide range of input resistances in ethmoid neurons (83 to 1102 MΩ). This wide range may represent a variability in resting conductances or different soma sizes in the ethmoid nucleus. Membrane input resistance can provide insight into somata size because small neurons typically have high input resistance values and vise versa. Hence, the wide range of input resistances in ethmoid neurons may suggest the possibility of different neuron types.

4.3 Direct current evoked spikes

From membrane potentials near -60 mV, ethmoid neurons generated LTSs on termination of hyperpolarizing current pulse injections. The LTSs were associated with the generation of one or two spikes. The burst firing characteristics of ethmoid neurons were similar to other dorsal thalamic neurons (Steriade et al., 1997).

A striking feature of tonic firing in ethmoid neurons was the large variability in spike amplitude, duration and dV/dt. The first spike evoked in a train shared many characteristics with neuronal Na^+ action potentials, although the duration was slightly longer (cf. Turner et al., 1997; Schwarz and Puil, 1998). An interesting feature of the tonic firing activity was the presence of spikes with small amplitudes, long durations and slow dV/dt values. Although it is possible that this variability is artifactual, it is also

likely that the differences in spike characteristics reflect differences in their generation or contributions from other membrane currents, such as slow Na^+ or slow Ca^{2+} currents. Ca^{2+} entry during Na^+ action potentials may have produced a progressive contribution to the observed spikes. This depolarizing Ca^{2+} contribution would compromise the Na^+ inactivation process and repolarization. Hence, the observed spikes would undergo a reduction in amplitude and an increase in duration (cf. Tennigkeit et al., 1998).

4.3.1 Effects of QX-314 on tonic firing

Intracellular application of QX-314 had significant effects on the membrane properties of ethmoid neurons. QX-314 hyperpolarized neurons and increased their input resistance. These effects were consistent with the blockade of a persistent Na^+ current (I_{Nap}). I_{Nap} activate at membrane potentials positive to ~ -65 mV (cf. Crill, 1996). At membrane potentials utilized in this thesis (i.e., ~ -60 mV), blockade of I_{Nap} would result in a decreased Na^+ conductance at rest, leading to a more hyperpolarized membrane potential. Furthermore, the reduced number of resting channels available to conduct ions also accounts for the increased input resistance during intracellular application of QX-314 (cf. Crill, 1996).

During application of QX-314, we did not observe the typical spike configurations during tonic firing. Spikes generated in the tonic mode had a smaller mean amplitude, longer mean duration and slower mean dV/dt. Assuming that QX-314 blocks all fast, slow and persistent Na^+ currents, these data suggested that other currents may be involved in generating spikes in the tonic mode.

4.3.2 Effects of Ni^{2+} on tonic firing

Ni^{2+} is an antagonist of R-and T-type channels. It is unlikely that T-type Ca^{2+} currents are involved in tonic firing because thalamic neurons must be hyperpolarized below approximately -65 mV for several tens of milliseconds to remove T-type Ca^{2+} inactivation (Carbone et al., 1984; Nowycky et al., 1985; Huguenard et al., 1992). Hence, it seemed likely that the Ni^{2+}-sensitive component of the evoked spikes was due to blockade of R-type Ca^{2+} channels. However, one should bear in mind that inactivation of T-channels may have different properties in ethmoid neurons than in other thalamic neurons.

High voltage activated R-type Ca^{2+} channels were originally identified as currents resistant to blockade by known Ca^{2+} channel antagonists including, ω-conotoxin MVIIC, ω-conotoxin- GVIA, ω-agatoxin IVA, and the dihydropyridines (Zhang et al., 1993; Randall and Tsien, 1995). Depending on the type of neuron, R-type currents activate at membrane potentials between -35 and -45 mV (Foehring et al., 2000). R-type channels expressed on somata and dendrites generate Ca^{2+}-dependent action potentials (Deschênes et al., 1984; Jahnsen and Llinás, 1984). For example, Ni^{2+}-sensitive Ca^{2+} spikes evoked by current pulse injection in neurons of the lateral amygdala are approximately 40 mV in amplitude and 15 ms in duration (Humeau and Lüthi, 2006). The amplitude, duration and Ni^{2+} sensitivity of Ca^{2+} spikes is similar to the broad spikes generated during tonic firing in ethmoid neurons. Interestingly, Ni^{2+} had no effects on the characteristics of the first spike evoked in a train, suggesting that the first spike was unlikely mediated by Ca^{2+}

currents. In summary, it seems possible that R-type Ca^{2+} currents make a progressive contribution to spikes evoked during tonic firing.

4.4 Medial lemniscal evoked depolarizations

Stimulation of the medial lemniscus generated depolarizations in all recorded ethmoid neurons (n = 44). It should be noted that the described group I and group II depolarizations may not represent the full range of responses that can be evoked by medial lemniscal stimulation. The differences in latency between group I and group II depolarizations were significant. This may reflect stimulation of a heterogeneous axon population, which would produce differences in axonal propagation velocity.

