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CHOLINERGIC CONTROL OF SENSORY SYNAPTIC TRANSMISSION IN PRIMARY AND NONPRIMARY AUDITORY THALAMUS OF RAT

BY

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(B.Sc.)

This thesis is submitted as a partial fulfillment of the Ph.D. program in the Neuroscience Graduate Program.

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Dedication

This thesis is dedicated to my parents.

Author's contributions

Chapter 2 *Muscarine induces an anomalous inhibition of synaptic transmission in rat auditory thalamic neurons in vitro.*

Performed the extracellular experiments that revealed the neuronal population responses to muscarine, analyzed population data, and provided the data for figures 1, 2, and 3. Wrote the first draft of the paper and prepared figures 1 and 3.

Chapter 3 *Differential resting membrane potential regulation in primary and nonprimary auditory thalamus by muscarinic receptor activation*

Performed all experiments with the exception of the whole-cell patch recordings. Analyzed all the data. Wrote first draft of paper and prepared all illustrations.

Chapter 4 *Low threshold calcium spike-induced membrane afterhyperpolarization and its modulation by muscarinic receptors in rat auditory thalamus*

Performed all experiments and analyzed all data. Wrote the results section and figure legends of the paper and prepared all illustrations.

ABSTRACT

A large body of literature now indicates that behavior-dependent functional adaptations in the central auditory system are in large part regulated by a cholinergic modulatory input from the midbrain reticular formation. The central hypothesis behind this thesis is that acetylcholine is capable of modulating auditory synaptic transmission in a synaptic pathway-specific manner.

The auditory thalamocortical system consists of primary (lemniscal) and nonprimary (nonlemniscal) auditory thalamocortical projections that originate from two segregated cell populations located in the ventral (MGv) and dorsal (MGd) divisions of the medial geniculate body (MGB). The two pathways are known to engage in different aspects of auditory function. A thalamic explant preparation was employed for the study, in which the parallel MGB pathways, together with their sensory afferents, can be maintained and accessed in vitro for comparative electrophysiological studies. The following summarizes the main findings described in this thesis.

1. When the MGB explant is persistently exposed to acetylcholine, the evoked sensory synaptic responses from MGv neurons consist almost exclusively of a single action potential and are able to follow high-frequency stimuli. In contrast, nonprimary or MGd neurons responded to sensory pathway stimulation with a stereotyped burst of action potentials which failed to follow a stimulation frequency higher than 5 Hz.

2. Intracellular recordings revealed that muscarine induces a slow membrane depolarization in ventral neurons, which helps EPSPs trigger single action potentials but prevents them from eliciting low threshold Ca^{2+} spike (LTS)-burst complexes. An

opposite, membrane hyperpolarization response was observed in dorsal cells where muscarinic receptor activation promotes coupling between the EPSP and the LTS -burst.

3. Muscarine blocks a leak and a Ca^{2+}-dependent K^+ membrane conductance $K_{(ca)}$ in ventral MGB neurons whereas in dorsal cells it opens leak K^+ channels and largely has no effect on $K_{(ca)}$.

It is concluded that a differential modulatory mechanism mediated by muscarinic receptor activation helps create and maintain a fast synaptic responding mode in the primary thalamocortical pathway for high-fidelity sensory relay and a slower but robust bursting transmittal mechanism in the nonprimary pathway suitable for detection of sensory events and induction of long term synaptic plasticity in target cells.

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List of Abbreviations

ACh	acetylcholine
AChE	acetylcholinesterase
ACPD	trans (1S, 3R)-1-amino-1,3-cyclopentanedicarboxylic acid
ACSF	artificial cerebrospinal fluid
ADP	afterdepolarization
AHP	afterhyperpolarization
AMPA	(S)-α-amino-2,3-dihydro-5-methyl-3-oxo-4-isoxazolepropanoic acid
4-AP	4-aminopyridine
ATP	adenosine triphosphate
BIC	brachium of the inferior colliculus
CD	caudodorsal nucleus

CNS	central nervous system
CV	coefficient of variance
DC	direct current
EGTA	ethylene glycol-bis(β-aminoethyl ether) –N,N,N',N'-tetraacetic acid
EPSP	excitatory postsynaptic potential
FSL	first spike latency
GABA	γ-aminobutyric acid
GIRK	G-protein-gated inward rectifier K^+ channel
HEPES	N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid)
IC	inferior colliculus
IPSP	inhibitory postsynaptic potential
$[K^+]$	potassium ion concentration

$[K^+]_o$	extracellular potassium ion concentration
K_{Ca}	calcium-activated potassium conductance
LTP	long-term potentiation
LTS	low-threshold calcium spike
LV	lateroventral nucleus
MCh	acetyl-β-methylcholine chloride (methacholine)
MGB	medial geniculate body
MGd	dorsal division of the medial geniculate body
MGm	medial division of the medial geniculate body
MGv	ventral division of the medial geniculate body
MRA	muscarinic receptor activation

NMDA	N-methyl-D-aspartate
PLC	phospholipase C
PPTg	pedunculopontine tegmental nucleus
RAS	reticular activating system
REM	rapid eye movement
RMP	resting membrane potential
SE	standard error
TEA	tetraethylammonium
TTX	tetrodotoxin
V_m	membrane potential

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Chapter 1. General Introduction

1.1 General anatomic structure of the auditory thalamus

The thalamus is an obligatory relay for most signals transmitted to the cerebral cortex. Each sensory modality is allocated its own thalamic relay nuclei. The thalamic nuclei that project to cortex are partly surrounded by a thin shell of inhibitory GABAergic (GABA: γ-aminobutyric acid) neurons known as the reticular thalamic nucleus. These neurons do not project to cortex but instead innervate the projection nuclei and are innervated by them via thalamocortical axon collaterals. Layer six of the cortex reciprocates the thalamocortical projection and gives collaterals to the reticular thalamic nucleus. In the dorsal medial geniculate body, (see below) input from cortex comes from layer five rather than six. Within the projection nuclei, there are GABAergic inhibitory interneurons that receive cortical, reticular thalamic and ascending inputs and synapse on thalamic projection neurons. Several neuromodulatory systems innervate the thalamus (see Steriade et al., 1990b, for a review). These originate from small brainstem or basal forebrain nuclei that have large innervation territories and secrete, depending on the nucleus, acetylcholine, serotonin, noradrenaline, dopamine, or histamine (see McCormick, 1992a, for a review).

The main auditory thalamic relay nuclei are found in the medial geniculate body (MGB), situated on the lateral midbrain. In mammals, the MGB contains three divisions: the ventral MGB (MGv), the dorsal MGB (MGd) and the medial MGB (MGm) (Winer et al., 1999). In certain species a fourth division, the suprageniculate, is recognized (Winer, 1992). The MGv and MGd receive auditory signals from the inferior colliculus (IC) via axons running in a superficial white matter tract known as the brachium of the inferior colliculus (BIC).

1.2 Parallel Pathways

1.2.1 Anatomy of parallel auditory pathways

In each of three sensory modalities (vision, touch, and hearing) signals ascend to cerebral cortex in a pair of largely segregated channels operating in parallel known as the lemniscal and extralemniscal pathways (Jones, 1985b and 1991). They are also known as the primary and nonprimary pathways and remain largely segregated at the thalamic level. In the auditory thalamus of rodents and carnivores, the two pathways differ in connectivity and response characteristics (Calford and Aitkin, 1983; Graybiel, 1972; LeDoux et al., 1987; Lennartz and Weinberger, 1992; Morest, 1965). The primary auditory pathway arises in the core nucleus of the inferior colliculus, which projects to the MGv, which in turn projects to the primary auditory cortex (Anderson et al., 1980; Clarey and Irvine, 1992a and 1992b; Clarey et al., 1992; Graybiel, 1972; LeDoux et al., 1988). The nonprimary pathway starts in the shell nuclei of the inferior colliculus, the midbrain tegmental region and the spinothalamic tract (Graybiel, 1972; Iwata et al., 1992; LeDoux et al., 1988; Winer, 1992), which project to nonprimary auditory thalamus (MGd, MGm, and suprageniculate). The afferents to nonprimary auditory thalamus are polysensory and can convey signals from noxious stimuli. Nonprimary auditory thalamus projects to limbic structures such as amygdala, associational auditory cortex and striatum (Anderson et al., 1980; Doron and LeDoux, 2000; Shinonaga et al., 1994; Winer 1992). Thalamic divisions in both pathways receive a modulatory afferent from the midbrain-pontine reticular formation that is predominantly cholinergic (Fitzpatrick et al., 1989; Jones, 1985a; Levey et al., 1987). This study used MGd to represent nonprimary auditory thalamus. The pathway to MGd starts in

the pericentral nucleus of the inferior colliculus and nucleus sagulum (Calford and Aitkin 1983), with an additional projection from the nucleus of the BIC (Kudo et al., 1984). MGd projects to associational auditory cortex and possibly to amygdala, but not to striatum (LeDoux et al., 1985; Shinonaga et al., 1994). A simplified picture of parallel auditory thalamic pathways is given in Fig. 1. Of particular interest here is the caudodorsal nucleus of the MGd located at the caudal tip of the MGB. This nucleus has intense reciprocal connections with the insular or temporal polar region of the temporal cortex where lesions produce pronounced deficits in auditory function in both animals and humans (see below). Our *in vitro* electrophysiological studies of nonprimary thalamus were mainly performed on this nucleus.

Figure 1 Simplified diagram of parallel ascending pathways in the mammalian upper auditory system. Not shown: feedback projections from cortex and reticular thalamic nucleus to the MGB.

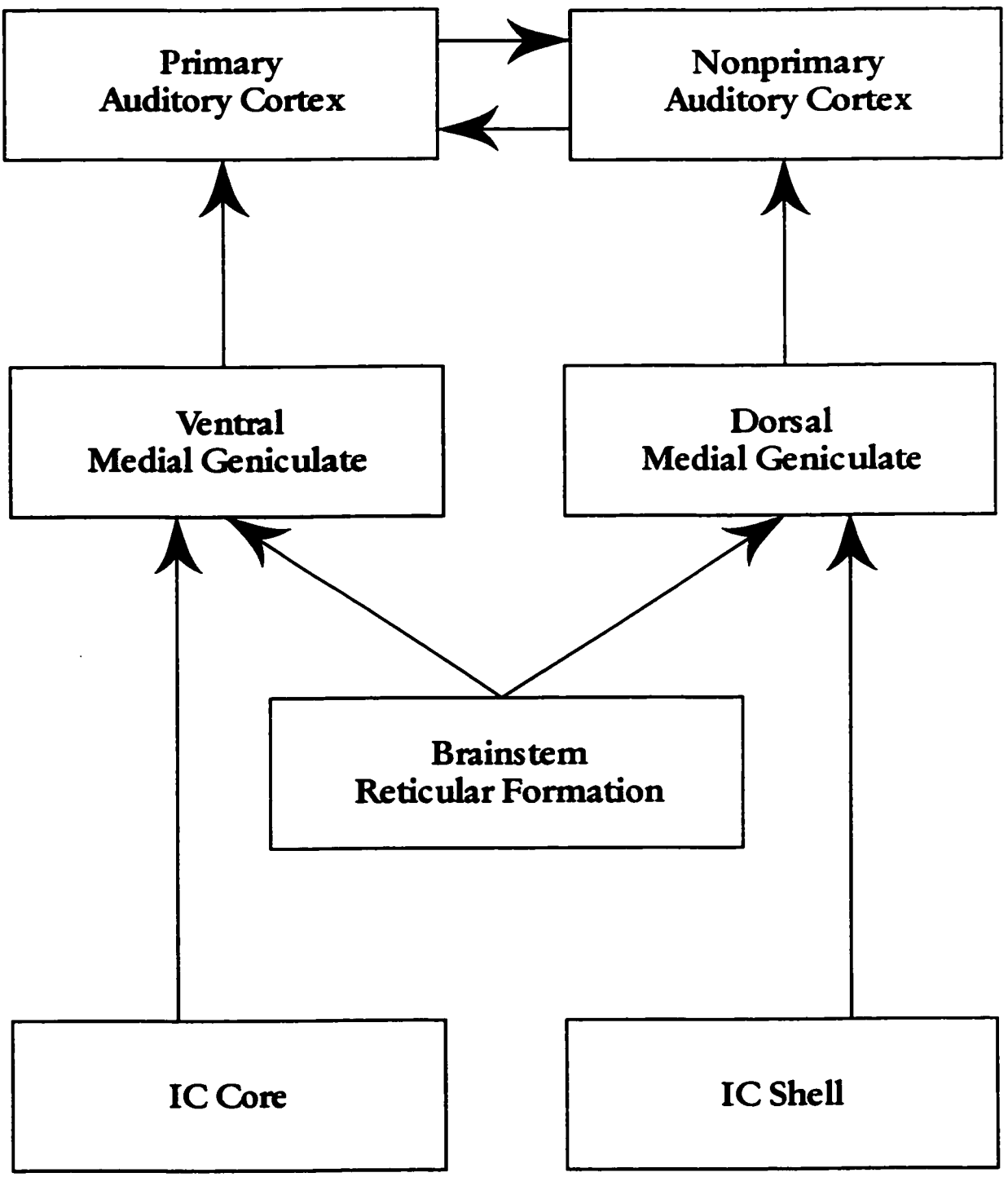
Primary
Auditory Cortex
Nonprimary
Auditory Cortex
Ventral
Medial Geniculate
Dorsal
Medial Geniculate
Brainstem
Reticular Formation
IC Core
IC Shell

Figure 1

1.2.2 Functional features of parallel auditory pathways

The primary auditory thalamocortical projection has a tonotopic map (Brugge, 1992). The maps associated with primary sensory pathways may provide a structural framework for information processing and plasticity (Diamond et al., 1999). More can be said about the function of the nonprimary auditory pathway. Lesions in the nonprimary auditory cortices are associated with severe deficits in discrimination of temporally structured sound patterns and complex sound processing (Kelly, 1973; Layton et al., 1979; Neff et al., 1975). Lesion of associational auditory cortex also impairs lexical retrieval in humans (Damasio et al., 1996). These results implicate nonprimary auditory thalamus in sound pattern recognition and lexical retrieval. Examples of complex sounds requiring nonprimary pathways for their discrimination would be species-specific vocalizations and combinations of acoustic cues (Buchwald et al., 1988; Suga et al., 1990). In bats, echo-pulse delay information is relayed to cortex by MGd. (Olsen, 1992; Suga, 1989; Suga et al., 1990). The nonprimary projection is also involved in polysensory integration (Brainard and Knudsen, 1993; Graybiel, 1972) and some forms of learning (Rogan and LeDoux, 1995; Weinberger, 1993). Such learning is initiated in nonprimary MGB where acoustic signals and unconditioned stimuli converge on single neurons (Davis, 1997; LeDoux, 1998). Lesion of nonprimary thalamus impairs auditory classical conditioning (LeDoux et al., 1986; McCabe et al., 1993; Poremba and Gabriel, 1997).

1.2.3 Single unit results in parallel auditory pathways

In vivo, primary and nonprimary thalamocortical neurons differ in many ways in their responses to sound stimuli (Calford, 1983; Kraus et al., 1994; Winer, 1992). Physiological

boundaries coinciding with anatomical boundaries have been defined within the MGB (Aitkin and Prain, 1974; Calford, 1983; Calford and Aitkin, 1983) and include a boundary between primary and nonprimary divisions. In addition to being tonotopic, responses in the primary pathway were modality specific, reliable, and fast, with a short constant latency (Aitkin and Webster, 1972; Calford, 1983; Lennartz and Weinberger, 1992; Rodrigues-Dagaeff et al., 1989). Nonprimary neurons, in contrast, showed a lower spontaneous and evoked spike activity (Aitkin and Prain, 1974; Calford, 1983; Calford and Webster, 1981; Lennartz and Weinberger, 1992) and sometimes a polysensory response (Graybiel, 1972; Lennartz and Weinberger, 1992; Pandya and Yeterian, 1985). Most MGd neurons responded with a relatively long and variable latency (Calford, 1983; Edeline et al., 1999; Hu, 1995) and many tended to habituate to repetitive stimuli (Calford, 1983; Calford and Webster, 1981). However, this last distinction between pathways also appeared at the collicular level (Aitkin et al., 1975). MGd neurons exhibited broader frequency tuning than MGv neurons (Edeline et al., 1999; Lennartz and Weinberger, 1992). The mismatch negativity was found in nonprimary auditory thalamus but not in primary auditory thalamus (Kraus et al., 1994). The mismatch negativity is obtained by recording cortical or thalamic field potentials evoked in response to a series of tone stimuli. Most of the stimuli are identical but they are interspersed with a rare deviant stimulus that has a slightly different pitch or loudness. The field potential evoked by the common stimulus is subtracted from the potential evoked by the deviant stimulus. The resulting curve of field potential difference against latency exhibits a broad negativity that indicates a neurophysiological response to stimulus change. Other phenomena peculiar to nonprimary auditory thalamus, as exemplified by the MGd, were a selective, strong response to kitten calls in adult cats (Buchwald et al.,

1988) and plasticity in frequency tuning in classical conditioning experiments (Edeline and Weinberger 1991; Weinberger, 1993).

As described below, connectivity constraints seem inadequate to completely account for the differentiation of the primary and nonprimary pathways. Thalamic relay neurons in different pathways still had distinct patterns of synaptic response after being isolated *in vitro* from the rest of the CNS (Hu, 1995; Hu et al., 1994)

1.3 *In vitro* findings on MGB physiology

Recent work from this laboratory has investigated the properties of synaptic transmission and the cellular basis of firing pattern transformation in rat MGB using an *in-vitro* brain explant preparation. The explants, maintained by artificial cerebrospinal fluid superfusion, retain in isolation from the rest of the CNS large parts of the MGv and the MGd together with their parallel axonal inputs from the brachium of the inferior colliculus. Synaptic responses can be evoked in both divisions with single BIC shocks at low frequency (1-2 Hz) and low intensity (1-2 times threshold). Two modes of response were observed: 1) a single or dual action potential, or 2) a high frequency, brief burst containing 2 to 5 action potentials (Hu, 1995; Hu et al., 1994). A given explant neuron responded in only one mode, although experimental interventions that changed resting membrane potential (RMP) could produce mode switching (see below). The single spike responses followed repetitive stimulation to above 10 Hz whereas burst mode neurons stopped responding when stimulation frequency was raised to 5 to 10 Hz. The single spike neurons had a narrow distribution of first spike latencies (FSL; 5-15 ms) with a small mean coefficient of variance (C.V.). The burst FSLs, however, were extremely variable from one stimulation to the next.

Burst FSLs were long compared to single spike FSLs and fluctuated from 10 to 70 ms with an averaged C.V. more than twice as large as that of single spikes (Hu, 1995)

When the threshold of cell discharge was measured extracellularly, the excitability of dorsal MGB neurons was found to be significantly lower than their ventral counterparts, consistent with previous *in vivo* observations (Calford, 1983; Calford and Aitkin, 1983; Clarey et al., 1992). However, when recorded intracellularly, BIC stimulation invariably elicited fast (latency < 3 ms), monosynaptic EPSPs in both ventral and dorsal cells with equal efficacy. Many dorsal cells actually exhibited a pure N-methyl-D-aspartate (NMDA) receptor-mediated EPSP. The apparent low excitability of these cells was partially caused by a more negative resting membrane potential (Vm, -65 to –75 mV), which prevented the EPSPs from reaching the threshold of Na^+ action potential generation (Hu, 1995; Hu et al., 1994; Senatorov and Hu, 1997). Such a negative resting membrane potential, however, apparently does not block the NMDA receptor response. This may be due to the fact that NMDA receptors expressed in thalamic neurons have a subunit composition (NR1/NR2D) less susceptible to voltage-dependent magnesium block (Dunah et al., 1998; Momiyama et al., 1996; Monyer et al., 1992). In subsequent experiments, it was found that the burst of fast action potentials is mediated by a low-threshold Ca^{2+} spike (LTS), which has a latency that may show wide interstimulus variations (Crunelli et al., 1989; Hu, 1995; Hutcheon et al., 1994; Jahnsen and Llinás, 1984). The induction of the LTS-burst complex in dorsal neurons depends upon two conditions: a more negative resting membrane potential, which allows de-inactivation of the LTS, and a relatively large EPSP, which acts as a trigger for LTS activation. This mechanism was termed EPSP-LTS coupling (Hu, 1995; Mooney et al., 1995;

Chapter 2) and has been shown to occur on distal dendrites of cortical and hippocampal neurons (Magee and Johnston, 1995; Markram and Sakmann, 1994).

The explant results are relevant to *in vivo* single unit and evoked potential results in MGB. Both bursts and single spike discharges can be evoked by sound stimuli *in vivo* (Aitkin and Dunlop, 1969; Calford, 1983; Dr. J. F. He, personal communication). The prominent latency jitter of BIC-evoked burst responses in explant (Hu, 1995) is paralleled by the jitter in the latency of sensory evoked potentials in auditory forebrain (Regan, 1989), and is consistent with *in vivo* single unit latency measurements in MGd (Calford, 1983; Dr. J. F. He, personal communication).

If the rate of acoustic stimulation is raised to 5-10 Hz, the middle to late latency components of the auditory evoked potentials in the thalamocortical system, but not their afferent response in the brainstem, are significantly reduced (Kraus and McGee, 1992; Regan, 1989). This is consistent with the fact that burst mode neurons do not follow 5-10 Hz stimulation in explant. When stimulation thresholds were measured in explant, the excitability of MGd neurons was less than that of MGv neurons (Hu, 1999), consistent with *in vivo* observations.

1.4 Control of resting membrane potential in MGB

Experimental interventions that change RMP produce mode switching in MGB. RMP regulation in MGB is due to intrinsic factors (ion channels and electrogenic transporters) and extrinsic factors (ligand gated receptor-channel complexes). The extrinsic factors, a focus of this dissertation, are covered more fully in the section on acetylcholine. Intrinsic differential factors are the level of expression of a hyperpolarization-activated inwardly rectifying current

known as I_h, (Hu, 1995; Senatorov and Hu, 2000) and the level of expression of the Na^+-K^+-ATPase or sodium pump (Senatorov and Hu, 1997; Senatorov et al., 1997). Thalamocortical cells utilize I_h to maintain a relatively depolarized membrane potential (above -70 mV) favoring the single-spike mode of firing (McCormick and Pape, 1990). In explant, the response mode difference between pathways is partly because MGd neurons lack I_h (Hu, 1995). The Na^+-K^+ transporter, which produces an outward electrical current as a byproduct of its operation, plays an important role in regulating the mode of synaptic response to sensory input in auditory thalamus. The pump current adds a 3 to 12 mV negative shift to the RMP of an MGB neuron. The density of alpha3 pump subunit in the plasma membrane domain was 40% higher in MGd than in MGv and the sodium pump current was significantly higher in MGd than in MGv (Senatorov and Hu, 1997).

Besides I_h and sodium pump expression, two additional mechanisms probably contribute to fewer burst mode neurons in MGv than in MGd: a stronger IPSP in MGv and a weaker NMDA component of the EPSP in MGv (Hu, 1995; Hu et al., 1994). The stronger MGv IPSP acts by shortening the EPSP to the point where it is ineffective in triggering a burst. (Rebound bursts can occur on recovery from the IPSP but such bursts have very long latencies, >200 ms, that suggest they do not contribute to sensory relay.) Glutamate acts on both NMDA and non-NMDA preferring receptors in MGB. MGd cells but not MGv cells exhibited a predominant slow EPSP due to NMDA receptors. Synaptic responses in MGd were blocked by both AMPA and NMDA antagonists. In contrast, responses in MGv were always sensitive to AMPA antagonists but were seldom sensitive to NMDA antagonists. The weaker expression of NMDA transmission in MGv appears to favor fast synaptic signaling (Hu et al., 1994), mediated by rapidly desensitizing AMPA receptors.

1.5 Acetylcholine

1.5.1 Anatomy

Afferents to thalamus are commonly subdivided into specific sensory systems and nonspecific modulatory systems (Steriade and Deschênes, 1984). The latter control the mode of thalamocortical signal transmission in various behavioral states (Jones, 1985a; Semba, 1991; Steriade and Deschênes, 1984; Steriade and Llinás, 1988; Steriade et al., 1990b; Williams et al., 1994). An important neuromodulatory system is the reticular activating system (RAS), with cell bodies located in the midbrain and pontine reticular formation. Electrical stimulation of the RAS has depolarizing (Deschênes and Hu, 1990) and disinhibitory (Hu et al., 1989) effects on thalamus. Input to thalamus from the RAS predominantly involves secretion of the neuromodulator acetylcholine (Levey et al., 1987; Steriade et al., 1990b). The cholinergic cell bodies are located near the brachium of the cerebellum and cholinergic inputs to thalamus are therefore sometimes referred to as peribrachial inputs. In rats, the equivalent reticulothalamic afferent originates in the pedunculopontine tegmental nucleus (PPTg) which contains both cholinergic and non-cholinergic neurons (Fitzpatrick et al., 1989; Hallanger et al., 1987).

1.5.2 Functional role of acetylcholine

Cholinergic neurons of the PPTg were shown in behavioral studies in cats to increase their firing rates in response to positive reinforcement (Dormont et al., 1998). PPTg neurons tended to have tonic firing patterns and none exhibited LTS-related bursting in any state of the wake-sleep cycle (Steriade et al., 1990a). Cholinergic PPTg neurons mediate paired pulse

inhibition of the acoustic startle response (Koch et al., 1993). The cholinergic PPTg projects to basal ganglia (Beninato and Spencer, 1987; Futami et al., 1995), and unilateral excitotoxic lesion of the PPTg induced hemiparkinsonism in monkeys (Kojima et al., 1997), possibly by removal of the cholinergic excitation of dopaminergic nigrostriatal neurons (Futami et al., 1995). Lesion studies utilizing excitotoxins also suggest that the PPTg is involved in the process by which reinforcers control purposive behavior (Lepore and Franklin, 1996), a conclusion supported by pharmacological manipulation of PPTg activity (Conde et al., 1998).

Associative learning occurs in primary auditory cortex when a tone is paired with activation of the cholinergic neuromodulatory nucleus basalis (Weinberger and Bakin, 1998), implicating acetylcholine in learning. Another kind of learning, behavioral sensitization to cocaine or methamphetamine, appears to utilize muscarinic cholinergic transmission (Heidbreder and Shippenberg, 1996; Ohmori et al., 1995). In a sustained attention task, destruction of cholinergic afferents to medial prefrontal cortex attenuated the increased neuronal firing rates associated with presentation of a visual distractor (Gill et al., 2000). This suggests that cholinergic transmission has a role in the mechanism of attention. Lesions in the PPTg and a similar nearby cholinergic nucleus known as the lateral dorsal tegmental nucleus result in loss of consciousness, implicating acetylcholine in maintenance of consciousness. The situation is complicated by the fact that drugs that block neuromodulatory cholinergic actions produce delirium but apparently not coma (reviewed in Smythies, 1997). Acetylcholine concentration in thalamus is relatively high during waking and paradoxical sleep and is relatively low during slow-wave sleep (Williams et al., 1994). Acetylcholine plays a role in producing certain states of consciousness such as waking and phenomena produced by cholinergic stimulation *in vitro* may therefore occur during these

states *in vivo*. Sherman and Guillery (1996) proposed that the thalamus could alternate between burst mode and tonic mode according to system requirements for detection versus analysis. (Burst mode appears adapted for event detection because the low spontaneous firing rate of this mode makes responses to discrete sensory stimuli stand out (Guido et al., 1995). Tonic mode appears adapted to supporting analysis by structures downstream from the thalamus because the firing rate of the thalamic neuron faithfully follows the input stimulus intensity, suggesting a linearity of response.) Sherman and Guillery suggested that RMP control by cholinergic peribrachial inputs to thalamus could be responsible for alternation between burst and tonic modes.

1.5.3 Actions and signal transduction pathways of acetylcholine in thalamus

In brain, acetylcholine acts on two classes of receptor: nicotinic and muscarinic. The nicotinic receptor combines a pair of receptor sites and a nonspecific cation channel in the same molecular assembly and mediates a fast depolarizing response to acetylcholine release. It is stimulated selectively by nicotine and blocked by hexamethonium ion. The muscarinic receptor is of the G-protein coupled class and affects membrane potential indirectly. The indirect pathways can involve second messengers. It is selectively stimulated by muscarine and acetyl-β-methylcholine chloride (methacholine) and is selectively blocked by atropine, pirenzepine (at one micromolar), scopolamine, and tropicamide. Five subtypes of the muscarinic receptor exist: m1 to m5. The m2 and m4 subtypes inhibit adenylyl cyclase and the remaining three activate phospholipase C. Other effector enzymes may also be activated (reviewed in Felder 1995). The resulting changes in second messenger concentration alter ion channel activity and can lead to a persistent change in RMP. In addition, the G-proteins

activated by the m2 and m4 subtypes can affect ion channel activity directly (Birnbaumer et al., 1990; Huang et al., 1995; Inoue and Yoshii, 1992). Action of acetylcholine at nicotinic receptors is classified as neurotransmission whereas action at muscarinic receptors is an example of neuromodulation.

In rat and cat, but not guinea pig, acetylcholine induces depolarization in thalamocortical neurons, consisting of a rapid onset, rapidly desensitizing nicotinic response and a persistent muscarinic component (McCormick and Prince, 1987a). The muscarinic component is caused by closure of a leak potassium channel (named for the linearity of the current when plotted against membrane potential) that removes EPSP-LTS coupling by means of a depolarization of the RMP (McCormick, 1989; McCormick and Prince, 1987a; Mooney et al., 1995; Chapter 2).

In rat lateral geniculate nucleus, the visual equivalent of the MGv, application of methacholine induced a slow depolarization in relay neurons. The depolarization had an early phase and a late phase, distinguished by amount of inward current and change in input resistance (Zhu and Uhlrich, 1998). The early phase was mediated by m3 receptors, activation of which led to both a decrease in the leak potassium conductance and an increase in I_h. The late phase was mediated by m1 receptors, activation of which led only to a decrease in the leak conductance (Zhu and Uhlrich, 1998). Immunohistochemical work later confirmed the presence of m1 and m3 receptors on rat lateral geniculate relay neurons (Plummer et al., 1999). In contrast, strong immunoreactivity to the m2 receptor was found in dendrites and somata of interneurons and in neurons of the reticular thalamic nucleus. These two cell types are hyperpolarized by muscarinic agonists (McCormick and Pape, 1988;

McCormick and Prince, 1986), implicating the m2 receptor in mediating thalamic hyperpolarizing responses to muscarinic agonists.

