Intrinsic membrane properties affecting signal transformation in auditory thalamic neurons

by

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Abstract

Neurons in the ventral medial geniculate body (MGBv) of the thalamus transform auditory signals and participate in corticothalamic oscillations, depending on the behavioral state. To study the role of intrinsic membrane properties and neuromodulation on the signal transformation of MGBv neurons, whole-cell patch-clamp recordings and pharmacological analyses were performed in rat brain slice preparations. The neurons were labelled and morphologically characterized by immunohistochemical methods.

In all mature MGBv neurons, current injections produced a range of voltage-dependent firing patterns. At depolarized membrane potentials, observed previously during wakefulness in vivo, the neurons fired tonically with different latencies. In contrast, at hyperpolarized potentials low-threshold burst firing was observed, which has been implicated in the generation of delta-oscillations during deep sleep. Na\(^+\), Ca\(^{2+}\), K\(^+\) and mixed cationic conductances were found to contribute to subthreshold rectification and the action potential patterns. High-threshold Ca\(^{2+}\)-spikes were identified for the first time in MGBv as part of the burst response and may play a role in dendritic signalling. Thus the intrinsic membrane properties influence timing and quality of spikes during the different tonic and burst firing modes, with important implications for auditory signalling and sleep oscillations.

The intrinsic membrane properties endow MGBv neurons with frequency filter properties. Using frequency analysis methods (ZAP analysis), a membrane resonance at 1-2 Hz was found at hyperpolarized potentials, due to an interaction of the low-threshold T-type Ca\(^{2+}\)-conductance with the passive membrane properties. At more depolarized potentials, the membrane exhibited low-pass filter characteristics. These frequency preferences were modulated by activation of subthreshold Na\(^+\) and K\(^+\)-conductances. Firing of Na\(^+\) and high-threshold Ca\(^{2+}\)-spikes occurred most readily at the preferred frequencies. Thus the resonance at 1-2 Hz confers properties of a band-pass frequency filter on MGBv neurons, which may contribute to the generation and synchronization of delta oscillations during slow-wave sleep.

Postnatal development in MGBv neurons changes dendritic morphology, intrinsic membrane properties, firing patterns and frequency preferences: the dendritic trees increased in size and complexity; the resting membrane potentials and input resistance decreased, while action potential and low-threshold Ca\(^{2+}\)-spike amplitude and rate of rise increased over the first two postnatal weeks. Immature neurons exhibited high-threshold Ca\(^{2+}\)-spikes appended to action potentials during tonic firing, but no low-threshold burst firing could be evoked.
The development was concluded by day 12-15, when full auditory function and the characteristic behavioral states of mature animals are observed.

Activation of metabotropic glutamate and GABA\(_B\) receptors change signal transformation in MGB\(_v\) neurons. Application of the selective metabotropic glutamate receptor agonist 1S,3R-ACPD reversibly depolarized MGB\(_v\) neurons, changing burst to tonic firing and the 1-2 Hz resonance to low-pass filter properties. This depolarization was produced by activation of a TTX- insensitive Na\(^{+}\)-dependent current without a change in input conductance between -40 and -85 mV, a novel finding in thalamic neurons. Actions on K\(^{+}\)-currents were ruled out, as the K\(^{+}\)-channel blockers Ba\(^{2+}\) and Cs\(^{+}\) and changes in extracellular K\(^{+}\) had no effects on the evoked 1S,3R-ACPD- current. The effects of 1S, 3R-ACPD were blocked by the metabotropic glutamate receptor antagonist MCPG. Irreversible activation or blockade of the 1S,3R-ACPD-current by intracellular application of the GTP analogs GTP\(_{\gamma}\)S and GDP\(_{\beta}\)S, respectively, suggested an involvement of G-proteins in intracellular signal transduction. These metabotropic glutamate effects may play a role in corticothalamic modulation of auditory signal transmission.

Activation of GABA\(_B\) receptors by the selective agonist baclofen reversibly hyperpolarized MGB\(_v\) neurons by activation of a Ba\(^{2+}\)-sensitive K\(^{+}\)-current, which increased the input conductance. The conductance increase shunted the 1-2 Hz resonance. The effect of baclofen was blocked by application of the GABA\(_B\) receptor antagonist CGP 35348. The occlusion and blockade of baclofen effects by the GTP analogs GTP\(_{\gamma}\)S and GDP\(_{\beta}\)S, respectively, confirmed an involvement of G-protein activation. The GABA\(_B\) receptors may mediate longer-lasting inhibitory potentials from the inferior colliculus and the thalamic reticular nucleus, which have been implicated in the modulation of sleep and pathological oscillations.

Application of the volatile anesthetic isoflurane reversibly hyperpolarized MGB\(_v\) neurons and shunted firing in both tonic and burst modes. Moreover, isoflurane eliminated the resonance at 1-2 Hz. These effects may play a role in the disruption of auditory signal transduction and oscillations by general anesthetics.

These investigations have identified for the first time intrinsic membrane properties and neuromodulatory effects in MGB\(_v\) neurons, which may explain the fundamental differences in auditory information processing during diverse behavioral states (wakefulness, sleep and anesthesia).
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Dedication

This thesis is dedicated to Donna and Agnes.
1. Introduction

Scope

Thalamic relay neurons in the ventral medial geniculate body (MGBv) encode auditory information as firing patterns and participate in sleep oscillations. The firing output of MGBv neurons contains phasic burst, sustained tonic and rhythmic firing patterns, depending on auditory stimulus parameters and behavioral state. The mechanisms producing these firing patterns were unclear. Also, neuromodulators and anesthetics have been proposed to change or disrupt auditory thalamic signalling. To this end, we studied for the first time the role of membrane properties and neuromodulation in signal transformation and oscillations of MGBv neurons. As intrinsic membrane properties, the variety of firing patterns and oscillatory tendencies should be evident in MGBv neurons in slice preparations in vitro, independent of synaptic activity. In particular, the following 4 questions were addressed:

1. What intrinsic mechanisms underlie the variety of output patterns in MGBv?
2. Do MGBv neurons exhibit frequency filter functions?
3. How do metabotropic glutamate and GABA receptors modulate signalling?
4. How do general anesthetics disrupt signalling in MGBv?

The auditory pathway

Auditory perception is essential for survival of animals and speech perception in humans. In order to analyse complex sounds, the auditory system needs to perform an extremely precise signal analysis of spectral, temporal and spatial aspects of sound within wide ranges of frequency (~20-15,000 Hz) and intensity (~120 dB). To achieve this task, sound information is processed along the auditory pathway, which is comprised of many hierarchically and parallel organized
nuclei, with complex synaptic connectivity and neurons with specialized membrane properties (see reviews in Edelman et al. 1988, Ehret and Romand 1997). The hair cells in the inner ear transform incoming sound waves into electrical signals, which are transmitted as action potential patterns to the cochlear nuclei. These patterns are further analysed by neurons in the auditory brainstem nuclei, where firing rate, phase locking and interaural spike latency differences are used to encode sound intensity, periodicity and location (see reviews by Clarey et al. 1991, Edelman et al. 1988). Sound frequency information is transmitted by way of anatomical pathways, evident from best frequency maps in the auditory system (cf. Schreiner 1995). The inferior colliculi integrate temporal and frequency information of the brainstem nuclei and may encode sound duration (Casseday et al. 1994).

The auditory thalamus performs dynamic gating functions. On the one hand, auditory information is relayed to the auditory cortex, on the other hand, MGBv neurons participate in thalamocortical oscillations, which may prevent this signal transfer (Ehret and Romand 1997, Steriade et al. 1990). These gating functions are controlled by brainstem neuromodulation and thalamocortical activity, both depending on and contributing to the behavioral state (Steriade and McCarley 1990). We chose the MGBv relay neurons to study how their intrinsic membrane properties would contribute to these diverse signalling requirements.

**Anatomy of the medial geniculate body**

The medial geniculate body (MGB) is the auditory thalamic nucleus. Three major divisions can be distinguished, based on neuronal density and morphology: the ventral, dorsal and medial divisions (Morest 1964). These areas contain two characteristic types of neurons - relay neurons, which project mainly to the primary auditory cortex, and interneurons which synapse within MGB. The relay neurons in the ventral medial geniculate body (MGBv) have relatively
large somata, often possessing a bitufted dendritic arborization and are arranged in a laminar organization. In contrast, the relay neurons in the dorsal and medial MGB (MGBd, MGBm) exhibit different types of morphology and no obvious spatial organization (Clerici et al. 1990, Morest 1964). The MGBm is also referred to as "magnocellular", as it contains neurons with large somata. The interneurons are smaller, exhibit radially projecting dendrites and contain γ-aminobutyric acid (GABA), implying an inhibitory function (Winer and Larue 1988). The number of interneurons in MGB varies widely, depending on the species (e.g., cat ~25%, rat ~1%; Winer and Larue 1996).

Connectivity of the MGB

The MGB is part of a complex network containing several parallel pathways and feedback loops. The three MGB divisions participate in different parallel auditory pathways from the inferior colliculus (IC) to the auditory cortex. Anatomical and functional studies have identified predominant projections connecting specific subdivisions on each level. The *lemniscal* pathway is characterized by its tonotopical organization at all levels of the tecto-thalamo-cortical pathway. The central nucleus of the IC (ICC) provides afferent input to the MGBv, which projects to the primary auditory cortex. In the *non-lemniscal* or non-tonotopic system, afferent input from the pericentral nucleus of the IC (ICP) is relayed to the MGd and on to the non-tonotopic secondary auditory cortex. The target of a third *multimodal* system is the MGm, responsive to inputs from the multimodal external nucleus of the IC (ICX). The projection from MGm to the cortex appears not specifically segregated and projects to different auditory cortical areas (see reviews by Edelman et al. 1988, Ehret and Romand 1997).

Thalamocortical connections are commonly reciprocal, e.g., cortical columns receiving input from MGB usually project back to the same area in MGB (Andersen et al. 1980, Jones
1985, Winer and Larue 1987). Combined anatomical and electrophysiological studies have shown that thalamocortical axons terminate in layer 4 of the auditory cortex, while pyramidal cells in layer 5 and 6 project back to the MGB (Mitani et al. 1985, Ojima 1994). An exception to these projections are the axons of the MGBm, which terminate in layer 1 of different auditory cortical fields (Mitani et al. 1987).

The auditory partition of the thalamic reticular nucleus (TRN) forms another feedback loop with the MGB, receiving collaterals of thalamocortical and corticothalamic fibers (Jones 1985, Ohara and Lieberman 1985, Rouiller et al. 1985, Shosaku and Sumitomo 1983). Other important projections to MGB include inputs from neuromodulatory brainstem nuclei (Fitzpatrick et al. 1989, Steriade et al. 1988). The projection from MGBm to the amygdala has been implicated in auditory fear conditioning (LeDoux et al. 1985). For detailed reviews, refer to Edelman et al. 1988, Ehret and Romand 1997, Jones 1985 and Winer 1992.

**Neurotransmitters in MGB**

Afferent auditory input from the inferior colliculus to MGB can be both excitatory and inhibitory, a finding unique among sensory thalamic nuclei. Fast excitatory responses are mediated by glutamate, which activates non-NMDA ionotropic receptors in MGBv and NMDA as well as non-NMDA ionotropic receptors in MGBd (Hu et al. 1994). The inhibitory component arises from GABAergic projection neurons in the IC (Winer et al. 1996), which activate fast GABA$_A$ and longer lasting GABA$_B$ mediated potentials in MGBv and MGBd (Peruzzi et al. 1997). Glutamate mediates the excitatory projection to the MGB from auditory cortex, including metabotropic responses (Bartlett and Smith 1995). Another inhibitory projection to the MGB arises from the GABAergic neurons in the TRN (Shosaku and Sumitomo 1983), but the receptor types activated in MGB neurons are unknown.
The MGB receives a variety of inputs from neuromodulatory brainstem nuclei, mediating cholinergic nicotinic and muscarinic responses from pedunculopontine and laterodorsal tegmental nuclei (Fitzpatrick et al. 1989, McCormick and Prince 1987, Mooney et al. 1995, Steriade et al. 1988, Tebecis 1972). Also, serotonergic inputs arise from raphe nuclei and noradrenergic inputs from locus coeruleus, mediating excitatory and inhibitory responses, which vary with the animal species and division in MGB (Fitzpatrick et al. 1989, McCormick and Prince 1988, Tebecis 1967, 1974).

Thalamocortical relay neurons in the MGB utilize glutamate as an excitatory transmitter (LeDoux and Farb 1991), which generates fast potentials in the auditory cortex (Cox et al. 1992, Metherate and Ashe 1993) and NMDA and non-NMDA mediated responses in the amygdala (Li et al. 1995).

Responses to auditory stimulation in MGBv

Auditory stimuli evoke a variety of excitatory, inhibitory and oscillatory responses in MGB. These responses include combinations of on/off burst firing, sustained tonic firing, suppression of spontaneous firing and rhythmic firing in awake animals (Aitkin et al. 1966, Aitkin and Prain 1974, Creutzfeldt et al. 1980). Most extracellular studies in MGB have been performed in animals under anesthesia, where neuronal responses are dramatically changed, depending on type and depth of anesthesia. During anesthesia, spontaneous and sustained responses are rare while burst responses have been commonly observed (Aitkin and Dunlop 1968, Zurita et al. 1994). The following characteristics of responses to auditory stimulation have been found in MGB neurons:

1. Response latencies vary to a large degree within MGB. Neurons in MGBv exhibit usually short latency responses (6-20 ms) to sound or click stimulation, while most MGBd neurons
exhibit longer latency responses up to 500 ms (Calford and Webster 1981). Some investigators of auditory representation in thalamus have interpreted the latency of phasic onset responses as a code for dynamic changes (Rouiller et al. 1983). However, the anesthesia-dependence of the phasic responses, and more elaborate sound coding in the thalamus of alert animals may invalidate such hypotheses (Creutzfeldt et al. 1980, Zurita et al. 1994).


3. *Frequency responses* differ also within MGB divisions. Sharp tuning curves for characteristic frequencies and an orderly tonotopical organization are found in MGBv (Imig and Morel 1985). In contrast, MGBd neurons are broader frequency-tuned and not tonotopically organized (Calford and Webster 1981). However, the precision of tonotopical organization in MGBv may be correlated with anesthetic states and has not been observed in awake animals (Whitfield and Purser 1972).

4. *Temporal responses* of MGBv neurons include the ability to phase-lock to low-frequency sounds up to 1000 Hz (Rouiller et al. 1979) and to follow amplitude- and frequency-modulation frequencies up to 100 Hz (Creutzfeldt et al. 1980). These responses exhibit a specific selectivity for bands of amplitude and frequency modulation (Gaese and Ostwald 1995).

5. *Binaural stimulation* in MGB elicits facilitatory, occluding or similar responses in an equal number of neurons compared with monaural stimulation, attributable to temporal interaction of excitatory and inhibitory inputs (Aitkin and Dunlop 1968).
6. Oscillations, observed as spontaneous or click-evoked rhythmic discharges, are a hallmark of the auditory thalamus and are not observed at lower stations of the auditory pathway (Aitkin et al. 1966, Galambos et al. 1952). MGB neurons can spontaneously oscillate at delta, theta and gamma range frequencies (Barth and MacDonald 1996, McCormick and Prince 1988, Narikashvili 1976). These different oscillation frequencies have been implicated in sleep states and sensory coding (Steriade et al. 1990, Singer 1993).

State-dependent firing patterns

Auditory thalamic firing patterns differ with behavioral states (Fourment and Hirsch 1979, Steriade et al. 1990, Steriade and McCarley 1990). During wakefulness, tonic firing patterns at depolarized membrane potentials prevail, while slow oscillatory burst firing at more hyperpolarized potentials is observed during deep sleep (Fourment and Hirsch 1979). During application of anesthetics (Ketamine and Pentobarbital), MGB neurons reduce their spontaneous activity and change their response patterns to auditory stimuli (Zurita et al. 1994). An assessment of auditory coding by specific firing patterns would require systematic studies on auditory responses during wakefulness, sleep states and anesthesia.