4.4.1 Effects of kynurenate

An interesting finding was that medial lemniscal evoked depolarizations were only partly sensitive to kynurenate. Since glutamate mediates synaptic depolarization at many synapses activated by medial lemniscal stimulation, it is possible that co-transmission and/or co-release of glutamate with an another neurotransmitter and/or neuromodulator may have occurred. We also cannot exclude the possibility of co-stimulation of non-medial lemniscal fibers, such as zona incerta or spinothalamic fibers, which traverse the area between the medial lemniscus and the ethmoid nucleus (cf. Paxinos and Watson, 1986).

4.4.2 Effects of Ni^{2+}

Interestingly, it appeared that Ni^{2+}-sensitive Ca^{2+} currents contributed to the medial lemniscal evoked depolarization of ethmoid neurons. Ni^{2+} decreased the mean amplitude of group I depolarizations and abolished or significantly reduced the second peak in group II depolarizations. As stated above, it is likely that the effects of Ni^{2+} are on R-type currents because T-type Ca^{2+} currents are inactivated in thalamocortical neurons at the membrane potentials utilized in this thesis (Carbone et al., 1984; Nowycky et al., 1985; Huguenard et al., 1992).

R-type Ca^{2+} channels are located on cell bodies, dendrites and nerve terminals (reviewed by Catterall, 2000). Channels located on cell bodies and dendrites generate Ca^{2+}-dependent action potentials. However, R-type channels located on nerve terminals are involved in neurotransmitter release (Catterall, 2000). Hence, Ni^{2+} may be blocking postsynaptic R-type Ca^{2+} channels and/or decreasing transmitter release by antagonism of presynaptic R-type Ca^{2+} channels.

4.4.3 Effects of phentolamine

Phentolamine application had diverse effects on group I and group II depolarizations. On group I depolarizations, phentolamine increased or decreased the amplitude. On group II depolarizations, phentolamine decreased both the first and the second peak. Possible reasons for phentolamine's diverse effects may have resulted from multiple sites of action.

α_1-adrenergic receptors are located predominantly on postsynaptic membranes and mediate excitation in neuronal membranes (Hardman et al., 2001), whereas α_2-adrenergic receptors are predominantly presynaptic and activation of these receptors can decrease neurotransmitter release (Aantaa et al., 1995; Lakhlani et al., 1997). Furthermore, phentolamine can also have a local anesthetic action by blocking Na^+ channels (Ledda and Marchetti, 1972; Northover, 1983).

In summary, it appeared that channels sensitive to phentolamine are involved in modulating depolarizations evoked by stimulation of the medial lemniscus. Phentolamine's diverse effects may have resulted from a pre- and/or post-synaptic antagonism of α-adrenergic receptors and/or from blockade of Na^+ currents.

4.5 Effects of carbenoxalone

Many neurons in the CNS communicate through electrical synapses, defined as gap junction-mediated connections (Connors and Michael, 2004). The results of our investigations of the gap-junction blocker, carbenoxalone, were inconclusive. Carbenoxalone decreased input resistance in two out of four neurons, suggesting the possible closure of low resistance gap junctions. Carbenoxalone did not affect ethmoid firing evoked by direct current injection, suggesting that these spikes were not generated from neurons coupled to the patched neuron. Carbenoxalone also increased the amplitude of two group I depolarizations. These effects were likely due to the concomitant increase in input resistance. Additional studies are required to examine the possibility of electrical coupling in the ethmoid nucleus.

4.6 Future directions

Further studies on ethmoid neurons will help provide insight into the physiological function of these neurons and their potential role in ventrobasal inhibition. An important question to answer is whether the depolarization evoked by medial lemniscal stimulation is synaptic. Other forms of transmission that could account for the depolarization include gap junction coupling and/or glial release of neurotransmitter and/or neuromodulator (cf. Bezzi et al., 2004; Connors and Michael, 2004) Hence, it is necessary to stimulate the medial lemniscus in the presence of tetrodotoxin (TTX), a substance that antagonizes Na^+ channels from the extracellular surface (Narahashi, et al., 1964). In the presence of TTX, stimulation of the medial lemniscus should not generate responses in ethmoid neurons if the connection is synaptic. Understanding if the connection between the medial lemniscus and ethmoid neurons is mono- or polysynaptic will also provide further insight into the nature of the medial lemniscal evoked strychnine-sensitive inhibition of ventrobasal neurons. Furthermore, dual recording of ethmoid and ventrobasal neurons will also confirm whether ethmoid neurons can generate strychnine-sensitive inhibition in ventrobasal neurons.

4.7 Conclusions

This thesis revealed several interesting features of ethmoid neurons. Ethmoid neurons had passive properties similar to other neurons of the dorsal thalamus. Ethmoid neurons also generated spikes in the tonic and burst firing mode, a feature common to most thalamocortical neurons.

We also demonstrated that stimulation of the medial lemniscus evoked depolarizations in all recorded ethmoid neurons. Pharmacological characterization of the evoked depolarizations were only partly sensitive to kynurenate. Furthermore, the depolarizations were not sensitive to strychnine and partly sensitive to bicuculline, Ni^{2+} and phentolamine. From these data, it seems feasible that ethmoid neurons could participate in a kynurenate-insensitive circuit between the medial lemniscus and neurons of the ventrobasal thalamus

The studies described in this thesis represent the first exploration in a previously uncharted nucleus. The observations provide evidence for functional similarities of ethmoid neurons to other thalamic neurons, as well as evidence for a novel input from the major somatosensory pathway, the medial lemniscus.

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