1.5.4 Effect of acetylcholine on the afterhyperpolarization

Muscarinic receptor activation (MRA) has other effects in the brain besides RMP control. It blocks a slow intracellular calcium-activated afterhyperpolarization (AHP) that follows action potential trains in various limbic structures (reviewed in Halliwell, 1990). This action may be mediated by phospholipase C activation. In hippocampal CA1 (Cole and Nicoll, 1983 and 1984) and subicular neurons, application of a muscarinic agonist blocks AHPs, thereby facilitating neuronal firing by reducing spike accommodation and/or unmasking a depolarization potential (Bal and McCormick, 1993; Kawasaki et al., 1999). In the thalamus, the AHP plays an important role in the genesis of a slow (1-5 Hz) or delta membrane oscillation. The oscillation occurs when the AHP interacts with a low-threshold spike (LTS) mediated by a T-type of Ca^{2+} conductance (Steriade et al., 1991). A direct test of the susceptibility of the thalamic AHP to block by MRA seems lacking (reviewed in Sherman and Koch, 1986).

1.6 Experimental approach

The rat auditory thalamus was chosen for this study because primary and nonprimary pathways lie in close proximity, the sensory afferents are accessible, and the synaptic circuitry is simple. Neurons in the MGB are almost entirely relay cells (Mugnaini and Oertel, 1985; Winer et al., 1999; Winer and Larue, 1988) and do not produce collaterals within the MGB (Jones, 1985b). The sensory afferent running in the BIC is connected monosynaptically

to the projection neurons (Hu et al., 1994). An explant preparation was used because the objective was to study primary and nonprimary pathways comparatively, requiring BIC, MGv, and nonprimary MGB in the same preparation with intact connections. The slice preparation is unsuitable because the three structures do not lie in the same plane. The explant preserves large parts of the MGv and caudodorsal MGd together with their axonal inputs from the brachium of the inferior colliculus. The result is a single sensory synapse in isolation, notably isolation from reticular thalamic, cortical and uncontrolled neuromodulatory inputs. Neuromodulatory inputs can be imitated by using drugs such as muscarine, dissolved in the superfusate. Synaptic transmission can be studied by recording in MGB with intracellular or extracellular electrodes while applying single-shock stimulation to the axons in the BIC. Selective chemical stimulation of BIC can alternatively be used. The former method provides well-timed inputs useful for the study of transmission latency. A further rationale for the use of single-shock stimulation of the BIC comes from *in vivo* results and answers concerns that such stimulation is misleading because it activates axons synchronously. When the auditory system of the bat was provided with amplitude-modulated signals that approximate passively heard natural sounds, some categories of IC neuron responded with action potentials time-locked to the modulation (Condon et al., 1996). It is inferred that this time locking can synchronize action potentials from different IC projection neurons.

The free surface of the MGB is occupied largely by the lateroventral nucleus (LV) of the MGv and the caudodorsal nucleus (CD) of the MGd (Clerici and Coleman, 1990; LeDoux et al., 1987). Viable tissue in an explant is restricted to its outer 500 microns. Since

this is about the thickness (in a horizontal plane of section) of CD or LV in rat, both structures may be presumed intact.

The research is focussed on muscarinic rather than nicotinic mechanisms because the reticular stimulatory effect is blocked by muscarinic antagonists (Hu, 1988; Hu et al., 1986 and 1989; Steriade et al., 1990b). Additionally, the nicotinic effect desensitizes relatively rapidly (McCormick and Prince, 1987a and 1987b), suggesting that it lacks the ability to maintain a sustained neuromodulatory effect on synaptic transmission.

Both extracellular single-unit recording and intracellular sharp electrode recording were employed. Extracellular recording avoids all interference with the internal conditions and membrane properties of the neuron, but only registers action potentials. It is useful for obtaining the effect of drugs or altered extracellular ionic concentrations on synaptic transmission. Intracellular recording interferes with RMP and membrane conductance to some extent but reveals the mechanistic details underlying alterations in synaptic transmission.

1.7 Objective

The main objective of the research described herein was to test the hypothesis that acetylcholine is capable of modulating auditory synaptic transmission in a synaptic pathway-specific manner.

Chapter 2.

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Title: **Muscarine Induces an Anomalous Inhibition of Synaptic Transmission in Rat Auditory Thalamic Neurons *in Vitro*[1]**

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Abbreviations: BIC, brachium of the inferior colliculus; MGB, medial geniculate body; MGv, ventral division of the medial geniculate body; EPSP, excitatory postsynaptic potential; LTS, low-threshold spike; $[K^+]$, potassium ion concentration; $[K^+]_o$, extracellular potassium ion concentration; ACSF, artificial cerebrospinal fluid; GABA, γ-aminobutyric acid; ACh, acetylcholine; MCh, methacholine (acetyl-β-methylcholine chloride).

ABSTRACT

Muscarinic receptor-mediated inhibition of central synaptic transmission was studied in a monosynaptic pathway connecting the inferior colliculus and the auditory thalamus in *in vitro* rat brain explants. Extra- and intracellular synaptic responses were recorded using sharp electrode and whole-cell patch clamp techniques in the ventral division of the medial geniculate body following electrical stimulation of the brachium of the inferior colliculus. Stimulation of tectal afferents evoked either a high-frequency burst or a single-spike synaptic response in ventral geniculate neurons. Bath application of muscarinic receptor agonists abolished responses consisting of a high-frequency burst, but not responses consisting of a single spike. In the majority of single-spike cells, muscarinic agonists induced a synaptic facilitation. The burst blocking effect was mimicked by a moderate elevation of extracellular potassium. Intracellular recordings showed that burst synaptic responses similar to those recorded extracellularly were induced by an excitatory postsynaptic potential. This synaptic potential, by first activating a low-threshold spike, was able to evoke a burst of sodium spike discharges. Muscarinic agonists caused a slow membrane depolarization that inactivated the low-threshold spike, leading to a blockade of the burst response. This mechanism is tentatively termed here EPSP-LTS decoupling. Our results therefore support the hypothesis that part of the muscarinic receptor-mediated synaptic inhibition previously reported in anesthetized animal preparations *in vivo* represents a membrane depolarization rather than pre- or postsynaptic inhibition.

INTRODUCTION

The non-auditory innervation of the medial geniculate body (MGB) of the thalamus by the midbrain reticular formation is part of an important neuromodulatory system that controls thalamocortical signal transmission (reviewed by Steriade and Deschênes, 1984; Steriade and Llinás, 1988). A major component of the ascending reticular input arises from a population of cholinergic cells located at the mesopontine junction (Levey *et al.*, 1987). In rat and cat visual thalami, both *in vivo* and *in vitro* studies show that acetylcholine (ACh) excites thalamocortical relay neurons by inducing a fast nicotinic and a slow muscarinic receptor-mediated membrane depolarization, and that it inhibits GABAergic (GABA: γ-aminobutyric acid) interneurons by inducing a membrane hyperpolarization (Hu *et al.*, 1989; McCormick and Pape, 1988). These membrane mechanisms seem to act in parallel to control the excitability of thalamocortical synaptic communication (Steriade *et al.*, 1990). Though much is known about the effects of ACh in visual thalamus, its effects on synaptic transmission in other thalamic nuclei remain uncertain. A number of laboratories have reported a robust inhibitory action of ACh *in vivo* (MacLeod *et al.*, 1984). For instance, local iontophoretic application of ACh in cat MGB may reduce spontaneous synaptic activity in about 30% of cells (Tebécis, 1970). Inhibition may occur in up to 60% of neurons in the anterior pulvinar, lateral posterior, ventromedial and certain midline thalamic nuclei (Godfraind, 1975; MacLeod *et al.*, 1984). Marks and Roffwarg (1993) recently reported that in somatosensory thalamic relay neurons in the barbitalized rat, excitatory muscarinic effects were observed following local application of ACh or the cholinergic agonist carbachol in only 29% of cells, the commonest observation being no effect. Furthermore, these agonists inhibited the excitation produced by iontophoretic application of glutamate. To account for apparent cholinergic depressive actions, Duggan and Hall proposed

that ACh acts on presynaptic terminals to inhibit spontaneous synaptic activity in thalamic relay neurons (Duggan and Hall, 1975).

The purpose of this study was to examine the cellular mechanisms underlying muscarinic inhibition of synaptic transmission in auditory thalamus. We were particularly interested in the burst response to synaptic stimulation, a pattern of activity commonly encountered in previous *in vivo* studies (Marks and Roffwarg, 1989; Duggan and Hall, 1975; MacLeod *et al.*, 1984; Marks and Roffwarg, 1993). An *in vitro* thalamic explant preparation containing an isolated but largely intact auditory tectothalamic pathway was employed (Hu *et al.*, 1994).

The rat auditory thalamus consists primarily of relay neurons (Winer and Larue, 1988). Previous study has shown that electrical stimulation of tectal efferents evokes either a burst or a single-spike synaptic response in the MGB (Hu *et al.*, 1994). In the present study, we first addressed the question of whether ACh has a direct "inhibitory" effect on the burst synaptic response. We then investigated possible pre- and postsynaptic mechanisms underlying this "inhibition". We have focused our experiments on the lemniscal, or ventral division of the MGB, a region where the co-existence of both burst and single-spike response types provides an internal control for studying cholinergic effects. We now report that application of muscarinic agonists reversibly blocked synaptic responses in nearly two-thirds of burst, but not single-spike, cells in the ventral division of the MGB (MGv). Intracellular and whole-cell recordings suggested that the burst synaptic responses were mediated by an excitatory postsynaptic potential (EPSP) triggering a low-threshold spike or LTS (Jahnsen and Llinás, 1984). Muscarinic receptor agonists induced a slow membrane depolarization, which led to a partial inactivation of the LTS, thereby causing an "anomalous" or depolarization-mediated

burst blockade. The functional implication of such a modulatory mechanism is discussed.

METHODS

Tissue Preparation. The general methodology of the explant preparation has been described in detail elsewhere (Hu *et al.*, 1994). Briefly, male Long-Evans rats (150 - 175g.) were decapitated and the brain removed. The front and back of the brain were squared with coronal sections through the optic chiasm and middle of the cerebellum. The left half of the brain was isolated and pinned to the Sylgard base of a humidified recording chamber (School of Pharmacy, University of London). The temporal-occipital lobes and hippocampal tissues adjacent to the MGB and the brachium of the inferior colliculus (BIC) were aspirated to expose the free surfaces of the MGB and BIC. A silicone tube stationed dorsoposteriorly to the MGB delivered a gravity feed of warmed (34 ± 1°C), oxygenated (95% 0_2; 5% CO_2) artificial cerebrospinal fluid (ACSF) at a flow rate of 3.5-13.5 ml/min. The superfusion tube was adjusted so that the free surface of the MGB and the rostral two thirds of the BIC were totally submerged in the ACSF.

The composition of the ACSF was (in mM): NaCl, 122; KCl, 3; $MgCl_2$, 1.3; $NaHCO_3$, 25.9; $CaCl_2$, 0.8 (extracellular recording) or 2.5 (intracellular recording); glucose, 11. Where superfusate having an elevated (8-15 mM) potassium ion concentration ($[K^+]$) was used, the extra potassium replaced sodium on an equimolar basis. Final pH after saturation with the oxygenation gas: 7.4 ± .02. Final osmolality: 295 ± 2 mOsm/kg.

The stimulating electrode was inserted in the BIC 1 - 2 mm from the MGB. The electrode was bipolar, consisting of two chlorided silver wires 120 μm in diameter. The wires were spaced to take in most of the width of the BIC.

Recording and Stimulation. The entire free surface of the MGB provided a total working area of about 1.0 x 0.5 mm. The MGB is seen as a large, hemispherical body of gray matter of which the ventral part is occupied largely by the lateroventral nucleus of the MGv. This nucleus constitutes the major component of the lemniscal auditory thalamus. The locations of our recordings were restricted to the ventral division.

Extracellular recording micropipettes were prepared on a Narishige vertical puller and filled with either KCl (2.5M), K acetate (2.5M) and KCl (1.5M), or NaCl (3M). Signals were conventionally amplified (Axon Instruments Inc.), high-pass filtered at 200-300 Hz, and digitized (Neuro-corder, DR-384) at 44 kHz for videotape recording, or were captured on-line with *pClamp* software. Intracellular recording micropipettes and patch electrodes were made with borosilicate glass capillary tubing (1.2 mm o.d., WPI, USA) on a Flaming-Brown puller. Sharp electrodes contained K acetate (3.5M) or in some experiments, K acetate (2.5M) and KCl (1.5 M). DC resistance was 60 to 120 MΩ. Patch electrodes, which had a tip diameter < 1 μm, were filled with (in mM): potassium gluconate, 130; KCl, 10, $CaCl_2$, 1; Na_3EGTA, 11; HEPES, 10; $MgCl_2$, 2; Na_2ATP, 2. The pH of the solution was adjusted to 7.4 with NaOH.

The extracellular recording electrode was lowered manually until it touched the surface of the tissue, as evidenced by an electrical transient appearing on the monitoring oscilloscope. The electrode was then lowered using an electronic driver (Burleigh) in steps of 2.5 μm to search for neurons responding to BIC stimulation. Stimulation was delivered at a repetition rate of 1 to 2 Hz and consisted of 20 to 40 V pulses of 0.1 ms duration. When a neuron was found, the voltage was reduced to near threshold. Threshold was defined as the stimulation intensity that provoked a synaptic response in about 30-50% of trials during 10-20 consecutive stimuli. Following the identification of threshold, the stimulation voltage was raised just sufficiently

to obtain adequately frequent responding. Blind whole-cell patch clamp recording was performed according to the method described by Blanton *et al.* (1989) using an Axopatch-200A amplifier (Axon Instruments Inc.). Briefly, the electrode was lowered into the fluid over the MGB. The amplifier was set to voltage-clamp mode and to continuously deliver a seal-test pulse (5 mV) at line frequency. The capacity transient of the electrode was compensated and the electrode potential was set to zero. The electrode resistance in the fluid was 3-8 MΩ. When the electrode was slowly advanced into the tissue and was adjacent to a cell (as judged by a small negative D.C. shift, a sudden decrease of current amplitude and occasionally action potential firing), a small negative pressure was applied to the electrode via a 10-ml syringe attached by soft plastic tubing to the electrode holder. This resulted in a steady increase in electrode resistance to a value larger than 1GΩ, usually within 1-2 minutes. At this point, a larger suction was applied, which resulted in a rapid breakdown of patch membrane as evidenced by a sudden drop of electrode resistance and by the appearance of a resting membrane potential. All cells were recorded within a layer of tissue extending from the ventral free surface of the MGB to a depth of 500 μm. This layer included the marginal zone, a thin layer of cells about 75 μm thick overlying the MGv. The surface location of each neuron, along with the depth of the recording position below the surface of the tissue as registered by the Burleigh electrode advancer, were recorded. No significant run-down of EPSPs occurred during whole-cell recording mode.

Drug Application. Muscarine, methacholine, atropine, scopolamine, hexamethonium, picrotoxin and bicuculline were all purchased from Research Biochemicals International (RBI, Natick, MA, USA). The chemicals were prepared as stock solutions (0.5-10 mM) and stored at 4°C. All antagonists were added as stock solutions to bath medium contained in an

oxygenated side-bath to produce the final concentrations indicated in the text. However, the drug concentration in the bath could be significantly higher than that in the interstitial fluid, due to problems such as tissue barriers and/or degradation (see below). Antagonists were applied by switching the source of the superfusate from the main reservoir to the sidebath. Agonists were usually applied by steadily infusing them into the medium through a small catheter inserted into the superfusion tube proximal to the exit point over the explant. The volume and timing of agonist infusions were controlled by an electronic timer connected to a two-way syringe pump (Harvard Apparatus). The stated agonist concentrations were obtained by dividing the product of the stock solution concentration and the pumping rate by the sum of the pumping rate and the perfusion rate. Tests in which response recovery was not observed were excluded from the analysis. In 77% of cells, testing with the muscarinic agonist was performed at least twice.

RESULTS

Muscarine-mediated blockade of synaptically evoked burst responses. The database of this study consisted of recordings from 118 cells that responded to stimulation with a burst (fig. 1A) and 64 cells that responded with a single spike. A burst response was defined as a response having two or more spikes, a latency to the first spike of greater than 10 ms, a variable first-spike latency, a first inter-spike interval of less than 5 ms, and an inability to follow stimulation at 10 Hz (Hu, 1995). An additional four cells responded with a dual spike pattern that could not be defined as burst. These cells were classed as single-spike for purposes of analysis. In 66% of the burst neurons, application of muscarine (0.5-45 μM for 10-1000 sec) or the muscarinic agonist methacholine (12-625 μM for 5-60 sec.) caused a reversible, transient

response blockade (fig. 1), which in a subpopulation of cells was followed by a period of spontaneous firing (fig. 3). During the period of spontaneous firing, BIC stimulation could elicit single-spike responses (fig. 3). The burst blocking effect of muscarine was reproduced by 100-200 μM of ACh (n = 6). The apparent variability and difference in effective drug concentrations between MCh, ACh and muscarine were likely caused by a combined factor involving both compound hydrolysis by acetylcholinesterase (AChE; for ACh) and tissue barriers that may significantly reduce drug diffusion from the surface into the tissue core (Sarantis et al, 1993). Indeed, after addition of an AChE inhibitor (eserine) into the bath medium, a significantly lower concentration of ACh induced similar response in MGv neurons (n = 4). The above muscarinic effects were abolished in a bath medium containing 5-50 μM of muscarinic antagonists atropine or scopolamine (n = 4) but not 450 μM of the nicotinic antagonist hexamethonium (n = 6), suggesting the observed effects were primarily mediated by muscarinic cholinergic receptors. It is noteworthy that the antagonistic effect of scopolamine on muscarinic or methacholine responses was extremely long lasting. A 5-min application of scopolamine could abolish the muscarinic effects for hours. In agreement with a previous study that found that GABAergic synaptic transmission does not mediate the evoked burst response (Hu *et al.*, 1994), we found that synaptic burst blockade by muscarine persisted in a bath medium containing GABAergic receptor antagonists (picrotoxin or bicuculline, 30 μM each, n = 5).

In contrast to the burst-response cells, muscarine or MCh inhibited significantly fewer neurons that had a baseline response to BIC stimulation consisting of single or dual spikes (fig. 2; $P < 0.01$). The predominant effect (66% of single/dual-spike cells) was excitation, as evidenced by response facilitation (a reduction of failures to respond to synaptic stimulation, a shortening of response latency, and/or a reduction of latency variability) and/or the

appearance of spontaneous, usually epileptiform activity (Mooney and Hu, 1994).

(fig. 1 near here)

(fig. 2 near here)

(fig. 3 near here)

Membrane depolarization mimics muscarinic "inhibition". Given the known membrane depolarizing effect of muscarine in the rat thalamus (see Introduction), we examined the possibility that membrane depolarization *per se*, such as that induced by an elevated extracellular K^+ concentration ($[K^+]_o$), can produce an "inhibitory" effect on burst synaptic responding. In each of 9 neurons tested, an increase of $[K^+]_o$ (to 8-15 mM) invariably caused a transient blockade of extracellular burst responses evoked by BIC stimulation (fig. 4). This change in $[K^+]_o$ produced a membrane depolarization of 20-30 mV in MGv cells in whole-cell recordings (Senatorov and Hu, unpublished results). Shortly after onset of the burst blockade, single-spike synaptic responses with a constant latency appeared in five of these neurons, indicating that synaptic transmission was not unspecifically impaired in the high-potassium medium (fig. 4, see also Hu, 1995).

(fig. 4 near here)

EPSP-LTS coupling and muscarinic blockade. Our intracellular analysis of evoked synaptic responses was based on 26 neurons, seven of which were recorded using patch-clamp

electrodes. All recorded cells had a membrane potential negative to -55 mV and spike amplitudes larger than 62 mV. The apparent input resistance of these cells ranged from 45 to 176 MΩ in sharp electrode recordings and 78 to 340 MΩ in whole-cell recording mode. In the presence of 20 μM of picrotoxin, stimulation of BIC induced an EPSP in most MGv neurons, which in turn triggered a burst of action potentials similar to that recorded extracellularly. A representative example of such a response is illustrated in fig. 5. In this cell, the BIC-evoked response exhibited a dual peak: an early EPSP followed by a large depolarization hump (fig. 5B). A burst of action potentials was elicited by the delayed depolarization hump, but not by the early EPSP. As documented previously (Jahnsen & Llinás, 1984; Scharfman *et al.*, 1990; Hu, 1995), this delayed depolarization is apparently mediated by an LTS rather than by a prolonged component of the EPSP itself, a conclusion supported by three observations. 1) The delayed response is abolished upon membrane depolarization to -60 mV. At this membrane potential, the occurrence of the early EPSPs is unaffected. 2) The delayed response exhibits a high degree of fluctuation in peak latencies. 3) The depolarization is followed by an unusually long refractory period (Hu, 1995). In seven cells, bath application of muscarine or MCh evoked a slow membrane depolarization (5 to 10 mV), which was accompanied by a 10-20% increase in membrane resistance (n = 5). During the depolarization, the EPSP became either ineffective in eliciting an LTS, or the EPSP-LTS complex was no longer able to trigger a burst of fast action potentials (fig. 5B). In another 3 cells, we observed a brief (5 to 10 seconds) membrane hyperpolarization following muscarine application and coming in advance of the slow depolarization (data not shown). Sustained membrane hyperpolarizing responses were uncommon. These data suggest that conventional postsynaptic cell inhibition and shunting inhibition do not underlie muscarine-mediated burst blockade in MGv cells. It is noteworthy,

however, that the amplitude of depolarization induced by muscarine or MCh varied markedly from cell to cell. In about 30 to 40% of neurons, we could not obtain a clear membrane response. Furthermore, because EPSP-LTS decoupling occurs in a somewhat all or none fashion after usually a small membrane depolarization, an accurate dose-response curve cannot be confidently constructed. This issue is further addressed in the Discussion.

(fig. 5 near here)

It has been reported that muscarine, in addition to inducing a membrane depolarization, can also reduce the size of EPSPs by depressing release of glutamate from presynaptic terminals (Sugita *et al.*, 1991). In 9 cells, we have compared in the same cell the peak amplitudes of BIC-evoked EPSPs before and after application of MCh (100-200 μM; n = 5) or muscarine (50 μM; n = 4). These EPSPs were evoked with low-intensity stimuli to ensure that they were devoid of LTSs or action potentials. During the phase of muscarine-induced membrane depolarization, the evoked EPSPs all showed a reduction in peak amplitudes (fig. 5B). However, a large part of this amplitude reduction is likely caused by decreased EPSP driving force rather than inhibition of glutamate release (fig. 6). Indeed, when muscarine-induced membrane voltage was brought back to the control level via direct negative current injection (fig. 6a), the reduction in EPSP amplitudes became insignificant in comparison with the controls (fig. 6, $P > 0.06$; two sample Student t-test; n = 3). Furthermore, as illustrated in fig. 6b, the peak potential of evoked EPSPs showed a linear, and largely overlapping, variation in relation to membrane voltage changes before and following drug application (n = 5). The slope of this linear relationship and the extrapolated reversal potential (about -46 mV) of the

evoked EPSPs were largely unchanged by muscarine, suggesting a lack of a clear presynaptic inhibitory event. In the remaining 4 cells, either a 20% increase (n = 2) or a 10 to 15% decrease (n = 2) of EPSP height was observed based on the corresponding EPSP-holding potential plots. In contrast to the EPSP, we found that the inhibitory postsynaptic potentials in these cells were largely abolished by muscarinic agonists (Hu *et al.*, 1993).

(fig. 6 near here)

DISCUSSION

A mixed excitatory and inhibitory effect of muscarinic agonists on synaptic transmission has been described in many early *in vivo* iontophoretic studies conducted in the thalamus (see introduction). With the auditory tectothalamic pathway as a model, we have shown that activation of muscarinic receptors *in vitro* selectively suppressed burst synaptic transmission, but not the transmission mediated by single-spikes. Three lines of evidence suggest that this blockade of the synaptic burst is not mediated by conventional pre- or postsynaptic inhibition mechanisms. First, unlike many drug-induced inhibitory phenomena, the effects induced by muscarine show a striking dependence on the mode of synaptic response. Interestingly, a similar mode-dependent modulation of cell responses by muscarinic receptors has also been found *in vivo* in the rat ventromedial thalamic nucleus (MacLeod *et al.*, 1984). Second, the "inhibitory" effect of muscarine can be mimicked by an elevation of extracellular potassium and therefore, as would be expected, by membrane depolarization. Third, muscarinic agonists did not produce in MGv cells any membrane hyperpolarization with a phase or duration matching that of burst blockade, or any evident failure of EPSPs that could be ascribed

to presynaptic inhibition.

Our intracellular recordings showed that a burst of action potentials similar to that recorded extracellularly could be evoked by an EPSP-triggered LTS. The LTS is a well-characterized membrane voltage response due to opening of T-type calcium channels (Jahnsen and Llinás, 1984; Crunelli, *et al.*, 1989; Coulter *et al.*, 1989). T-type calcium channels can be blocked by low concentrations of nickelous ion, which blocks the LTS in isolated thalamic neurons (Suzuki and Rogawski, 1989). However, blocking experiments were not feasible in our preparation because all known blockers of the T-current have low specificity, affecting calcium channel types other than T-type, including L and N-type channels that mediate presynaptic transmitter release. In thalamic relay cells, T-type calcium channels de-inactivate upon membrane hyperpolarization to below -65 mV. At negative membrane potentials, activation of the LTS can be induced by a depolarization event (e.g., an EPSP) that is subthreshold for the fast sodium spike (Jahnsen and Llinás, 1984; Scharfman *et al.*, 1990; Hu, 1995). Depending on the level of resting membrane voltage and the size of the triggering EPSP, the LTS may vary significantly in magnitude and peak latency (Scharfman *et al.*, 1990; Hu, 1995). A large LTS invariably surpasses the action potential threshold and triggers a high-frequency spike burst, but imposing a small membrane depolarization often leads to a partial or incomplete LTS inactivation. Several kinetic features may be used to distinguish the LTS from its underlying EPSP within an EPSP-LTS complex. For instance, unlike EPSPs, the delayed depolarizations we identify as LTSs are often reduced and are sometimes blocked in a highly non-linear or a somewhat all-or-none fashion by increases in resting potential to -60 mV (Hu, 1995). Moreover, when activated synaptically, the delayed depolarizations exhibit a time to peak that fluctuates widely and activation is often followed by a refractory period

lasting several tens of milliseconds. These features are not commonly observed for direct synaptic responses (Jahnsen and Llinás, 1984; Scharfman *et al.*, 1990; Hu, 1995). Our results show that the muscarine-mediated blockade of the burst synaptic response is mainly mediated by a slow membrane depolarization, which led to a full or partial inactivation of the LTS. The latter then becomes ineffective in triggering action potentials. We tentatively term this inhibitory mechanism EPSP-LTS decoupling. Under our experimental conditions, presynaptic inhibition of EPSPs does not seem to underlie the blockade of the burst synaptic response, although an increase in membrane input resistance induced by muscarine (see below) may partially mask the presynaptic inhibitory effect of muscarine that has been reported for other brain regions (Sugita *et al.*, 1991). Further experiments using voltage-clamp techniques will help to clarify this issue. Interestingly, we found that the GABAergic inhibitory synaptic potentials evoked in MGv cells are consistently inhibited by muscarinic agonists (Hu *et al.*, 1993). Such a depression of GABAergic inhibition by muscarine, together with its membrane depolarizing effect, may contribute to the muscarine-induced epileptiform firing we observed in MGv cells.

The muscarinic membrane depolarization is primarily mediated by closure of a leak potassium conductance, involving a pertussis toxin-insensitive G-protein coupled receptor mechanism (McCormick, 1992). In agreement with this, we observed in this study either a small (about 20%) increase or no change in the membrane input resistance during the period of muscarine-induced membrane depolarization. Because the leak current declines linearly as the reversal potential for potassium (-80 to -90 mV) is approached, the amplitude of muscarinic depolarization is small in cells having a relatively negative membrane voltage. This may partially explain why muscarinic agonists had only a weak or insignificant effect in a

subpopulation of intracellularly recorded (30-40%) or extracellularly recorded (18%) MGv neurons. Extracellular recordings showed more consistent cholinergic effects than did intracellular recordings. Part of the variability of the intracellular results may simply reflect cell membrane damage due to sharp electrode penetration and the resultant alteration of the leak membrane conductance (Spruston *et al.*, 1993). Under physiological conditions, the occurrence of the EPSP-LTS complex in lemniscal sensory pathways has been observed to begin with the onset of slow-wave sleep and membrane hyperpolarization (Fourment *et al.*, 1984; Hirsch *et al.*, 1983). During hyperpolarization episodes, spontaneously occurring EPSPs were often seen to trigger LTSs. It is conceivable that a similar situation may occur in anesthetized animals, where EPSP-LTS coupling would induce sporadic spontaneous bursting activity resembling that observed *in vivo* (Marks and Roffwarg, 1993). In cells having a sufficiently depolarized resting membrane potential, the decoupling mechanism is not expected to be operational. Indeed, it has been reported that in lightly anesthetized animals, the ACh-mediated inhibition of many thalamic neurons often ceased to occur if the cells showed spontaneous or glutamate-induced tonic single-spike firing (MacLeod *et al.*, 1984; Duggan and Hall, 1975).

Although muscarine induces a large membrane hyperpolarization in guinea pig MGB, hyperpolarizing responses seem uncommon in rat MGB neurons (McCormick and Prince, 1987; but see Chapter 3). In the cat MGB, the effect of ACh on identified thalamocortical projection neurons is mainly excitatory (McCormick and Prince, 1987). In a subpopulation of rat MGB cells where a transient membrane hyperpolarization did occur, we found that the time course and the magnitude of the hyperpolarization were often insufficient to lead to burst blockade. We also noticed that at a resting potential of -70 to -75 mV, negative current injection sufficient to produce at least 10 mV of membrane hyperpolarization was required to prevent

an EPSP-LTS complex from triggering a spike burst. A hyperpolarizing muscarinic response of such amplitude was not observed in the present study. Further study is needed to determine whether the difference between the observation of a low incidence of hyperpolarizing responses in rat MGB and the observation of a high incidence in guinea pig can be related to a species difference in the distribution of particular muscarinic receptor subtypes.

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Footnotes

1. This work was supported by the Parkinson Foundation of Canada, the Ontario Mental Health Foundation and the Medical Research Council of Canada (MRC). B.H. is a MRC scholar.