Mechanisms of pattern generation in MGB

Several mechanisms have been proposed for the generation of the firing patterns observed in vivo (see above). Interacting excitatory and inhibitory synaptic transmission has been implicated in the suppression of spontaneous firing and complex binaural interactions (Aitkin and Dunlop 1968, Nelson and Erulkar 1963).

Differences between fast, single-spike responses in the MGBv and longer latency bursts in MGBd neurons were attributed to an interaction of synaptic inputs with intrinsic membrane
properties (Hu 1995). Inputs to the more depolarized MGBv neurons were closer to threshold, while the delay in the more hyperpolarized MGBd occurred via an interaction with a low-threshold calcium spike (LTS). However, these studies were only performed at the resting membrane potential in this in vitro study. A stationary membrane potential has not been observed in vivo (Fourment and Hirsch 1979).


Spindle oscillations, observed during drowsiness and light barbiturate anesthesia, depend on interactions of the intrinsic membrane and synaptic properties of neurons in the dorsal thalamus-thalamic reticular network (Bal et al. 1995, Contreras and Steriade 1996, von Krosigk et al. 1993). Spindle oscillations are observed at less hyperpolarized membrane potentials than delta-oscillations, in the main activation range of the hyperpolarization-activated cation current, $I_H$ (McCormick and Pape 1990b). This current has been shown to shape the waxing and waning of spindles due to its modulation by intracellular calcium ions (Bal et al. 1995, Bal and McCormick 1996, Lüthi and McCormick 1998). The different voltage ranges of sleep spindles and delta-oscillations may explain their apparent incompatibility (Nunez et al. 1992).

Gamma oscillations have been observed in corticothalamic networks (Barth and MacDonald 1996, Neuenschwander and Singer 1996, Ribary et al. 1991, Steriade et al. 1996) and
may facilitate synchronized thalamic firing, which could promote feature binding (cf. Alonso et al. 1996, Engel et al. 1992, Sillito et al. 1994). Thalamic gamma oscillations may require activation of dendritic \( \text{Ca}^{2+} \) spikes at depolarized membrane potentials (Pedroarena and Llinás 1997) and are facilitated by excitatory cholinergic brainstem activation (Steriade et al. 1991, Pinault and Deschenes 1992).

**Organization of this study**

Using patch-clamp recordings and pharmacological techniques in MGBv slices *in vitro* (Chapter 2), we studied, how intrinsic membrane properties produced the different firing patterns and filter properties in MGBv neurons. Square pulse stimuli were employed to investigate the input-output relationship of MGBv neurons (Chapters 3 and 4). The study of membrane filter properties required oscillatory stimuli to characterize the frequency preferences at different membrane potentials and their dependence on intrinsic conductances (Chapter 5).

Auditory signal transformation and oscillations undergo major changes during postnatal development (Mares et al. 1982, Pujol et al. 1970). To identify the mechanisms for some of these changes, we studied the neuronal membrane properties over a range of ages to establish a developmental profile of firing patterns and oscillatory tendencies (Chapter 6).

Neuromodulators change the signal transformation in the MGBv and other thalamic nuclei (McCormick 1992b, Steriade et al. 1990). Glutamate and GABA, the fast auditory neurotransmitters in the MGBv, were also thought to intrinsically modulate auditory signal transformation via activation of metabotropic receptors (Bartlett and Smith 1995, Peruzzi et al. 1997, Shosaku and Sumitomo 1983). Here we studied the mechanisms for such an intrinsic modulation in MGBv, which have not been addressed previously (Chapters 7 and 8).
To study how anesthetics would disrupt auditory signal transformation at the thalamic level (cf. Knijević and Puil 1997, Ries and Puil 1993), we tested the effects of the general anesthetic, isoflurane, on MGBv neurons (Chapter 9). An identification of anesthetic mechanisms in the MGBv also was required for the better understanding of in vivo results which were usually performed under anesthesia.
2. Methods

All experiments followed protocols approved by the Committee on Animal Care of the University of British Columbia.

Preparation of MGBv slices

Sprague-Dawley rats (5-24 days old, n = 111) were decapitated during deep anesthesia with halothane. The brain was rapidly removed from the cranium and submerged in cold (4 °C) artificial cerebrospinal fluid (ACSF). The ACSF contained (in mM) 124 NaCl, 26 NaHCO₃, 10 glucose, 4 KCl, 2 CaCl₂, 2 MgCl₂, and 1.25 KH₂PO₄, and had a pH of 7.3, maintained by a continuous saturation with 95 % O₂ : 5 % CO₂. Using a Vibroslicer (Campden Instruments, London, England), we cut 300-400 µm thick coronal slices of the MGB. Before recording, the slices were incubated at 22-25 °C for at least 3 h.

Patch-clamp recording conditions

Whole-cell patch-clamp electrodes were pulled (Narishige, Model PP83) from borosilicate glass (WP-Instruments, Sarasota, FL). The electrode solution (pH 7.3) contained (in mM) 140 K-gluconate, 10 N-2-hydroxyethylpiperazine-N-2-ethanesulfonate (HEPES), 5 KCl, 4 NaCl, 3 adenosine 5'-triphosphate (Na₂ATP), 0.3 guanosine 5'-triphosphate (Na₃GTP), 10 ethylene glycol-bis-(β-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA) and 1 CaCl₂ (~10 nM Ca²⁺, calculated using Max Chelator software). For experiments with intracellular bis-(o-aminophenoxy)-ethane-N,N,N',N'-tetraacetic acid (BAPTA), 10 mM BAPTA replaced EGTA. Similarly, when studying the effects of GTP-analogs, 0.3 mM guanosine 5'-O-(3-thiotriphosphate) (GTPγS) or 0.3 mM guanosine 5'-O-(2-thiodiphosphate) (GDPβS) replaced GTP in the electrode solution. For
solutions of low extracellular Na\(^+\)-concentration (26 mM), we replaced NaCl with N-methyl-D-
glucamine chloride (NMDGCl) of equimolar amounts. In the case of Li\(^+\)-applications, we 
replaced 50 mM NaCl with equimolar LiCl. In 109 cases, we added 0.7% \textit{N}-(2-aminoethyl)
biotinamide hydrochloride (Neurobiotin; Vector Laboratories, Burlingame, CA) to the electrode 
solution for staining the recorded neurons after the experiment (see below), verifying, 
histologically, the location of neurons in MGBv and characterizing their morphology (Chapter 3).

The electrode tip was exactly positioned in the MGBv, using translucent illumination 
(100x magnification, Hoffman contrast objective). Whole-cell patch-clamp recordings (Blanton 
et al. 1989, Hamill et al. 1981) were made from 280 presumed relay neurons at 21-25°C with an 
Axoclamp 2A amplifier (Axon Instruments, Foster City, CA) in current-clamp or discontinuous 
single-electrode voltage-clamp mode (current-voltage switching frequency 4-5 kHz, 30 \% duty 
cycle, gain 3-5 nA/mV, phase shift and optimized anti-aliasing filter, frequent monitoring of the 
voltage clamp performance). Data acquisition, storage and analysis were controlled using 
pClamp 5 software (Axon Instruments) running on a PC. The experiments were continuously 
recorded on a chart recorder (Gould) and videotape (super beta, Sony). Recording conditions for 
neurons were commonly stable for several hours. In three experiments, the temperature was 
raised to 33 ± 1°C, heating the ACSF and the bath with a light bulb and measuring the 
temperature with a thermoprobe (Chapter 5).

Capacitance compensation and bridge balance techniques were used to compensate for the 
access resistances in the range of 10 to 15 M\(\Omega\) for voltage-clamp experiments and <25 M\(\Omega\) for 
current-clamp mode. For voltage-clamp experiments, tetrodotoxin (TTX, 300 nM) and Cd\(^{2+}\) (0.1 
mM) were added routinely to block spikes and synaptic activity. The voltage/current (V/I) 
relationships of the 1S,3R-ACPD- or baclofen-evoked currents were determined with slow 
voltage-ramp protocols: Neurons were held for 400 ms at -50 or +10 mV, followed by a ramp at
60 ms/mV to -110). This procedure yielded similar results compared to steady-state current responses measured at the end of 500 ms voltage-step protocols (n = 3, data not shown). Input conductance was calculated from the voltage response to injections of hyperpolarizing current pulses (cf. Chapter 3) or the slope of V/I relationships. All voltage values were corrected for a measured junction potential of -11 mV.

**Frequency-domain analysis**

As described previously in detail (Hutcheon et al. 1996; Puil et al. 1986, 1994b), we analyzed the frequency responses of MGBv neurons as impedance amplitude profiles (ZAP), represented by the ratio of fast Fourier transforms of the voltage responses to swept-sinewave current inputs, sweeping through a chosen frequency range. The swept-sinewave current input was adjusted to produce < 15 mV peak to peak voltage responses to ensure approximate linearity. For an assessment of frequency selective firing in MGBv, the amplitude of the swept-sinewave current was systematically increased to reach firing threshold under various conditions (Chapters 5 and 9). We used sinewave current functions sweeping from 0.1 to 20 Hz, or in some cases up to 50 Hz, and plotted the magnitude of the complex-valued impedance as a function of frequency in a range of 0.1 and 10 or 20 Hz where the frequency-responses of MGBv neurons are voltage-dependent and subject to modulation by drugs (Tennigkeit et al. 1994, 1997). Frequency response curves (FRCs) at higher frequencies (20 to 200 Hz) did not exhibit voltage-dependence or high-frequency resonance and decayed asymptotically (not shown). The electrode did not contribute to these frequency responses, as control sweeps in the bath without a cell exhibited completely flat responses. Sampling frequency was adjusted to the maximal ZAP frequency, e.g. for 20 Hz, 8192 samples were taken at 4.69 msec intervals and filtered at 100 Hz. Frequency responses of single ZAP sweeps (noise < 5% of impedance magnitude) were plotted after 5 point
averaging for clarity, which yielded similar results as averaging several sweeps (cf. Hutcheon et al. 1996).

**Drug application**

Unless noted otherwise, all drugs were prepared as stock solutions in water, usually at 1000 times the final concentration. They were applied diluted in ACSF, perfused at a rate of 2 ml/min through a submersion-type chamber with a volume of ~ 0.3 ml. Ion channel blockers and receptor antagonists were applied in the ACSF for a minimum of 10 min before measurements to ensure their equilibrium in the tissue.

Application of the mGluR agonist, 1S,3R-ACPD at 50 μM for 30-60 sec, produced a reversible response in 56 out of 67 experiments (see Chapter 7, Results). This protocol was used to investigate the mechanism of action of mGluR activation. In 10 experiments, 50 μM D-APV and 10 μM CNQX were added throughout the experiment. However, we did not observe differences in experiments without these ionotropic glutamate receptor antagonists, confirming the selectivity of 1S,3R-ACPD for mGluRs (cf. Pin and Duvoisin 1995).

Concentrations of isoflurane are given as volume percent in the gas delivered from a calibrated vaporizer (Ohio Medical, Toronto, Ontario) and equilibrated with the ACSF. At 22 °C, the solubility of isoflurane in ACSF is approximately 2-fold greater than at 37 °C. The concentrations used in this investigation would correspond to twice the concentrations of *in vivo* anesthesia.
Materials

4-aminopyridine (4-AP), ATP, baclofen, BAPTA, EGTA, GDPβS, GTP, GTPγS, HEPES, L-glutamate, NMDGCl, TTX, tetraethylammonium (TEA) and inorganic salts (e.g. CdCl₂, NiCl₂, BaCl₂, CaCl₂) were purchased from Sigma (St. Louis, MO). The following glutamate receptor agonists and antagonists were purchased from Precision Biochemicals (Vancouver, BC): 1S,3R-1-aminocyclopentane-1,3-dicarboxylate (1S,3R-ACPD), (RS)-α-methyl-4-carboxyphenylglycine (MCPG), D-2-amino-5-phosphonovalerate (D-APV) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). CGP 35348 was a generous gift of Novartis AG (Basel, Switzerland). Isoflurane was purchased from Abbott Laboratories (Montreal, Quebec).

Histological methods

Following a recording session, the slices containing neurobiotin-filled cells were fixed overnight at 4°C in 4% paraformaldehyde and 20% sucrose, diluted with phosphate buffer (0.1 M, pH 7.2). The following day, the slices were re-sectioned to ~90 μm on a freezing microtome (Reichert, Austria), processed as described by Kita and Armstrong (1991) and counterstained with thionine. Camera lucida drawings were made of the labeled neurons and the dendritic trees reconstructed from adjacent sections (Chapters 3 and 6).

Statistical analysis

Quantitative data are presented as means ± S.E., unless noted otherwise. Responses under different conditions were compared using Student’s t test. Differences were considered significant for p < 0.05.
3. Signal transformation and firing modes in MGBv

3.1. INTRODUCTION

In auditory processing, a primary function of thalamocortical relay neurons in the MGBv is to encode sound signals as different sequences of action potentials. In thalamocortical processing, however, distinct firing patterns of thalamocortical relay neurons signify states of alertness and sleep, i.e., the information transferred to the cortex is dramatically different. Most of the studies on thalamocortical signalling have been performed in the dorsal lateral geniculate nucleus (LGNd), the major visual thalamic relay nucleus (reviewed by McCormick 1992b; Steriade et al. 1990). The signal transformation of MGBv and LGNd during wakefulness is supposedly very different. The eyes are closed during sleep, whereas the ears are open. In the sleeping brain, neurons of the auditory pathway must remain responsive to specific sounds when they serve as wake-up calls, requiring attention.

During alertness, the relatively depolarized membrane potentials of the relay neurons induce a tonic mode of firing. This permits the transformation of synaptic inputs into modulated spike firing rates which precisely represent the sensory signals. During some sleep stages, MGB and other thalamocortical neurons at more hyperpolarized potentials markedly change their behavior to the burst firing mode (cf. Fourment and Hirsch 1979; Hirsch et al. 1983). The spike bursts typically occur at the onset of a depolarizing stimulus, on the depolarizing slope of inhibitory postsynaptic potentials (IPSPs), and in rhythmic patterns that appear to represent a non-alert state rather than the precise nature of the sensory signal (McCormick and Feeser 1990; Steriade and Llinás 1988).

The distinct tonic and burst patterns of thalamocortical firing are a consequence of the interactions of voltage-dependent conductances with the passive membrane properties.
(Steriade et al. 1993). From hyperpolarized potentials, for example, the activation of a T-type Ca\textsuperscript{2+} current (I\textsubscript{T}) and the slow deactivation of a hyperpolarization-activated H-type current (I\textsubscript{H}) initiates burst firing. When I\textsubscript{T} undergoes inactivation at depolarized potentials, a depolarizing stimulus produces tonic firing of action potentials. In the alert or sleeping brain, non-sensory transmitter systems can modulate the resting potential and thereby control the firing modes (McCormick 1992b). In brain slices, current injection into the neuron can impose different firing modes. This permits a study of the intrinsic membrane properties in isolation from potentially confounding parameters (e.g., synaptic connectivities) that exist in the intact brain and tissue explant preparations (cf. Hu et al. 1994).