Figure 1 Muscarinic inhibition of bursting synaptic responses. A, successive extracellular recordings of typical synaptically evoked bursts under control conditions. B, the burst responses were completely blocked after bath application of MCh at 390 μM for 10 seconds. C, recovery. The burst response recovered 77 seconds after the end of drug application. Note that the latency of the burst response varied, in this case from 16 to 34 ms.

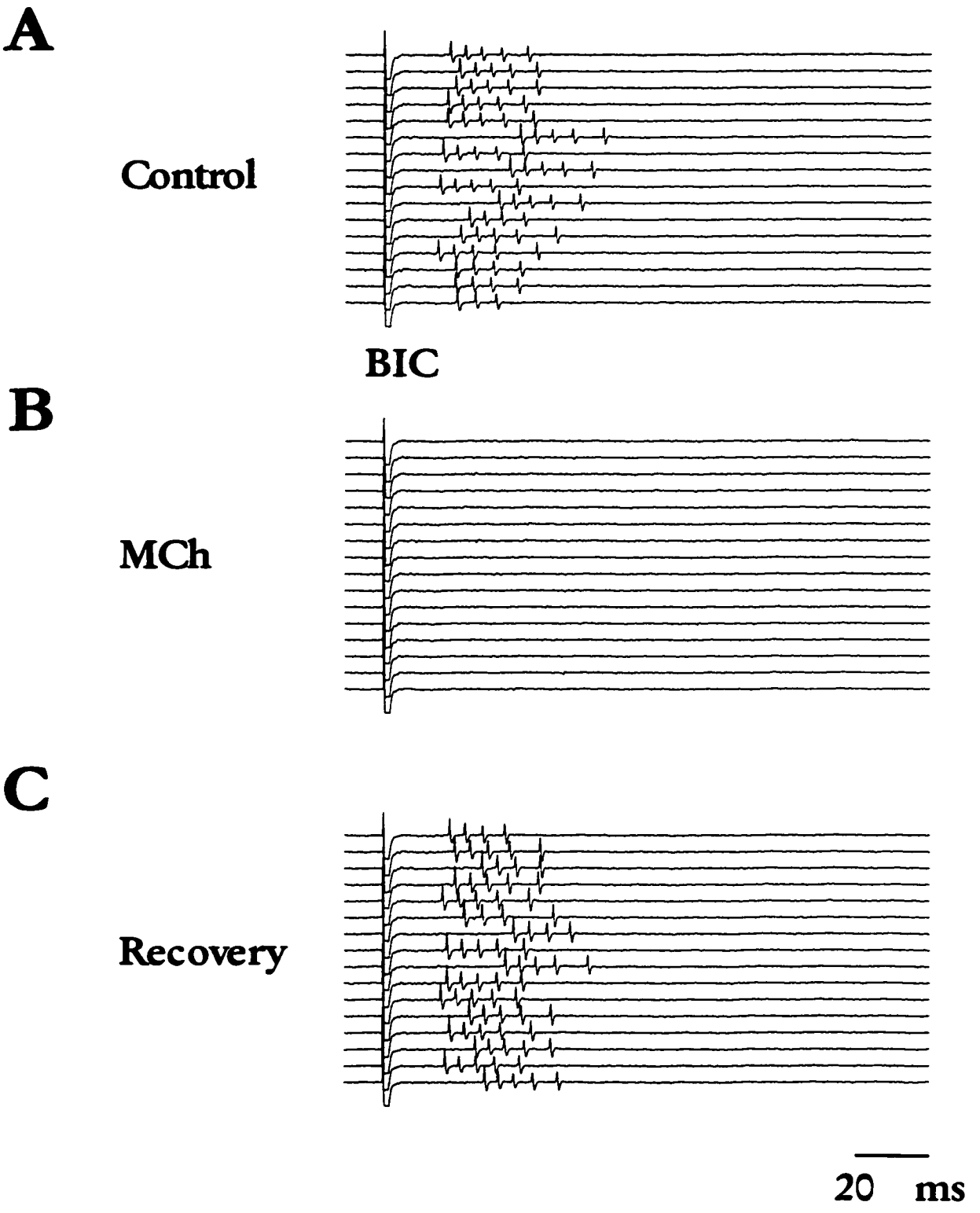

Figure 1

Figure 2 Summary of the effects of muscarinic receptor agonists on BIC-evoked synaptic responses in MGv cells. The muscarinic effects were classed into four categories. The blocked/excited group represents cells with an initial response blockade followed by spontaneous firing (see text). Burst (A) and single-spike (B) cells were statistically compared with each other within each category of muscarinic response with the nonparametric two-sample proportion test. Note that the muscarinic synaptic "inhibition" occurs mainly in burst responders but not in neurons discharging a single-spike to BIC stimulation. Asterisk: $P < 0.05$; Double asterisk: $P < 0.01$.

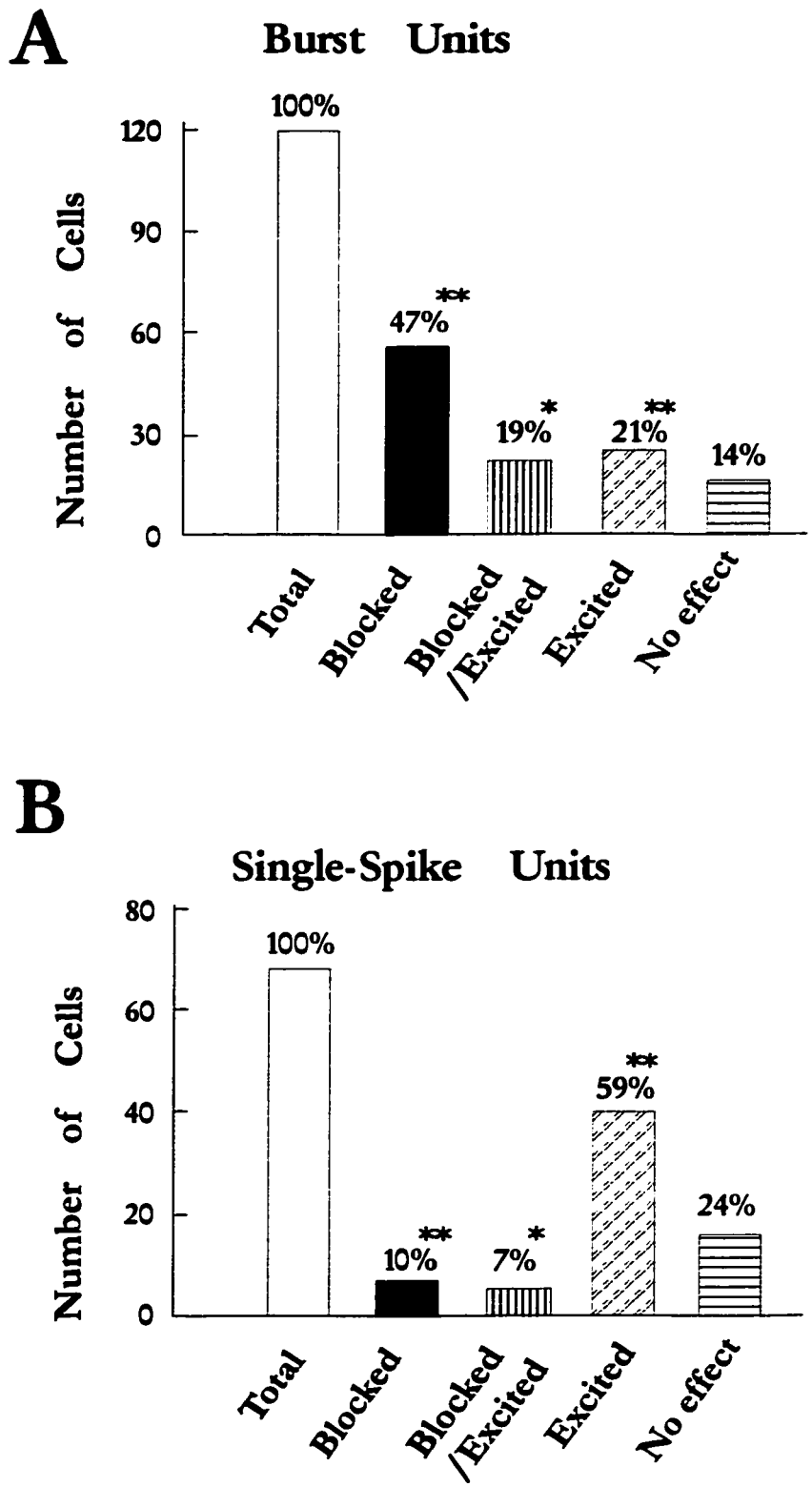
A
Burst Units
Number of Cells
120
90
60
30
0
100%
47%
**
19%
*
21%
**
14%
Total
Blocked
Blocked /Excited
Excited
No effect
B
Single-Spike Units
Number of Cells
80
60
40
20
0
100%
10%
**
7%
*
59%
**
24%
Total
Blocked
Blocked /Excited
Excited
No effect

Figure 2

Figure 3 Transformation of a burst synaptic response into a single-spike discharge by a muscarinic agonist in a representative MGv cell. Asterisks denote omitted segments. A, synaptically evoked bursts in normal ACSF. B, the burst responses were blocked following application of MCh at 400 μM for 10 sec. C, during the same test shortly after the burst blockade, BIC stimulation started to elicit single-spike responses. Note also the occurrence of sporadic spontaneous firing.

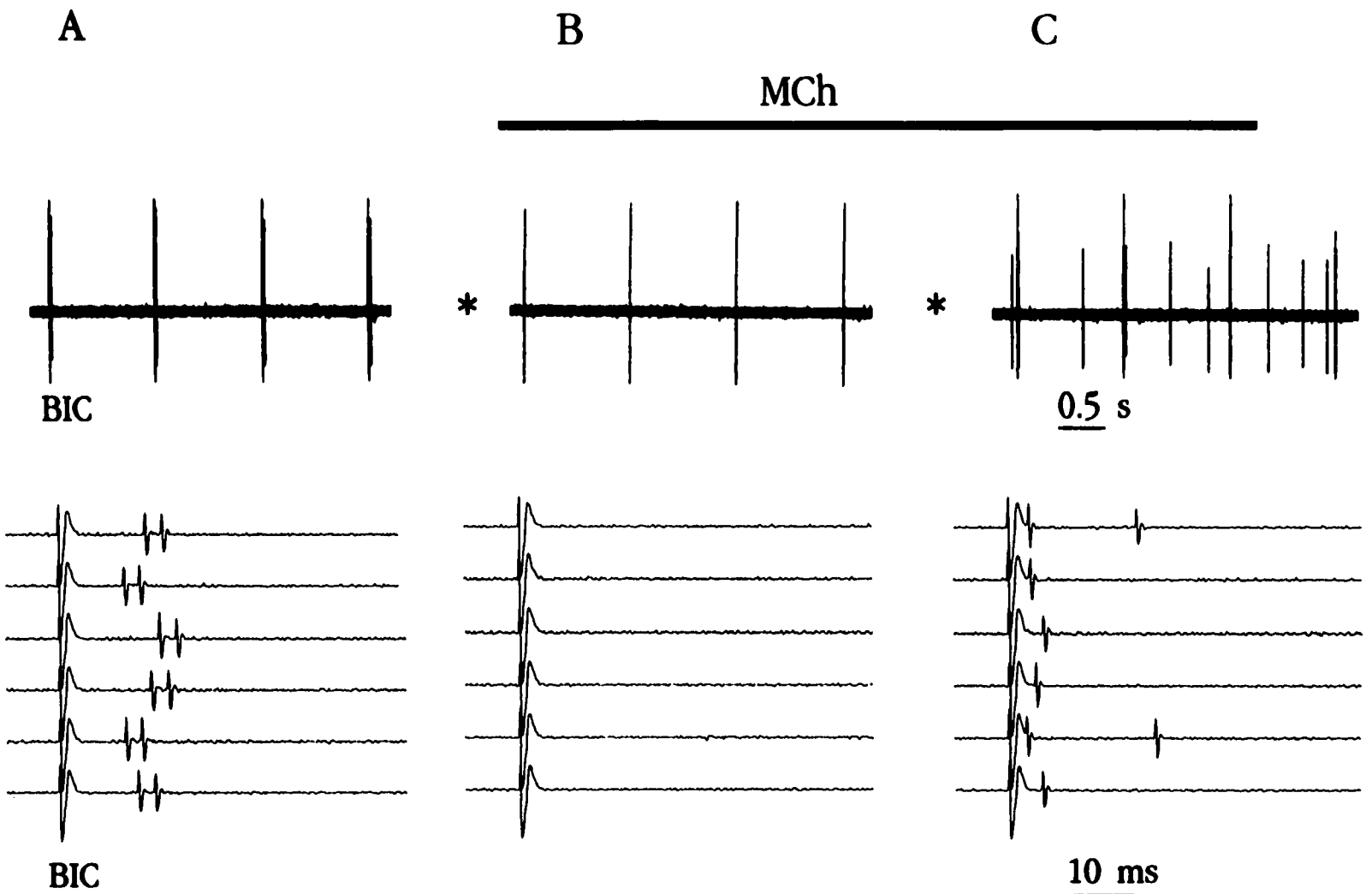

Figure 3

Figure 4 K^+-induced membrane depolarization mimicked muscarinic "inhibition" of synaptic bursts. Backslashes indicate omitted segments. A, control burst responses in normal ACSF. B, replacement of the ACSF by bath medium containing elevated KCl (8 mM). Evoked synaptic responding was completely blocked. C, in the same test shortly after response blockade, BIC stimulation started to elicit single-spike responses. At 10 mM of K^+ (data not shown), this response could be accompanied by sporadic spontaneous discharge.

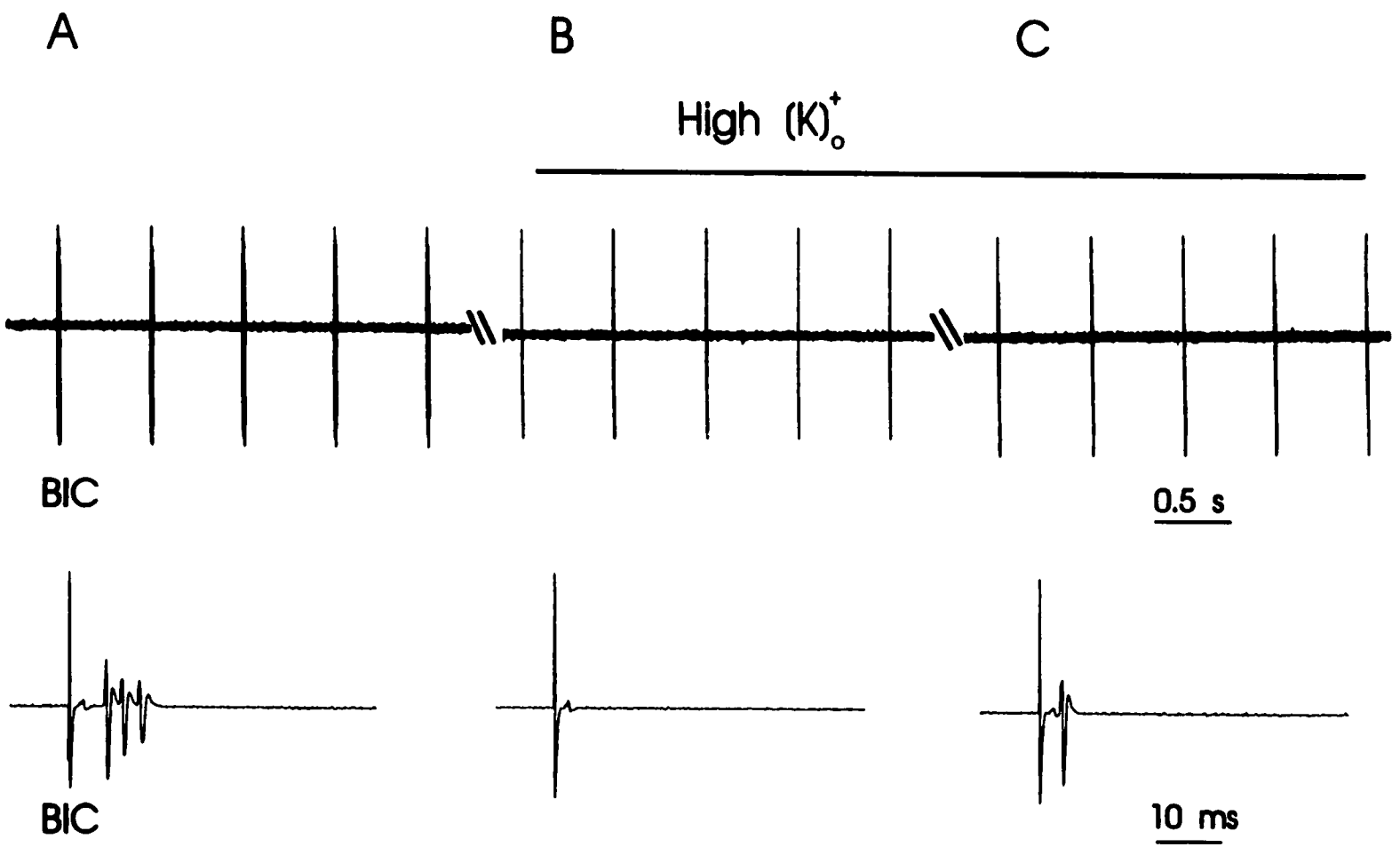

Figure 4

Figure 5 Slow membrane depolarization and EPSP-LTS decoupling produced by MCh. A, upper panel: A compressed chart recording from a MGv cell illustrating a slow membrane depolarization induced by bath application of MCh (100 μM). The regular upward voltage deflections were BIC-evoked synaptic responses, partially truncated for clarity of illustration. Downward deflections were due to stimulus artifacts. Asterisk denotes the omission of approximately 20 seconds. Lower panels: Expanded segments of the chart recording showing details of synaptic responses. Dotted line indicates the resting potential. 1: Baseline. BIC stimulation evoked an EPSP and a delayed EPSP-LTS burst complex. 2: During MCh-induced membrane depolarization. BIC stimulation evoked only an EPSP. 3: Recovery. After membrane repolarization, the EPSP-LTS complex recurred. B: The EPSP-LTS complex illustrated in "A" is expanded and shown superposed on a pure EPSP recorded during depolarization by MCh. Note the lower amplitude of the EPSP during the depolarization.

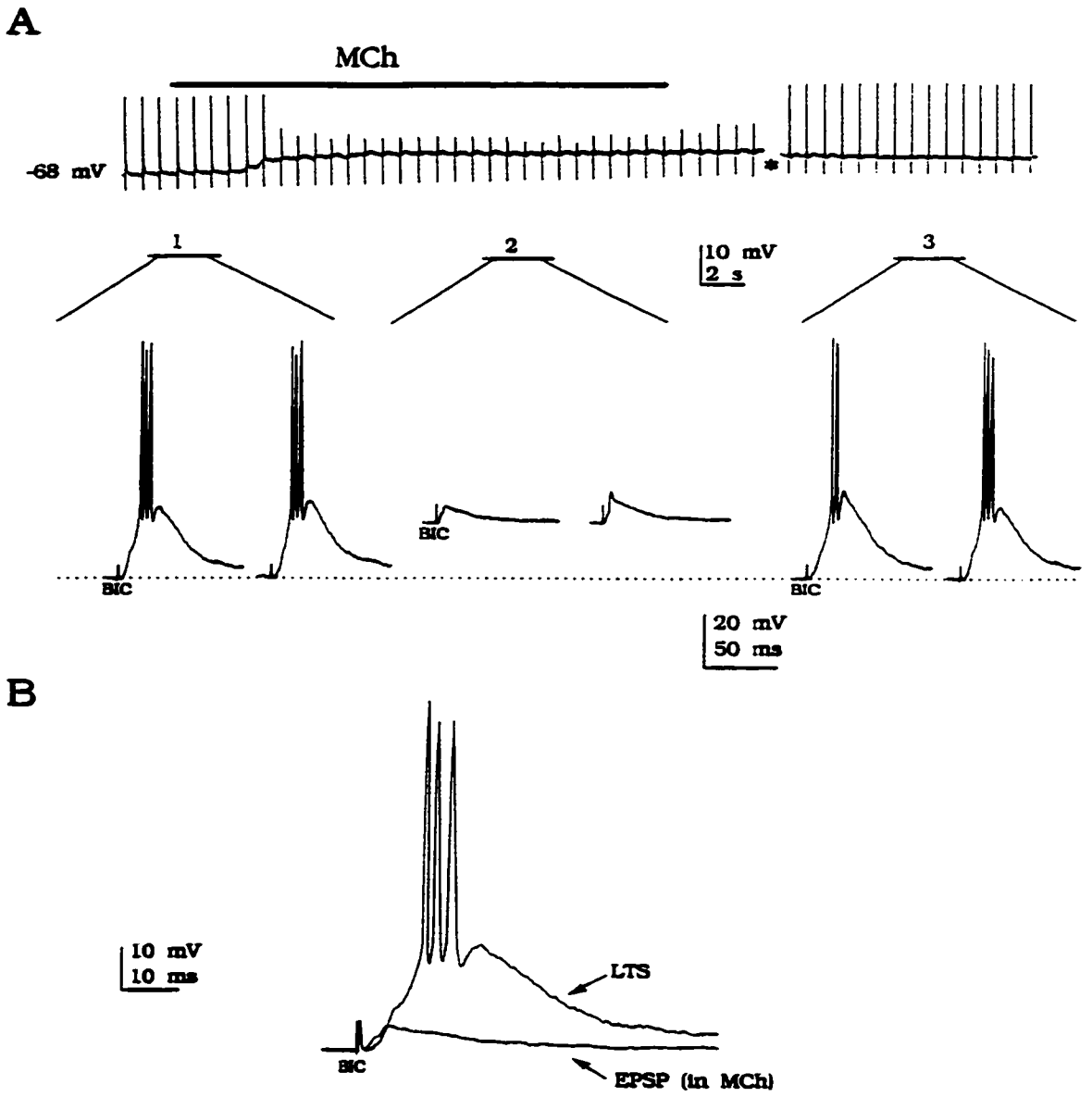
A
MCh
-68 mV
1
2
3
10 mV
2 s
BIC
BIC
BIC
20 mV
50 ms
B
10 mV
10 ms
LTS
BIC
EPSP (in MCh)

Figure 5

Figure 6 Lack of apparent inhibitory effects of muscarinic agonists on BIC-evoked EPSPs. Whole-cell recordings obtained from two MGv cells are illustrated in *a* and *b*, respectively. In each cell, a pool (20-50) of EPSPs were obtained before (control), during and after drug application (left panel). The graphs shown on the right are produced by plotting the same intracellular data against resting potential. *a*. EPSPs measured at a fixed resting potential artificially held to around -58 mV by negative current injection. The peak amplitudes of the EPSPs evoked both during control and drug application periods exhibited a similar scattered distribution ($P > 0.06$). *b*. The effects of muscarine (50 μM) on EPSPs were examined at different membrane holding potentials. The evoked EPSPs obtained both before and during drug application periods show a linear variation in amplitude in relation to membrane voltage. Muscarine exerts no clear effect on the slope of this linear relationship and on the apparent reversal potential (about -46 mV based on extrapolation) of the evoked EPSPs. Data was best-fit by linear regression. Note that the Y-axes of both figures represent absolute voltage readings of individual EPSPs, rather than baseline-subtracted EPSPs. Such a plot helps to ensure the evoked EPSPs are not contaminated by LTSs whose occurrence often imposes a clear non-linearity on a cell's V-I relationship because of the voltage dependence of LTS activation/deactivation.

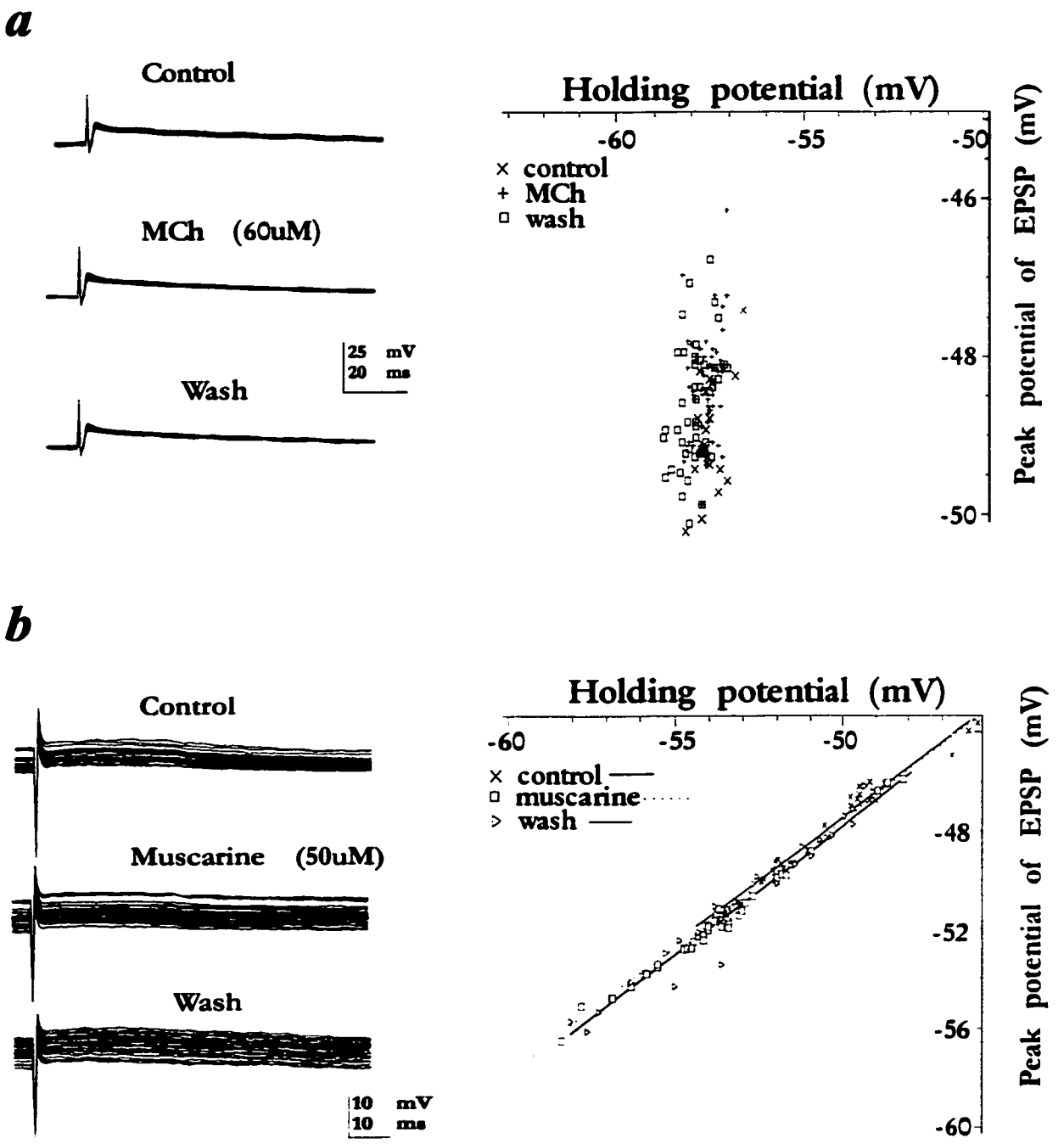
a
Control
MCh (60uM)
Wash
25 mV
20 ms
Holding potential (mV)
-60
-55
-50
× control
+ MCh
□ wash
-46
-48
-50
Peak potential of EPSP (mV)
b
Control
Muscarine (50uM)
Wash
10 mV
10 ms
Holding potential (mV)
-60
-55
-50
× control
□ muscarine
> wash
-48
-52
-56
-60
Peak potential of EPSP (mV)

Figure 6

Index Terms

Muscarine; inhibition; low-threshold spike; rat; auditory thalamus; EPSP-LTS coupling.

Chapter 3.

Mooney, D. M., Senatorov, V. V., and Hu, B. Differential resting membrane potential regulation in primary and nonprimary auditory thalamus by muscarinic receptor activation. To be submitted to Proc. Natl. Acad. Sci. USA.

Title: **Differential Resting Membrane Potential Regulation in Primary and Nonprimary Auditory Thalamus by Muscarinic Receptor Activation**

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Running title: Path-specific muscarinic modulation

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Abbreviations: ACh, acetylcholine; ACSF, artificial cerebrospinal fluid; 4-AP, 4-aminopyridine; BIC, brachium of the inferior colliculus; GIRK, G-protein-gated inward rectifier K^+ channel; LTP, long-term potentiation; LTS, low-threshold spike; MGB, medial geniculate body; MGd, dorsal division of the medial geniculate body; MGv, ventral division of the medial geniculate body; MRA, muscarinic receptor activation; RMP, resting membrane potential; TTX, tetrodotoxin; V_m, membrane potential.

ABSTRACT

We investigated the cellular mechanisms by which acetylcholine (ACh) modulates synaptic responses in primary and nonprimary auditory thalamic pathways. A thalamic explant preparation was employed, which allowed the parallel medial geniculate body (MGB) pathways, together with their sensory afferents, to be maintained and accessed *in vitro* for comparative electrophysiological studies. Application of single shocks to the sensory afferents of the MGB produced either a single action potential or a high-frequency burst response in MGB neurons, which can be recorded extracellularly. When the MGB explant was persistently exposed to acetylcholine, the sensory synaptic responses evoked from the ventral division of the MGB (MGv) consisted primarily of a single action potential that was able to follow high-frequency stimuli. In contrast, neurons of the dorsal division of the MGB (MGd) responded to sensory pathway stimulation exclusively with a burst of action potentials, which failed to follow a stimulation frequency higher than 5 Hz. Intracellular recordings revealed that muscarine induces a slow membrane depolarization in MGv neurons and a decrease in membrane conductance, which facilitates the triggering of a single action potential by an EPSP. An opposite effect was found in MGd. A membrane hyperpolarization response, accompanied by an increase in membrane conductance, was observed in MGd cells where muscarinic receptor activation (MRA) promotes coupling between EPSPs and the low-threshold calcium spikes (LTS) that underlie burst responding. This differential cholinergic effect was blocked by muscarinic receptor antagonists and was not clearly replicated by agonists acting at the receptors of other modulator candidates such as noradrenaline. Therefore, ACh may modulate auditory thalamocortical sensory processing by promoting

parallel modes of synaptic transmission: a fast synaptic responding mode in the primary thalamocortical pathway for high-fidelity sensory relay and a slower but robust bursting transmittal mechanism in the nonprimary pathway suitable for the induction of synaptic plasticity in target cells.

INTRODUCTION

The mammalian thalamocortical system consists of parallel, often distinct functional pathways (Jones, 1985 and 1991; Calford and Aitkin, 1983). In the auditory system, the primary or lemniscal pathway originates from the ventral (MGv) division of the medial geniculate body (MGB) and projects to the primary auditory cortex. It maintains a tonotopical organization and relatively fast synaptic transmission mechanism, allowing a high-fidelity relay of acoustic signals arising from the central nucleus of the inferior colliculus (Anderson et al., 1980; Clarey et al., 1992; Clarey and Irvine, 1990a and 1990b; Graybiel, 1972). In contrast to this lemniscal organization, the nonprimary thalamocortical system exhibits a relatively sluggish synaptic response to acoustic input but remarkable modifications in its activity and plasticity in response to novel stimuli or during behavioral conditioning (Aitkin and Prain, 1974; Calford, 1983; Edeline and Weinberger, 1991; Kraus et al., 1994; Weinberger, 1993; Winer, 1992). The nonprimary thalamocortical neurons are located in the dorsal (MGd) and medial (MGm) divisions of the MGB. They are reciprocally connected with the associational auditory cortices (e.g. superior temporal gyrus and temporal polar region) and, in the case of MGm, project to the lateral amygdala (Anderson et al., 1980; LeDoux et al., 1985; Shinonaga et al., 1994; Winer, 1992). Lesions of the nonprimary auditory system are often associated with profound deficits of auditory learning and memory in animals and humans (Kelly, 1973; Layton et al., 1979; LeDoux et al., 1986; McCabe et al., 1993; Neff et al., 1975; Poremba and Gabriel, 1997; Semrud-Clikeman, 1997; Damasio et al., 1996).

A large body of literature now indicates that changes in behavioral states such as selective attention, arousal, and the sleep-waking cycle are accompanied by a significant

transformation in the firing pattern of thalamic neurons and such functional adaptive responses are primarily governed by the brainstem reticular formation (Kraus et al., 1992; Singer, 1979; Steriade and Deschênes, 1984; Steriade and Llinás, 1988; Steriade et al., 1990; Velasco et al., 1968). Reticulothalamic afferents originate mainly from a group of cholinergic neurons located in the mesopontine reticular formation (Levey et al., 1987; Steriade et al., 1990). A significant rise in ACh level in the thalamus was observed during states of waking and rapid eye movement (REM) sleep (Williams et al., 1994). Activation of cholinergic afferents can lead to arousal and enhanced sensory synaptic transmission (Francesconi et al., 1988; Steriade et al., 1990). Such cholinergic modulatory effects are primarily mediated by a slow membrane depolarization in thalamic relay neurons through muscarinic receptor activation (Deschênes and Hu, 1990; McCormick, 1992a).

Despite the availability of considerable information on the role of cholinergic control of vigilant states, the conception that cholinergic effects constitute a rather global neuromodulatory mechanism seems incompatible with the marked functional differentiation and diverse patterns of synaptic activity seen in the central auditory system. Recent *in vitro* electrophysiological studies indicate that primary and nonprimary MGB neurons exhibit distinct modes of synaptic response to sensory afferent stimulation with the former discharging a short-latency single spike and the latter firing a high-frequency burst (Hu, 1995). Interestingly, Clugnet and LeDoux (1990) reported that stimulating the MGB with high frequency bursts, but not tonic pulses, leads to a long-term potentiation (LTP) of the synaptic response in the lateral amygdala. Such an LTP may result from an enhancement of glutamate release from MGB efferents (Rogan and LeDoux, 1995; McKernan and Shinnick-Gallagher, 1997). One possible scenario could be that cholinergic afferents exert their

modulatory effect in a synaptic pathway dependent manner, enhancing rather than diminishing the physiological differentiation of parallel sensory pathways.

In the present study, we investigated the membrane effect of ACh and its impact on synaptic responses in both primary and nonprimary auditory neurons from MGB explants maintained *in vitro*. Our results demonstrate that activation of muscarinic receptors in the MGB leads to opposite membrane effects in parallel thalamocortical pathways. In MGv neurons, it induces a membrane depolarization and a facilitation of single spike synaptic responses following sensory pathway stimulation; whereas in MGd cells MRA elicits a membrane hyperpolarization and high-frequency synaptic burst responses. Therefore, ACh may help to create and maintain a fast synaptic responding mode in the primary thalamocortical pathway for high-fidelity sensory relay and a slower but robust bursting transmittal mechanism in neurons of the nonprimary pathway suitable for LTP induction in cortical target cells. Parts of these results have been previously published in abstract form (Mooney and Hu, 1995 and 2000).

METHODS

Tissue Preparation. The general methodology of the explant preparation has been described in detail elsewhere (Hu et al., 1994). The experimental protocol was approved by the hospital animal care committee, which ensures conformity with guidelines set by the Canadian Council on Animal Care. Male Long-Evans rats (50-80 g shipped weight) from Charles River Laboratories were used. Age when used: 39 ± 1 d (intracellular sharp-electrode work) or 47 ± 2 d (extracellular work). Averages are given ± S.E. Briefly, the rat was decapitated and the brain removed. The front and back of the brain were squared with

coronal sections through the optic chiasm and middle of the cerebellum. The left half of the brain was isolated and pinned to the Sylgard base of a humidified recording chamber (School of Pharmacy, University of London). The temporal-occipital lobes and hippocampal tissues adjacent to the MGB and the brachium of the inferior colliculus (BIC) were aspirated to expose the free surfaces of the MGB and BIC. The pia and blood vessels were then peeled off the MGB and BIC using two fine (#5) forceps. A silicone tube stationed dorsoposteriorly to the MGB delivered a gravity feed of oxygenated (95% O_2; 5% CO_2) artificial cerebrospinal fluid (ACSF) at a flow rate of 4.5 ± 0.09 ml/min (intracellular sharp-electrode work) or 6.1 ± 0.23 ml/min (extracellular work). For some of the extracellular work, the flow rate was 4 ml/min. The ACSF was warmed (equilibrated with a water bath at 33.5 ± 0.05 °C) during intracellular sharp-electrode and extracellular recordings. The superfusion tube was adjusted so that the free surface of the MGB and the rostral two thirds of the BIC were totally submerged in the ACSF.

The composition of the ACSF was (in mM): NaCl, 122; KCl, 3; $MgCl_2$, 1.3; $NaHCO_3$, 25.9; $CaCl_2$, 0.8 (extracellular recording), 3.0 (intracellular sharp-electrode recording), or 2.0 (whole-cell recording) and glucose, 11. Final pH after saturation with the oxygenation gas: 7.2. Final osmolality: 295 ± 2 mOsm/kg.

The stimulating electrode was inserted in the BIC 1-2 mm from the MGB. The electrode was bipolar, consisting of two chlorided silver wires 120 μm in diameter, insulated except for about 1 mm at the tips. The wires were spaced to take in most of the width of the BIC. To allow for stabilization, recording did not start until at least two hours after the preparation was made.

Some experiments were done in whole-cell patch recording mode with voltage

clamp. These experiments utilized tissue slices rather than explants. 400 μm horizontal or coronal MGB slices were cut on a Vibratome at 0°C and then stabilized 1-2 hr. at room temperature in moving, oxygenated ACSF. During recording, slices were maintained under flowing (2 ml/min), oxygenated ACSF at room temperature.

Recording and Stimulation. The entire free surface of the MGB in explant provided a total working area of about 1.0 x 0.5 mm. The MGB is seen as a large, hemispherical body of gray matter. The locations of our recordings were restricted to the dorsal and ventral parts of the MGB, avoiding borderline areas. Dorsal recordings were predominantly in the caudodorsal division, a major component of the nonprimary auditory thalamus. Ventral recordings were in the lateroventral division, a major component of the primary auditory thalamus.

Extracellular recording micropipettes were prepared on a Narishige vertical puller from borosilicate glass capillary tubing (1.5 mm o.d., 0.84 i.d., with filament, WPI, FL, USA) and filled with 3 M NaCl. DC resistance with this fill was 26 ± 1 MΩ (37% of the extracellular database was recorded with electrodes filled with 4 M K acetate + 0.15 M KCl.). Intracellular sharp recording micropipettes were made with borosilicate tubing (1.5-mm o.d., 0.84 i.d., with filament, WPI, FL, USA) on a Flaming/Brown puller, Model P-87. Sharp electrodes contained K acetate (1-4 M) and KCl (0.04-0.15 M). DC resistance was 96 ± 6 MΩ with a fill of 4 M K acetate + 0.15 M KCl. Signals were conventionally amplified (Axon Instruments Inc.; Axoclamp-2A for intracellular and extracellular recording), high-pass filtered at 200-300 Hz if extracellular, and digitized (Vetron Model 400) at 22 kHz for videotape recording, or were captured on-line with pClamp software.

The recording electrode was lowered manually until it touched the surface of the

tissue, as evidenced by an electrical transient appearing on the monitoring oscilloscope. The electrode was then lowered using an inchworm motor and electronic driver (Burleigh) in steps of 2.5 μm (extracellular) or 3.5 μm (intracellular sharp-electrode) to search for neurons. All neurons were recorded within a layer of tissue extending from the ventral free surface of the MGB to a depth of 500 μm. This layer included the marginal zone, a thin layer of cells about 75 μm thick overlying the MGv. The surface location of each neuron, along with the depth of the recording position below the surface of the tissue as registered by the Burleigh electrode advancer, were recorded. In extracellular work, stimulation was delivered to the BIC at a repetition rate of 1 Hz and consisted of 39 ± 5 V biphasic pulses (range 7-80 V) of 0.1-0.2 ms duration. In the project in which the MGB was exposed to acetylcholine continuously, monophasic constant-current pulses of 10 mA were used. When a neuron (action potentials following the stimulus artifact) was found, the voltage was reduced to near threshold. Threshold was defined as the stimulation intensity that provoked a synaptic response in about 30-50% of trials during 10-20 consecutive stimuli. Following the identification of threshold, the stimulation voltage was raised just sufficiently to obtain adequately frequent responding. In intracellular sharp-electrode work, positive current pulses of 0.75 nA and 100 ms were applied to the recording electrode at 1.4 Hz and the proximity of a cell detected by a rise in voltage during the pulse. The capacitance neutralization circuit was oscillated for 1 ms to effect impalement. Negative current was injected to maintain V_m less than -100 mV, and was reduced as the seal developed. Unless otherwise indicated, experiments were performed in the absence of injected DC currents. After electrode withdrawal, an extracellular DC potential was recorded and was subtracted from the intracellularly measured V_m to give a more accurate value of V_m. Cells were required to have

the LTS characteristic of thalamic projection neurons, and to have a membrane resistance of at least 30 MΩ to be included in the statistical database. Membrane resistance was measured using current pulses of -0.1 to -0.4 nA, 140 or 300 ms in duration, at 0.5 or 0.2 Hz. Changes in membrane conductance due to muscarine application were measured with current pulses of -0.2 to -0.25 nA (a relatively linear part of the V/I curve), or with current ramps, measurements being taken at times corresponding to -0.2 nA. Conductance measurements were done under manual voltage clamp to avoid the activation of voltage-sensitive currents. The manual part of the injected current was not included in the current used for the conductance calculation.

In whole-cell voltage clamp experiments, patch electrodes were made from borosilicate glass capillary tubing (1.5 mm o.d., with filament, WPI, USA) on a Flaming/Brown puller, Model P-87. Electrodes were filled with (in mM): K gluconate, 130; NaCl, 10; KCl, 10; Na-HEPES, 10; acid EGTA, 1 and Mg-ATP, 4. The pH of the solution was adjusted to 7.4 with 1 M KOH. The DC resistance of the patch electrodes was 4-8 MΩ. Signals were recorded using Axopatch-200A amplifiers (Axon Instruments Inc., USA) and were digitized and videotaped with a Vetron PCM Recorder (Vetron Technology. Inc., USA) or were digitized with a Digidata 1200 Interface (Axon Instruments Inc., USA) for on-line acquisition using pClamp software. Seal resistance was 2-5 GΩ. In all experiments, pipette capacitance was compensated before breaking the cell membrane. Up to 80% of the series resistance was compensated. The liquid junction potential was measured by a published technique (Neher, 1992) and found negligible.

Drug Application. Acetylcholine chloride, 4-aminopyridine, buspirone hydrochloride, (+)-muscarine chloride, (-)-norephedrine, (-)-phenylephrine hydrochloride,

and pirenzepine 2HCl were purchased from Research Biochemicals International (RBI; Natick, MA, USA). Atropine sulfate, carbachol, EGTA, hexamethonium bromide, magnesium ATP, (±)-muscarine chloride, neostigmine bromide, K gluconate, tetrodotoxin (TTX) with citrate buffer, and tropicamide were purchased from Sigma. ACSF salts and K acetate were purchased from Fisher. Na-HEPES was from ICN. Trypan blue was from BDH. Except for TTX and the electrode fill salts, the chemicals were prepared as stock solutions (0.006-10 mM) in ACSF free of glucose and calcium, and stored at 4° C or -10° C. TTX stock solution, electrode fill solutions and ACSF were made up in distilled, deionized water. All antagonists were added as stock solutions to ACSF contained in an oxygenated side-bath to produce the final concentrations indicated in the text. Antagonists were applied by switching the source of the superfusate from the main reservoir to the sidebath. Agonists were applied by steadily infusing them into the ACSF through a small catheter inserted into the supply tube immediately upstream from a small jet-and-chamber mixer, the outlet of which was stationed over the explant. An electronic timer connected to a two-way syringe pump (Harvard Apparatus) controlled the volume and timing of agonist infusions. For whole-cell recording mode, the mixer was omitted. The stated agonist concentrations were obtained by dividing the product of the stock solution concentration and the pumping rate by the sum of the pumping rate and the superfusion rate. 4-aminopyridine (4-AP) was applied by injecting a 0.5 mM stock solution into the BIC at a rate of 0.2 µl/min, using a syringe pump. The micropipette used for this was beveled at a 30° angle to produce an opening 10-15 µm in diameter, and was inserted at a 45° angle to the tissue surface to an estimated depth of 250 µm (half the thickness of the viable layer). Pumping at 1.0 µl/min was maintained during insertion. The stock solution also contained trypan blue dye at a calculated concentration of

5 mM (no correction for purity). This allowed real-time visualization of the spread of the drug from the injection site so that pumping rate and time and injection location could be adjusted to avoid spread of drug to postsynaptic elements in the MGB. In experiments in which 4-AP was injected, the explant preparation was turned 180° in a horizontal plane before experimentation and re-pinned, so that the flow of ACSF was such as to carry any 4-AP seepage from the BIC away from the MGB. The nonparametric Fisher's 2x2 exact test was used for statistical testing unless otherwise indicated in the text.

RESULTS

Extracellular synaptic responses under persistent cholinergic activation. A mixture of acetylcholine (50 to 150 μM) and neostigmine bromide (10 to 20 μM) was bath-applied to the explant continuously, starting at least ten minutes prior to the start of extracellular recording. The purpose of the neostigmine was to suppress hydrolysis of the acetylcholine by cholinesterases in the tissue. Under this condition, BIC stimulation induced exclusively burst responses in MGd (n = 10) and primarily single-spike responses in MGv (10/12), indicating a significant difference in responding mode ($P < 0.0005$; Fig. 1). Higher concentrations of acetylcholine (100-150 μM) could produce spontaneously rhythmic bursts of action potentials (n = 4), similar to an epileptiform activity previously observed with MRA (Mooney and Hu, 1994).

Membrane effects of MRA. The database for our electrophysiological and pharmacological investigation of resting membrane potential (RMP) and conductance modulation consisted of intracellular recordings from 42 neurons in the MGd and 49 neurons in the MGv. 87% of the neurons had overshooting or nearly overshooting action potentials

and 79% had membrane potentials more negative than -55 mV. The average membrane potential of MGd neurons was –60 ± 1 mV and the average potential for MGv neurons was –62 ± 1 mV. The membrane potential numbers must be seen in light of recent studies in hippocampal slices. RMP measured with sharp electrode was found to be 10-15 mV more positive than with whole-cell patch recording (Staley et al., 1992), a difference caused by a nonselective leak conductance introduced by impalement. The average membrane resistance of MGd neurons was 92 ± 5 MΩ and of MGv neurons was 89 ± 7 MΩ.

Application of (±)-muscarine (50 μM) or (+)-muscarine (50 μM) for 20 s or occasionally 40 s usually resulted in hyperpolarization of MGd neurons and depolarization of MGv neurons (Fig. 2 and Fig. 3). The muscarine concentration ranges used in MGv and MGd were almost the same. The histogram of Fig. 3 was based on 26 MGd neurons and 36 MGv neurons. The proportion of MGd neurons that hyperpolarized was significantly higher than the proportion of MGv neurons that did so ($P < 0.001$). The proportion of MGv neurons that depolarized was significantly higher than the proportion of MGd neurons that did so ($P < 0.001$). The fact that a few neurons supposedly in MGv hyperpolarized and a few neurons supposedly in MGd depolarized, contrary to what the majority did, may be due to errors in placement of the recording electrode. In 9 experiments, MGv-neuron depolarization and MGd-neuron hyperpolarization were observed in the same explant. Neither MGd-neuron hyperpolarizations ($n = 3$) nor MGv-neuron depolarizations ($n = 6$) induced by muscarine were affected after synaptic transmission was blocked by TTX (0.3 or 1.0 μM), indicating that muscarine acted directly on the recorded neuron (Fig. 4).

The dose-response relationship of membrane potential changes had a great deal of scatter among different neurons. However, the dose-response curves from individual neurons

could be compared if normalized. Such data is shown in Appendix 2 for seven neurons. Curves with negative slopes were omitted. The data show an increasing trend in the amplitude of the membrane potential change in the range 10-80 μM.

In MGv the average peak depolarization was 7.7 ± 0.7 mV (n = 15). With a 20 s application period the depolarization had an average duration (development plus recovery) of 390±40 s. In MGd the average peak hyperpolarization was -5.2 ± 0.5 mV (n = 16) and, with a 20 s application period, the hyperpolarization had an average duration of 160 ± 10 s. This duration was significantly shorter than that of MGv neuron depolarizations ($P < 0.01$; nonparametric Mann-Whitney test). Muscarine-induced hyperpolarization in MGd neurons (n = 3) and muscarine-induced depolarization in MGv neurons (n = 3) were reversibly blocked to the extent of 70-100% (avg. 88 ± 6%) by the muscarinic antagonists pirenzepine (1 μM), atropine (5 μM), or tropicamide (50 or 100 μM) (Fig. 5). Recovery from the blockade was about 69 ± 14% of the control.

Conductance changes induced by MRA. During muscarinic hyperpolarizations in MGd neurons, the neuron's membrane conductance increased by an average of 41 ± 13% (n = 4). During muscarinic depolarizations in MGv neurons, conductance decreased by an average of 21 ± 5% (n = 8). These measurements were done under manual voltage clamp. MGd conductance changes were significantly different from those found in MGv ($P < 0.01$).

The MGd hyperpolarizing response had a reversal potential that averaged –96 ± 4 mV (n = 2), suggesting an opening of K^+ channels. Reversal potential was measured either by observing the absence of a muscarinic effect under conditions of membrane hyperpolarization due to artificial current injection, or by using current pulses or a ramp to obtain the V/I curve with and without muscarine present, and observing the voltage at which

the curves crossed. That the potassium conductance was specifically the voltage-independent leak conductance that was observed in an earlier study (McCormick, 1992b), was suggested by the finding that under manual voltage clamp the curves of chord conductance versus membrane potential with and without muscarine were roughly parallel for a considerable range of potentials (e.g.: from -65 to -105 mV; n = 3; Fig. 6A). Lack of parallelism in this voltage range would have indicated muscarinic action on a rectifying (i.e.: voltage dependent) conductance such as GIRK1 (Ponce et al., 1996). The leak conductance is distinguished by its lack of rectification.

For MGv neurons both intracellular and whole-cell recordings were used to estimate the reversal potential of the muscarinic current. The most typical outcome of conductance studies with manual voltage clamp in MGv was that the two curves of conductance against membrane potential almost coincided, despite the presence of a depolarizing response to (+)-muscarine under current clamp (3/4; Fig. 6B). In whole-cell recording mode, muscarine induced an inward current (5/6) in responding neurons (consistent with the muscarinic response of MGv neurons). The response was associated with a decrease in slope conductance and a reversal potential range from -110 to –87 mV, indicating closure of a potassium conductance (Fig. 6D). In 3/4 neurons, the conductance change was independent of membrane voltage, indicating the involvement of a leak potassium conductance. However, in three neurons showing the muscarinic inward current, extrapolation of the difference current/voltage data suggested the existence of a zero-current potential at relatively positive values (positive to -17, -10, and -35 mV respectively). This suggests that in some experiments muscarine may induce a persistent voltage-gated sodium conductance (Gola et al., 1998).

MRA-induced modulation of synaptic responding mode. In the next series of experiments, we specifically examined the role of MRA in modulating synaptic response mode. In the absence of continuous ACh treatment, BIC stimulation induces a synaptic burst response in 89% of responding MGd neurons (n = 56) and 62% of responding MGv neurons (n = 251). A burst response was one having 2 or more spikes, a first interspike interval of less than 5 ms, and typically exhibiting an inability to follow 10 Hz, a latency to the first spike greater than 10 ms, and a variable first-spike latency (Hu, 1995). Application of (±)-muscarine to most burst responding neurons from MGd or MGv either blocked the response or had no effect. At low doses (2-30 μM), the proportion of MGv neurons that were blocked was significantly greater than the proportion of MGd neurons that were blocked ($P < 0.001$). At high doses (>30 μM), the proportion of MGd neurons that showed no effect was significantly greater than the proportion of MGv neurons ($P < 0.001$) showing no effect (Fig. 7). In 21% of the MGv burst neurons the burst blockade was followed by single-spike responding. Only one MGd burst neuron showed this combination of effects. The results show that muscarine differentially regulated the mode of synaptic transmission dorsally versus ventrally in the MGB, enhancing rather than reducing the dissimilarity between the two pathways. Under muscarine, MGd burst neurons continued to respond to synaptic stimulation with bursts, while the comparatively less-frequent MGv neurons that responded with bursts were silenced and sometimes converted to single-spike mode.

In a separate group of neurons (mean V_m = 59 mV, input resistance = 106 MΩ), we found that carbachol (0.5 mM), in the presence of 100 μM hexamethonium to suppress nicotinic effects, hyperpolarized MGd cells, similarly to what is seen with muscarine. During the hyperpolarizing response, single shock BIC stimulation at 0.5 Hz or less elicited an EPSP

that coupled to an LTS-burst complex (6/6). Such EPSP-LTS coupling was reversibly abolished if the membrane potential was temporarily returned to baseline during the hyperpolarization by injecting positive current (Fig. 8). In 4/4 neurons, carbachol treatment also suppressed shock-induced IPSPs. Whether this effect is also necessary to coupling is not known.

In the next set of experiments, in which recording positions were confined to the MGd, asynchronous action potentials were induced in BIC afferent fibers by pumping a solution of the potassium channel blocker 4-AP into the BIC. Hyperpolarization by manual current injection was used to imitate the MRA effect in MGd. (IPSPs produced by the 4-AP injection method were small to nonexistent so IPSP suppression was not a consideration in imitating MRA). In 4 neurons the drug-induced EPSPs were large enough that coupling of EPSPs to LTS-burst complexes occurred. In each of the 4 neurons coupling was only observed during manual hyperpolarization (Fig. 9). In two of these neurons, the duration of the recording was sufficient for a demonstration of recovery from manual hyperpolarization: LTS-burst complexes ceased to occur when injected current was reduced. A membrane potential of about –70 mV produced the most coupling. The results obtained by simulating MRA with manual current injection were confirmed in a fifth MGd neuron by the use of real MRA (n = 1). A hyperpolarization due to carbachol application caused coupling of 4-AP induced EPSPs to LTS-burst complexes (Fig. 11). This effect was replicated.

The EPSP-LTS-burst complexes typically had multiple shoulders on the rising side of the depolarization with the highest shoulder higher than the tops of non-coupling EPSPs or compound EPSPs. The factors determining whether coupling occurred appear to be large EPSP amplitude and/or multiple EPSPs close together in time. To further characterize EPSP

temporal summation and induction of the LTS burst, we have calculated the average number of spontaneous EPSPs that occurred within a 100 ms time window just preceding an LTS burst and this was then compared with the average number of EPSPs occurring in 100 ms windows containing at least one EPSP but not followed by burst events. As shown in Fig. 12, the mean number of "burst-associated" EPSPs per window is significantly higher than that in non-burst periods ($P < 0.0001$, two-sample Student's t-test). The rational for using a 100 ms time window is that the latency of shock-induced bursts in explant is in the range 10 - 70 ms (Hu, 1995). The neurons used to make Fig. 12 were recorded in MGd and produced EPSP-LTS-burst complexes in response to asynchronous 4-AP induced synaptic input, in the presence of hyperpolarization due to current injection or carbachol.

The hyperpolarization-activated cation conductance known as I_h triggers spontaneous LTS-burst complexes in thalamic slice preparations (McCormick and Pape, 1990), raising the question of whether the LTS-burst complexes seen in this study were triggered by I_h. Four of the five neurons exhibiting coupling were tested for the presence of I_h and all four were shown not to express I_h, as evidenced by the absence of a depolarizing sag on hyperpolarizations to below –100 mV (range: -103 to –124) induced by 140 ms current steps injected through the recording electrode.

In MGv the depolarization effect caused by (±)- or (+)-muscarine promoted non-burst responses (action potentials more widely separated than 5 ms) to spontaneous synaptic input (Fig. 10). Neurons that did not output action potentials under baseline conditions showed spontaneous action potentials after depolarization by muscarine ($n = 7$). In 2 of these neurons, spontaneous EPSPs could be seen under baseline conditions. The presumed EPSPs persisted if the baseline was manually hyperpolarized to -81 or -84 mV, showing that they

were not fast prepotentials. In the remaining 5 neurons, observation of spontaneous action potentials was taken as supporting evidence that muscarinic activation promotes single-spike responding to EPSPs in this division, since spontaneous action potentials and single-spike responding were shown to occur together in a previous extracellular study (Mooney et al., 1995). Inactivation of synaptically triggered LTSs during muscarinic depolarizations in MGv has been more amply demonstrated in a previous study (Mooney et al., 1995).

Given the co-existence of serotonergic, noradrenergic and cholinergic afferents in reticulothalamic projections (Fitzpatrick et al., 1989), we further examined whether agonists acting on noradrenergic α-1 (norephedrine/ phenylephrine, about 20 μM) and serotonergic 1A (buspirone) receptors may also promote burst synaptic responses in MGd neurons in a synaptic pathway dependent manner. Consistent with a previous study (McCormick, 1992b), our extracellular (n = 9) and intracellular (n = 5) recordings showed that these agonists mainly blocked the burst response in the MGB through a small, albeit inconsistent, membrane depolarization. There was, however, no clear differential effect exerted by these receptor agonists on MGv and MGd neurons.

DISCUSSION

Our extracellular study established that a strong differential in synaptic response mode between the MGd and MGv occurs *in vitro* under steady stimulation by acetylcholine. Burst mode responses were the only response type found in MGd whereas single-spike responses strongly predominated in MGv. A similar pattern of response was found earlier *in vitro* without the use of cholinergic agents, (Hu et al., 1994; Hu, 1995), which may reflect a differential expression of Na^+-K^+-ATPase and I_h between the two cell divisions (Hu, 1995;

Senatorov et al., 1997; Senatorov and Hu, 2000). However, we cannot exclude the possibility that a residual cholinergic effect may remain in the explants. An earlier paper (Mooney et al., 1995) demonstrated a strong tendency of MRA to selectively block the responses of burst mode neurons in MGv, but clearly, it was not blocking the burst responses of MGd neurons in our study to the extent of interfering with the response mode differential. The reason for the preservation of the response mode differential under acetylcholine was sought in intracellular recordings of MGd and MGv neurons briefly exposed to muscarine.

We observed in rat auditory thalamus differential RMP regulation by a direct action on postsynaptic muscarinic receptors, depolarizations occurring in MGv and hyperpolarizations occurring in MGd. The difference was not due to variations in the condition of the preparation because the differential effect was demonstrated in single explants. A role in regulation was suggested by the fact that RMP effects were modest but greatly outlasted the drug application period. Conductance was increased in MGd and decreased in MGv, with the leak potassium conductance apparently involved in both kinds of response. Our finding that the muscarinic depolarization was due to closure of a leak conductance agrees with an earlier study (McCormick, 1992b). However, we also found that opening of what appeared to be the leak conductance was responsible for muscarinic hyperpolarization. This is at variance with the earlier study that found activation of a rectifying potassium current during periods of outward current mediated by a muscarinic agonist (McCormick, 1992b). This may be due to a species difference (rat versus guinea pig) or to a difference in recording position (MGB versus dorsal lateral geniculate body).

Different muscarinic effects on membrane potential or current have been reported in various brain regions (Gabel and Nisenbaum, 1999; Greene et al., 1989; McCormick, 1992b;

McCormick and Prince, 1987; McQuiston and Madison, 1999; Nunez et al., 1997; Segal, 1982; Wakamori et al., 1993; Xi-Moy et al., 1993). In lateral geniculate nucleus of cat, projection neurons depolarized in response to a muscarinic agonist (with some neurons exhibiting a small preceding hyperpolarization), whereas reticular thalamic neurons and inhibitory interneurons exhibited only muscarinic hyperpolarizations (McCormick and Pape, 1988; McCormick and Prince, 1986 and 1987). However, the present results represent the first clear finding of opposite muscarinic effects on membrane potential and synaptic response mode in parallel thalamocortical projection pathways. Muscarinic hyperpolarization in MGd promoted synaptic transmission in burst mode, whereas muscarinic depolarization in MGv promoted transmission in tonic mode. The burst promoting effect has also been found previously in thalamic reticular neurons (McCormick and Prince, 1986). Transmission in burst mode could be driven by asynchronous drug-induced EPSPs, suggesting that EPSP-LTS coupling in the MGd is not an artificial phenomenon requiring unrealistic synchronous firing of innervating axons induced by electrical shock. The factors determining whether coupling occurred appeared to be large EPSP amplitude and/or multiple EPSPs close together in time. The importance of multiple EPSPs is shown in Fig. 12. This suggests that EPSP-LTS coupling in MGd can be utilized to detect coincidental synaptic events. It may be asked why burst firing in MGd neurons serves as a mechanism that is superior to single spike firing for detecting coincidences. The answer to this question may be threefold. 1. Our modeling study (see below) shows that bursts are triggered preferentially by large EPSPs that are mediated by NMDA receptors. The NMDA glutamate receptor has kinetic properties suitable for temporal integration of coincidental synaptic inputs (Seeburg et al., 1995; Binns and Salt, 1996). 2. The present study demonstrates that bursts tend to be triggered by multiple

EPSPs occurring close together in time. However, in tonic mode single spikes would be triggered by all EPSPs, leading to a high background activity, reducing the detectability of coincidence events. 3. Spontaneous bursts in thalamic neurons may elicit spikes in cortical neurons with higher reliability than thalamic single spikes (Swadlow and Gusev, 2001). This indicates that coincidences encoded by bursting could be communicated to cortex with higher reliability than coincidences encoded by single spikes.