MGBv neurons in an in vitro preparation at rest respond to synaptic excitation with single action potentials or bursts, whereas in the dorsal partition, they respond predominantly with bursts of action potentials (Hu et al. 1994). The capacity of MGB neurons to produce different firing patterns has not been the subject of detailed investigation. Here, we used whole-cell patch-clamp recordings in the current-clamp mode to describe a variety of distinct spike output patterns at different membrane potentials in MGBv neurons in in vitro preparations. We show that these neurons have the burst and tonic modes of firing, suggesting that these cells encode sound differently in waking and sleep states. However, MGBv neurons have special mechanisms that may promote an efficient throughput of sensory signals to the cortex. These include an ability to fire action potentials and low- as well as high-threshold calcium spikes, linked by the activation of various conductances. Such properties could endow MGBv neurons with an ability to switch modes during a sensory stimulus in deep sleep and produce an alerting reaction which initiates precise signal coding.
3.2. RESULTS

Morphological characteristics of MGBv neurons

During the recording, MGBv neurons were filled with neurobiotin and stained after the experiment (cf. Chapter 2). Figure 3.1. shows camera lucida drawings of typical relay neurons and their approximate locations in the ventral partition. In both instances, an ellipsoid cell body and multiple tufted dendritic trees typify the neurons, as in previous observations in rats and other mammals (Clerici et al. 1990; Morest 1964). Figure 3.1.B also shows that, from contiguous locations, the neurons extended their dendritic trees inwardly towards deeper locations within the MGBv. Note that the axon (open arrows), emanating from the soma in Figure 3.1.B, followed a path in the dorsomedial direction without exhibiting collaterals. We observed a few dendritic spines in several neurons. Occasionally, a lesion in the soma or proximal dendrite pointed to the presumed location of the electrode during whole cell recording.

Fig. 3.1. Camera lucida drawings of representative MGBv neurons and their positions in the ventral partition of the medial geniculate body.
A: Note the characteristic tufted dendritic trees. B: In neurons at the border, most dendrites were oriented towards the center of the nucleus. The axon of the neuron (arrows) left the MGBv dorsomedially in the direction of the primary auditory cortex.
Passive membrane properties and membrane rectifying properties

The average resting membrane potential ($V_r$) was $-68.6 \pm 4$ mV (mean ± S.D., $n = 74$). In order to minimize contributions of active properties, input resistance ($R_i$) was calculated from the voltage responses ($<10$ mV) near the end of 1 s hyperpolarizing pulses. The input resistance ranged from 79 to 525 MΩ and averaged $226 \pm 100$ MΩ in 74 neurons. We estimated the membrane time constant ($\tau_m$) from a least squares fit of one or two exponentials to the above voltage responses. The averaged $\tau_m$ was $40 \pm 17.6$ ms ($n = 71$, single exponential fit).

Hyperpolarization or depolarization of MGBv neurons resulted in pronounced membrane rectification. Figure 3.2.A shows the voltage responses to hyperpolarizing current pulses (1 s duration) in a neuron at a $V_r = -70$ mV. Such responses in most neurons did not have a depolarizing sag, characteristic of an H-current ($I_H$, McCormick and Pape 1990b). However, despite a quasilinear relation between $V_r$ and $-85$ mV in the corresponding current-voltage (I-V) plot (Figure 3.2.B), an inward (depolarizing) rectification occurred at more hyperpolarized membrane potentials. Extracellular application of Ba$^{2+}$ (100-200 μM, $n = 7$), a blocker of the inward rectifier ($I_{KIR}$, Travagli and Gillis 1994), markedly decreased membrane conductance in the range between rest and $-130$ mV (Figure 3.2.A,B). A depolarization (1-15 mV) usually accompanied the decrease in conductance, as previously attributed to concomitant blockade of voltage-independent $K^+$-currents by Ba$^{2+}$ (Jahnsen and Llinás 1984b; Sutor and Hablitz 1993). The voltage dependence, activation range and sensitivity to blockade by Ba$^{2+}$ are consistent with the hypothesis that a fast activating $K^+$-current such as $I_{KIR}$ was a major contributor to the rectification, as described previously (Constanti and Galvan 1983; Scroggs et al. 1994).
Fig. 3.2. Inward rectification and current-voltage (I/V) relationship in MGBv neurons.
A: responses to injection of hyperpolarizing current pulses under control and Ba\(^{2+}\) (100 µM) containing conditions from the resting membrane potential (-70 mV). Application of Ba\(^{2+}\) increased input resistance below rest, blocked the fast inward rectification below -85 mV and revealed sags in the voltage responses, that were blocked by co-application of Ba\(^{2+}\) and Cs\(^{+}\) (1.5 mM). Application of Cs\(^{+}\) depolarized the neuron by -10 mV and hyperpolarizing DC was injected to hold it at -70 mV. B: plot of potential at the end of the pulse versus the injected current (from A). An increase in slope resistance is evident during application of Ba\(^{2+}\) (100 µM). A slow inward rectification, unmasked by Ba\(^{2+}\)-application, was blocked during co-application of Ba\(^{2+}\) and Cs\(^{+}\) (1.5 mM), resulting in a linear I/V relationship. C: subthreshold voltage responses evoked by 40 pA depolarizing current steps from rest (-63 mV) show a pronounced ramplike voltage response in the control condition (1), that is reduced to different degrees due to various conditions (2-5). D: plot of potential at the end of the pulse versus injected current (I/V relationship) in the subthreshold range of the neuron in C. Application of TTX (300 nM) decreased the input resistance (2), compared to control (1). Additional replacement of Ca\(^{2+}\) with Mg\(^{2+}\) in the perfusate (3, “0 Ca\(^{2+}\”) further reduced the slope of the I-V relationship. Co-application of 4-AP (100 µM) and TTX increased the input resistance (4) above the control values, which was reduced by replacement of Ca\(^{2+}\) with Mg\(^{2+}\) (5).
In MGBv neurons, furthermore, the application of Ba\(^{2+}\) unmasked a sag in the hyperpolarizing voltage responses, suggestive of an \(I_H\) (Figure 3.2.A, middle). This slower, inward rectification appeared at potentials of less than -90 mV. The voltage sags were sensitive to blockade by Cs\(^+\) (1.5 mM, \(n = 3\)) when co-applied with Ba\(^{2+}\), resulting in a linear I-V relationship over a wide range of potentials (Figure 3.2.B). The hyperpolarized potential (usually below -77 mV) required for activation of the sags and the susceptibility to blockade by Cs\(^+\), but not Ba\(^{2+}\), are consistent with an involvement of a hyperpolarization-activated inward current, such as \(I_H\). This type of rectification contributes to the resting potentials of LGNd neurons (McCormick and Pape 1990a,b). In MGBv neurons, application of Cs\(^+\) depolarized the neurons usually by several millivolts. Therefore, we did not find evidence in these experiments for an involvement of \(I_H\) in the maintenance of \(V_r\) (cf. Hu 1995).

An inward rectification, which was markedly time dependent, was also present at depolarized potentials, as deduced from the effects of selective ion channel blockers on the voltage responses to current pulses. It appeared as a gradual depolarization, forming a voltage ramp as observed in different thalamic neurons (Jahnsen and Llinás 1984a). As shown in Figure 3.2.C, application of tetrodotoxin (TTX, 300 nM) reduced the ramp slope and amplitude of the voltage responses to depolarizing current pulses in a subthreshold range. The present results are consistent with an activation of a voltage-dependent, persistent Na\(^+\)-conductance which would contribute to the depolarizing voltage responses (Jahnsen and Llinás 1984b). We also found evidence for Ca\(^{2+}\)- and K\(^+\)-involvement in this rectification. During application of TTX, the extracellular replacement of Ca\(^{2+}\) with Mg\(^{2+}\) (\(n = 2\)), or application of Cd\(^{2+}\) (50 \(\mu\)M, \(n = 2\)), or Ni\(^{2+}\) (600 \(\mu\)M, \(n = 1\)) produced a reduction in the voltage responses, nearly eliminating the rectification (Figures 3.2.C,D and 3.10.B). These observations are similar to descriptions of a Co\(^{2+}\)-blockade of a Ca\(^{2+}\)-conductance that
contributes to the rectifying properties of cortical neurons (Hotson et al. 1979). An additional contribution of a $K^+$ conductance also seems likely in MGBv neurons; the co-application of a selective $K^+$-channel blocker, 4-aminopyridine (4-AP; 100 µM) with TTX exaggerated the rectification (Figure 3.2.C, D). In summary, a variety of voltage-dependent $Na^+$, $Ca^{2+}$, and $K^+$-conductances interacting with the passive properties produce the rectification in the range depolarized from rest. Functionally, this property presumably provides a steady and adjustable, net inward current that modulates the delay to firing and plateau potentials (see below and Figure 3.4.D).

Action potential firing patterns

The threshold for action potentials was $-51.6 \pm 3$ mV. The neurons exhibited an ability to fire action potentials in various patterns, depending on the membrane voltage or current input. Figure 3.3. displays the different firing modes elicited by depolarizing current pulse injections in two neurons from several membrane potentials. Depolarization from resting or more positive potentials induced tonic firing (Figure 3.3.A) whereas similar current injections from more negative potentials induced phasic firing of either action potential bursts (Figure 3.3.B) or single fast spikes, riding on low threshold $Ca^{2+}$ spikes (LTSs; Figure 3.3.C). On strong hyperpolarization, high threshold $Ca^{2+}$ spikes (HTSs) followed the phasic response (Figure 3.3.B,C; see below).
Fig. 3.3. Firing modes of MGBv neurons at different membrane potentials.
A: Tonic firing mode. The current pulse (+100 pA) elicited a ramplike delay to firing from rest (-70 mV). A short-latency train of action potentials was evoked on DC-depolarization to -62 mV. B: Burst firing mode. In the same neuron, but hyperpolarized with DC injection to -80 mV, the current pulse evoked a burst of action potentials on the crest of a low threshold spike (LTS burst firing). Note the broader high threshold spike (HTS), when the LTS was evoked from a lower membrane potential (-86 mV). C: In another neuron, only one action potential was elicited on top of the LTS. The number of action potentials did not vary with the initial membrane voltage or current pulse amplitude, but again a HTS could be evoked on top of the LTS from a lower membrane voltage (-82 mV).
**Tonic firing modes**

At $V_r = -70$ mV, the neuron of Figure 3.3.A exhibited a long delay to firing, characteristic of most MGBv neurons at rest depolarized to near threshold by current pulses. A slowly rising ramplike increase in the voltage response, seen in the subthreshold voltage traces (Figure 3.2.C, control), preceded the first action potential (Figures 3.3.A and 3.4.A). The application of 4-AP (100 μM, n = 3), or depolarization by DC-injection, markedly reduced the duration of the ramplike voltage change and promoted firing of short latency (Figure 3.4.D), whereas application of tetraethylammonium (TEA, 2 mM, n = 3) did not significantly influence the delay to firing (Figure 3.4.D). This provides evidence for the presence of a slowly inactivating, A-type $K^+$-conductance as described for lateral geniculate and striatal neurons (McCormick 1991; Nisenbaum et al. 1994). In MGBv neurons, therefore, the latency to firing appears to be regulated by the membrane potential prior to excitation as well as by an interplay of a TTX-sensitive, persistent Na$^+$-conductance, a Ca$^{2+}$-dependent rectifier and a slowly inactivating, A-type $K^+$-conductance.

The MGBv neurons responded at short latency with trains of action potentials, with little or no spike frequency adaptation on depolarization with large amplitude current pulses or DC-injection from depolarized membrane potentials (-62 mV in Figure 3.3.A; cf. Figure 3.4.B.D). The firing frequency was roughly proportional to the amplitude of injected current (Figure 3.5.C). Prominent afterhyperpolarizations (slow AHPs) did not appear after trains of action potentials, but a fast afterhyperpolarization (fAHP) characterized each action potential in a spike train (Figure 3.5.A,B). The replacement of extracellular Ca$^{2+}$ with Mg$^{2+}$ (n = 3) or application of Cd$^{2+}$ (50 μM, n = 4), a blocker of the high threshold Ca$^{2+}$ current in thalamic neurons (Hernandez-Cruz and Pape 1989), reversibly blocked the fAHPs and reduced the interspike intervals. This increased the firing frequency in these neurons (Figure 3.5.).
Fig. 3.4. Mechanisms producing the delay to firing.

A (left): Injection of current pulses of two amplitudes (70 and 80 pA) from the resting potential (-71 mV) elicited firing after a pronounced ramplike delay (arrow). A (right): Application of 4-AP (100 μM) dramatically reduced this delay (arrow; 50 and 70 pA current pulses). Note the increase in input resistance, blockade of spike repolarizing conductances and spike doublets. 4-AP-application depolarized the neuron by ~3 mV and hyperpolarizing DC injection was used to return the potential to -71 mV. B: DC-depolarization by about 10 mV reduced the delay to firing in the same neuron (50 and 70 pA current pulses). C: A plot of latency to the first spike (measured from pulse onset) versus injected current for the conditions in A and B shows the reduction of first spike latency with depolarization (either by pulse or DC-injection) or due to 4-AP application. D: The delay to firing in another neuron (current pulses, 90 and 110 pA; rest -74 mV) was not affected by 2 mM TEA (compare traces marked by asterisks). Spike repolarizing conductances were blocked as well, resulting in spike doublets and plateau potentials.
Fig. 3.5. Effects of extracellular replacement of Ca$^{2+}$ with Mg$^{2+}$ on tonic firing frequency.
A: Fast AHPs of single spikes during tonic firing, evoked by a 100 pA rectangular current pulse, are blocked by replacement of Ca$^{2+}$ with Mg$^{2+}$ in the perfusate ("0 Ca$^{2+}$", compare traces marked by arrow and asterisks in A and B). This reversibly reduced the interspike interval and increased the firing frequency, shown in plot of C. In all conditions, note the monotonic increase in firing frequency with increased current amplitude.
Fig. 3.6. Effects of high threshold Ca\textsuperscript{2+} channel blockade on the repolarization and duration of action potentials.

A: Injection of a 190 pA rectangular current pulse into a neuron elicits tonic firing. Application of 50 µM Cd\textsuperscript{2+} resulted in a train of action potentials that inactivated progressively, reaching a plateau potential at the end of the pulse. The action potentials and the plateau potential were abolished by application of 300 nM TTX. B: Action potential train elicited by injection of a 300 pA current pulse. Note the difference between the first and subsequent action potentials in control. During the pulse, the duration of consecutive action potentials increased; this increase was partially blocked by application of 50 µM Cd\textsuperscript{2+}. The plot compares the action potential width under both conditions. The inset shows superpositions of the first and third action potentials under control and Cd\textsuperscript{2+}-conditions.
During applications of Cd$^{2+}$, larger depolarizing pulses elicited a train of action potentials of rapidly decrementing amplitude which led to a plateau depolarization. Application of TTX completely blocked the action potentials and plateau depolarization (Figure 3.6.A). The alteration in firing pattern due to Ca$^{2+}$ removal from the extracellular media, or Cd$^{2+}$ application and total blockade by TTX imply that a Ca$^{2+}$-activated K$^{+}$ conductance contributes to the repolarizing phase of action potentials in MGBv neurons.

The first action potential elicited in a train usually was much shorter in duration than subsequent spikes. The blockade of high threshold Ca$^{2+}$-channels by Cd$^{2+}$ application decreased the duration of the falling phase of consecutive spikes in an evoked train (Figure 3.6.B). The effects become evident on superposition of the first and third spikes in the train under control and Cd$^{2+}$ conditions. Whereas the first action potentials are almost congruent, Cd$^{2+}$ application significantly reduced the amplitude and width of the third spike (Figure 3.6.B, plot inset). Therefore, high threshold Ca$^{2+}$-conductances have an important role in regulating the firing frequency, width and repolarization of action potentials in tonic firing patterns.