In an unpublished computer modeling study (Hu et al., 1995; Hu and Mooney, 1998), we showed that thalamic neurons can selectively transmit a pattern of large EPSPs in the presence of randomly occurring smaller EPSPs, based on membrane hyperpolarization and the presence of the T-type Ca^{2+} current. A pattern made up of large EPSPs was concealed within a stream of random, smaller EPSPs. Both kinds of EPSP triggered action potentials when the simulated neuron was relatively depolarized, leading to a noisy output in which no pattern was visible. When the neuron was somewhat hyperpolarized, simulating the action of MRA, only the large EPSPs triggered action potentials, by first coupling to the LTS. The hidden pattern was thereby revealed. No such selective, differential transmission was seen in the absence of the T-current responsible for the LTS. In this model, the muscarinic hyperpolarization may exert a dual effect: to prevent small EPSPs from reaching action potential threshold, and to de-inactivate the T-current, thereby enabling coupling between a large EPSP and the LTS.

The signal transduction mechanism underlying differential muscarinic effects on the leak conductance was not addressed in this study. It is possible that different muscarinic receptor subtypes are expressed dorsally versus ventrally in MGB. Activation of the M2 subtype has been associated with hyperpolarization (Leonard and Llinás, 1994; Mochida,

1990). Activation of the M1 subtype has been associated with depolarization (Hsu et al., 1996; Klink and Alonso, 1997). A comparative study of receptor subtype expression in MGv and MGd is needed. Co-application of subtype-specific antagonists such as AF-DX 116 with muscarine is one possible experimental approach (Mochida, 1990). The differential muscarinic effect may be accompanied by differences in signal transduction pathways. Additional studies are needed to address this issue. For instance, differential involvement of the phosphoinositol/protein kinase C pathway would be revealed if intracellular application of a specific protein kinase C inhibitor such as a bisindolylmaleimide (Toullec et al., 1991) abolished the muscarinic effect in one pathway but not in the other.

Firing pattern transformation is an essential element of sensory synaptic signaling. It has been proposed that in visual thalamus the burst mode is used for detection of stimuli, especially non-optimal stimuli, whereas the tonic mode is used for analysis (Sherman and Guillery, 1996). However, Reinagel et al. (1999) found that in primary visual thalamus both bursts and tonic spikes encode stimulus information efficiently, which raises the question of why the thalamocortical system should require these two seemingly redundant firing modes. The answer may be that burst mode is entered in order to enable synaptic plasticity in cortex. A possible advantage of this kind of enabling effect is that its spatial resolution could be higher than that of other controllers of plasticity such as cortical cholinergic excitation (Weinberger and Bakin, 1998), as the result of control of thalamic membrane potential by GABAergic reticular thalamic inputs. However, our focus is on entry into burst mode by means of global muscarinic effects in MGd, a mechanism that may take advantage of the burst channel to add a pathway-specific component of plastic change. Long-term changes in synaptic plasticity between interconnected limbic structures often require patterned activities

(Holscher et al., 1997; Lynch, 1998; Thomas et al., 1998). Thus, stimulation of the perforant pathway with theta rhythm bursts (an EEG pattern associated with exploratory behavior) is particularly effective in inducing LTP in the hippocampus (Holscher et al., 1997; Thomas et al., 1998). LTPs also develop in the lateral nucleus of the amygdala and associational auditory cortex during fear conditioning and associative learning (LeDoux et al., 1988; Quirk et al., 1997; Rogan and LeDoux, 1995). In the MGB-amygdala pathway, however, neither the standard hippocampal stimulation paradigms nor tonic stimulation mimicking a normal MGB neuronal firing pattern was effective in LTP induction (Clugnet and LeDoux, 1990). The only MGB output pattern effective for LTP induction was short bursts consisting of 30 pulses at 400 Hz, delivered once per second (Clugnet and LeDoux, 1990). The physiological basis of such a robust phenomenon is unknown, although transmitter release at axonal terminals may be affected by a firing pattern initiated at the soma or axon hillock. This conjecture is consistent with the finding that MGB-amygdala LTP may result from an enhancement of glutamate release from MGB projecting axons (McKernan and Shinnick-Gallagher, 1997). The present finding that MGd neurons maintain a strong tendency to fire bursts of action potentials after ACh exposure supports the notion that the output of the MGd may play an important role in the induction of synaptic potentiation in temporal nonprimary auditory cortex. This conjecture is also consistent with a recent observation that muscarinic receptor blockade prevented rats from learning an auditory discrimination task but did not affect maintenance of learned signal discriminations (Kudoh et al., 2000).

In conclusion, our results show that cholinergic control of auditory thalamocortical sensory processing consists of a differential modulatory mechanism mediated by muscarinic receptor activation. This mechanism helps create and maintain a fast synaptic responding

mode in the primary thalamocortical pathway for high-fidelity sensory relay and a slower but robust bursting transmittal mechanism in the nonprimary pathway. The latter may be suitable for detection of sensory events and induction of long-lasting changes in synaptic strength in target cells.

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Figure 1 Synaptic responses recorded in the MGB under steady stimulation by acetylcholine in the presence of neostigmine. Traces show extracellular recordings of responses of four representative neurons to single-shock stimulation of the BIC, with stimulation times aligned. The time of stimulation is shown by the arrowhead. Stimulus artifacts have been deleted for clarity. Horizontal bars on the BIC indicate stimulation electrodes. Filled circles (•) suggest the distribution of recording positions in MGd and MGv. High-frequency burst responses were recorded in MGd, (top two traces) whereas single-spike responses were recorded in MGv (bottom two traces). Schematic of MGB adapted from Fig. 1 in Hu et al., 1994.

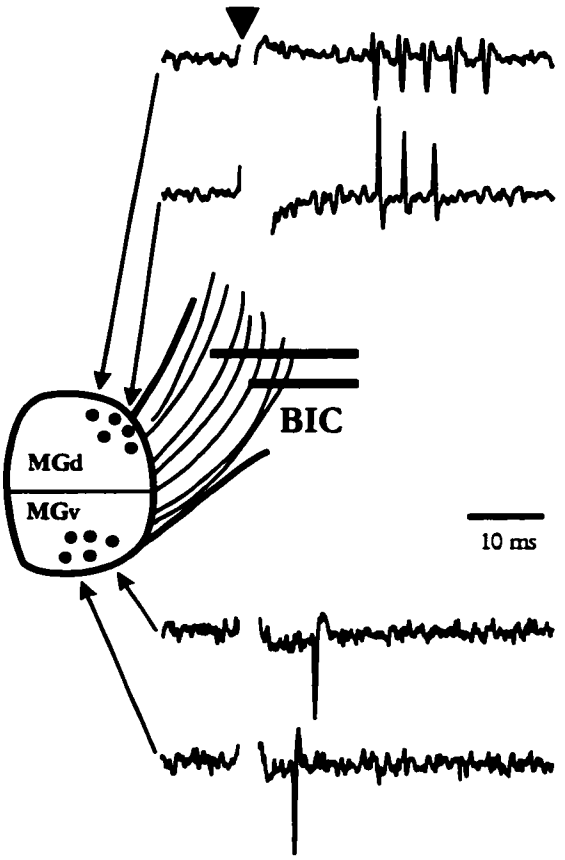


Figure 1

Figure 2 Change in RMP due to application of (±)- or (+)-muscarine, measured at the time of maximum change. Each diamond is one neuron. The X-axis is an artificial variable used to group data according to anatomical location. Scatter in RMP change did not appear to derive from variations in the depth of the recorded neuron below the surface of the tissue. There were 5/7 cases of non-reproducibility of membrane potential change in a given neuron at a given concentration. In the five cases of non-reproducibility, membrane potential changes were consistent in direction but inconsistent quantitatively.

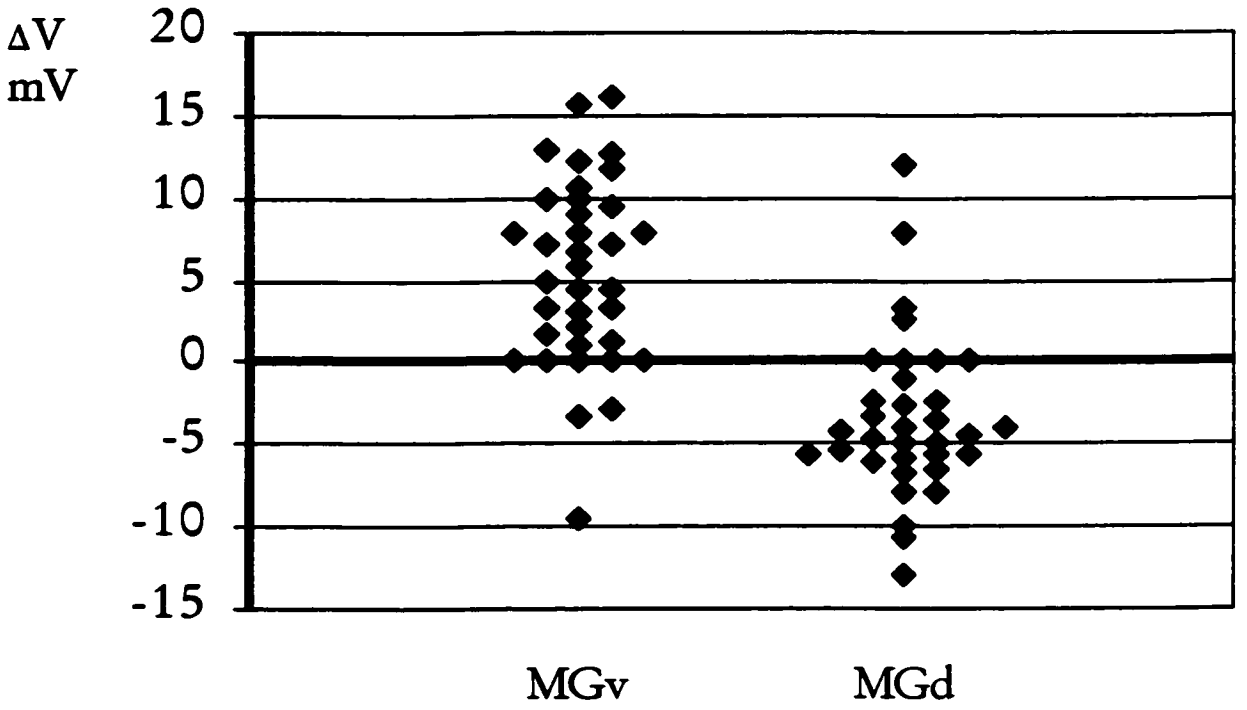


Figure 2

Figure 3 Differential regulation of membrane potential by (±)- or (+)-muscarine in the MGd and MGv divisions of the MGB. Bars above traces of membrane potential give application periods. Numbers above bars give concentration of (+)-muscarine. Dots in schematic of MGB give representative recording positions. Schematic of MGB adapted from Fig. 1 in Hu et al., 1994. Bottom MGd trace and middle MGv trace are from same explant. Histogram: "hyper." = hyperpolarization; "depol." = depolarization; "biph." = biphasic response, with hyperpolarization followed by depolarization. MGd and MGv neurons were statistically compared with each other within each category of muscarinic response using the nonparametric two-sample proportion test. Note that the percentage of hyperpolarizing MGd neurons was significantly greater than the percentage of hyperpolarizing MGv neurons, and that the percentage of depolarizing MGv neurons was significantly greater than the percentage of depolarizing MGd neurons. Asterisks: $P < 0.001$. In 10 neurons, there was no effect of muscarine and the "no effect" category is not included in the figure. Neurons in which the muscarine effect was not replicated were not used to make the frequency histogram, unless the effect was strong (i.e.: absolute value of membrane potential change $\geq$ 5 mV). 89% of neurons used to make the histogram had a replicated effect. Neurons showing more than one kind of effect were classified according to the stronger or more replicated effect. Neurons in which membrane potential did not show recovery toward baseline following muscarine application or in which the muscarine effect was both weak and inconsistent were excluded from the analysis.

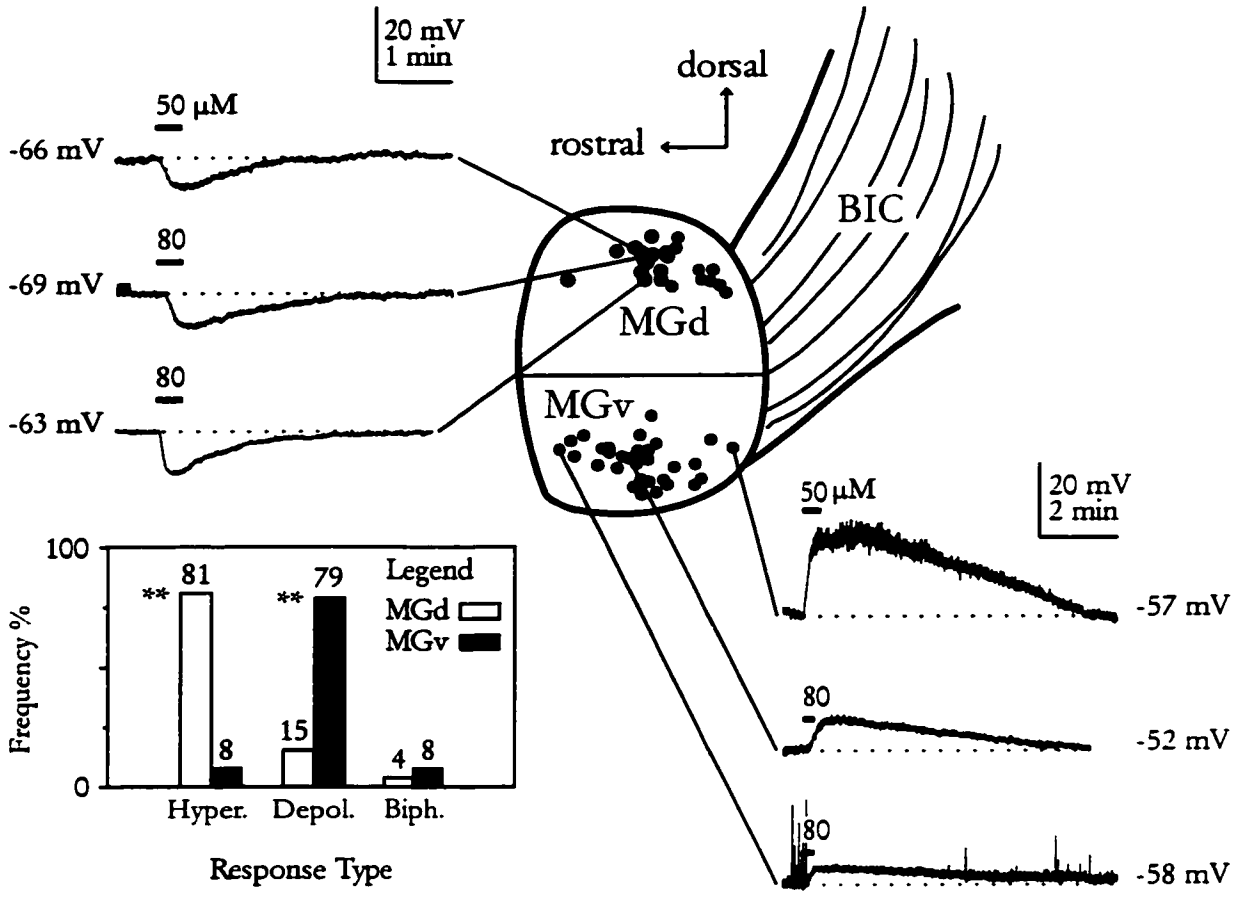

20 mV
1 min
dorsal
rostral
50 µM
-66 mV
80
-69 mV
80
-63 mV
BIC
MGd
MGv
50 µM
20 mV
2 min
-57 mV
80
-52 mV
80
-58 mV
100
0
Frequency %
81
**
8
79
**
15
4
8
Legend
MGd
MGv
Hyper.
Depol.
Biph.
Response Type

Figure 3

Figure 4 The effect of muscarine on MGd and MGv neurons was direct and not mediated by synaptic transmission. The smaller traces above the muscarinic responses show the synaptic response of the neuron to BIC stimulation. In each case, synaptic transmission was blocked under TTX, whereas the effect of muscarine on RMP persisted. The two neurons shown were recorded in MGd and MGv divisions of the same MGB. The reduction in the amplitude of the muscarinic depolarization under TTX that was seen for the ventral neuron was atypical: usually no reduction or a slight reduction was seen (4/5).

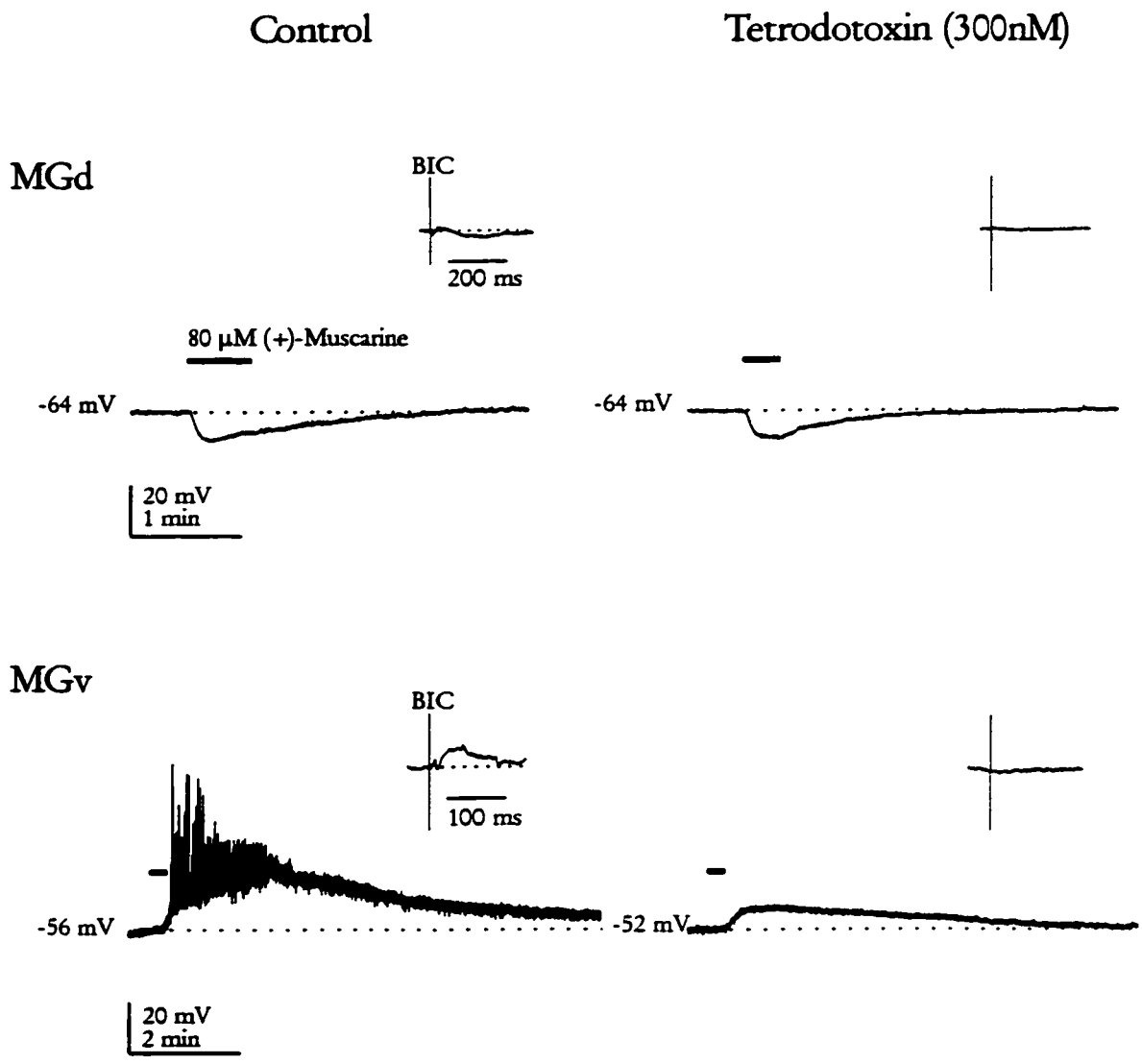
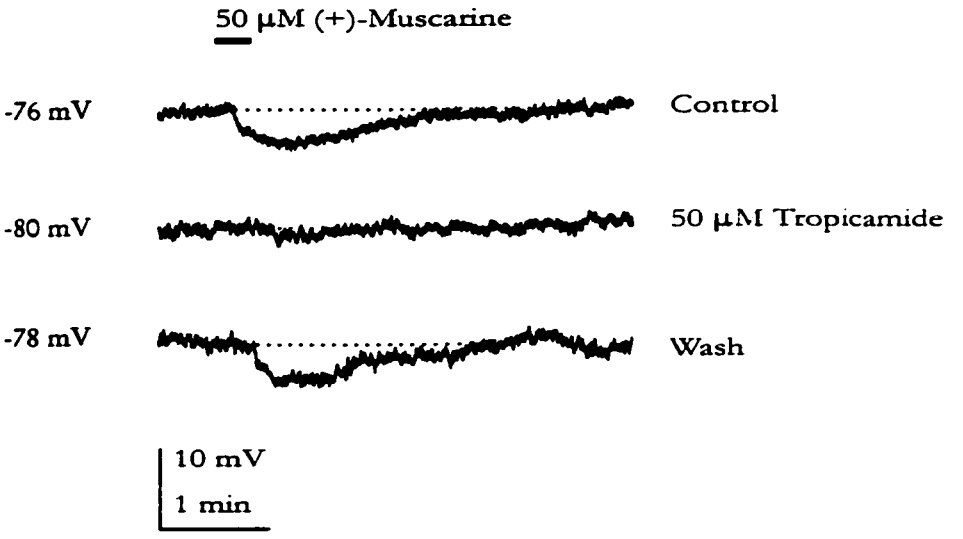

Control
Tetrodotoxin (300nM)
MGd
BIC
200 ms
80 µM (+)-Muscarine
-64 mV
-64 mV
20 mV
1 min
MGv
BIC
100 ms
-56 mV
-52 mV
20 mV
2 min

Figure 4

Figure 5 RMP regulation was reversibly blocked in both MGd and MGv in the presence of the short-acting muscarinic antagonist tropicamide. Note that in the MGv control trace, muscarine application terminated a train of spontaneous LTSs.

MGd



MGv

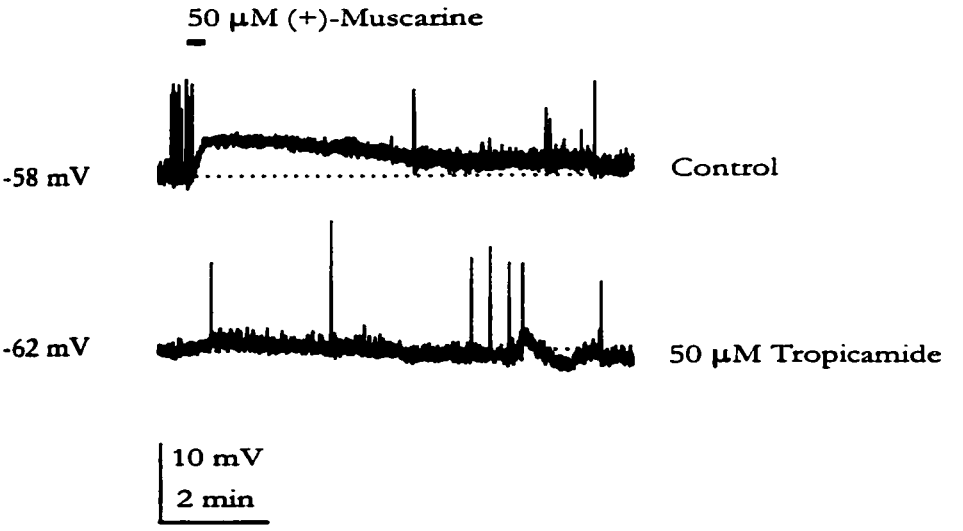


Figure 5

Figure 6 A: Muscarinic stimulation with manual voltage clamp caused a conductivity increase in MGd neurons over a range of voltages, consistent with enhancement of a leak conductance. Open circles: control; filled circles: 80 μM (+)-muscarine. Vertical bars show the net conductance induced by muscarine. B: Muscarine typically caused little conductivity change in MGv neurons by the method of varied current injection under manual voltage clamp. C: The conductance upregulated by muscarine in the MGd was probably potassium-selective. Voltage responses to current ramps with and without muscarine present coincided at the equilibrium potential for potassium (about -98 mV). The current ramp runs from –1 nA to 1 nA. D: Whole-cell voltage clamp results from slice, probably in MGv. Muscarinically induced inward current (difference of the two curves) was linear over the range -110 to –20 mV. Reversal potential was –89 mV (average of 3 trials with same neuron). These data are consistent with a muscarinically induced closure of the leak potassium conductance.

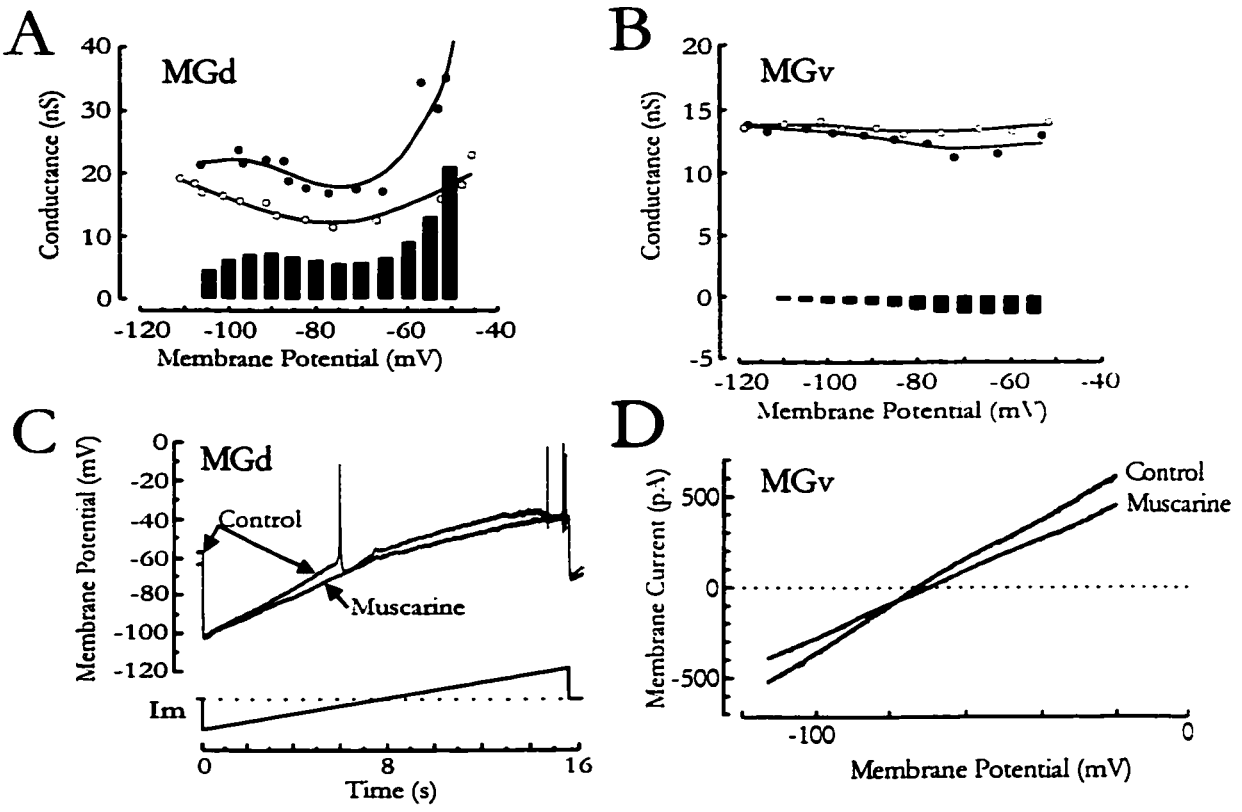

A
MGd
Conductance (nS)
40
30
20
10
0
-120
-100
-80
-60
-40
Membrane Potential (mV)
B
MGv
Conductance (nS)
20
15
10
5
0
-5
-120
-100
-80
-60
-40
Membrane Potential (mV)
C
MGd
Membrane Potential (mV)
0
-20
-40
-60
-80
-100
-120
Control
Muscarine
Im
0
8
16
Time (s)
D
MGv
Membrane Current (pA)
500
0
-500
Control
Muscarine
-100
0
Membrane Potential (mV)

Figure 6

Figure 7 Synaptic responses under (±)-muscarine. Comparing MGd and MGv burst-neuron populations, a significantly higher proportion of MGv neurons showed response blockade under low doses of muscarine, while a significantly higher proportion of MGd neurons showed no effect even at high doses. "Block" includes neurons that began responding with a single spike following a block of responding. "No Eff." = no effect. Burst responding continued unchanged under muscarine. "Incon." = Inconclusive. Tests with muscarine were inconclusive (n = 11). "Replc." = Replacement. The burst response was replaced with some other response type with no intervening block (n = 5). Asterisks: $P < 0.001$. Percentages were calculated separately for dorsal and ventral populations and were based on the population sizes given in the legend. Tests in which a synaptic response did not return after blockade were excluded from the analysis. 61% of neurons were tested with at least two concentrations of muscarine. Neurons were classified into either a low-dose category (2-30 μM of (±)-muscarine) or a high-dose category (over 30 μM). The basis of the classification was the lowest dose that produced a block or replacement, unless no effect was seen. In cases of no effect, the highest dose tried was used as the basis of classification. In the "no effect" group the highest dose tried was sometimes in the low-dose category, raising the question of whether a block would have been seen had higher doses been tried. However, only 5% of the extracellular database was in the "no effect" low-dose category. In the MGd high-dose "no effect" category, the average maximum dose tried was 80 ± 7 μM. The corresponding figure for the MGv was 60 ± 8 μM. The difference is in a direction tending to increase the number of "no effect" neurons in MGv relative to MGd, yet a lower proportion of MGv neurons was found to exhibit no effect.

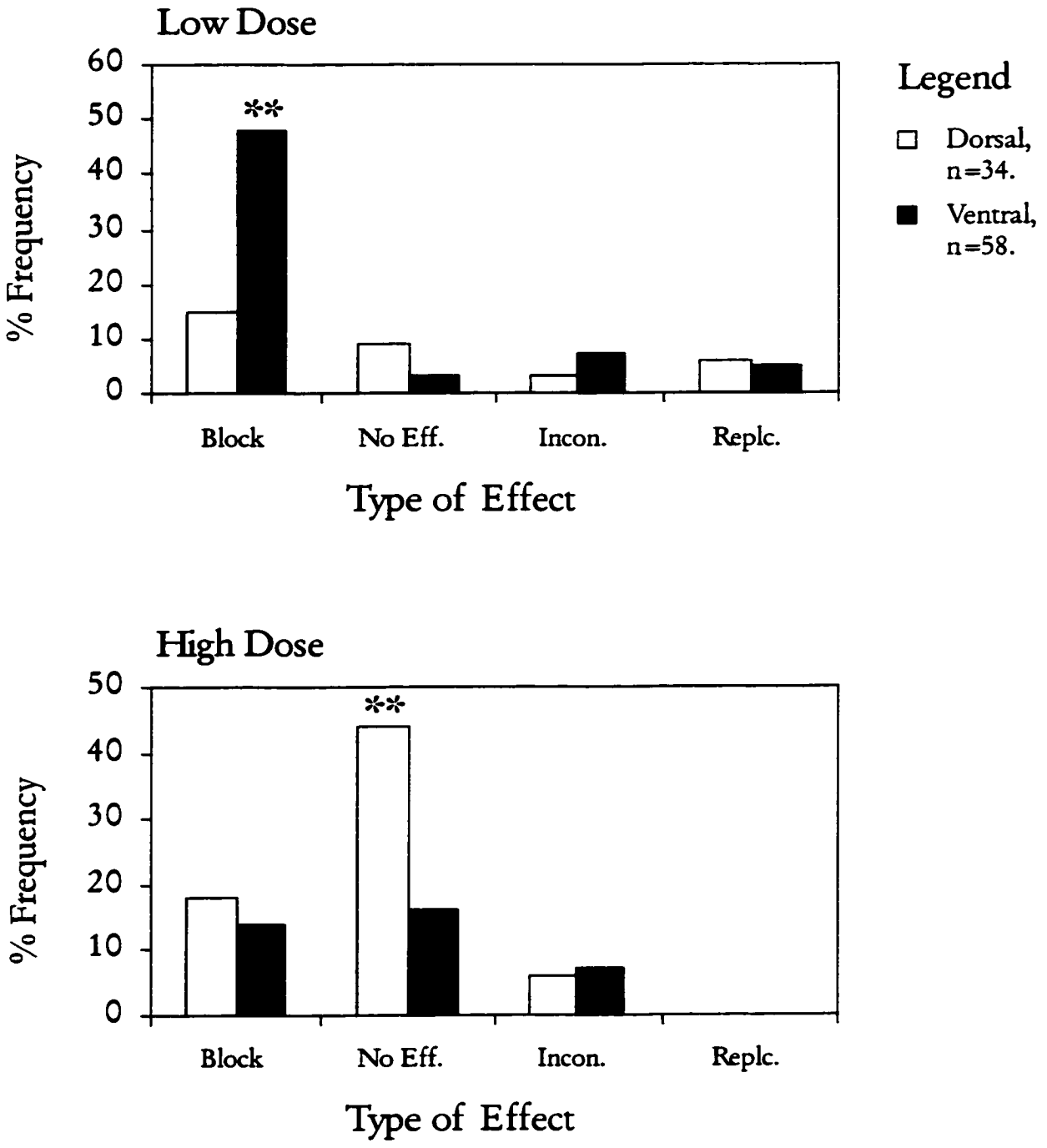

Low Dose
60
50
40
30
20
10
0
% Frequency
**
Block
No Eff.
Incon.
Replc.
Type of Effect
Legend
Dorsal, n=34.
Ventral, n=58.
High Dose
50
40
30
20
10
0
% Frequency
**
Block
No Eff.
Incon.
Replc.
Type of Effect

Figure 7

Figure 8 The hyperpolarization induced in MGd neurons by MRA caused coupling of stimulated EPSPs to LTS-burst complexes (tallest spikes in top trace). Temporary manual return of membrane potential to baseline during the hyperpolarization reversibly eliminated coupling, showing that hyperpolarization is necessary to coupling. The three traces at bottom are details of the top trace shown on an expanded time scale. A: control. B: muscarinic hyperpolarization. C: during manual return to baseline potential. Notice that MRA also suppressed a large IPSP, present under baseline conditions but not present during manual return to baseline potential.

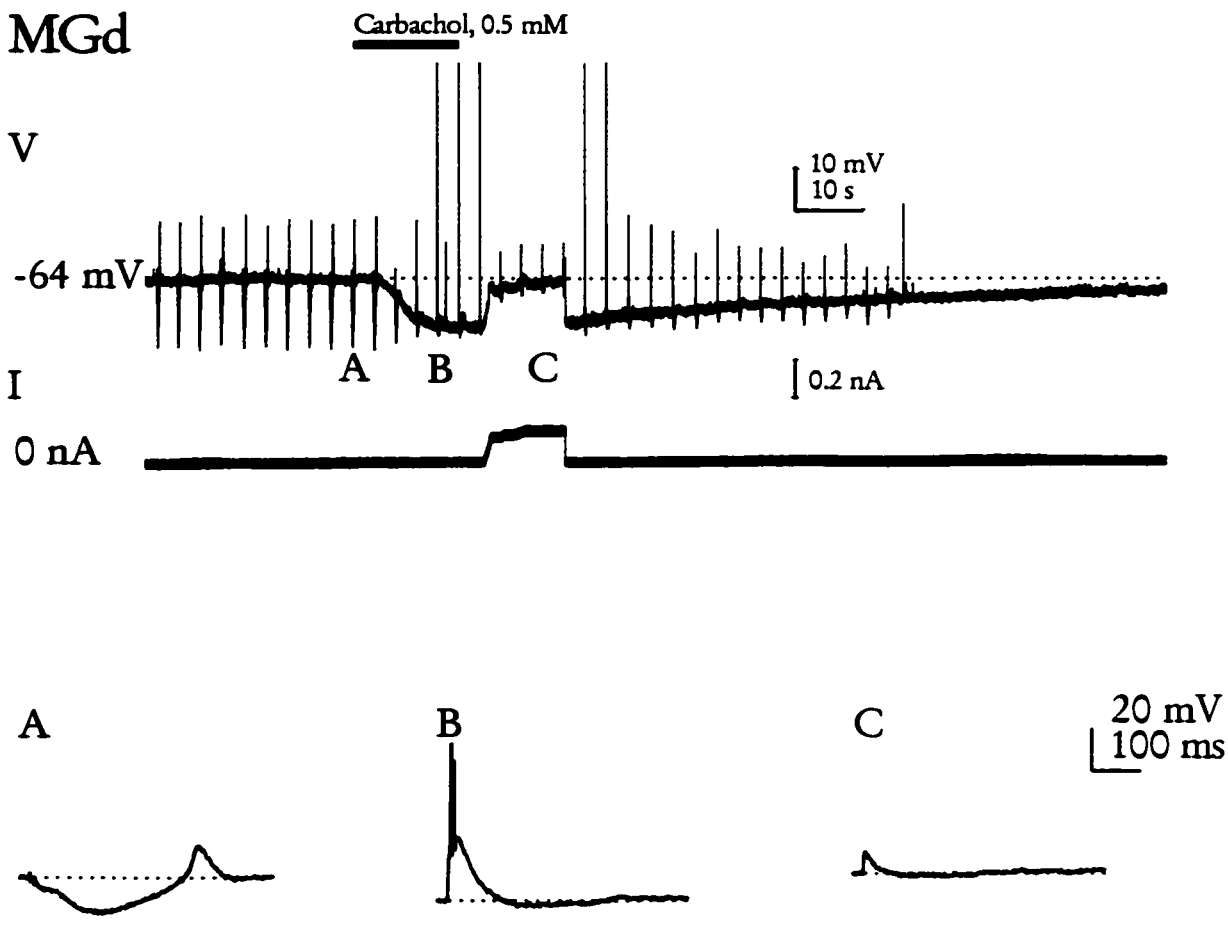

MGd
Carbachol, 0.5 mM
V
10 mV
10 s
-64 mV
A
B
C
I
0.2 nA
0 nA
A
B
C
20 mV
100 ms

Figure 8

Figure 9 Upper two traces: when EPSPs were present in MGd neurons due to treatment of afferent axons with 4-AP, manual hyperpolarization to imitate MRA caused the larger EPSPs or clusters of EPSPs to couple to LTS-burst complexes (tall spikes in voltage trace). Slashes indicate omitted segments. Bottom traces: EPSP-LTS-burst complexes from three different MGd neurons recorded during manual hyperpolarization are shown on an expanded time scale. Short vertical lines indicate the onsets of EPSPs. Note the relatively close EPSP spacing just before the LTS-burst complexes.

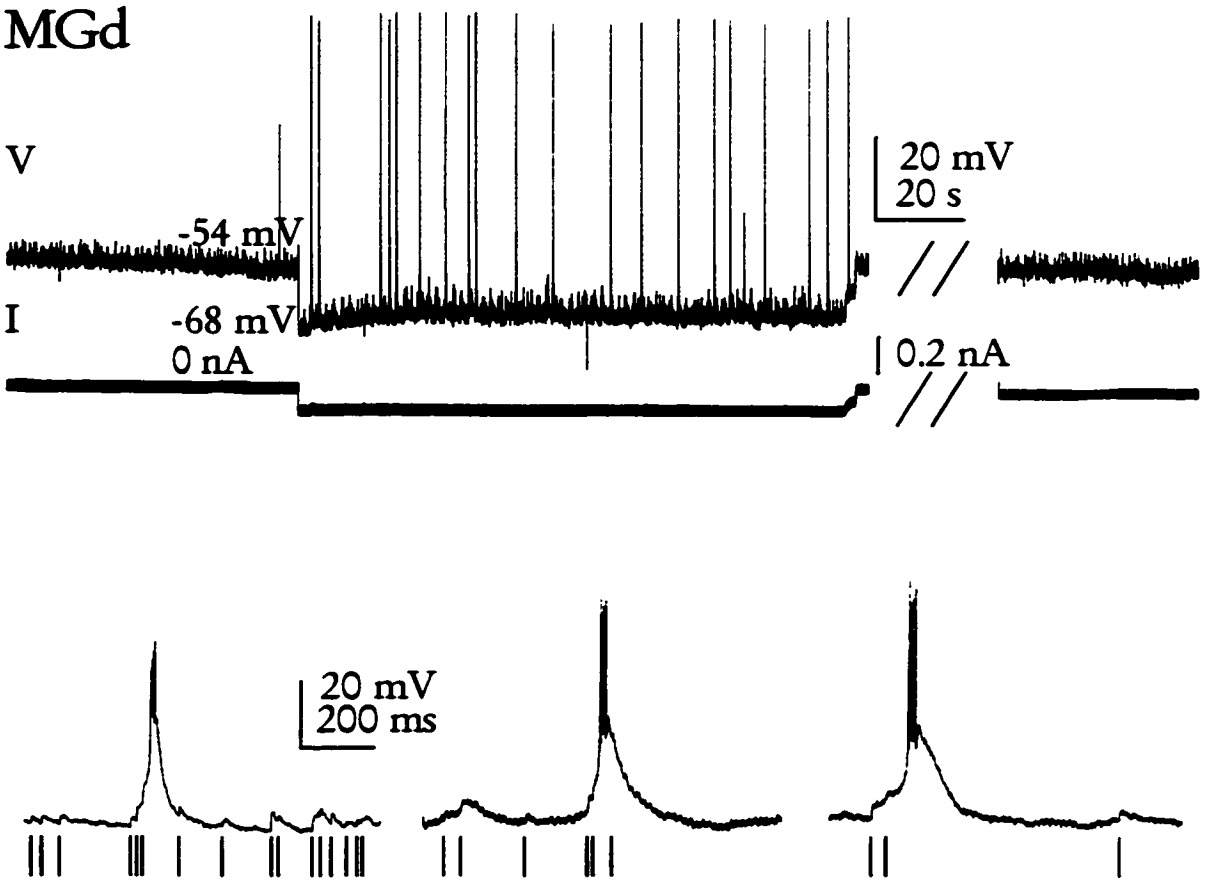


Figure 9

Figure 10 The depolarization induced in MGv neurons by MRA sometimes caused spontaneous EPSPs to couple directly to action potentials, giving rise to single spikes. Under each condition, four consecutive traces are shown, separated by offsets for clarity.

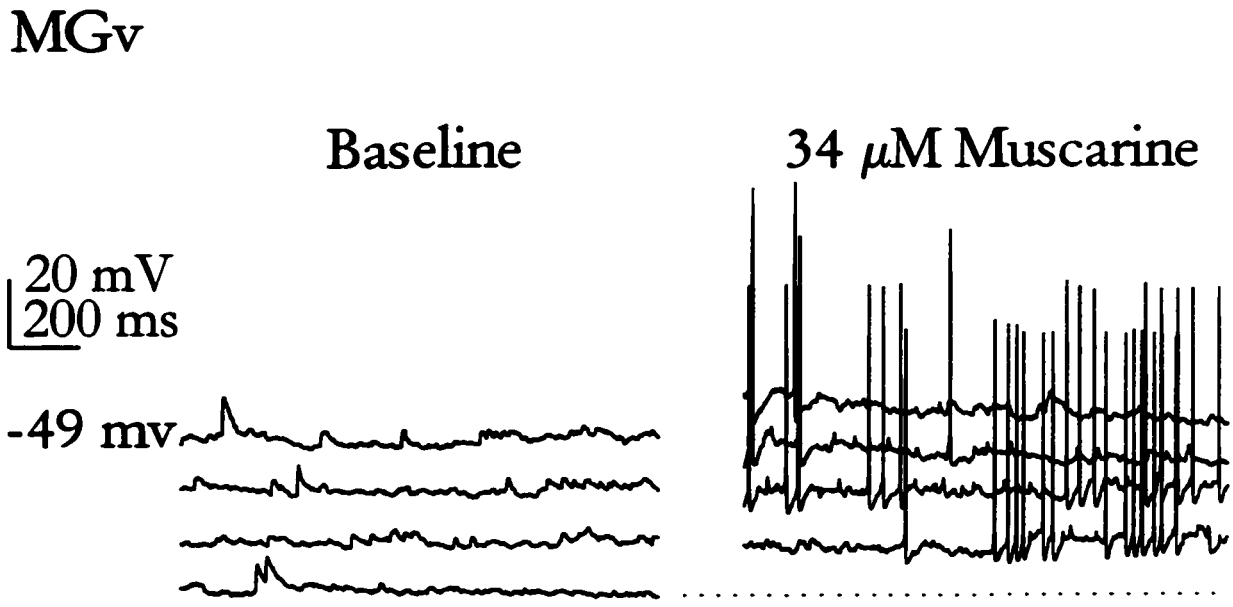


Figure 10

Figure 11 The hyperpolarization induced by MRA in MGd neurons could cause drug induced EPSPs to couple to LTS-burst complexes. Under each condition, five consecutive traces are shown, separated by offsets for clarity. Traces at bottom show typical responses on an expanded time scale. Under both baseline and carbachol conditions a small amount of DC current (+0.1 nA) was injected through the recording electrode.

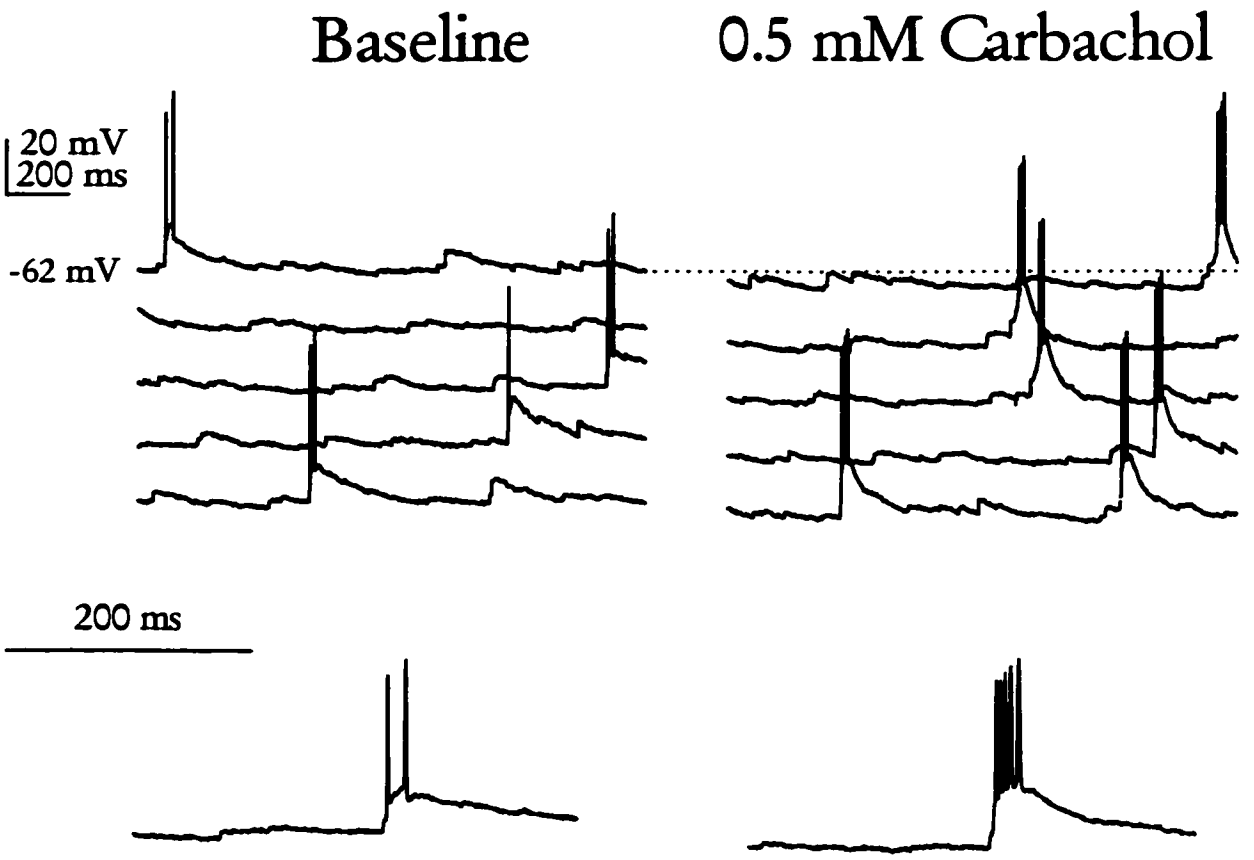


Figure 11

Figure 12 Coincidence detection by the bursting mechanism. Left bar shows average number of EPSPs per 100 ms for events not followed by bursts (100-ms windows containing at least one EPSP). Right bar shows average number of EPSPs in the 100 ms preceding a burst. Error bars are the 95% confidence limits. Data was pooled from three neurons, giving a total of 44 burst events and 100 non-burst events. Asterisks indicate $P < 0.0001$.

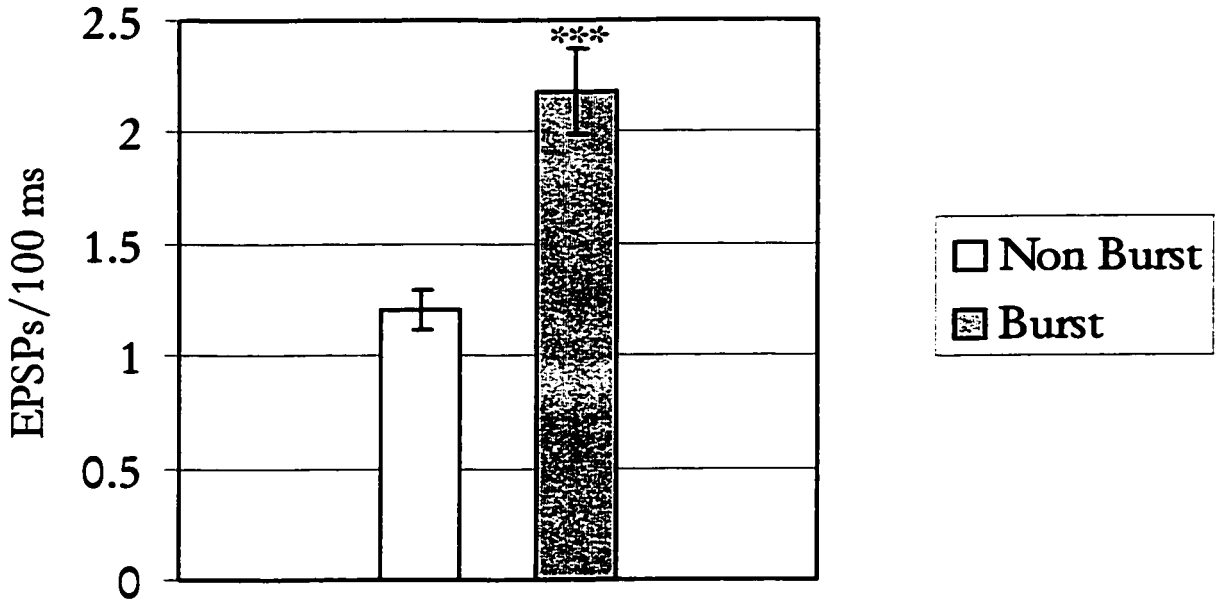


Figure 12

Chapter 4.

Mooney, D. M. and Hu, B. Differential muscarinic modulation of a low threshold calcium spike-induced slow afterhyperpolarization in rat lemniscal and non-lemniscal auditory thalamus. To be submitted to J. Neurophysiol.

Title: **Differential Muscarinic Modulation of a Low Threshold Calcium Spike-Induced Slow Afterhyperpolarization in Rat Lemniscal and Non-lemniscal Auditory Thalamus**

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Running title: Muscarinic Modulation of afterhyperpolarization

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ABSTRACT

Cholinergic modulation of thalamic neuronal activity plays an important role in sensory information processing. In this study, we investigated the effects of muscarinic receptor activation on a Ca^{2+}-dependent K^+ conductance which has been shown to mediate slow membrane potential oscillations during deep sleep. The experiments were conducted in an explant preparation that preserved the thalamic segments of lemniscal and non-lemniscal auditory pathways. It was found that activation of the low-threshold Ca^{2+} spike in neurons from both pathways, with or without eliciting Na^+ action potentials, could lead to a prominent membrane afterhyperpolarization, which lasted for seconds. Bath application of muscarine reversibly abolished this afterhyperpolarization in lemniscal neurons whereas it largely had no effect on the afterhyperpolarization in non-lemniscal cells. These data indicate that acetylcholine may differentially modulate the intrinsic membrane excitabilities and the coupling between the low threshold spike and the afterhyperpolarization in thalamocortical neurons in a synaptic pathway-dependent manner.

INTRODUCTION

The cholinergic innervation from the mesopontine reticular formation to the thalamus regulates thalamocortical sensory relay through activation of muscarinic receptors in a behavioral state dependent fashion (Steriade, 1995). Muscarinic receptors are G-protein coupled membrane proteins linked to two major second messenger systems: phospholipid-dependent signal transduction (M1, 3) and G-protein mediated inhibition of adenylyl cyclase (M2, 4) (Felder, 1995). In thalamic neurons, cholinergic modulation is characterized by two main types of cellular events: a slow membrane depolarization and a membrane hyperpolarization response (McCormick, 1992; McCormick and Prince, 1987; Chapter 3). The muscarinic depolarization, which promotes tonic firing and synaptic transmission, is dominantly seen in thalamic sensory relay neurons and is caused by a closure of the leak K^+ membrane conductance (I_{leak}) coupled to M1 and M3 receptor subtypes (Zhu and Uhlrich 1998). The muscarinic hyperpolarization is mediated by M2 and M4 receptors, which results in an opening of I_{leak} (McCormick and Prince, 1986; Chapter 3). This latter response has been demonstrated in thalamic reticular neurons and in GABAergic interneurons (Hu et al., 1989; McCormick and Pape 1988; McCormick and Prince 1986). Thalamic neurons also express other types of channels sensitive to muscarinic receptor activation, not the least of which is a Ca^{2+}-dependent K^+ conductance or $K_{(ca)}$ (Halliwell, 1990). This channel is widely expressed in central and peripheral nervous systems and is typically activated following a single or a train of action potentials, which gives rise to a membrane afterhyperpolarization or AHP (Halliwell, 1990). The duration of the AHP can range from 10 milliseconds to several seconds, depending on the quantity of Ca^{2+} influx and the $K_{(ca)}$ kinetics (Halliwell, 1990). In the thalamus, the AHP plays an important role in the genesis of a slow (1-5 Hz) or delta

membrane oscillation by interacting with a low-threshold Ca^{2+} spike mediated by the T-type Ca^{2+} conductance (Steriade et al., 1991 and 1993).

There is a lack of study addressing the muscarinic modulatory effects on AHPs in thalamic neurons and particularly on the membrane afterhyperpolarization induced through low-threshold Ca^{2+} channels. Suppression of an AHP induced by the low threshold Ca^{2+} spike (LTS) has, however, been reported in cat thalamic nuclei *in vivo* following stimulation of mesopontine cholinergic afferents, which leads to a blockade of slow membrane potential oscillations (Steriade et al., 1991). Using a rat explant preparation of the medial geniculate body (MGB) containing ventral (MGv) lemniscal and dorsal (MGd) non-lemniscal auditory neurons, we here report that activation of the LTS, with or without eliciting Na^{+} action potentials, produced a slow AHP in MGB neurons that lasted for seconds. The slow hyperpolarization, which we here term the AHP_{lts}, was reversibly blocked by muscarine or carbachol. This inhibitory effect, however, occurred primarily in lemniscal MGB neurons but it was absent in non-lemniscal thalamic cells. These data support the notion that acetylcholine (ACh) may differentially modulate intrinsic membrane excitability and LTS-AHP coupling in thalamocortical neurons in a synaptic pathway-dependent manner.

MATERIALS AND METHODS

Experiments were performed on thalamic neurons obtained from 275-325g male Long-Evans rats. The methodologies of preparing thalamic explants and distinguishing between ventral and dorsal regions have been described in previous papers (Hu, et al., 1994; Mooney et al., 1995). Briefly, after decapitation, the brain was quickly removed, and a block of tissue containing the MGB was isolated on ice and then transferred to a recording

chamber, where it was superfused with carboxygenated artificial cerebrospinal fluid (ACSF) at about 32°C.

Intracellular recording electrodes were filled with 4 M KAc, 0.15 M KCl (final resistance 80-120 MΩ). Muscarine or carbachol was diluted from stock solution and applied in the bath medium. Carbachol was run in the presence of 100 μM hexamethonium to suppress nicotinic effects. Manual voltage clamp was used for conductance measurement during drug application. An Axoclamp-2A amplifier with 0.1 head-stage gain was used for intracellular recordings. Signals were digitized (Model 400 by Vetron, Rebersburg PA) at 22 kHz and recorded on videotape. On-line data capture at a 10 kHz sampling rate was performed using an A-D interface (DigiData 1200) operated by PClamp (Axon Instruments, Foster City, CA). The MGB neurons included in the database had a mean resting membrane potential negative to –60 mV and a membrane resistance higher than 90 MΩ. Analysis was performed using PClamp software. Statistical examination was conducted using Fisher's nonparametric 2x2 proportion test.

RESULTS

In roughly 30% of all stably impaled MGv neurons (n = 62) and 50% of all MGd neurons (n = 90), injection of negative current pulses induced a rebound LTS-burst complex followed by a slow AHP or AHP_{LTS}, which had an amplitude that could exceed 10 mV and a time constant of recovery of 2.0 ± 0.1 seconds (n = 11, Fig. 1). Complete recovery of the AHP could require seven seconds. The AHP_{LTS} persisted in the presence of tetrodotoxin (TTX; 300 nM; n = 4) and could be induced by the isolated rebound LTS, unaccompanied by a burst of action potentials (Fig. 2).

In 2/11 MGd neurons (18%) and in 20/20 MGv neurons, application of (±)-muscarine (50 μM), (+)-muscarine (50 μM), or carbachol (500 μM) caused a slow reversible depolarization of the resting membrane potential (RMP); in all these cells, the AHP_{LTS} was reversibly blocked (Fig. 3). During the AHP blockade, there was little change in the peak voltage of rebound LTSs. In 36% of muscarinically depolarized neurons, the RMP depolarization and AHP blockade were accompanied by the temporary appearance of an afterdepolarization (ADP) following the rebound LTS (Fig. 3). In contrast to MGv neurons, applications of muscarinic agonists had no appreciable effect on the AHP in 82% of MGd neurons (n = 11), but caused a slow reversible hyperpolarization (Fig. 4). The contrasting effect of muscarinic agonists on the AHPs between MGv and MGd neurons is highly statistically significant ($P < 0.00001$).

To determine whether agonist-induced membrane depolarization or hyperpolarization contributes to the muscarinic effects on the AHPs, the slow RMP changes induced by muscarinic agonists were compensated by manual current injection. Under this manual voltage clamp the AHPs were still apparent in MGd (n = 2) whereas muscarinic blockade of AHPs persisted in MGv (n = 4), indicating that the muscarinic effects on AHPs are independent of slow RMP changes in both MGd and MGv (Fig. 4). The voltage-clamp experiments also revealed an increase in apparent membrane conductance in MGd and a decrease in MGv neurons during AHP blockade (Fig. 4).

DISCUSSION

In the present study, we have demonstrated that a long-lasting membrane afterhyperpolarization can be induced in thalamic auditory neurons following a low threshold

Ca^{2+} spike. The burst of action potentials typically associated with an LTS is not necessarily required for AHP induction. We therefore term this membrane response the AHP_{lts}. We further showed that the AHP_{lts} was abolished by muscarine in ventral lemniscal but not in dorsal non-lemniscal thalamocortical neurons. This original finding of a pathway-specific neuromodulatory effect may have important functional implications.