*Low-threshold spike (LTS) firing modes: LTS burst firing*

We observed action potentials on top of a large, slowly rising hump of depolarization following injections of hyperpolarizing current pulses (Figure 3.2.A) or during depolarizing pulses injected from hyperpolarized membrane potentials (Figure 3.3.B,C). These potentials appeared identical to LTSs. An activation of a transient T-type Ca$^{2+}$-conductance probably produces the LTS, as described in various neurons of the mammalian thalamus (Crunelli et al. 1989; Jahnsen and Llinás 1984a,b). We confirmed the following aspects of this hypothesis:

1. the LTS persisted after application of TTX (300 nM, n = 11; Figure 3.7.A), whereas omission of Ca$^{2+}$ from the external perfusing solution (n = 5; Figure 3.7.B) or application of
Ni²⁺ (600 µM, n = 5; not shown) eliminated the LTS; (2) the amplitude of the LTS depended on the amplitude of the preceding hyperpolarization. However, the duration of the LTS did not depend on the membrane potential or the current pulse magnitude (Figure 3.7.A); (3) by plotting the maximal rate of rise of the LTS against the potential at the end of the preceding hyperpolarizing pulse (Figure 3.7.A), we observed a sigmoidal relationship reflecting the voltage-dependence of LTS inactivation (Figure 3.7.C). This plot shows that the LTS may not be evoked from above ~-73 mV where the T-conductance, likely, is completely inactivated. On further hyperpolarization, the conductance progressively deinactivated, reaching a maximum near -95 mV (Figure 3.7.C). The LTSs produced single, double, or bursts of action potentials at frequencies up to 160 Hz, when elicited from below -75 mV. We recorded burst responses typical for thalamic neurons during deep sleep (3-7 spikes; cf. Fourment and Hirsch 1979; Hirsch et al. 1983; McCarley et al. 1983) in about 50% of MGBv neurons (Figure 3.3.B).

Low-threshold spike (LTS) firing modes: LTS single (or double) spike firing

In the other neurons (Figure 3.3.C), only one or two action potentials appeared on a LTS hump. In such cases, injections of hyperpolarizing DC or current pulses over a wide range of values did not change the number of action potentials or burst firing patterns. This was not due to insufficient depolarization because, starting from lower potentials, we could evoke high threshold Ca²⁺ spikes, but not more action potentials (see below). We also observed that application of 4-AP (100 µM, n = 4), but not of TEA (2 mM, n = 3), led to an increase in the number of action potentials on the LTS in these neurons (Figure 3.10.A).
Fig. 3.7. The low threshold spike (LTS).
A: Hyperpolarizing current pulses from rest (-68 mV) elicited large rebound spikes at the pulse offset, after blockade of voltage-dependent Na\(^+\) conductances by TTX (300 nM). B: Replacement of extracellular Ca\(^{2+}\) with Mg\(^{2+}\) ("0 Ca\(^{2+}\)") blocked the LTS. C: Voltage dependence of LTS inactivation, characterized by plotting the maximal rate of rise of the rebound spikes in A versus the potential at the end of the hyperpolarizing current pulses. Note that the LTS could only be elicited from potentials below -80 mV and was completely inactivated above -74 mV.
The high-threshold Ca\(^{2+}\) spike (HTS)

During hyperpolarization that removed LTS inactivation, and injection of high-amplitude current pulses into neurons capable of either single or multiple firing on the LTS, we usually observed a spike of long duration (25 to 70 ms) following single or multiple action potentials. This broad spike was evoked from potentials that were 10-15 mV higher than the threshold for action potentials and consisted of one or more all-or-nothing components (Figures 3.3.B,C; 3.8. and 3.10.). We refer to this type of spike as a high threshold spike or HTS. Under control conditions, most neurons exhibited the HTS, except for nine neurons that were capable only of a single action potential on the LTS. The HTS depended on Ca\(^{2+}\) influx because (1) HTSs could not be evoked on removal of external Ca\(^{2+}\) (n = 5) and (2) application of Cd\(^{2+}\) (50 \(\mu\)M, n = 3) selectively blocked the HTS (cf. Hernandez-Cruz and Pape 1989), leaving the LTS and the first action potential relatively unaffected (Figure 3.8.).

Under conditions of TTX-blockade, depolarizing current steps from depolarized membrane potentials evoked a series of HTSs after a delay (Figure 3.9.A). A Ca\(^{2+}\)-dependent inwardly rectifying current presumably contributed to this delay because the removal of extracellular Ca\(^{2+}\) or application of Cd\(^{2+}\) reversibly blocked the HTSs as well as the rectification (Figure 3.9.B).

Under control conditions, we observed HTSs as part of, or in association with, the LTS bursts (Figure 3.3.B,C) and during tonic firing of action potentials following blockade of K\(^+\) conductances with 4-AP (100 \(\mu\)M, n = 4; Figure 3.4.A) or TEA (2 mM, n = 3; Figure 3.4.D). The firing threshold for the HTS (-41.0 ± 2.7 mV, n = 9) was significantly higher than for action potentials (-51.6 ± 3.0 mV, n = 12).
Fig. 3.8. The high threshold spike (HTS).
A: Using a protocol similar to that in Figure 7 (shows voltage during and after hyperpolarizing current pulses, as indicated below), rebound LTSs and one action potential were elicited after sufficient hyperpolarization from -76 mV (first column) to -86 mV (middle column, LTS restricted burst). Further hyperpolarization to -94 mV elicited a HTS (arrow) at a higher threshold, on top of the LTS. Application of Cd$^{2+}$ (50 μM) selectively and reversibly blocked this HTS, without greatly affecting the action potential and LTS (right column, middle trace).
Fig. 3.9. Blockade of tonic HTSs by Cd\(^{2+}\) application.
A: Tonic HTS firing (arrows) was evoked by depolarizing current pulses (increasing to the right: 40, 60 and 80 pA) from a depolarized potential (-56 mV) during application of TTX (300 nM). They were abolished by additional Cd\(^{2+}\) application (50 μM), as shown in the middle row of traces. Note the ramp-like delay to firing in traces of the middle vertical column. B: Application of Cd\(^{2+}\) (50 μM) reversibly blocked an inward rectification, as shown in the I-V plot for the neuron in A (measured at the end of the voltage deflections, subthreshold to firing).
Fig. 3.10. Effects of 4-AP on LTS bursting and the HTS.
A: A MBGv neuron, exhibiting limited LTS bursting (left trace), discharged a spike burst, including an HTS at the offset of a hyperpolarizing current pulse, after application of 4-AP (100 μM, right trace). B: After application of TTX (300 nM), a HTS could not be elicited on top of a LTS (left traces), indicating an involvement of Na⁺-dependent mechanisms in the coupling of these spike types. However, after application of 4-AP (100 μM, right traces) and hyperpolarization to -103 mV, the HTS coupled to the LTS. Note the prolonged falling phase of the HTS due to blockade of repolarizing currents by 4-AP (top trace).
After TTX-application, we could not evoke the HTS on top of a LTS (Figure 3.10.B), indicating that voltage-dependent Na$^+$ conductances may have a role in the initiation of the HTSs and in linking the HTS to the LTS. Blockade of K$^+$ conductances by 4-AP (100 μM) promoted the linking of LTS to HTS by an increase of the rising phase and amplitude of the LTS. These K$^+$ conductances also modulated the time course of the HTS because 4-AP increased the HTS duration during the phasic mode (Figure 3.10.B), similar to its effects on action potentials during the tonic mode of firing (Figure 3.4.A). The increased duration of resulting spikes varied widely, possibly due to a partly dendritic localization of high threshold Ca$^{2+}$ channels (Hell et al. 1993; Jahnsen and Llinás 1984b; Westenbroek et al. 1992).

However, blockade of K$^+$-conductances produced similar spikes in a preparation of acutely dissociated lateral geniculate neurons (cf. Figure 13 in Hernandez-Cruz and Pape 1989).

**LTS to tonic firing mode transitions**

We observed combined firing modes of MGBv neurons at hyperpolarized potentials, depending on the amplitude of the injected current pulse. From -86 mV, for example, a depolarizing pulse elicited a single action potential on an LTS (Figure 3.11.A). An increase in the pulse amplitude resulted in a single action potential on top of the initial LTS, followed by a depolarizing ramp (Figure 3.11.B). A further increase in the pulse amplitude produced an LTS, an action potential, and tonic firing on the ramp (Figure 3.11.C).
Fig. 3.11. Transitions of firing modes in MGBv.
A: Injection of a 110 pA depolarizing current pulse from a DC- hyperpolarized potential (-86 mV) evoked a single action potential on the LTS (see text) in a neuron. B: Increasing the amplitude of the current pulse to 170 pA elicited a ramp (arrow) after the LTS and action potential. C: A current pulse of 190 pA elicited a single action potential on the LTS, followed by a voltage ramp, that reached threshold for the tonic firing of two action potentials.

Interneurons in MGBv

We also recorded from two neurons, which exhibited the typical features of thalamic interneurons like fast action potentials and high firing frequency, high input resistance and no LTS burst responses (cf. Pape and McCormick 1995, Pape et al. 1994). Further studies on interneurons were not possible, due to their scarcity in rat MGBv (cf. Winer and Larue 1988).
3.4. DISCUSSION

Signal transformation in MGBv relay neurons

The stimulus-response patterns in the medial geniculate body (MGB) as described in numerous studies imply important contributions of the thalamic membrane properties to auditory perception. We have observed a rich complement of voltage-dependent conductances in MGBv neurons that can profoundly influence the conversion of afferent inputs to a variety of spikes and thalamocortical firing patterns. These have importance in the generation of output signals and during resting conditions or “spontaneous” activity. The various rectifications in the relationship of the current stimulus to the voltage response reflect these properties in a subthreshold range of membrane potentials. According to previous investigations, such conductances are subject to modulation by intra- and extracellular substances and messengers (McCormick 1992b). This raises our expectations that the phasic and tonic firing modes, which format signal transformation in thalamocortical neurons, are subject to fine tuning in the primary thalamic auditory relay neurons, depending on the behavioral state and other factors.

The electrophysiological parameters of MGBv neurons that correspond to a particular behavioral state would determine the output pattern of firing from the auditory thalamus. Our results suggest that the coding for sound differs dramatically during alertness and sleep when the MGBv neurons would perform different types of signal transformation. The precise codes for sound parameters in MGBv response patterns remain uncertain, but certainly the interactions of intrinsic properties differs in various behavioral states. Hence, the functional organization of the auditory thalamocortical system derived from previous recordings, including the effects of sleep and anesthesia, requires assessment and re-investigation. The properties of MGBv neurons that we observed in the slice preparations are probably not a
specialization of the immature brain, because auditory transmission, as evident in the behavior of young rats after postnatal day 13, is similar to that of adults (Ehret 1983; Rubel 1978).

**Physiological properties**

Despite some obvious differences in function of auditory and visual thalamus, most intrinsic properties of MGBv neurons were similar to those of LGNd neurons measured with whole-cell patch clamp techniques (cf. Ramoa and McCormick 1994). Depolarization from rest produced tonic responses and hyperpolarization below -75 mV by DC-injection was sufficient to initiate the burst mode. These values correspond to the membrane potentials in other thalamic neurons during deep sleep (Hirsch et al. 1983; McCarley et al. 1983). Like other thalamic relay neurons, MGBv neurons responded to the same excitatory current input with signal patterns that were different in the burst and tonic firing modes (Jahnsen and Llinás, 1984a,b). However, specific alerting sounds should lead to wakefulness and stimulus induced conversion of firing modes in MGBv.

**Rectification at hyperpolarized potentials**

We observed an inward rectification in a range hyperpolarized from the observed V_r of the MGBv neuron. A Ba^{2+}-sensitive component, likely an inward rectifier such as I_{KIR} (Travagl and Gillis 1994) which activated at potentials near the K^+ equilibrium potential (-85 mV), dominated this nonlinearity. An I_{KIR} at such hyperpolarized membrane potentials would not normally contribute to signal transformation, but modulation due to transmitters or other substances could affect the activation range of I_{KIR} (see Grigg et al. 1996; Williams et al. 1988). Also, changes in the internal [Mg^{2+}] (Matsuda et al. 1987; Vandenberg 1987), or intrinsic
polyamines (Ficker et al. 1994; Lopatin et al. 1994) may have interfered with the regulation of the observed rectification or blocked it at depolarized potentials.

Strong hyperpolarization by current pulse injection into MGBv neurons produced a Cs$^+$ sensitive voltage sag, evident only after a Ba$^{2+}$ blockade of an I$_{KIR}$-like rectifying component. The sag was largest at potentials more negative than the K$^+$ equilibrium potential and may represent an inward rectifier such as I$_H$ (McCormick and Pape 1990b). This Ba$^{2+}$ insensitive component may have resulted from a negative shift in the activation range of an I$_H$ in the whole-cell recording from neurons in slices. For example, recordings using sharp microelectrodes in tissue explants have provided evidence for activation of I$_H$ in MGBv neurons at potentials hyperpolarized to ~ -70 mV (Hu 1995). Consistent with this possibility, neuromodulators like adenosine, histamine, noradrenaline and serotonin can shift the activation range of I$_H$ (McCormick and Pape 1990a; McCormick and Williamson 1991; Pape 1992). This would allow interactions of I$_H$ with currents generating IPSPs, or greater contributions of the I$_H$ tail current to burst responses in MGBv neurons. Hence, a change in the I$_H$ activation range also could contribute to an alerting effect of a stimulus during deep sleep (see below).

Excitation of MGBv neurons

Depolarization of the resting MGBv neuron due to current pulse injection often produced a ramp depolarization that gradually increased and led to delayed discharge. The delay to the first action potential depended on the membrane potential, the current pulse amplitude, and hence, the net depolarization. Depending on the initial $V_r$, the same depolarizing stimulus could evoke a train of action potentials with vastly different delays. Unless $V_r$ were constant during different states of attention, an unlikely condition for thalamocortical neurons (Steriade et al. 1990), a response latency to an auditory stimulus could not encode identifying features of sound.
The ramp-induced delay in firing, however, confers a voltage-controlled low pass filter property to MGBv neurons. The corner frequency of the filter would vary widely in individual neurons, depending on the membrane potential. Consequently, a simple temporal relationship of sound parameters to spike firing, as observed with extracellular recording, may lead to erroneous conclusions about auditory coding in thalamocortical systems. The filter property, for example, that has been interpreted in terms of a selectivity of MGB neurons for amplitude modulation frequency of sound (cf. Preuss and Mueller-Preuss 1990) would vary, therefore, with the behavioral state.

The injection of current pulses of sufficient amplitude into MGBv neurons evoked brisk tonic firing of fairly constant, short latency (cf. Figures 3.3.A, 3.4. and 3.5.). The synchronous stimulation of many fibers in the brachium of the inferior colliculus that produces large excitatory postsynaptic potentials (EPSPs), leads to relatively constant latencies for single action potentials in the MGBv (Hu et al. 1994). Although typical of phasic firing in ~50% of MGBv neurons (see below), single spike responses to impulse inputs may reflect either tonic or burst modes. We also observed that current pulse injections into MGBv neurons evoke singlet action potentials on the depolarizing ramp (Figures 3.3.A and 3.4.A).

An important, perhaps clinically relevant consequence of the depolarizing ramp and spike delay is that the latencies of cortical evoked potentials (EPs) to sound stimuli should change over a wide range, as a function of the membrane potentials in MGBv neurons. In contrast to the auditory brainstem responses, the peak latencies of auditory EPs are, indeed, highly variable and depend on the state of alertness (Kraus and McGee 1991).
**Ionic mechanisms of the depolarizing ramp**

We conclude from our pharmacological studies that the interactions of voltage-dependent, persistent Na\(^+\), K\(^+\), and Ca\(^{2+}\) conductances produced the depolarizing ramp (Figure 3.2.C,D). The fast activating K\(^+\) conductance I\(_{\text{As}}\), sensitive to 4-AP blockade, appears to counteract the initial depolarization. Presumably, a subsequent decrease in this conductance due to its slow inactivation results in a depolarizing slope (Nisenbaum et al. 1994). The activation of a persistent Na\(^+\) conductance at ~ -50 mV (Stafstrom et al. 1985) likely produces a regenerative, depolarizing contribution to the ramp. Further amplification of the ramp depolarization by the activation of a Ca\(^{2+}\) conductance was evident during TTX blockade of the persistent Na\(^+\) conductance (Figure 3.2.C,D).

**Shapes of action potentials**

Despite stable, quite negative resting potentials, we observed a wide variety of action potential shapes, often with long durations (>5 ms). The observation of prolonged action potentials in this study seemed attributable largely to the slower kinetics of the membrane currents in the slices at room temperature (cf. Thompson et al. 1985). However, action potential and HTS firing patterns, similar to this study, have been observed previously with intracellular recordings in *in vitro* slices of adult animals at physiological temperatures (Jahnsen and Llinás, 1984a; see below). The lower temperature probably was instrumental for observations of the complex nature of MGBv spikes. The age of the animals presumably plays no major role, as action potential parameters and firing patterns exhibited no major changes from day 13 up to day 50 in a developmental study of rat ventrobasal neurons (Velazquez and Carlen 1996).
The initial action potentials in a tonic pattern were always much shorter in duration than subsequent spikes, inferring that time-varying active properties may explain the different action potential shapes. Similar observations have been reported previously in thalamocortical neurons (cf. Figure 10 Jahnsen and Llinás, 1984a). It is possible that electrode tip positions were often far from the axon hillock. Similar prolonged spike durations have been recorded from identified dendrites (Callaway and Ross 1995; Stuart and Sakmann 1994). However, we were unable to ascertain a dendritic or somatic location of the recordings since, in most cases, there was no indicator (e.g., dendritic swelling, Kim and Connors 1993) for the recording site on neurobiotin filled neurons. The likelihood of dendritic recordings may be greater than assumed previously when using the patch clamp technique, given the appreciable extensions of dendritic tufts in MGBv neurons (Figure 3.1.). If the recordings were somatic for example, an initiation of HTSs in the proximal dendritic tufts, triggered by action potentials, would produce complex potentials of long duration. This scenario is consistent with the observed differences between the initial and subsequent spikes in a tonic train, assuming that the initial action potential ‘prepares’ the dendritic membrane for HTS firing.

MGBv neurons exhibited evidence for contributions of a Ca\(^{2+}\)-dependent K\(^{+}\)-conductance to spike repolarization and to the fast AHP (Figures 3.4.D, 3.5. and 3.6.). Such Ca\(^{2+}\)-dependent currents have been observed previously in other thalamic neurons (cf. Budde et al. 1992). During application of TEA or 4-AP, in low concentrations, current pulses elicited HTSs or prolonged plateau depolarizations following action potentials (Figure 4), which made investigations of effects on repolarization and the fast AHP difficult. This indicates an important role for K\(^{+}\)-conductances in shaping the action potentials and controlling the contributions of high-threshold Ca\(^{2+}\)-conductances (including HTSs) to complex firing patterns (cf. Turrigiano et al. 1995).
High threshold spikes (HTSs)

An important finding of our studies on MGBv neurons is an ability to generate HTSs of long duration (>50 ms), activating above ~45 mV (cf. Hernandez-Cruz and Pape 1989). These spikes are similar to those observed during dendritic recordings in thalamic and cortical neurons (Jahnsen and Llinás 1984b; Kim and Connors 1993; Wong et al. 1979). Removal of Ca\(^{2+}\) from the extracellular medium abolished both LTSs and HTSs. We selectively blocked the HTSs with Cd\(^{2+}\) in low micromolar concentrations (Figure 3.8.). This implicated a dependence on high-threshold Ca\(^{2+}\) currents (Fox et al. 1987; Hernandez-Cruz and Pape 1989). We did not observe isolated HTSs during tonic firing of Na\(^{+}\)-action potentials. However, repetitive (tonic) firing of HTSs occurred following pharmacological suppression of Na\(^{+}\)-action potentials. Also, after imposed hyperpolarization, MGBv neurons fired an HTS appended to the LTS, as observed previously in other thalamocortical neurons (cf. Figure 9 Jahnsen and Llinás, 1984a).

A detailed investigation of the interactions between the LTS, HTS and Na\(^{+}\)-action potentials may require simultaneous recording of these responses in soma and dendrites (Stuart and Sakmann 1994), as well as neuronal modelling, since the Ca\(^{2+}\) and Na\(^{+}\) currents producing these voltage transients probably have their maximal densities at different locations in the neuron. The Na\(^{+}\) current density probably is maximal in the initial axon segment whereas the high threshold Ca\(^{2+}\) currents may flow predominantly at dendritic locations. We propose, therefore, that MGBv neurons elicit Na\(^{+}\)-mediated action potentials with various Ca\(^{2+}\)-components or HTSs, depending on spatial, temporal and voltage-dependent integration.

Activation of high-threshold Ca\(^{2+}\)-channels would markedly change dendritic responsiveness (Amitai et al. 1993). The Ca\(^{2+}\) influx can trigger release from intracellular Ca\(^{2+}\)-stores (Dupont et al. 1991), change synaptic plasticity (Malenka et al. 1988) and modulate gene expression (Murphy et al. 1991, Sheng et al. 1990). The differential spatial and temporal regulation of
action potentials and Ca\textsuperscript{2+} influx into the dendrites is of major importance for the generation of firing patterns and nonlinear synaptic integration (Constantine-Paton et al. 1990; Jaffe et al. 1992; Koch and Poggio 1992; Segev and Rall 1988). In sensory nuclei of the dorsal thalamus, neurons commonly have dendrodendritic synapses (Jones 1985), implying a need for calcium-triggered presynaptic transmitter release. The HTSs are well suited for this role.

*Excitation of hyperpolarized MGBv neurons*

MGBv neurons display a phasic (burst) mode of firing when depolarized from membrane potentials negative to \~\(-75\) mV. This characterizes the firing behavior of projection neurons of the dorsal thalamus during deep sleep (Fourment and Hirsch 1979, Steriade et al. 1990). In MGBv neurons, the phasic mode does not likely represent a special facility for temporal coding of sound. Roughly one half of the neurons in this study exhibited firing of only one or two action potentials on top of the LTS. This is in agreement with previous findings using different methodology in MGBv (cf. Figure 8A Hu 1995) and other thalamocortical neurons (cf. Figures 2,4,6,7 Jahnsen and Llinás, 1984a). In contrast, other MGBv neurons showed LTS bursts, consisting of several action potentials in response to current pulse injections. For this reason, the term “phasic” seems preferable to “burst” in descriptions of this firing mode in MGBv neurons. The application of 4-AP to MGBv neurons converts single-spike, phasic responses to a burst of two or more spikes by blocking a K\textsuperscript{+}-conductance, presumably of an A-type (Budde et al. 1992). The 4-AP blockade also increased the probability of HTS firing following the burst. This indicates that the 4-AP afflicted K\textsuperscript{+}-conductance served as a voltage-dependent regulator of action potential and HTS firing in the phasic mode. The HTS always occurred after single spikes or spike bursts, provided a strong hyperpolarization enabled firing of a large LTS. Thus, MGBv neurons at hyperpolarized potentials during deep sleep may respond to sensory input with
depolarizations, extended by the HTS, producing a pronounced Ca$^{2+}$ inflow. Depending on the features of the depolarizing input (amplitude, duration and location on the denritic tree), MGBv neurons can convert from the phasic mode to tonic firing through activation of the depolarizing ramp (cf. Figure 3.11.). The 4-AP sensitive K$^+$-conductance that regulates the phasic response and participates in the production of the ramp, may have a crucial role in the transition between firing modes during states of sleep and alertness. Thus, neurons in the MGB may have a special ability to use single auditory stimuli to convert from bursting behavior during deep sleep to the tonic mode, for auditory perception during wakefulness.
4. Modulation of bursts and high-threshold calcium spikes

4.1. INTRODUCTION

Neurons of the auditory thalamus, under *in vivo* and *in vitro* conditions, exhibit tonic and burst firing patterns of action potentials (Fourment and Hirsch 1979, Chapter 3). Whereas tonically firing neurons faithfully transmit auditory and other signals to the cortex, phasically bursting neurons change the quality of this information transfer (Creutzfeldt et al. 1980, McCormick and Feeser 1990). The modulation and extent of bursting in thalamic neurons are important in the generation of synchronized oscillations during sleep and pathological states (Huguenard and Prince 1994, Steriade et al. 1993).

Previous investigations in dorsal thalamic nuclei have identified the burst as fast transient potentials triggered by a low threshold Ca\(^{2+}\)-spike (LTS). Investigators often assume that the fast potentials are entirely Na\(^{+}\)-dependent because of their blockade by tetrodotoxin (TTX, cf. Jahnsen and Llinas 1984b, Steriade et al. 1993). In MGBv neurons, however, a high threshold Ca\(^{2+}\)-spike (HTS) often complements the firing on top of the LTS, resulting in a range of burst firing patterns (Chapter 3) as observed in various thalamic neurons under *in vivo* and *in vitro* conditions (Jahnsen and Llinás 1984b, Lu et al. 1992). The HTS occurs towards the end of the burst in MGBv neurons, much like in CA3 hippocampal pyramidal neurons (Wong and Prince 1978). At low concentrations the Ca\(^{2+}\)-channel blocker, Cd\(^{2+}\), is a pharmacological tool for selective blockade of the HTS without greatly affecting action potentials and LTS (Hernandez-Cruz and Pape 1989, Tennigheid et al. 1996).

In the course of our studies on the discharges of MGBv neurons, we observed that, paradoxically, TTX-application also blocked the HTS. In this chapter, we test a hypothesis that the HTS may depend on an amplification of the LTS by a persistent Na\(^{+}\)-conductance.
This conductance activates in a subthreshold voltage range, resulting in a modulation of neuronal excitability (Llinás 1988, Stafstrom et al. 1985). In MGBv neurons, the persistent Na\(^+\)-conductance mediates a prominent inward rectification on depolarization from rest and contributes to a ramp-like depolarization during the delay to firing (Tennigkeit et al. 1996). The persistent and transient Na\(^+\)-conductances are both sensitive to blockade by TTX. This introduces difficulties in the assessment of the persistent current’s involvement in firing patterns. We attempted to resolve the selectivity issue, similar to that presented by the lidocaine derivative QX-314 (Stafstrom et al. 1985), by using low concentrations of TTX to partly block the persistent Na\(^+\)-conductance, retaining largely intact Na\(^+\)-action potentials.

Other subthreshold currents also may control the voltage-coupling of LTS to HTS in MGBv neurons. Various neurons possess slowly inactivating, subthreshold K\(^+\)-currents with a high sensitivity to blockade by 4-aminopyridine (4-AP) and dendrotoxin, but not to tetraethylammonium (TEA). These currents modulate the onset to firing, synaptic integration, temporal fidelity and receptive field properties (Brew and Forsythe 1995, McCormick 1991, Nisenbaum et al. 1994, Storm 1990). In MGBv neurons, applications of 4-AP at low concentrations which block slowly inactivating A-type K\(^+\)-channels (McCormick 1991), shorten the delay to firing and increase spike duration in the tonic and burst firing modes (Tennigkeit et al. 1996). Here, we confirmed a second hypothesis that blockade of a K\(^+\)-current such as I_{As} results in a recoupling of the HTS to the LTS, despite prior blockade of Na\(^+\)-conductances.

Using whole-cell patch-clamp recordings in the current-clamp mode and application of ion channel blockers, we show here that both persistent Na\(^+\)- and K\(^+\)-conductances modulate the coupling of the various spikes, providing a variability in the burst pattern that can profoundly influence the cellular responses to auditory inputs.
4.2. RESULTS

*High-threshold spikes in neurons firing in tonic and burst modes*

A majority of MGBv neurons generated HTSs when depolarized by current pulse injection from hyperpolarized potentials (Tennigkeit et al. 1996). Under normal extracellular conditions, the HTS elicited by pulse injection had, characteristically, a slower rate of rise, lower amplitude, longer duration, and higher threshold than action potentials (Figs. 1B and 3A). After complete blockade of a neuron's ability to fire action potentials by application of 300 nM TTX, positive current pulses or DC-injection evoked a tonic pattern of HTSs in the depolarized voltage range (Fig. 4.1A). On shifting a neuron to the burst firing mode by DC-hyperpolarization, we observed an HTS appended to one or more action potentials on top of an LTS (Fig. 4.1B). We sought confirmation that the HTSs were dependent on Ca\textsuperscript{2+} conductances by applying Cd\textsuperscript{2+} (50 μM), a Ca\textsuperscript{2+} -channel blocker (Fig. 4.1.). When a neuron was in the burst firing mode, the blockade of Ca\textsuperscript{2+}-conductances by Cd\textsuperscript{2+} did not significantly affect the LTS and action potentials. However, Cd\textsuperscript{2+}-application (50 μM) completely blocked the HTSs in neurons firing in either mode. We considered the possibility that MGBv neurons generated the HTS at a dendritic location. With this in mind, we proceeded to investigate the coupling of the LTS to HTS by interfering with Na\textsuperscript{+}-conductances.

*TTX-blockade of the HTS and its coupling to the LTS*

We studied the concentration-dependent effects of TTX in an initial series of experiments. Very low concentrations (3-6 nM) of TTX did not significantly change the membrane potential, input resistance, action potentials or firing patterns. Application of 30 nM for 5 to 20 minutes,
Fig. 4.1. TTX-insensitive HTSs and their selective blockade by Cd$^{2+}$.

A: From a depolarized membrane potential (-56 mV), HTSs were evoked by depolarizing current pulses, after complete blockade of Na$^+$-currents by TTX (300 nM). Application of Cd$^{2+}$ (50 μM) reversibly blocked these HTSs. Note that they were not evoked by injection of higher amplitude current pulses, compensating for a decrease in membrane resistance. B: Under control conditions, an HTS was evoked on top of an LTS, following an action potential. The HTS can be distinguished from action potentials by its slower rate of rise, lower amplitude and longer duration. Application of Cd$^{2+}$ (50 μM) selectively and reversibly abolished the HTS, leaving the LTS and action potentials intact.
or 300 nM for 2 to 4 minutes ("low" concentration), decreased depolarizing voltage responses and eliminated the HTS on the LTS without greatly altering the ability to generate action potentials. The application of low TTX-concentrations also raised the current-requirement for evoking tonic firing and reduced the number of spikes on a rebound LTS (Fig. 4.2.A). During the application, an increase in DC-current injection was necessary to hold the membrane potential at the depolarized control level for the tonic mode. Therefore, the steady-state, subthreshold amplification produced by a persistent Na\(^+\)-conductance was blocked by low TTX-concentrations. This blockade interfered with action potential- and HTS generation in both tonic and burst firing modes.

Figure 4.2.B shows that TTX-application at low concentrations (n = 8) produced a blockade of the HTS on the LTS, leaving action potential firing intact (cf. Fig. 4.2.A). Note the paradoxical similarity to Fig. 4.1.B, where the HTS is eliminated as a consequence of Cd\(^{2+}\)-application. However, during the TTX-application, removal of T-type Ca\(^{2+}\)-current inactivation by increasing hyperpolarization to ~ -100 mV increased LTS amplitude and produced a return of the HTS on the rebound LTS. Application of higher TTX-concentrations (300-1000 nM) for >5 minutes ("high or maximal" concentration) decreased maximally depolarizing voltage responses and eliminated all firing on the LTS and reduced its magnitude (n = 42; Fig. 4.2.B). Increased hyperpolarization to near -105 mV did not provide conditions sufficient for returning the HTS on the rebound-LTS, but strong depolarizing pulses to beyond -40 mV still evoked the HTS (cf. Fig. 4.1.A). Assuming that TTX did not affect Ca\(^{2+}\)-channels involved in HTS generation, these results are consistent with a modulatory role of a persistent Na\(^+\)-conductance in amplifying the LTS and thus coupling multiple action potentials and the HTS to the LTS.
Fig. 4.2. Modulation of tonic and burst firing by different concentrations of TTX.
A: Depolarizing and hyperpolarizing current pulses of same amplitude evoked tonic and rebound burst firing, including an HTS, from a depolarized membrane potential (-62 mV, left). After application of 30 nM TTX, tonic and burst firing were completely abolished upon injection of identical current pulses (middle). Increased positive DC current was injected to keep the membrane potential at control level. Current pulses of larger amplitude still elicited tonic firing, but only a single action potential on the LTS (right). B: In another neuron, the burst response on a rebound LTS (left), including an HTS, was reduced to a single action potential (middle) after application of the low concentrations of TTX. The HTS returned on depolarization from a more hyperpolarized potential (-100 mV). Maximal TTX application (300 nM, > 5 min.) abolished all firing on the LTS and reduced the LTS amplitude even on rebound from a very hyperpolarized potential (-110 mV, right).
The voltage-dependent, persistent Na\textsuperscript{+}-conductance - an active bridge to the HTS

From the above results, we considered the possibility that TTX blocked both a subthreshold, persistent Na\textsuperscript{+}-conductance, and the transient Na\textsuperscript{+}-conductance. Therefore, we examined the effects of TTX on the subthreshold and threshold voltage responses evoked by current pulses.