There are two main types of calcium dependent potassium conductances, known as big and small K_{ca} channels, involved in AHP generation (Halliwell, 1990). The former can be activated following a single or train of action potentials and are sensitive to the K^+ channel blocker tetraethylammonium (TEA) (Halliwell, 1990). The small K_{ca} is, however, selectively abolished by apamin and appears to play an important role in regulating rhythmic burst firing patterns in forebrain neurons (Bal and McCormick, 1993; Hu and Bourque, 1992; Kawasaki et al., 1999). Depending upon the kinetics and K_{Ca} involved, AHPs can be described as either fast, medium or slow, with a duration raging from ten milliseconds to seconds (Halliwell, 1990). There are apparently two types of slow AHP, one of comparatively short duration and not blocked by muscarine and the other blocked by muscarine (Halliwell, 1990). Our data demonstrates that the AHP found in the MGd has a slow time course of recovery but is resistant to muscarinic blockade, which may represent an anomalous example of the first type (Schwindt et al., 1988). Kawasaki et al. (1999) have reported a TTX –sensitive, burst-induced AHP that was suppressed by carbachol in rat subiculum. The duration of the observed AHP was, however, two orders of magnitude shorter than those observed in our study and it was probably mediated by a different channel, the medium-duration AHP channel (Halliwell, 1990). In guinea pig and cat thalamic slices, neurons of the parataenial nucleus had unusually large AHPs in comparison to cells in other thalamic nuclei

(McCormick and Prince, 1988). Norepinephrine selectively reduced the AHP, an action accompanied by a decrease in spike frequency accommodation and the emergence of a slow afterdepolarization.

Our data indicate that muscarinic receptor activation induces a powerful inhibitory mechanism acting on $K_{(ca)}$, consistent with findings from many other brain regions (Baraban et al., 1985; Halliwell, 1990; Malenka et al., 1986; Muller et al., 1992; Kawasaki et al., 1999). The onset of the muscarine effect is rather rapid but its effect may outlast drug washout. In addition, we found that nearly the entire AHP_{lts} is blocked by muscarine, suggesting that kinetically the AHP_{lts} may be derived from a homogenous population of $K_{(ca)}$ channels. Because blockade of AHP_{lts} can be induced without membrane depolarization, the involvement of a muscarine-sensitive and depolarization-dependent K^+ conductance (the M-current, I_m) in AHP_{lts} is unlikely (Halliwell, 1990). In a subpopulation of neurons, blockade of AHP_{lts} unmasked a depolarization potential. This afterdepolarization response has been found in other brain regions and is suggested to be mediated by a non-selective cation conductance that can be induced by rising intracellular Ca^{2+} (Bal and McCormick, 1993; Kawasaki et al., 1999; McCormick and Prince, 1988; Schwindt et al., 1988). Furthermore, we consider that the muscarinic blockade of AHP is unlikely to be a result of potentiation of a hyperpolarization-activated inward current, I_h, which can be enhanced by neuromodulators such as norepinephrine (McCormick and Pape, 1990). An enhanced I_h could produce a depolarizing tail current at the offset of the hyperpolarization pulse used to induce the LTS, and this tail current could mask the AHP. This is unlikely to be the explanation of muscarinic block of the AHP because AHPs evoked by a depolarization pulse reaching membrane

potentials outside the I_h activation range (e.g.: > –65 mV) were also abolished by muscarinic receptor activation (Mooney and Hu, unpublished observation).

Our data is consistent with previous studies showing that low threshold calcium channel activation can lead to the occurrence of an AHP in the absence of Na^+ action potentials (Bal and McCormick, 1993; Perez Velazquez and Carlen, 1996; Williams et al., 1997). Medium-duration AHPs in nucleus basalis have been found to depend on Ca^{2+} influx through the T-type Ca^{2+} channels underlying the LTS (Williams et al., 1997). Bal and McCormick have previously shown that a TTX-insensitive LTS-induced AHP in GABAergic thalamic reticular cells is blocked by apamin (Bal and McCormick, 1993). The intimate coupling between LTS and $K_{(ca)}$ suggests a possible close membrane adjacency between the two conductances, and thus a limited ion diffusion barrier for Ca^{2+}. Further studies are needed to address this issue.

The family of muscarinic receptors consists of five subtypes that can be differentiated based on their affinities for various antagonists and their differential coupling to second messenger systems (Ladinsky et al., 1990). Activation of m2 and m4 receptors leads to G-protein-mediated phosphorylation and production of cGMP (Tsuga et al., 1998; Waid et al., 2000; Wang et al., 1999) as well as inhibition of the synthesis of cyclic AMP; whereas m1, m3 and m5 receptor activation produces various lipid metabolites through phospholipase C (PLC) activation (Felder, 1995). The second-messenger mechanism underlying muscarinic modulation of K_{ca} remains poorly defined. Studies in hippocampal CA1 neurons have suggested a role for PLC, protein kinase C, Ca^{2+}/calmodulin-dependent protein kinase II (Baraban et al., 1985; Halliwell, 1990; Malenka et al., 1986; Muller et al., 1992; Alberi et al., 2000) and the second messenger c-GMP (Krause and Pedarzani, 2000). Thalamic relay

neurons in the lateral geniculate body express primarily m1 and m3 receptors where muscarine produces a slow membrane depolarization by blocking a K^+ conductance (Zhu and Uhlrich, 1998). m2 receptors are mainly found in lateral geniculate interneurons and in reticular nucleus cells where muscarine hyperpolarizes the cells by opening a K^+ conductance (McCormick and Pape 1988; McCormick and Prince 1986; Plummer et al. 1999). These observations raise the possibility that the muscarinic responses in lemniscal and non-lemniscal auditory neurons may be mediated by different receptor subtypes.

Steriade and co-workers (1991) have found that stimulation of mesopontine cholinergic nuclei reduced or suppressed a slow (1-4 Hz) membrane potential oscillation consisting of LTS-AHP sequences in cat thalamus. The robust inhibitory effect of muscarine on AHPs described in this article may provide a partial explanation for this phenomenon. Furthermore, stimulation of brainstem cholinergic afferents to the ventral lateral thalamic nucleus was found to induce a slow membrane depolarization and a decrease in membrane conductance, an observation consistent with the results reported in this study (Deschênes and Hu, 1990). Taken together, these data indicate that the cholinergic input may serve as an essential, albeit not the only, mechanism for regulating intrinsic membrane potential oscillations and EEG desynchronization (Steriade et al., 1991).

The auditory thalamus in most species features a ventral primary (lemniscal) and a dorsal secondary (non-lemniscal) thalamocortical pathway, each of which is involved in different aspects of auditory function (Anderson et al., 1980; Clarey et al., 1992; Graybiel, 1972; Winer, 1992). The predominant occurrence of AHP blockade in ventral, but not in dorsal non-lemniscal, thalamocortical relay neurons indicates that ACh may preferentially

enhance intrinsic membrane excitability in the primary sensory pathway and differentially modulate LTS-mediated cellular events in a synaptic pathway-dependent manner.

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Figure 1 Slow afterhyperpolarization in MGd and MGv recorded under current clamp.

A: a) The response of an MGv neuron to hyperpolarizing current pulses delivered through the recording electrode. A sufficiently large hyperpolarization step induced a rebound LTS-burst complex that was followed by a slow AHP (sag in baseline between pulses). b) Detail of the voltage response to one current pulse. V: membrane potential. I: injected current.

B: a) The response of an MGd neuron to hyperpolarizing pulses. b) Detail of the voltage response to one current pulse, showing the sequence: hyperpolarizing step, LTS-burst complex, slow AHP.

Note: in both A: a) and B: a) the response to a variety of current pulse amplitudes is illustrated. The current pulse was made progressively smaller on successive elicitations of the AHP, so that the LTS amplitude and therefore the AHP amplitude decline in going from left to right in the membrane potential traces.

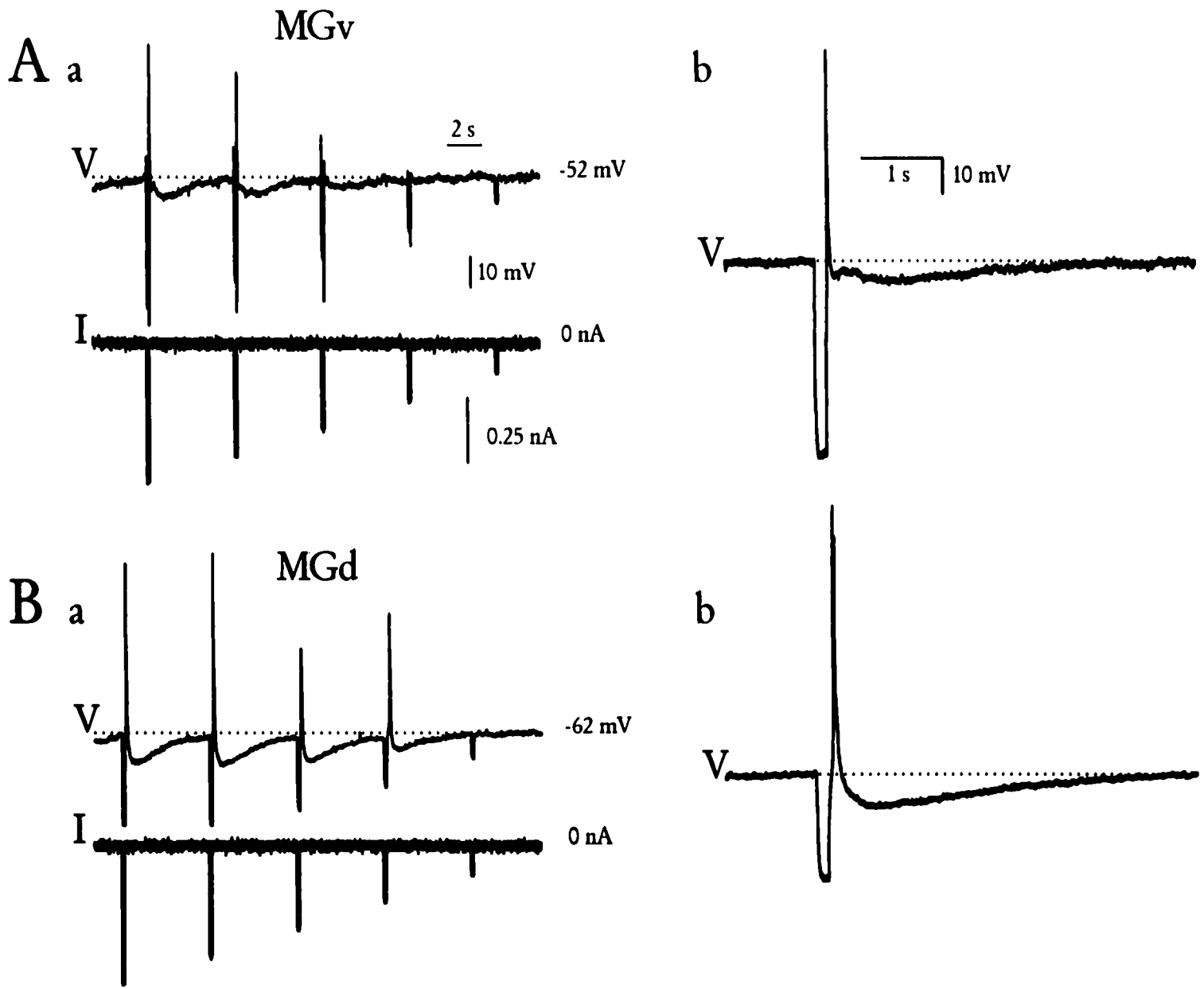


Figure 1

Figure 2 The AHP induced by an isolated LTS under TTX.

The LTS-burst-AHP complex in normal ACSF (top) compared with an LTS-AHP complex in ACSF containing 300 nM TTX (bottom) in the same MGv neuron, showing that a majority of the AHP amplitude was due to activation of LTS. The AHPs decayed to baseline (not shown).

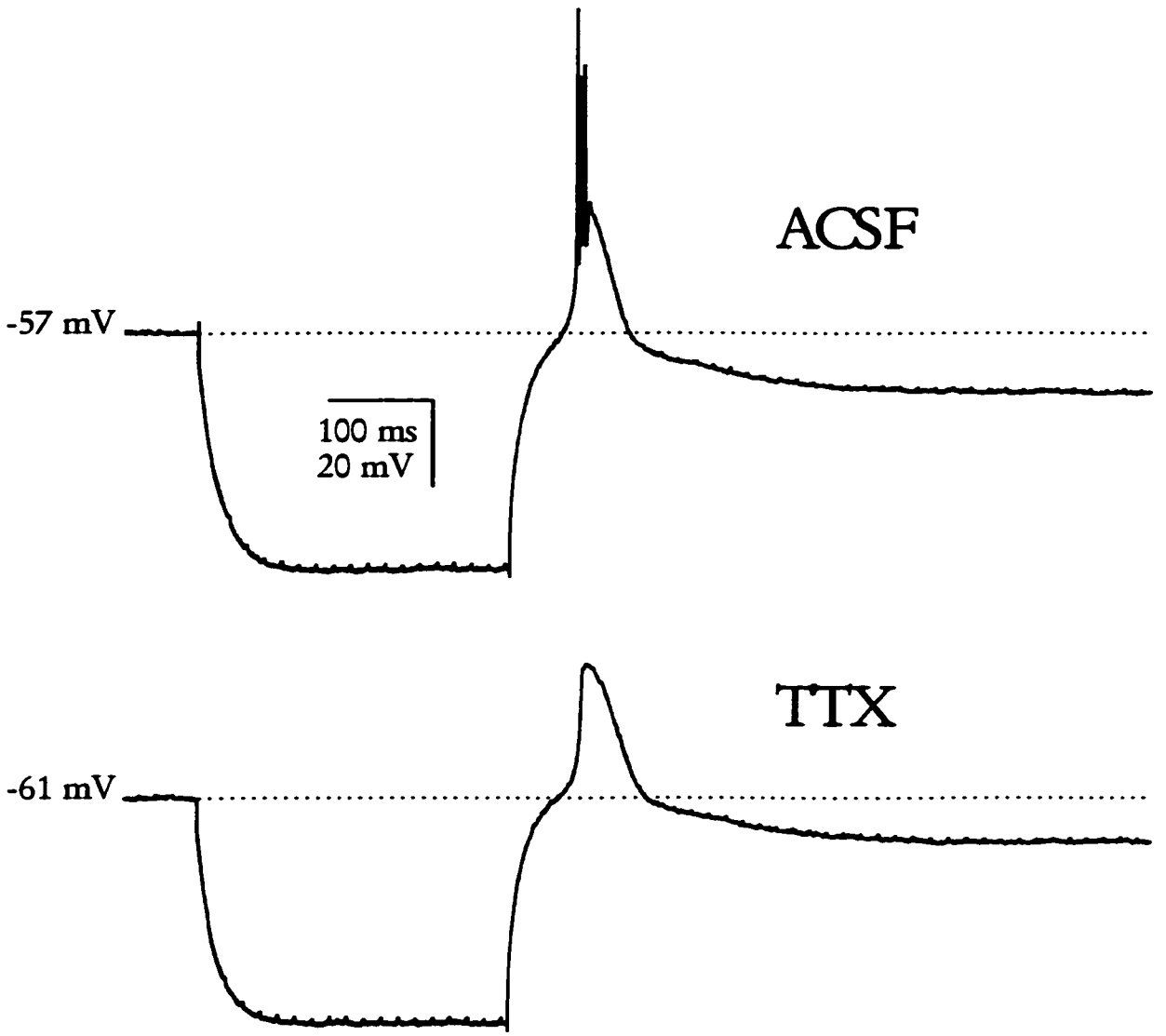


Figure 2

Figure 3 Blockade of the AHP in MGv by muscarine. A: Application of 83 μM (±)-muscarine in the ACSF for 20 s (heavy bar over middle trace) depolarized the resting membrane potential, blocked the AHP, and caused the appearance of an ADP, all changes being reversible. Left trace: expanded detail of the AHP under control conditions. Right trace: expanded detail of AHP recovery. Middle trace: downward arrows indicate segments expanded in B. TTX was present in the ACSF throughout the experiment to block action potentials.

B: Same experiment, showing an expanded rebound LTS and AHP in the presence of TTX dissolved in the ACSF (top trace). With the addition of muscarine, the AHP is replaced by an ADP apparently induced by the rebound LTS. Both recover to baseline before the next pulse. Hyperpolarizing voltage steps truncated for clarity.

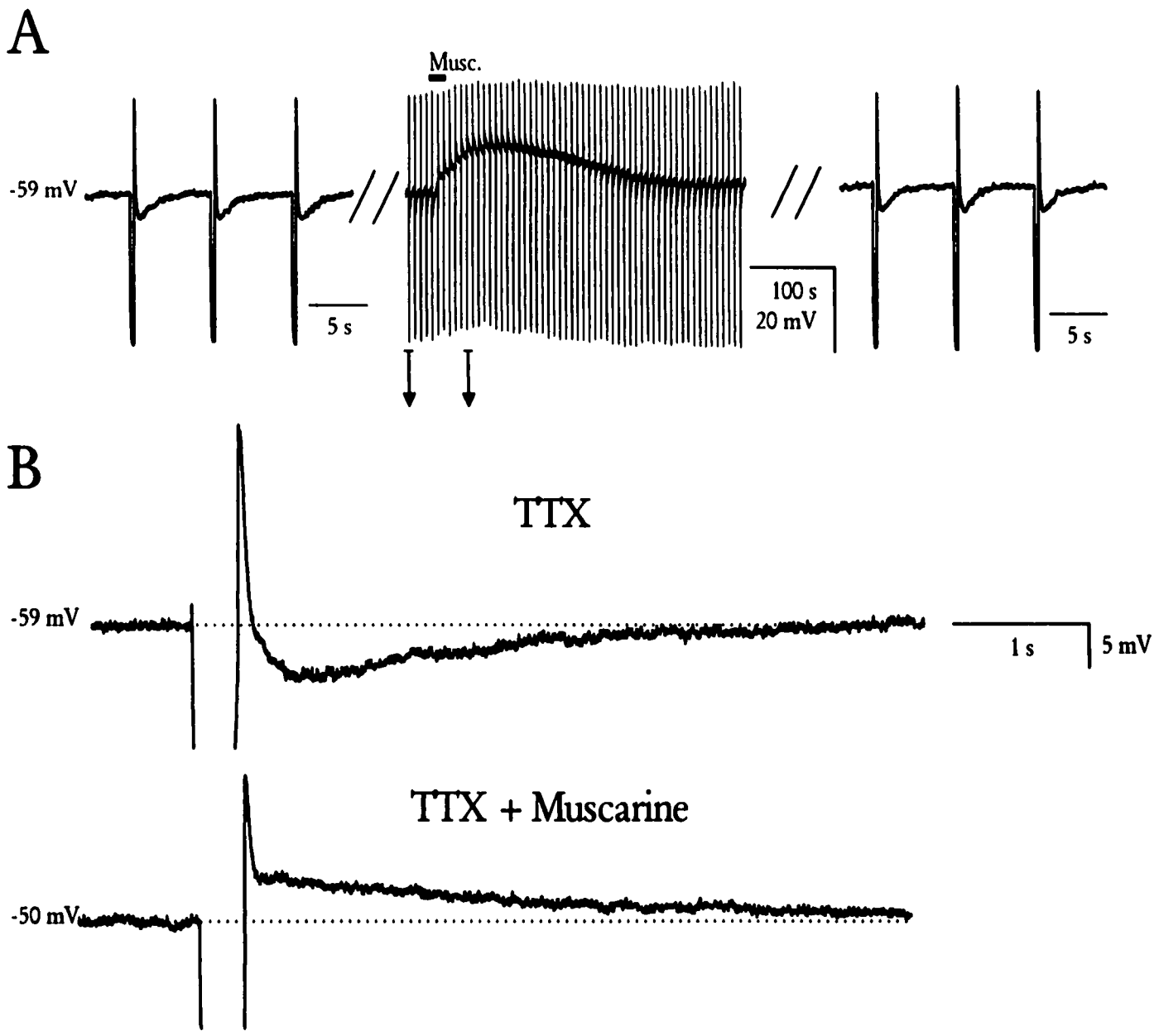

A
Musc.
-59 mV
5 s
100 s
20 mV
5 s
B
TTX
-59 mV
1 s
5 mV
TTX + Muscarine
-50 mV

Figure 3

Figure 4 Muscarinic receptor activation blocked the AHP in MGv but not in MGd. A: a) In the middle trace, application of 83 μM (±)-muscarine for 20 s to an MGv neuron produced a depolarization of the resting membrane potential. The depolarization was compensated about 40 s into the experiment by manual injection of repolarizing current. Left trace: AHP under control conditions. Right trace: AHP recovery. TTX was present throughout the experiment. b) Same experiment. An expanded control trace showing the AHP is superimposed on a trace taken under muscarine and manual voltage clamp. The difference in the sizes of the voltage steps indicates a reduction of membrane conductance under muscarine.

B: a) 500 μM of the nicotinic and muscarinic agonist carbachol was bath-applied to an MGd neuron for 15 s in the presence of 100 μM hexamethonium to suppress nicotinic effects. TTX was not used in this experiment. Manual voltage clamp was briefly applied after a muscarinic hyperpolarization of the resting membrane potential had developed (plateau in trace). The absence of a block of the AHP under muscarinic receptor activation in MGd did not depend on resting membrane potential. Action potentials and hyperpolarizing voltage steps are truncated for clarity. b) Same experiment. A control trace is superimposed on a trace recorded under carbachol and manual voltage clamp, showing that under carbachol there is no reduction of the AHP and a slight increase in membrane conductance.

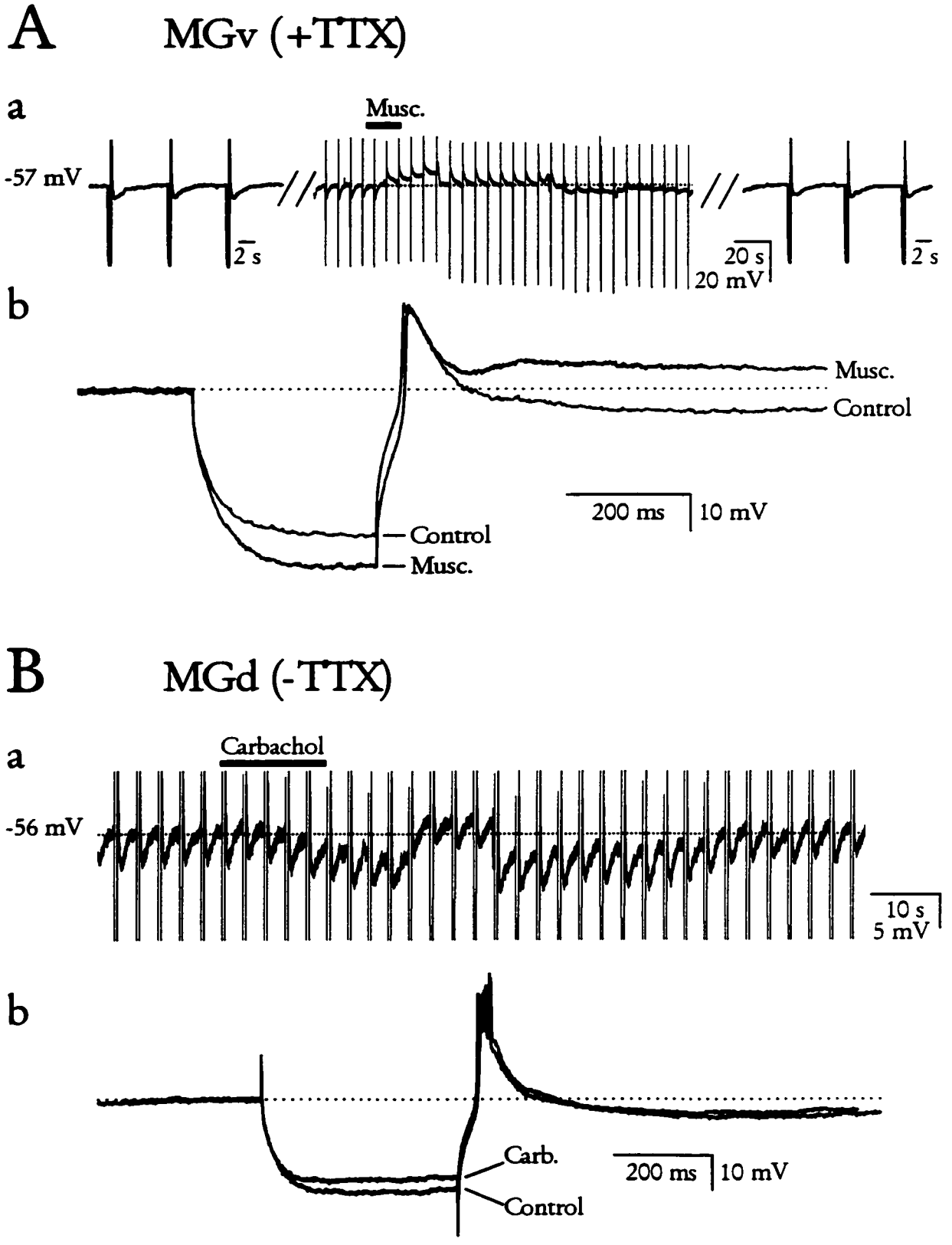

A
MGv (+TTX)
a
Musc.
-57 mV
2 s
20 s
20 mV
2 s
b
Musc.
Control
200 ms
10 mV
Control
Musc.
B
MGd (-TTX)
a
Carbachol
-56 mV
10 s
5 mV
b
Carb.
Control
200 ms
10 mV

Figure 4

Chapter 5. General Discussion

The central auditory system exhibits remarkable changes in the pattern of neuronal activity under different behavioral states involving the sleep-waking cycle, changes in arousal, selective attention and conditional learning. Such a functional adaptation in auditory thalamus is in large part regulated by a cholinergic modulatory input from the midbrain reticular formation. The present thesis has proposed that muscarinic receptor activation can create and maintain a parallel sensory synaptic signaling mechanism in auditory thalamocortical pathways by a differential modification of membrane ion conductances. The experimental evidence collected throughout this thesis study strongly supports this hypothesis and illustrates an original concept concerning synaptic information processing, which may have far-reaching theoretical implications.

5.1 Principal findings of the present studies

5.1.1 Muscarine selectively inhibits the burst response of MGv neurons by inducing a membrane depolarization.

A mixed excitatory and inhibitory effect of muscarinic agonists on synaptic transmission has been described in many early *in vivo* iontophoretic studies conducted in the thalamus (see Chapter 2, Introduction). Using the auditory tectothalamic pathway as a model, it has been shown that activation of muscarinic receptors *in vitro* selectively suppressed burst synaptic transmission, but not the transmission mediated by single-spikes. This blockade of synaptic burst is not mediated by conventional pre- or postsynaptic inhibition mechanisms. A burst of action potentials similar to that recorded extracellularly can be evoked by an EPSP-triggered low-threshold spike or LTS. A large LTS invariably surpasses the action potential threshold and triggers a high-frequency spike burst, but imposing a small membrane

depolarization often leads to a loss or reduction of the LTS due to a voltage dependent inactivation of the underlying conductance, the calcium T-current. Our identification of the LTS in our recordings is based on the observation of several kinetic features that distinguish the LTS from its underlying EPSP within an EPSP-LTS complex. Our results show that the muscarine-mediated blockade of the burst synaptic response is mainly mediated by a slow membrane depolarization, which led to a full or partial inactivation of the LTS. The latter then became ineffective in triggering action potentials. The muscarinic membrane depolarization is primarily mediated by closure of a leak potassium conductance, involving a pertussis toxin-insensitive G-protein coupled receptor mechanism (McCormick, 1992b).

5.1.2 Acetylcholine differentially regulates RMP in primary and nonprimary auditory thalamus, controlling response mode.

Our extracellular study established that a strong differential in synaptic response mode between the MGd and MGv occurs *in vitro* under steady stimulation by acetylcholine. Burst mode responses were the only response type found in MGd whereas single-spike responses strongly predominated in MGv. Much the same pattern of response was found earlier *in vitro* without the use of cholinergic agents (Hu et al., 1994). This may be partly due to differential expression of electrogenic pumps and I_h among MGv and MGd neurons (Senatorov and Hu, 1997). However, the possibility that a residual cholinergic effect remains in an acutely prepared and well-maintained explant cannot be ruled out.

A differential RMP regulation by a direct action on postsynaptic muscarinic receptors was observed in rat auditory thalamus, depolarizations occurring in MGv and hyperpolarizations occurring in MGd. The difference was not due to variations in the

condition of the preparation because the differential effect was demonstrated in single explants. A role in regulation was suggested by the fact that RMP effects were modest but greatly outlasted the drug application period. Conductance was increased in MGd and decreased in MGv, with the leak potassium conductance apparently involved in both kinds of response. The conclusion that the leak conductance was involved in the MGd effect is tentative because it rests solely on current clamp experiments with manual voltage clamp of the slow muscarinic hyperpolarization. Further support from whole-cell voltage clamp experiments would be desirable.

Muscarinic hyperpolarization in MGd promoted synaptic transmission in burst mode. This effect has precedent in the literature: it has been shown previously that in thalamic reticular neurons a hyperpolarization induced by MRA promoted the occurrence of burst discharges (McCormick and Prince, 1986). In our work, transmission in burst mode could be driven by asynchronous drug-induced EPSPs, suggesting that EPSP-LTS coupling in the MGd is not an artificial phenomenon requiring unrealistic synchronous firing of innervating axons induced by electrical shock. The factors determining whether coupling occurred appeared to be large EPSP amplitude and/or multiple EPSPs close together in time. This suggests that the function of EPSP-LTS coupling in MGd is to detect such events. The effects of muscarine on MGv/d neurons were further examined through extracellular recordings of synaptically evoked responses. In MGv, the burst synaptic response elicited by stimulation of collicular input fibers was largely abolished by muscarine. In contrast, the bursting synaptic response in the majority of MGd neurons remained unblocked. Hence, MRA seems capable of regulating the resting membrane potentials and the synaptic responses of thalamic relay neurons in a pathway-specific manner.

The present results represent the first clear finding of opposite muscarinic effects on membrane potential in approximately the same cell type (thalamic projection neurons) in different pathways. Previous reports of opposite muscarinic effects on membrane potential or current exist, but in those studies the two classes of response did not correlate with anatomical division, being due instead to a mixture of response types at the same site and/or to sequential hyperpolarization-depolarization effects in individual neurons (Gabel and Nisenbaum, 1999; Greene et al., 1989; McCormick, 1992b; McCormick and Prince, 1987a; McQuiston and Madison, 1999; Nunez et al., 1997; Segal, 1982; Wakamori et al., 1993; Xi-Moy et al., 1993)

5.1.3 The slow afterhyperpolarization was blocked by MRA in primary but not in nonprimary auditory thalamus.

In a considerable proportion of MGB neurons, it was noticed that negative current pulse injection would give rise to the following sequence: a negative voltage step, followed by a rebound LTS-burst complex, followed by an afterhyperpolarization (AHP). The AHP was notably slow, with a time constant of recovery of 2 s. It was reversibly blocked by MRA in all MGv neurons but was unchanged by MRA in a large majority of MGd neurons. In 36% of cases where blockade occurred, an afterdepolarization was unmasked. The AHP persisted under blockade of action potentials by tetrodotoxin, showing that it could be elicited by the LTS alone. However, positive current pulses, which produced trains of action potentials without an LTS, also induced the AHP. Blockade or non-blockade of the AHP by the muscarinic agonist was independent of concurrent RMP changes. The conclusion that MRA differentially affects the primary and nonprimary auditory thalamus is therefore supported

by the observation of two differential effects: AHP blockade and direction of RMP change. Furthermore, our results indicate that the muscarinic block of the AHP may mediate the blockade of slow membrane oscillations, which depend upon AHP-LTS-AHP cycling, induced by stimulation of reticular cholinergic afferents *in vivo* (Steriade et al., 1991).

5.2 Issues concerning the mechanisms of *in vitro* responses

Previous studies showed that intracellularly recorded bursting responses to BIC stimulation had two phases: an early EPSP and a later, larger depolarization having the form of a very broad spike or hump. The latency of the latter varied from one stimulus to the next. A burst of action potentials was triggered by the broad spike, but no action potentials were triggered by the earlier EPSP (Hu, 1995). The delayed broad spike is an example of a voltage-dependent, low-threshold spike (LTS). The LTS is caused by the regeneration and the subsequent depolarization-induced inactivation of the calcium T-current (Coulter et al., 1989; Crunelli et al., 1989; Jahnsen and Llinás, 1984). Experimentation revealed that the EPSP triggered the LTS in a somewhat all-or-nothing fashion and this triggering is termed EPSP-LTS coupling. In thalamus, the mode of synaptic response is critically linked to the exact value of the RMP (Hu, 1995; McCormick and Feeser, 1990; Steriade and Deschênes, 1984; Steriade and Llinás, 1988; Turner et al., 1994). In burst mode, the RMP is too low to allow EPSPs to reach action potential threshold directly, and therefore rules out single spike responding. However, an EPSP can reach threshold indirectly by triggering an LTS, resulting in a burst, because T-type calcium channels de-inactivate on hyperpolarization to below -65 mV. EPSP-LTS coupling requires not only a relatively negative RMP to de-inactivate the T-current (Coulter et al., 1989; Crunelli et al., 1989; Hu, 1995; Jahnsen and Llinás, 1984;

Mooney et al., 1995; Chapter 2) but also a relatively large EPSP, to serve as a trigger for LTS activation (Hu, personal communication, and Chapter 3). The predominance of burst responding in MGd is due to a more negative RMP (Senatorov and Hu, 1997) and a larger EPSP (Hu, personal communication). EPSP-LTS coupling has been found, both *in vivo* and *in vitro*, in several thalamic nuclei (Deschênes and Hu, 1990; Jahnsen and Llinás, 1984; McCormick and Feeser, 1990; Scharfman et al., 1990; Steriade et al., 1993). A hyperpolarization that was followed by a bursting synaptic response has been found in zebra finch auditory forebrain neurons selectively excited by autogenous song (Lewicki, 1996). EPSP-LTS coupling is also found in cortical and hippocampal neurons (Magee and Johnston, 1995; Markram and Sakmann, 1994), and RMP heterogeneity occurs in hippocampus (Spruston and Johnston, 1992). These facts emphasize the possibility that RMP may dictate the pattern of synaptic signal transmission by means of its effect on coupling.

Although differences in membrane potential among heterogeneous neuronal populations have precedents in hippocampus and amygdala (Spruston and Johnston, 1992; Sugita et al., 1993), experimentally it is by no means an easy task to demonstrate such differences. The tendency toward a more negative membrane potential, in the "absence" of acetylcholine (ACh), in dorsal MGB neurons has been ascribed to an electrogenic effect of the sodium pump and I_h (Hu, 1995; Senatorov and Hu, 1997). A number of methodological factors affect the outcome of attempts to quantitatively compare RMPs between MGv and MGd neurons. For instance, it was found that a more negative RMP is usually obtained if a bursting response can be first recorded extracellularly and conversely a more positive RMP is usually obtained for single spike neurons (Hu, 1995). Furthermore, different recording techniques (intracellular versus whole-cell) (Staley et al., 1992), proper identification of the

caudodorsal nucleus and the condition of the explant preparation may all affect RMP measurement. The latter factor is complicated by the possibility that some muscarinic effect may be residual in explant neurons after they are isolated from the intact brain. In this regard, it is noteworthy that a recent study using MGB slices has found no significant difference between MGv and MGd cells (Bartlett and Smith, 1999). The most plausible explanation here is that because the MGv population consists of mixed single spike and burst neurons, the average RMP measured from a sample will not reveal the more positive RMP usually associated with single spike neurons (Hu, 1995). A similar argument may also apply to the pooled RMP data obtained in the present study. Additional factors contributing to the discrepancy may be difficulties associated with nucleus identification, extracellular recording of single spike versus burst response, and the harsh tissue treatment required for the preparation of brain slices. See footnote 1.

LTS-mediated bursts in thalamus are usually associated with slow-wave sleep or sensory de-afferentation. Sleep-related bursts are not the same as bursts resulting from EPSP-LTS coupling, because the former occur spontaneously and rhythmically in the form of LTS-rebound excitation (Leresche et al., 1991; Steriade and Llinás, 1988). Burst firing of thalamic relay cells *in vivo* that is not restricted to a sleep stage has been reported (Lu et al., 1992; Steriade et al., 1993). LTS-evoked bursts have been found in the thalamus of humans during the waking state (Radhakrishnan et al., 1999; Raeva et al., 1999). The reduction of the middle to late latency components of the auditory evoked potentials in the thalamocortical system during 5-10 Hz acoustic stimulation (Kraus and McGee, 1992; Regan, 1989) could be tentatively explained by the fact that the refractory period following a thalamic LTS

prevents it from following 5-10 Hz stimulation (Jahnsen and Llinás, 1984; McCormick and Feeser, 1990).

Experiments done in whole cell patch clamp mode found that RMP was significantly lower in MGd than in MGv (-74 versus -68 mV) (Senatorov and Hu, 1997), which could explain the excitability difference between divisions. Differential GABAergic inhibition is not the cause: this inhibition is weaker in MGd than in MGv (Winer and Larue, 1988), yet MGd has the lower excitability (Hu et al., 1994). The ventral MGB receives a much larger corticothalamic innervation than does the dorsal or medial division (Winer and Larue, 1987). Therefore, the cortical deafferentation that occurs during explant preparation should produce a larger hyperpolarization ventrally than dorsally. Since hyperpolarization promotes burst responding, deafferentation seems inadequate to account for the predominance of burst responses dorsally.

5.3 Differential RMP control by acetylcholine

Numerous differences in the properties of responses to sound have been found between the anatomical divisions of the MGB *in vivo* (e.g.: Calford, 1983; Kraus et al., 1994). This raises the question of the cellular basis of these differences, which the present work partially answers. Intrinsic membrane properties were shown in previous work to contribute to differences in RMP and synaptic response (Hu, 1995; Senatorov and Hu, 1997), but the role of extrinsic factors such as cholinergic neuromodulation was not explored. The results show how cholinergic neuromodulation can help create the two dissimilar signaling pathways in thalamus by inducing differential RMP responses in different divisions. Muscarinic RMP changes in the MGB and lateral geniculate nucleus have previously been

found to interact with intrinsic neuronal membrane properties to control the mode of response to current pulses (McCormick and Prince, 1987). This is consistent with the hypothesis that differential RMP modulation by MRA underlies the synaptic response mode differences observed between dorsal and ventral MGB.

In cats, stimulation of the peribrachial area, known to contain cholinergic neurons projecting to thalamus, induced a slow membrane depolarization in thalamic neurons that was accompanied by increased reactivity to a corticothalamic volley (Deschênes and Hu, 1990). This suggests that cholinergic effects can be significant for synaptic transmission, but raises two questions: is the slow depolarization indeed cholinergic or muscarinic, and does muscarinic receptor activation affect the response to ascending principal afferent stimulation as opposed to corticothalamic stimulation. The present work based on muscarinic agonists *in vitro* demonstrates a cholinergic modulatory mechanism that is pathway and mode dependent. In recent work from another laboratory, acoustic responses of neurons in various divisions of the MGB were recorded in waking guinea pig (Edeline et al., 1999). Waking and paradoxical sleep both feature elevated levels of acetylcholine in thalamus (Williams et al., 1994). Edeline et al. found no significant difference in the spontaneous firing rates comparing MGv and MGd. This seems to conflict with results from this work, which would predict a lower spontaneous firing rate in MGd, due to membrane hyperpolarization by acetylcholine. Possibly, the MGd in the work of these authors had stronger spontaneous depolarizing inputs from cerebral cortex or IC that masked the hyperpolarizing effects of acetylcholine. Alternatively, the lack of differential spontaneous activity seen in the work of Edeline et al. may have been species-specific. This possibility is clearly raised by McCormick and Prince who showed that MRA is associated with a membrane depolarization

response in cat MGB neurons whereas in guinea pigs it induces a depolarization-hyperpolarization complex (McCormick and Prince, 1987a). Edeline et al. found long (>30 ms) response latencies in MGd but never in MGv, which agrees with in vitro results. Another point of agreement was that latency variability in MGv was only half that of the other subdivisions. (See section 1.3.)

5.4 Functional Significance of the findings

5.4.1 Cholinergic modulation in the brain

Suppression of the reticular activating system (in the upper brainstem and paramedian diencephalon) leads to coma (Hess and Bassetti, 1994; McCandless, 1981). Approximately ninety percent of brainstem projections to the thalamic sensory nuclei are cholinergic (Bentivoglio and Steriade, 1990; De Lima and Singer, 1987). ACh plays an important role in modulating thalamocortical communication (Hu et al., 1989; McCormick, 1992b). Acetylcholine is also an important transmitter for learning and memory (Aigner, 1995; Barkai and Hasselmo, 1997; Jerusalinsky et al., 1997; but see Blokland, 1995). The results presented in this thesis suggest that our current concept of cholinergic reticular modulation will have to change from a nonspecific global excitation or disinhibition to a more specifically acting influence, by which functional differences in parallel synaptic pathways are enhanced. Parallel synaptic transmission is highly conserved in mammalian thalamus across species and modalities (Graybiel 1972). The parallel anatomical arrangement is thought to be important for integrative processing of hearing (Calford, 1983; Graybiel, 1972; Lennartz and Weinberger, 1992). Burst mode responding, a feature correlated with

pathway type *in vitro*, is an interesting subject of study because of its possible use in sensory event detection and classical conditioning (see below).

5.4.2 Specific Roles of EPSP-LTS coupling and burst firing

5.4.2.1 Sensory event detection

When sensory signals are transmitted across central synapses, regardless of their physical traits, they must first be transformed into synaptic potentials: analog waveforms of varying size and/or frequency. How sensory information encoded by such analog signals is deciphered by postsynaptic cells and transmitted to the next level of the processing network is a hotly debated topic. Models such as "integrate-and-fire" or "random walk" (Shadlen and Newsome, 1994; Softky and Koch, 1993) are appealing mechanisms but they remain inadequate to answer the question of how neurons discriminate "meaningful" events from a continuous stream of apparently noisy synaptic bombardment, a problem commonly known as coincidence detection (Softky and Koch, 1993). We propose that the primary and secondary auditory thalamocortical pathways exemplify two distinct types of operating synaptic system. Since MGv neurons are like cortical neurons, the signaling mechanism that relates their input to output will lie somewhere on a continuum between temporal integration and coincidence detection, depending on the input pattern (Kisley and Gerstein, 1999). The dorsal MGB pathway, however, utilizes an unconventional synaptic mechanism characterized by LTS bursts, which provides a low-noise pathway for meaningful event detection.

Numerous *in vivo* studies indicate that the primary auditory thalamocortical pathway is tonotopically organized, generally has a higher excitability and is capable of following relatively rapid acoustic stimuli (Brugge, 1992; Calford, 1983; Calford and Aitkin, 1983;

Clarey et al., 1992). Muscarinic receptor activation in the thalamus would help maintain such a high-fidelity mode of synaptic transmission by membrane depolarization and increased membrane excitability that promotes tonic synaptic responses (McCormick, 1992b). On the other hand, the dorsal MGB pathway exhibits three salient features that are not evident in ventral neurons: a relatively hyperpolarized membrane potential, a large NMDA receptor-dominated EPSP and a prominent occurrence of EPSP-LTS coupling (Hu et al., 1994; Hu, 1995; Senatorov and Hu, 1997). The hyperpolarization serves a dual function: to ensure that the LTS remains in a state of at least partial de-inactivation and simultaneously to reduce synaptic noise by preventing small EPSPs from reaching firing threshold. When the incoming EPSPs become sufficiently large, which may result from synchronized synaptic firing and/or a synaptic response that has been potentiated in a specific behavioral context, a burst discharge occurs as the result of EPSP-LTS coupling. Hence, dorsal neurons are capable of eliminating "irrelevant" synaptic noise while selectively transmitting "relevant" signals. This synaptic type is here termed the "Differential Synapse". The work with drug-induced stimulation of the BIC reported here supports this notion. Those EPSPs that lead to EPSP-LTS-burst complexes by asynchronous afferent action potentials typically had multiple shoulders on the rising side of the depolarization, indicating temporal summation of individual synaptic events.

Based on their studies in visual thalamus, Sherman and Guillery proposed that the burst responding mode supports detection and tonic responding supports analysis of visual stimuli. In the auditory system, similar tasks appear to be carried out in parallel synaptic pathways, suggesting that a fundamental difference exists in the functional organization of vision and audition. The idea that the MGd is involved in sensory event detection is

consistent with the finding that certain auditory events thought to be involved in learning and attention (e.g.: the mismatch auditory potential) predominantly occur in the MGd (Di and Barth, 1992; McGee et al., 1992; McPherson and Davies, 1995; Naatanen and Teder, 1991).

5.4.2.2 Synaptic plasticity and short-term memory

Behavioral research conducted in animals and humans clearly indicates that lesions in nonprimary, but not primary auditory thalamocortical pathways, lead to most significant deficits in acoustic learning, short-term memory, and recall (Damasio et al., 1996; Kelly, 1973; Layton et al., 1979; Neff et al., 1975). For instance, Damasio and co-workers showed that patients with a restricted lesion in the temporal polar region of the insular cortex were unable to recall object names learned previously under auditory instruction (Damasio et al., 1996). In cats, bilateral lesions of this very area, which also causes a total degeneration of the caudodorsal MGB, resulted in a permanent loss of the ability to recognize sound sequences (Kelly, 1973). Is there a unique neural mechanism that may specifically account for such high-order auditory function?

The central auditory system exhibits classical conditioning of acoustic signals in MGd (Edeline and Weinberger, 1991) and lesions in the MGd and MGm significantly disrupted fear conditioning (LeDoux et al., 1986; McCabe et al., 1993; Poremba and Gabriel, 1997). Such learning is initiated in nonprimary MGB where acoustic signals and unconditioned stimuli co-terminate (Davis 1997; LeDoux, 1998). McKernan and Shinnick-Gallagher recently reported that fear conditioning of tone pips in rats resulted in a long-term potentiation (LTP) of synaptic responses in lateral amygdala neurons that is apparently due to an enhancement of the presynaptic release of glutamate from MGB

efferents (McKernan and Shinnick-Gallagher, 1997; Rogan et al, 1997). The induction of a lasting increase in synaptic strength along the MGB-limbic cortex pathway requires not only an enhanced activity in MGB neurons but also patterned stimuli. Clugnet and LeDoux found that to induce LTP in the lateral amygdala, paradigms used in the hippocampus for LTP induction or tonic stimuli mimicking the normal MGB neuronal firing pattern were ineffective. The only effective MGB stimulus pattern was a high frequency burst (30 pulses with an intraburst frequency of 400 Hz) delivered once per sec (Clugnet and LeDoux, 1990). This phenomenon suggests that the effectiveness of transmitter release at axonal terminals may be affected by the firing pattern initiated at the soma. The phenomenon of EPSP-LTS coupling and the resulting burst response may therefore play an essential role in a providing a patterned stimulus necessary for induction of LTP in downstream limbic structures. A single burst (3-5 spikes with an intraburst frequency of 300-500 Hz) by an MGB neuron has much fewer than 30 action potentials. However, at the population level longer sequences of high frequency action potentials could result from burst responses of variable latency among a group of cells (Hu, 1995). The finding that PPTg cholinergic neurons have positive reinforcement related activity (Dormont et al., 1998) implicates acetylcholine in the process of auditory learning.

5.4.2.3 Burst firing and signal encoding in other systems

In halothane/nitrous oxide anesthetized cats, a spike train consisting of a mixture of bursts and tonic spikes was recorded from each of 35 neurons of the primary visual thalamus (Reinagel et al., 1999). The trains were a response to a bar of randomly varying contrast situated in the neuron's receptive field. Burst firing did not occur exclusively for extended

periods of time, suggesting that burst mode was not completely under the control of neuromodulatory effects such as those studied here. Neuromodulation by ACPD reduced the burst fraction but did not significantly change coding capacity, information transmission rate, or coding efficiency. There was no evidence that burst mode encoded a special feature of the stimulus as in Gabbiani et al. (1996). However, this negative finding may be specific to primary sensory pathways and does not contradict our hypothesis about feature detection by bursts in nonprimary auditory thalamus. Reinagel et al. found that the coding efficiency of bursts and tonic spikes was roughly similar, but a burst event could transmit slightly more information than a tonic spike. This is consistent with prior findings that bursts have selective advantages for detection of stimuli (Guido et al., 1995). Primary visual thalamic neurons synapse in layers 4 and 6 of striate cortex. The layer 4 synapses exhibit paired-pulse depression and high transmission probability, enabling them to transmit both bursts and tonic spikes, thereby maximizing the recovery of visual information (Reinagel et al., 1999).

Swadlow and Gusev (2001) found that the paired-pulse depression of thalamocortical synapses onto layer 4 GABAergic interneurons interacted with the long ($\geq$ 100 ms) pause in firing that precedes a burst. (This pause is due to the hyperpolarization necessary to deinactivate the t-current.) The result was that the first spike in a burst had a much higher probability of firing the postsynaptic neuron than most tonic spikes, because synaptic depression dissipated during the pause. This means that at least one kind of layer 4 thalamocortical synapse preferentially transmits burst mode firing. The hypothesis of this thesis tends to take the focus off prompt transmission, however. The findings of Swadlow and Gusev imply that in thalamocortical transmission there can be a greater postsynaptic depolarization in response to bursts than in response to tonic spikes. The burst-induced

depolarization can be interpreted as the postsynaptic component of a Hebbian learning condition by the hypothesis advanced in this thesis, namely that one of the roles of burst firing is induction of synaptic plasticity in target neurons.

Lisman (1997) proposed a neural code consisting of synchronous short bursts, as an alternative to temporal integration or coincidence detection based on isolated spikes. The prevalence in the brain of unreliable synapses was interpreted as a device to selectively transmit the burst component of mixtures of bursts and isolated spikes. Bursts were shown to carry more information than isolated spikes in some preparations and isolated spikes could be noise (Cattaneo et al., 1981). Bursts were effective in inducing long term plastic changes in synapses in the carbachol-activated hippocampal slice (Huerta and Lisman, 1995) and have the properties necessary to reliably read out the information stored in the postsynaptic modification of long term potentiation. Thus, bursts were suggested to be useful for encoding, storing, and retrieving information. The plasticity results with hippocampal slice and the results of Cattaneo et al. are consistent with theories advanced in the present thesis that the functional significance of bursting is detection of behaviorally relevant stimuli and induction of synaptic plasticity.

Gabbiani et al. (1996) found that distinctive features of synaptic input to pyramidal neurons of the electrosensory lateral line lobe of weakly electric fish were more reliably signaled by short bursts than by isolated spikes. This agrees with one of our hypotheses about the functional significance of bursting in nonprimary auditory thalamus, which is to detect behaviorally relevant stimuli.

5.5 Conclusions

An important aspect of cholinergic control of auditory thalamocortical sensory processing is a differential modulatory action of muscarinic receptors on K^+ channels, which leads to opposite membrane and synaptic responses. This has helped create and maintain a fast synaptic responding mode in the primary thalamocortical pathway for high-fidelity sensory relay and a slower but robust bursting transmittal mechanism in the nonprimary pathway suitable for sensory event detection and for induction of long term changes in synaptic strength in limbic cortex during learning.

5.6 Further work suggested by the present results

The present results raise the issue of the molecular mechanism of the pathway-specific effects. One approach to mechanism is to identify the postsynaptic muscarinic receptor subtypes in MGv and MGd to determine whether a difference in subtype distribution underlies the differential RMP response to muscarinic receptor activation. In thalamus, the m1 and m3 subtypes are implicated in muscarinic depolarizations whereas the m2 subtype is implicated in muscarinic hyperpolarizations (McCormick and Pape, 1988; McCormick and Prince, 1986; Plummer et al., 1999; Zhu and Uhlrich, 1998). Another issue is the relevance of explant work to the waking state, in which other neuromodulators besides acetylcholine are secreted into the thalamus. The pathway-dependence of their effects on MGB neurons is still an unknown, raising the question of whether they would obscure the pathway-specific acetylcholine effects or strengthen them. Therefore, another project would be to determine the effect of other neuromodulators on synaptic response mode in MGv and MGd in explant. See footnote 2. Other unknowns are the effect of substances that may be co-secreted with

acetylcholine and the effect of subcellular localization of secreted substances. This calls for an experiment to establish *in vivo* or in slice that stimulation of the PPTg induces atropine-sensitive depolarizations in MGv neurons, leading to tonic firing, and atropine-sensitive hyperpolarizations in MGd neurons. The same experiment could be used to confirm *in vivo* the finding of differential response mode in primary and nonprimary auditory thalamus of rat previously found in explant. Finally, to support theories of the functional significance of burst mode responding, it would be desirable to demonstrate LTS-burst dependent LTP in cortical neurons receiving MGd afferents.

5.7 Footnotes

1. Even in the absence of muscarinic receptor activation (MRA), neurons in the MGd are on average somewhat more hyperpolarized than neurons in the MGv when measured from extracellularly identified bursting neurons (Hu, 1995) or under whole-cell recording conditions (Senatorov and Hu, 1997). MRA can be expected to enhance this difference. A recent *in vitro* intracellular sharp-electrode study that did not explore MRA (Bartlett and Smith, 1999) did not find the baseline difference in RMP between the two divisions. This study accords with earlier work from our laboratory in finding EPSP-LTS coupling and in finding expression of I_h more in ventral than in dorsal MGB divisions (Hu, 1995; Senatorov and Hu, 2000). We believe that the non-observation of the baseline difference in membrane potential is because impalement with sharp electrodes introduces a variable shunt conductance. RMP measured with sharp electrodes was 10-15 mV more positive than with whole-cell patch recording (Staley et al., 1992), which was caused by a nonselective leak conductance introduced by impalement. This conductance can introduce scatter into the

membrane potential and homogenize dorsal and ventral populations, obliterating the modest 10-mV difference in membrane potential on which signaling mode depends. Note that the intracellular part of the Chapter 3 study also failed to find significant membrane potential differences between the two divisions. The extracellular part of the Chapter 3 study found a highly significant difference in signaling mode between the two divisions, however. In the absence of MRA, the proportion of responding neurons that responded with a burst was significantly greater ($P < 0.0001$) in MGd than in MGv (89% versus 62%). Since extracellular response mode correlates with RMP (Hu, 1995) this suggests that the MGd population was on average more hyperpolarized. Our extracellular work also shows clearly that MRA tends to enhance the difference in preferred signaling mode between MGd and MGv, as would be expected from its effect on RMP.

2. Some data is available from our studies on the effect of the non-cholinergic neuromodulators (e.g.: noradrenaline, serotonin) released into MGB during waking. Extracellular recordings of the effect of phenylephrine, an adrenergic agonist, suggested that this agent depolarizes MGv neurons. No information is available on the effect of this particular agent on MGd neurons. 9 burst-responding cells were exposed to 10-145 μM of phenylephrine (usually 20 μM) for up to 4 min and 6 showed response changes. There was either block of the burst (n = 4), block with change to single spike responding (n = 1), or change to single spike responding (n = 1). 2/2 single spike-responding cells showed no effect. This pattern of response change has previously been associated with a membrane depolarization (Mooney et al., 1995; Chapter 2). In intracellular recordings, bath application of norephedrine (an α-1 adrenergic agonist) or buspirone (a 1A serotonergic agonist) at 24 to 194 μM for 20 s appeared to cause conductance changes and a depolarization

(norephedrine) in 1 of 3 MGv neurons and in 0 of 2 MGd neurons. This limited data is consistent with a picture of sporadic to consistent effects in MGv, depending on the agonist, and no effects in MGd. However, a clear differential modulatory action between dorsal versus ventral pathways has not yet been observed for serotonergic or noradrenergic agonists.

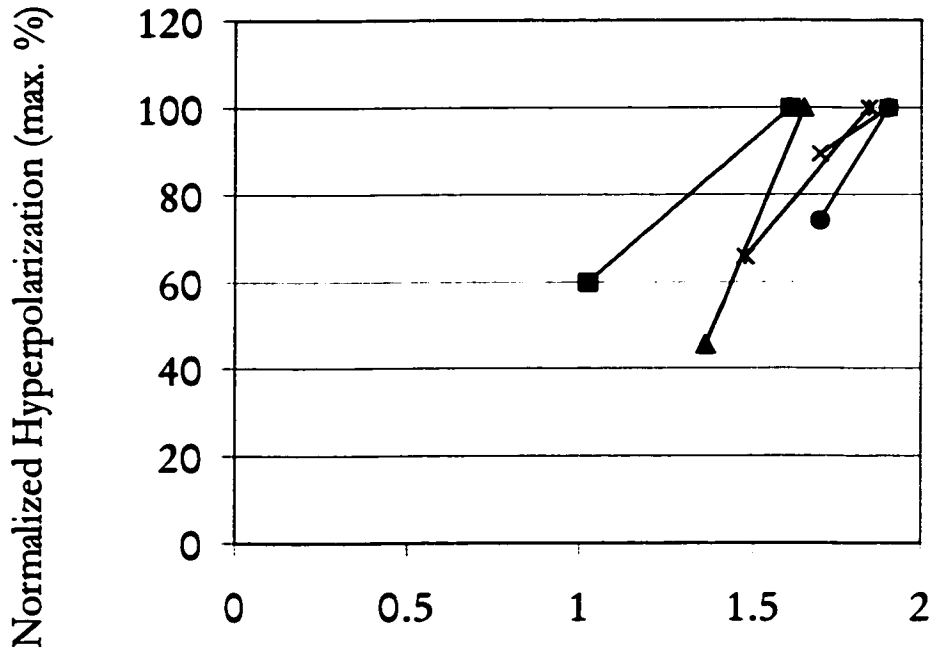
APPENDIX 1

During the course of our experiments, we noticed that the shipped age of the rats might influence muscarinic responses. 15/23 or 65% of MGd neurons in the statistical database (Chapter 3) responded to (+)-muscarine with a hyperpolarization of 4 mV or more. Data on the response of MGd neurons to muscarinic agonists from another study, not included in the statistical database of the Chapter 3 study, found only 7/41 or 17% of neurons responding with hyperpolarizations of 4 mV or more, the remainder showing small (+3 to -3 mV), or no membrane potential changes. The discrepancy cannot be attributed to rat age when used, ACSF makeup, temperature, or muscarinic agonist dosage. However, increasing the shipped age of the rats from 24 to 40 or 68 days resulted in a 23/46 or 50% success rate in producing muscarinic hyperpolarizations of 4 mV or more in MGd neurons.

APPENDIX 2

The illustration on the following page gives dose-response information for muscarinic RMP regulation in MGB (Fig. 1). Within a panel, different neurons are indicated by different symbols. Peak membrane responses were measured following exposure to (+)-muscarine. Data for each neuron has been normalized so that the largest amplitude of depolarization (MGv) or hyperpolarization (MGd) in the dose-response curve is 100%.

MGd



MGv

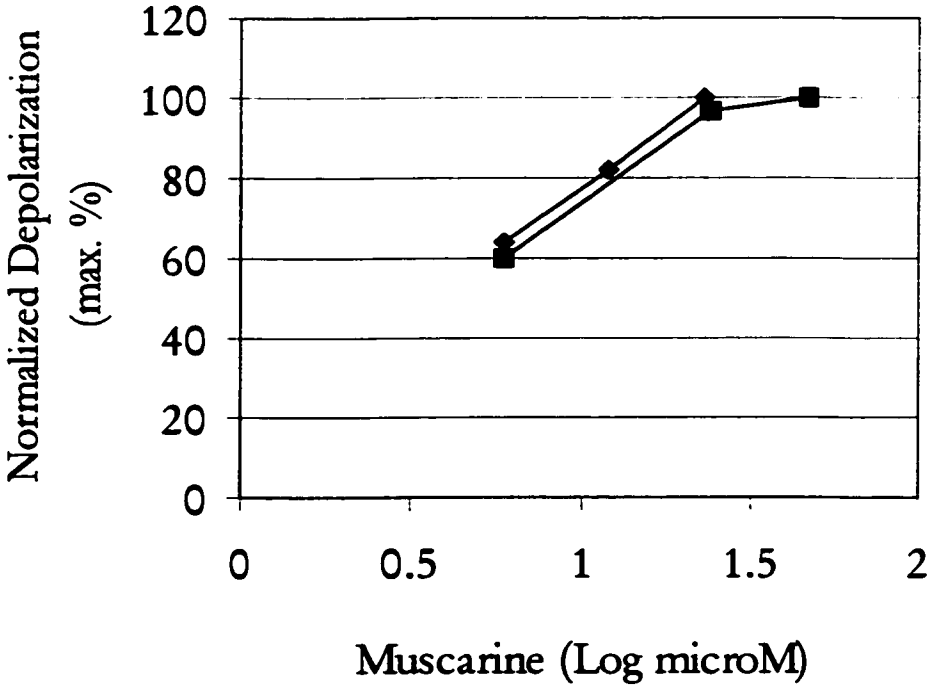


Figure 1

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