Application of low TTX-concentrations (30 nM, n = 3; 300 nM for 2-4 min; n = 5) significantly decreased the subthreshold depolarizing voltage responses as well as the maximal rate of rise and amplitude of the action potentials (Fig. 4.3.A). The average steady-state voltage amplification due to a persistent Na\textsuperscript{+}-conductance was reduced by 50% with low concentrations and completely blocked after maximal TTX application (n = 8, Fig. 4.3.C). After application of low TTX-concentrations, action potentials could be evoked with a significant decrease in the average maximal rate of rise. They were blocked completely after maximal TTX application (Fig. 4.3.A,D).

Application of the high TTX-concentration produced an apparent higher conductance state in a potential range that was subthreshold to HTS generation (Fig. 4.3.A,B). This state presumably was attributable to the blockade of depolarization by both transient and persistent Na\textsuperscript{+}-conductances. Depolarization beyond ~-40 mV evoked the HTS, with its characteristically slower rate of rise, smaller amplitude and longer duration, compared to action potentials (Fig. 4.3.A,D). It seemed likely, therefore, that the blockade of amplification, due to activation of a persistent Na\textsuperscript{+}-current (I_{Na,p}), resulted in the failure of the LTS to couple to the HTS generator.
Fig. 4.3. Concentration-dependent changes of subthreshold rectification and action potential firing after application of TTX.

A: Depolarizing current pulses of increasing amplitude evoked voltage responses, leading to firing of an action potential after a voltage ramp (Control, left). Upon application of 30 nM TTX, action potential firing is elicited by injection of current pulses of increased amplitude (middle). Note the decreased maximal rate of rise of the action potential. After application of 300 nM TTX for longer than 5 minutes, the generation of action potentials was completely blocked, but an HTS could be evoked after injection of large amplitude current pulses (note different threshold, maximal rate of rise, amplitude and duration; right).

B: The steady-state I/V relationship, obtained at the end of the subthreshold pulses in A, shows the concentration-dependent effect of TTX application on the subthreshold membrane rectification.

C: Summary graph shows mean (± SE) voltage responses to the maximal subthreshold control current pulse (n = 8). Significant reduction was observed after low concentrations of TTX (30 nM or 2-4 min. 300 nM) and after application of the maximal TTX concentration (> 5 min., 300 nM).

D: Summary graph shows mean (± SE) maximal rate of rise for the first evoked spike (cf. A, same neurons as in C). The mean maximal rate of rise was significantly reduced after low concentrations of TTX. After maximal doses of TTX, all action potentials were blocked. The HTSs exhibited ~10% of the maximal rate of rise of action potentials.
**A K⁺-conductance modulates coupling of the LTS to HTS generation**

There was good reason to investigate the hypothesis that K⁺-conductances also modulate the ability of an LTS to trigger the HTS. Our previous studies demonstrated that MGBv neurons possess a repertoire of conductances, including putative A-type K⁺-conductances that are sensitive to blockade with 4-AP (Chapter 3). For example, many MGBv neurons fired only one action potential in a phasic response, but on application of 4-AP (50-100 μM, n = 5), discharged multiple action potentials and an HTS on top of the LTS. After several minutes of application, 4-AP slowed HTS-repolarization, transforming the HTS into a plateau potential, lasting several hundreds of milliseconds (Fig. 4.4.A). The coapplication of Cd²⁺ (50 μM) and 4-AP eliminated the HTS and plateau potential, confirming a dependence on high threshold Ca²⁺-conductances.

To determine if an A-type K⁺-conductance had a modulatory effect, we first applied the maximal TTX-concentration to abolish action potentials and to uncouple the HTS generator from the LTS. We then applied 4-AP in serially increasing concentrations. With increasing K⁺-channel blockade, the solitary LTS increased in amplitude and duration; on 4-AP-application at 100 μM (n = 5), the LTS reached a threshold for HTS generation. A co-application of Cd²⁺ (50 μM) with 4-AP annihilated the plateauing HTS (Fig. 4.4.B). In contrast, the application of TEA, a K⁺-channel blocker with little effect on A-type conductances (McCormick 1991), did not change the voltage responses to hyperpolarizing current pulses, the rebound LTS, or result in LTS to HTS coupling (2-10 mM; n = 5; Fig. 4.4.C left). However, during co-application of TEA (10 mM) and 4-AP (100 μM), the same hyperpolarizing current pulses evoked a plateauing HTS on the rebound LTS (n = 2, Fig. 4.4.C right).
Fig. 4.4. Application of 4-AP, but not TEA, coupled the HTS to an LTS by an LTS increase.

A: An action potential on top of an LTS was elicited on the rebound from a hyperpolarizing pulse. After application of 4-AP (100 μM), an identical pulse evoked a burst of action potentials, followed by a plateau potential of several hundreds of milliseconds duration. This plateau potential was blocked by Cd²⁺ (50 μM), indicating its dependence on high threshold Ca²⁺-currents. B: In Control, a hyperpolarizing pulse evoked rebound burst firing including an HTS on the LTS (left). On the rebound from identical pulses, application of TTX (300 nM) eliminated burst firing on the LTS. With increasing doses of 4-AP, the LTS increased (50 μM) and a high threshold Ca²⁺-plateau potential was evoked on the LTS after application of 100 μM 4-AP (middle). This high threshold Ca²⁺-plateau potential also was blocked by coapplication of Cd²⁺ (50 μM; right). C: After application of TTX (300 nM, left), a rebound LTS was evoked by a hyperpolarizing pulse (Control). Coapplication of TTX and TEA (10 mM) did not significantly change the voltage response. After coapplication of 4-AP (100 μM, right), an identical pulse evoked a high threshold Ca²⁺-plateau potential on the LTS.
It seemed likely, then, that a pharmacologically distinct $K^+$-conductance modulated the coupling of the LTS to the HTS and its transformation to a plateau potential.

4-AP application changes input resistance in a range subthreshold to HTS

The 4-AP-sensitive coupling of the LTS to the HTS generator always occurred between membrane potentials of -65 mV and the threshold voltage for the HTS (-40 to -35 mV). Therefore, we considered the possibility that a slowly inactivating $K^+$-current, like the $I_{As}$ in dorsal lateral geniculate neurons (McCormick 1991), was active in the same range of potentials and modulated the coupling. After eliminating the $Na^+$-conductances and HTSs with TTX (300 nM for >5 min) and $Cd^{2+}$ (50 µM), we applied 4-AP (50-100 µM) to MGBv neurons (n = 8). The application increased their voltage responses to current pulses in a range positive to -55 mV (Fig. 4.5.). Note that 4-AP application also increased the magnitude and the duration of the LTS (Fig. 4.5.A-C). This voltage dependence of 4-AP blockade was consistent with the activation range of the slowly inactivating $K^+$-current $I_{As}$ (Fig. 4.5.D). All neurons exhibited an increased steady-state voltage response at the end of 500 ms depolarizing current pulses (Fig. 4.5.C-E), which was expected due to the slow inactivation time-constants of $I_{As}$ (McCormick 1991, Nisenbaum et al. 1994). The 4-AP-induced voltage-amplification was characterized by a gradual growth over 200-300 msec in 4 neurons (Fig. 4.5.B,C). However, in another 4 neurons the response increased to a maximum close to pulse onset after 4-AP-application (100 µM, cf. arrows in Fig. 4.5.E). Application of a higher 4-AP-concentration (2 mM) further enhanced this response at pulse onset (n = 6, Fig. 4.5.F), presumably due to additional blockade of a fast-inactivating $I_A$. These observations are consistent with the voltage- and concentration-dependent 4-AP-blockade of $I_A$ and $I_{As}$ in previous studies (cf. Fig. 7 in Nisenbaum et al. 1994).
Fig. 4.5. Influence of 4-AP on rectification and the LTS.
A: Depolarizing and hyperpolarizing voltage responses were evoked by current pulses from rest (-68 mV) after coapplication of TTX (300 nM) and Cd2+ (50 μM) which blocked Na+- and high threshold Ca2+-currents. B: After coapplication of 4-AP (100 μM), depolarizing voltage responses and rebound LTS increased, whereas hyperpolarizing voltage responses were similar to control. C: Subtraction of A and B revealed the 4-AP-sensitive components. Note the late depolarizing voltage amplification in contrast to the fast onset amplification of the LTS. D: Steady-state I/V relationship, measured at the end of pulses (A,B), reveals the voltage-range of the 4-AP effect. E: In another neuron, voltage responses were increased at pulse onset during 4-AP application (arrows). F: In the same neuron, application of 2 mM 4-AP further enhanced the onset and steady-state voltage responses.
Ionic contributions to plateau potentials

As described above, the application of 4-AP initiated a change in conductance, transforming the HTS to a plateau potential. A question arose as to the interaction of conductances in the production of the plateau potential. During application of 4-AP (50-100 μM), a depolarization from rest with a current pulse, or a rebound depolarization on termination of a hyperpolarizing pulse, resulted in a plateau potential of long duration (n = 6, Fig. 4.4.A). The plateau potential duration was highly variable (50 ms to 3 s) and often greatly exceeded the pulse duration (Fig. 4.6.A). The plateau potentials did not, in any obvious way, depend on the magnitude of the injected current pulse. The plateau potential was never observed on co-application of TTX (300 nM) and Cd$_2^+$ (50 μM, n = 9, cf. Fig. 4.5.). In the absence of these blockers, the plateau potentials had an average voltage value of -12.6 ± 1.9 mV (n = 5, mean ± SD). After blockade of Na$^+$-conductances with a maximally effective TTX-concentration (300 nM), the plateau was not significantly different (-8.6 ± 5.3 mV, n = 8). After blockade of high threshold Ca$^{2+}$-conductances with Cd$^{2+}$-application, the plateau potentials evoked by depolarizing current pulses had significantly lower amplitudes at -22.3 ± 2.6 mV (n = 4, Fig. 4.6.). These data implied that an interaction of persistent Na$^+$- and high threshold Ca$^{2+}$-currents normally generate the plateau potential, although each current alone can sustain a plateau.
Fig. 4.6. High threshold Ca$^{2+}$-channel blockade reduces plateaus.
A: After application of 4-AP (100 μM) a depolarizing current pulse elicited a plateau potential, exceeding the pulse duration. Blockade of high threshold Ca$^{2+}$-channels by coapplication of 50 μM Cd$^{2+}$ reduced the plateau potential duration and the amplitude by about 8 mV. B: The average plateau potential amplitude (horizontal bar ± SE) and individual values at the end of a depolarizing current pulse after application of 4-AP (100 μM) are plotted for neurons without blockade of Na$^{+}$- and high threshold Ca$^{2+}$-channels (left column), after application of Cd$^{2+}$ (50 μM) to block high threshold Ca$^{2+}$-channels (middle column) and after application of TTX (300 nM) to block Na$^{+}$-channels (right column). Plateau potentials in neurons without blockade and blocked Na$^{+}$-channels were similar, whereas blockade of high threshold Ca$^{2+}$-channels reduced the amplitudes significantly.
4.3. DISCUSSION

Origins of the high threshold spike and burst pattern

In the present studies, the HTS was an outstanding feature and appeared frequently, when the MGBv neurons were in the burst firing mode. In contrast, the HTS did not normally accompany the output pattern of a neuron firing in the tonic mode. This drew attention to the genesis for each type of spike comprising a burst. An axonal origin close to the soma seems assured for the action potentials of most studied CNS neurons, such as for the first spike in a burst (Colbert and Johnston 1996, Johnston et al. 1996). On the other hand, dendritic regions in cortical and hippocampal neurons possess a capability for generating Na\(^+\) spikes (Johnston et al. 1996). Although K\(^+\)- and Ca\(^{2+}\)-conductances may account for a reduced amplitude and longer duration, the second spike in a burst also could result from a depolarization of dendrites subsequent to Ca\(^{2+}\)-channel activation. The HTS originates from the activation of high threshold Ca\(^{2+}\)-channels, located more distally in the dendrites of MGBv neurons, as suggested for neurons of the dorsal thalamus (Deschênes et al. 1984, Jahnsen and Llinás 1984b). Low threshold Ca\(^{2+}\) currents in both somatic and proximal dendritic regions appear to generate the LTS in dissociated thalamic neurons (Hernandez-Cruz and Pape 1989). The partial success obtaining voltage-control in thalamocortical neurons in slice preparations and modelling studies tend to emphasize an origin for the LTS in the distal dendrites (Destexhe et al. 1998, Ries and Puil 1993). Our investigations raise the likelihood that slowly inactivating Na\(^+\) - and K\(^+\)-conductances modulate a voltage-dependent coupling of polarizing currents, resulting in an output pattern of LTS, action potentials, and HTS.
An involvement of persistent Na\textsuperscript{+}-conductance in coupling LTS to HTS generation

An unexpected finding was the complete blockade of an HTS on top of the LTS by the application of TTX. We still could evoke the HTS by injection of depolarizing pulses or DC-depolarization. The simplest explanation for the paradoxical blockade of an HTS was that TTX blocked a persistent Na\textsuperscript{+}-conductance (Jahnsen and Llinás 1984b, Schwindt and Crill 1980, Tennigkeit et al. 1996), removing an amplification that affected voltage-coupling of the Ca\textsuperscript{2+}-dependent LTS and HTS. In an absence of TTX, the persistent Na\textsuperscript{+}-conductance apparently amplified the LTS which attained threshold for the HTS (~40 mV) in the dendrites. The coupling probably would not otherwise occur because of an inactivation of the T-type Ca\textsuperscript{2+}-channels as the membrane potential approached -45 mV (Jahnsen and Llinás 1984a). This view is consistent with the recent demonstrations of persistent Na\textsuperscript{+} channels in dendrites of cortical neurons (Schwindt and Crill 1995). On propagation to the soma, the HTS would append the action potential discharge, contributing to the LTS-burst.

An involvement of a persistent Na\textsuperscript{+} conductance in the coupling of LTS and HTS generation also was evident from applications of TTX at low concentrations. We observed significant reductions in the subthreshold depolarizing responses and the rate of rise of action potentials, implying partial blockade of both persistent and transient Na\textsuperscript{+}-conductances. We could still evoke action potentials at a higher threshold but the partial blockade enabled us to study the effects of a persistent Na\textsuperscript{+}-conductance in the subthreshold range and on the firing patterns (cf. Stafstrom et al. 1985). While there was no clear evidence for a differential sensitivity of Na\textsuperscript{+}-conductance channel-states to TTX-blockade, we attribute the differential effects of TTX-application at low concentrations to a potentially different cellular localization of the small population of slowly versus fast inactivating Na\textsuperscript{+}-channels (~1%, Alzheimer et al.
1993). Thus, the TTX-applications would have a pronounced effect on membrane rectification before abolishing action potential firing.

**Regulation of firing patterns by a slowly inactivating K⁺-conductance**

Our findings of 4-AP-induced changes in membrane rectification and the LTS imply a potential modulation of membrane excitability of MGBv neurons during tonic and burst firing. We interpret the increases in the subthreshold rectification and LTS amplitude on application of low concentrations of 4-AP as a blockade of the slowly inactivating K⁺-current IAS, as described in thalamic and other neurons (McCormick 1991, Nisenbaum et al. 1994, Storm 1990). While IAS should activate rapidly (~20 ms, cf. Storm 1990, Spain et al. 1991) and have a maximal effect at the onset of a depolarizing current pulse (cf. Fig. 5E), we observed a marked delay in the effect of 4-AP in some neurons (Fig. 4.5.A-C). This unexpected small initial effect, followed by a presumed blockade of IAS, could result from interactions of 4-AP with the K⁺-channel pore. A comparable biphasic effect of 4-AP has been reported for a slowly inactivating K⁺-current under similar conditions (Bouchard and Fedida 1995). The low 4-AP concentrations used to block the slowly inactivating K⁺-current in MGBv would not likely affect the fast inactivating IAF which is blocked by millimolar concentrations of 4-AP (Fig. 4.5.F), as in other neurons (Huguenard et al. 1991, Nisenbaum et al. 1994). An IAS was present in slice preparations, but not dissociated neurons of the dorsal lateral geniculate nucleus (Budde et al. 1992, McCormick 1991), which could indicate changes in channel properties due to the dissociation procedure or a predominantly dendritic location of this conductance.

The blockade of K⁺-channels with 4-AP retarded HTS repolarization in MGBv neurons, facilitating a transformation into a plateau potential. High threshold Ca²⁺- and persistent Na⁺-conductances can sustain a plateau potential for several hundreds of
milliseconds, as described in several other types of neurons after blockade of K\(^+\)-channels (Kim and Connors 1993, Llinás and Sugimori 1980a,b; Schwindt and Crill 1980). In our experiments, another blocker of K\(^+\)-channels, TEA, did not increase the LTS to reach HTS threshold, probably because the K\(^+\)-conductances that have sensitivity to TEA-blockade usually have activation ranges above the LTS peak (Huguenard and Prince 1991). However, current pulses in the depolarized range evoked plateau potentials during application of TEA, emerging from fast spikes (cf. Fig. 3.4.D in Chapter 3) or HTSs (not shown). While both 4-AP- and TEA-sensitive conductances make contributions to the control of membrane excitability, a 4-AP-sensitive K\(^+\)-conductance likely modulates the coupling of LTS to the HTS and its transformation to a plateau potential.

In MGBv neurons, a regulated balance of persistent Na\(^+\) and 4-AP-sensitive K\(^+\)-conductances may determine the pattern of phasic responses consisting of single action potentials or bursts with or without the HTS on the LTS. All these patterns occur under control conditions. The persistent Na\(^+\)- and K\(^+\)-currents share certain characteristics, including voltage ranges of activation, inactivation kinetics and partial dendritic location. Apart from causing local voltage shifts, activation of these currents would alter the coupling of dendritic responses to the somatic spike generator.

Modulation of A-type K\(^+\)-currents by serotonin and adenosine occurs in locus coeruleus and Purkinje neurons that exhibit prominent HTSs (Hounsgaard and Midtgaard 1988, 1989; Pan et al. 1994, Wang et al. 1992). Such neuromodulation of I\(_{As}\) could dramatically change thalamic firing patterns and the intraneuronal transfer of information.
Conclusion and functional significance

Our findings imply that inward and outward currents interact with the passive membrane properties of MGBv neurons, to modulate their spike output patterns in the tonic and burst firing modes. An increase in the persistent Na\(^+\)-conductance, or decrease in the slowly inactivating K\(^+\)-conductance, for example, can enhance LTS size and bursting intensity. As the tendency for oscillatory synchronization in epileptic states would change with burst intensity, drugs that alter these conductances could influence seizure activity. A reduction in LTS amplitude would have a similar effect as the modulation of the T-type Ca\(^{2+}\)-current by ethosuximide in the treatment of absence epilepsy (Coulter et al. 1989, Huguenard and Prince 1994). For example, a mechanism of anticonvulsive action of phenytoin may involve blockade of I_{Na,P} (Chao and Alzheim 1995).

Active dendrites participate in the integration of synaptic information (Hounsgaard and Midtgard 1989, Johnston et al. 1996, Llinás and Sugimori 1980a, Stuart and Sakmann 1994, Wong and Prince 1978). Local depolarization due to activated T-type Ca\(^{2+}\)-, persistent Na\(^+\)- and high threshold Ca\(^{2+}\)-conductances will tend to amplify synaptic inputs at peripheral dendritic segments and thus have a role in auditory signal transfer (cf. Hu 1995). Shunting effects will, however, dampen the excitatory transmission, particularly in the presence of co-activated outward currents. Of great interest is the discharge of the HTS, presumably of dendritic origin, as part of the burst under "normal" conditions. An implication is that MGBv neurons may be equipped to regulate a localized influx of Ca\(^{2+}\), occurring over brief time periods, possibly beyond the resolution of imaging techniques. Intracellular regulatory and metabolic consequences of this influx may be functionally important sequels of the oscillatory bursting activity during states of reduced awareness, e.g., sleep.
5. Frequency preference in MGBv: The role of membrane properties

5.1. INTRODUCTION

The MGBv neurons exhibit a wide range of voltage-dependent thalamic firing patterns, latencies and spike types, due to interaction of voltage-dependent membrane currents (cf. Chapters 3 and 4, Tennigkeit et al. 1996, 1998b). Thalamic intrinsic membrane properties also transform frequency-modulated inputs (McCormick and Feeser 1990, Mukherjee and Kaplan 1995, Puil et al. 1994b) and can oscillate at 0.5 - 5 Hz in vitro in the absence of sensory or cortical input (Leresche et al. 1991, McCormick and Pape 1990). The frequency preferences in thalamic neurons of several species have been investigated previously (Puil et al. 1994b, Ströhmann et al. 1994, Tennigkeit et al. 1994). An interaction of passive membrane properties with different time- and voltage-dependent conductances accounted for the subthreshold frequency preferences. In avian auditory thalamic neurons, as in rat neocortical neurons, a hyperpolarization-activated cationic conductance produces a resonance of 1-6 Hz (Hutcheon et al. 1996, Ströhmann et al. 1994), while in rat and guinea pig thalamic neurons the low-threshold T-type Ca\(^{2+}\)-conductance supports a resonance in a similar frequency range (Hutcheon et al. 1994, Puil et al. 1994b, Tennigkeit et al. 1994). In rat MGBv neurons, this resonance near 1 Hz prevails at hyperpolarized potentials, while low pass filter characteristics are observed at depolarized membrane potentials (Tennigkeit et al. 1997). Apart from the instrumental role of the low-threshold T-type Ca\(^{2+}\)-conductance in the generation of resonance (Hutcheon et al. 1994, Puil et al. 1994b, Tennigkeit et al. 1994), we postulated that other subthreshold conductances would modulate the frequency preference.

Using swept-sinewave stimuli and frequency domain analysis (ZAP, cf. Chapter 2), as well as similar pharmacological tools used in Chapters 3 and 4, we investigated the
mechanisms of intrinsic frequency selectivity in MGBv neurons and the coupling of different spike-types to differing frequency preferences.

5.2. RESULTS

Voltage-dependence of frequency preference

The voltage dependence of frequency preference in MGBv neurons is shown in Fig. 5.1. The voltage responses to the swept sinewave current input at depolarized (-60, -65 mV) and at very hyperpolarized (-85 mV) potentials decreased steadily from low to high frequencies. In contrast, at potentials of -70 (rest) to -80 mV, an increase at intermediate frequencies was observed (Fig. 5.1.A). The ratios of the Fourier transformed voltage responses and the current inputs yielded impedance amplitude profiles (ZAPs). These frequency response functions characterize the filter properties of the neuron at various membrane potentials (Fig. 5.1.B). In these plots, resonance is apparent as a maximum in the frequency responses near 1 Hz. Low-pass filter characteristics (at depolarized and at very hyperpolarized potentials) prevailed when the maximum frequency response was at the lowest frequencies (0.1 Hz, Fig. 5.1.C). Such voltage profiles of subthreshold frequency preferences were observed in all MGBv neurons of mature rats (> 14 days post natum, n = 106). The membrane properties and frequency preferences were different during development in the first two postnatal weeks (cf. Chapter 6). In three other neurons, we tested the effects of a raise in temperature on the frequency responses. A temperature increase of about 10 °C from normal room temperature (22 °C) did not evoke changes of the voltage-profile of frequency preferences, but raised in the maximum resonance to near 2 Hz in all cases (Fig. 5.1.D).
A: ZAP current input 
(0.1-20 Hz, 38.4 sec) 
+20 mV 
+20 pA

Voltage responses
-70 mV, -75 mV,
-60 mV, -65 mV, -70 mV, -75 mV, -80 mV, -85 mV,
+20 mV, +10 pA, 0 pA, -20 pA, -40 pA, -60 pA

Fig. 5.1. Voltage-dependent frequency preference in MGBv.
A: Injection of swept sinewave current (ZAP, left) evoked voltage-responses at different holding potentials. These responses decreased from low to high frequencies at depolarized (-60, -65 mV) and very hyperpolarized (-85 mV) potentials. In contrast, increased responses at intermediate frequencies were evoked at potentials of -70 (rest) to -80 mV. B: Frequency preferences are shown as impedance amplitude profiles (ZAPs), obtained by division of the fast Fourier transformed voltage responses by the current input. C: In this neuron (same as A,B) resonance was apparent as a maximal frequency response near 0.8 Hz (-75, -70 mV), while low-pass filter properties appeared at very depolarized (-65 mV) or hyperpolarized (-85 mV) potentials at room temperature (23 °C). Note logarithmic scaling of the frequency axis for better resolution in the lower frequency range. D: In another neuron, frequency responses were obtained at 33 °C. At rest (-73 mV), resonance was observed at 2 Hz. This resonance was reduced at -68 and -78 mV, while low-pass filter characteristics were observed at depolarized potentials (-63 mV).
The role of Na\(^+\)- and Ca\(^{2+}\)-conductances in frequency preference

Application of the Na\(^+\)-channel blocker tetrodotoxin (TTX, 300 nM) decreased the amplitude of the subthreshold frequency responses at depolarized potentials, but had only a small effect at hyperpolarized potentials (Fig. 5.2.A-C, n = 30). The subthreshold activation range and voltage dependence of the TTX effect are indicative of a greater role for the persistent Na\(^+\)-conductance in the amplification of low-pass filter responses at depolarized potentials than in the modulation of the resonance.

In contrast, replacement of extracellular Ca\(^{2+}\) with equimolar Mg\(^{2+}\) had only small effects at depolarized potentials, but completely abolished resonance at hyperpolarized potentials (Fig. 5.2.A-C, n = 9). Similar results were obtained with application of Ni\(^{2+}\) (1 mM, n = 4, not shown), a specific blocker of the low-threshold T-type Ca\(^{2+}\)-current (Hernandez-Cruz and Pape 1989). The resonance displayed the same voltage range of activation and Ca\(^{2+}\)-dependence as the T-type Ca\(^{2+}\)-current, apparent from activation and blockade of the low-threshold Ca\(^{2+}\)-spike (LTS; Fig. 5.2.D). These results indicate that activation of the T-type Ca\(^{2+}\)-conductance generates low-frequency resonance in MGBv, but does not contribute to low-pass filter properties at depolarized potentials, where this current is inactivated (Hernandez-Cruz and Pape 1989, Tennigkeit et al. 1994).
Fig. 5.2. Na\textsuperscript{+} - and Ca\textsuperscript{2+}-dependence of frequency preferences.
A: At a depolarized membrane potential (-62 mV), low-pass filter frequency preference was observed. Application TTX (300 nM) reduced the amplitude of the frequency response, but additional replacement of extracellular Ca\textsuperscript{2+} with Mg\textsuperscript{2+} (0 Ca\textsuperscript{2+}) had almost no effect. B: At the resting potential (-67 mV), a resonance near 1 Hz was present. Application of TTX largely reduced the amplitude of the response, leaving a small resonant hump intact. Additional replacement of extracellular Ca\textsuperscript{2+} with Mg\textsuperscript{2+} abolished this small resonance. C: In contrast, at hyperpolarized potentials (-72 mV), TTX had only a minor effect on resonance (~1 Hz), but additional replacement of extracellular Ca\textsuperscript{2+} with Mg\textsuperscript{2+} completely abolished the pronounced resonance. D: In the time-domain of the same neuron, application of TTX blocked all spikes on a rebound LTS. Additional replacement of extracellular Ca\textsuperscript{2+} with Mg\textsuperscript{2+} abolished the LTS.
A-type $K^+$-conductances modulate frequency filter properties

The contribution of A-type $K^+$-conductances to frequency responses was studied, as previous studies in MGBv and other thalamic neurons have implicated an activation of A-type $K^+$-currents in the subthreshold modulation of firing patterns (Chapters 3 and 4, McCormick 1991). The slowly inactivating current $I_{As}$, sensitive to low concentrations of 4-AP, has a similar activation range and kinetics as the persistent Na$^+$-current. Thus, both currents can interact and modulate the delay to firing and the coupling low-to high-threshold Ca$^{2+}$-spikes in MGBv (Chapter 4). After blockade of action potentials and synaptic transmission by application of TTX, coapplication of low concentrations of 4-AP (0.05-0.1 mM, n = 5) only slightly amplified resonance at hyperpolarized potentials, but dramatically increased the low-pass responses at depolarized potentials (Fig. 5.3.A). This effect did not depend on the presence of resonance and was also observed after removal of extracellular Ca$^{2+}$ (n = 2, Fig. 5.3.B).

The fast inactivating A-type $K^+$-current $I_{Af}$, blocked by millimolar concentrations of 4-AP, controls the activation of the T-type Ca$^{2+}$-current, as both exhibit similar activation and inactivation parameters (Huguenard et al. 1991). After blockade of action potentials and HTSs by coapplication of TTX and Cd$^{2+}$, coapplication of higher concentrations of 4-AP (1-2 mM, n = 6) greatly amplified resonance peaks at hyperpolarized potentials and the impedance of the low-pass frequency filter at depolarized potentials. These results are consistent with a lower activation threshold of $I_{Af}$ compared to $I_{As}$ (Huguenard et al. 1991, McCormick 1991) and an involvement of these currents in the control of resonance and low-pass filter properties in MGBv.
Fig. 5.3. 4-AP-sensitivity of frequency preference.
A: After application of TTX (300 nM), resonance near 1 Hz and low-pass filter frequency preference were observed at hyperpolarized and depolarized membrane potentials, respectively. Application of 4-AP (0.1 mM) slightly amplified resonance at hyperpolarized potentials, but dramatically increased low-pass filter responses at depolarized potentials. B: After complete blockade of resonance by additional replacement of extracellular Ca$^{2+}$ with Mg$^{2+}$, the effect of 4-AP-application (0.1 mM) was still small at hyperpolarized potentials, but large at depolarized potentials. C: In another neuron, after coapplication of TTX (300 nM) and Cd$^{2+}$ (0.1 mM) to abolish all spikes, a resonance of ~1 Hz is observed at -71 mV and a low-pass frequency preference at -61 mV. Coapplication of 4-AP (2 mM) amplified both the resonance and the low-pass frequency response (cf. A). D: In this neuron, the rebound LTS was also dramatically increased by application of 4-AP (2 mM).
Modulation of frequency preference by an inwardly rectifying $K^+$-conductance

To study the modulation of frequency responses by a fast-activating, Ba$^{2+}$-sensitive inwardly rectifying $K^+$-conductance ($I_{KIR}$), as identified previously in MGBv (Chapter 3), extracellular Ba$^{2+}$ was applied at small concentrations (0.2 mM, $n = 3$). Such applications increased the amplitude of frequency responses at all membrane voltages, presumably due to concomitant blockade of leak $K^+$-channels (Sutor and Hablitz 1993). However, the increase was maximal at very hyperpolarized potentials, where $I_{KIR}$ is activated (Fig. 5.4.). These results are consistent with a depression of impedance magnitude at these potentials by $I_{KIR}$, as observed in cortical neurons (Hutcheon et al. 1996). Application of Cs$^+$ (3 mM, $n = 2$) resulted in a similar amplification of frequency responses as observed with Ba$^{2+}$, also without shifts in resonance frequency (not shown). This effect was similar to the $I_{KIR}$-blockade with Ba$^{2+}$ and did not support a major role of the slowly-activating, Cs$^+$-sensitive hyperpolarization-activated cation conductance in the generation of resonance. This was expected, given that this conductance was activated at very negative potentials (<-85 mV) and thus below the activation range for $I_{KIR}$ in MGBv (Chapter 3, Tennigkeit et al. 1996).

Influence of membrane filter functions on firing

We examined the influence of differing frequency preferences on the firing output evoked from both depolarized and hyperpolarized membrane potentials in 18 neurons. We increased the ZAP current amplitude just beyond spike threshold and compared the responses to those obtained with single sinusoidal stimuli of matching amplitude at six test frequencies. In the DC-hyperpolarized range, both types of stimulation elicited spike firing on top of the periodic waves within the range of resonant frequencies (Fig. 5.5.A, top).
Fig. 5.4. Ba\(^{2+}\)-sensitivity of frequency preference.
A: Application of Ba\(^{2+}\) (0.2 mM) increased the low-pass filter response at -70 mV. B: A resonant response at -75 mV showed a greater increase after application of Ba\(^{2+}\). C: An even larger increase of a low-pass filter response was observed at a very hyperpolarized potential (-85 mV).
Sinusoidal stimulation of neurons held at depolarized potentials, however, evoked action potentials on top of the sinusoids only at the lowest frequency, in keeping with the neuron's lowpass filter functions. The action potential threshold of the neuron in Fig. 5.5. was \(-40\) mV (i.e. in tests from \(-50\) mV). However, a fast depolarization of neurons hyperpolarized to \(-70\) mV led to firing at \(-60\) mV (Fig. 5.5.). The discrepancy in apparent thresholds is explained by greater T-current activation at the end of the depolarizing phase of the sinewave (see Hutcheon et al. 1994). Consequently, an LTS can couple the depolarizing phase of the stimulus to the threshold for action potentials (cf. Fig. 4.2.D in Chapter 4).

After TTX-blockade of action potentials, MGBv neurons display an ability to fire high threshold Ca\(^{2+}\) spikes (HTSs) at thresholds near \(-35\) mV (Chapter 4). After TTX application (n = 18), the pattern of HTSs evoked by sinusoidal stimulation reflected, once again, the frequency selectivity evident in the subthreshold ZAPs (Fig. 5.5.B). At depolarized potentials (\(-40\) mV, Fig. 5.5.B, right), the peaks of the sinewave triggered HTSs only at the lowest frequency. In the hyperpolarized range (\(-70\) mV, Fig. 5.5.B) and despite the large voltage difference, an activation of I\(_T\) coupled the depolarizing phase of the sinusoidal voltage response to HTS threshold. At all DC-maintained voltages, larger amplitude stimuli caused firing over a wider band (not shown, cf. Hutcheon et al. 1996). The relationship between voltage amplitude and threshold directly determined firing in the depolarized range. On depolarization from hyperpolarized potentials, coupling by T-type Ca\(^{2+}\)-channels accounted for the subthreshold frequency selectivity which influenced the firing behavior of MGBv neurons.
Fig. 5.5. Frequency-selective firing.

A: Increasing the swept-sinewave current evoked firing, as the voltage swept through the resonant frequencies (~1 Hz) at the resting potential (-70 mV). Single sinewaves evoked by a current of same amplitude reached threshold for action potentials at 0.5 and 1 Hz, but not at lower or higher frequencies. Note different time-scales for the sinewave-responses. At depolarized potentials (-50 mV), swept-sinewave and single-sinewave current inputs evoked action potential firing only at very low frequencies. B: In the same neuron, after application of TTX (300 nM), HTSs were evoked by higher current injection. At rest (-70 mV), HTS firing was also evoked at resonant frequencies with swept-sinewave and single-sinewave current input. At depolarized potentials (-50 mV), HTSs were restricted to low frequencies.
5.3. Discussion

At potentials near spike threshold, MGBv neurons had membrane dynamics that behaved like a lowpass filter or RC-circuit. The input conductance, mostly due to a leak K\(^+\)-current, and a large capacitance, relatively fixed by the expansive dendritic tree, dominated the frequency responses in this potential range. A highly voltage-dependent Na\(^+\)-conductance, i.e., a persistent Na\(^+\) current (I\(_{\text{NaP}}\)), was activated during the depolarizing phase of the sinusoidal input, accounting for the apparent impedance amplification at membrane potentials of ≥-65 mV. As a consequence of this activation, there was considerable enhancement of the quality factor of the lowpass filter, without an effect on the range of preferred frequencies. The activation of voltage-dependent K\(^+\)-conductances attenuated the filter quality. For example, we observed that low concentrations of 4-AP increased impedance at low frequencies, implying that I\(_{\text{As}}\) normally attenuates the impedance in this frequency range (cf. trigeminal root ganglion neurons, Puil et al. 1986). Because I\(_{\text{As}}\) activates rapidly and its inactivation time constant is long (Storm 1990) compared to the sinusoidal periods used in this investigation, the preferred frequency range at depolarized voltages did not greatly change during blockade by low concentrations of 4-AP. Thus, the subthreshold voltage-dependent Na\(^+\)- and K\(^+\)-conductances interact with the resting properties to determine the filter quality - an interaction subject to regulation by neuromodulators (cf. Mittmann and Alzheimer 1998).

In a narrow hyperpolarized range, impedance increased and included a resonance that peaked near 1 Hz, producing bandpass filter properties in MGBv neurons. The voltage range for resonance overlapped approximately with the activation range of the T-current (i.e., near -65 mV). Indeed, we found that the application of Ni\(^{2+}\) or removal of extracellular Ca\(^{2+}\), i.e.,
procedures which would block $I_T$, eliminated the resonance. We also observed an increase in the peak resonant frequency with an increase in temperature, likely due to the high temperature dependence of T-current kinetics (Coulter et al. 1989, cf. Huguenard 1996). As in mediodorsal thalamic neurons (Hutcheon et al. 1994, Puil et al. 1994), it seems quite likely that an interaction of the voltage- and time-dependent T-type Ca$^{2+}$ current with the passive membrane properties produced this type of resonance in MGBv neurons.

Another finding of our investigations was that the activation of $K^+$-conductances attenuated the resonant hump amplitudes. The blockade of the fast A-channels (i.e., $I_{Af}$) with millimolar concentrations of 4-AP amplified the resonance, providing evidence that $I_{Af}$ normally subdues resonance. The inward rectifier, $I_{KIR}$, also suppressed the impedance amplitudes in the resonant band, as inferred from the amplification of the frequency responses during blockade of $I_{KIR}$ with Ba$^{2+}$. We show below that an increase in a voltage-independent (leak) $K^+$-conductance due to application of a general anaesthetic, isoflurane (Chapter 9, Tennigkeit et al. 1997), or the GABA_B-receptor agonist, baclofen (Chapter 8, Tennigkeit et al. 1998a) shunted resonance. Thus, in the hyperpolarized range, modulation through a variety of $K^+$ conductances can decrease the quality factor of the bandpass filter or the amplitude of resonant voltage oscillations.

The interactions of active and passive properties responsible for resonance endow thalamic neurons with an inherent tendency for oscillation (Hutcheon et al. 1994; Puil et al. 1994; Ströhmann et al. 1994). To a large extent, the intrinsic properties of thalamocortical neurons are responsible for the low frequency slow oscillations of delta sleep (Curró Dossi et al. 1992, Leresche et al. 1991, McCormick and Pape 1990b, Soltesz et al. 1991). The faster oscillations, such as sleep spindles, involve the intrinsic membrane and, additionally, network connections with reticular thalamic and cortical neurons (Steriade et al. 1985, von
Krosigk et al. 1993). The observed frequency selectivity for inputs and tendencies to oscillate at preferred frequencies would support synchronized oscillatory activity in such neuronal networks (Lampl and Yarom 1993, Gutfreund et al. 1995, Puil et al. 1994, Hutcheon et al. 1994, 1996). For example, the bandpass filter function of MGBv neurons would introduce a tuning element for coherent oscillations in thalamocortical networks.

In the subthreshold range between rest and spike threshold, the neuronal filter functions in the MGBv have upper cutoff frequencies in the order of a few Hz, far below audible auditory frequencies. Therefore, the membrane properties of MGBv neurons could not transfer, to any appreciable extent, a temporal code for sound frequency. However, we would expect the neuronal filter to have an important role in the integration of synaptic inputs conveying a temporal representation of auditory signal parameters. It seems likely that the cutoff frequencies are somewhat higher during synaptic activity at physiological temperatures (cf. Paré et al. 1998) than in our observations, compatible with a temporal representation of rhythmic properties of frequency- or amplitude-modulated sound. Near spike threshold and at the depolarized potentials that occur during alert states (Steriade et al. 1990), the lowpass filter properties would promote thalamocortical transfer of AM or FM rhythms of up to a few Hz (Gaese and Ostwald 1995). On the other hand, the resonant properties during maintained hyperpolarization in MGBv neurons may have little importance in auditory perception because membrane potentials more negative than ~65 mV characterize states of deep sleep. Hence, the resonance may support the oscillatory behaviour in thalamocortical circuits, reflected in the slow EEG waves (1-4 Hz) of deep sleep and other subconscious states.
6. Postnatal development of firing patterns and frequency preference

6.1. **Introduction**

In adult mammals, the intrinsic electrophysiological properties of MGBv neurones contribute to the observed complex firing patterns (Chapters 3 and 4, Clarey et al. 1991, Creutzfeld et al. 1980, Tennigkeit et al. 1996, 1998b). In contrast to adults, immature mammals exhibit markedly different responses to auditory stimulation (Pujol 1972, Pujol et al. 1970), before the onset of functional hearing, which occurs in rats around postnatal day 13 (P13), as determined from behavioral responses to sound (Ehret 1983, Rubel 1978). This age is also characterized by the onset of synchronized oscillations during deep sleep in the thalamocortical system (Jouvet-Mounier et al. 1970, Mares et al. 1982). While the development of morphology, intrinsic membrane properties, synaptic responses and spindle oscillations have been studied in detail in visual and somatosensory thalamus (McCormick et al. 1995, Pirchio et al. 1997, Ramoa and McCormick 1994, Perez-Velazquez and Carlen 1996, Warren and Jones 1997), the development of MGBv has not been studied. However, changes in morphology and intrinsic membrane properties are expected to affect the development of auditory function and sleep states in MGBv.

To this end, we used whole-cell patch-clamp recordings in the current-clamp mode combined with histological staining of the recorded neurons (cf. Chapter 2) to characterize the morphology, intrinsic membrane properties and firing patterns of MGBv neurons during postnatal development.
6.2. Results

Morphological changes

Major morphological changes of MGBv neurons were observed from the end of the first postnatal week (P5), when ventral and dorsal subdivision can first be distinguished in MGB, to the end of the second postnatal week. While immature neurons (< P13) already exhibited the typical multitufted denritic pattern of MGBv neurons (cf. Tennigkeit et al. 1996), their somata and dendritic fields were smaller (Fig. 6.1., P5, n = 14) than mature neurons (Fig. 6.1., P18, n = 31). The size of the soma and dendritic trees gradually increased, acquiring mature shape around P13. These findings are consistent with earlier observations in rat MGB (Coleman 1990).

Fig. 6.1. Morphology of MGBv neurons during postnatal development.
Camera-lucida drawings of typical neurobiotin-filled neurons at P5 and 18 show an increase in soma size, as well as extension and elaboration of the dendritic tree.
Subthreshold membrane properties

The subthreshold membrane properties of MGBv neurons change progressively during the first two postnatal weeks and remain stable thereafter, as demonstrated in Figures 6.2. and 6.3. In the immature neuron (P9), held at -72 mV with hyperpolarizing d.c. injection, hyperpolarizing pulses evoked a slowly developing sag (Fig. 6.2.A). The negative activation range and time course were reminiscent of an $I_H$ in mature neurons, as revealed after Ba$^{2+}$-blockade of $I_{KIR}$ (cf. Fig. 3.2.A in Chapter 3). A sag was observed in 84% of neurons before P13 (n = 44), but not in any neuron after P16. In a mature neuron (P20), hyperpolarizing pulses from rest revealed a prominent fast-onset rectification, due to activation of an $I_{KIR}$ (cf. Fig. 3.2.B in Chapter 3). In the I/V-relationships, obtained at the end of pulses, a steep linear slope from threshold to about -90 mV, indicates a high input resistance of the P9 neuron (Fig. 6.2.C). The appearance of the depolarizing sag corresponds to the inward rectification at potentials below -90 mV. Older neurons had a smaller input resistance, as indicated by decreased I/V slopes (Fig. 6.2.C). The fast-onset rectification of the P20 neuron appears below -80 mV. Subthreshold depolarizing current pulses in young neurons (P9) evoked smaller voltage responses after application of TTX (Fig. 6.2.D, n = 7). This reduction of an inward rectification indicates the subthreshold activation of a persistent Na$^+$-conductance, as in mature MGBv neurons (cf. Fig. 3.2.C in Chapter 3).
Fig. 6.2. Development of subthreshold membrane rectification.
A: In a P9 neuron, held at -72 mV, hyperpolarizing current pulses evoked a slowly developing inward rectification ("sag") at negative membrane potentials. B: In a P20 neuron at rest (-70 mV), hyperpolarizing current pulses evoked a prominent fast onset inward rectification at negative membrane potentials. C: I/V-relationships obtained at the end of current steps from A and B are linear at subthreshold membrane potentials and exhibit strong inward rectification below -90 mV in P9 and below -80 in P20. Note decrease in slope from P9 to P20, indicating a decrease in input resistance. D: In a different P9 neuron, application of TTX (300 nM) reduced the subthreshold voltage responses to current pulses.
Fig. 6.3. Development of passive membrane properties.
A: Resting membrane potentials gradually become more negative up to P14, remaining at these levels up to P21. Numbers of neurons per age are indicated below the bars and pertain also to graphs B and C. B: The input resistance (mean ± S.E. per day) decreased to mature levels up to P12, remaining constant up to P21. C: The time constant also decreased over a similar time course.
Development of passive membrane properties

We characterized the passive membrane properties of 192 MGBv neurons from age P5 to P21. The resting membrane potential was more positive in young neurons, declining over a similar time course as input resistance and time constant (Fig. 3). In mature neurons, the resting membrane potential was relatively hyperpolarized and often within the activation range of the T-type Ca\(^{2+}\)-current. The input resistance of MGBv neurons, calculated from small (<15 mV) voltage responses to current pulses, declined from P5 to P13 and remained stable thereafter (Fig. 6.3.B). A similar decrease was observed for the membrane time constant τ (Fig. 6.3.C), obtained from single exponentials fitted to the small voltage responses. The passive membrane properties and morphology changed over a similar developmental period and may be related (see Discussion).

Development of action potentials

Thresholds for the generation of action potentials were slightly increased in young neurons up to P9 (Fig. 6.4.A). Action potential shape was very different in young and mature neurons, with increased rates of rise and decay in mature neurons (Fig. 6.4.B). The action potential amplitude, measured from threshold was significantly increased in mature compared to young neurons (Fig. 6.4.D, n = 8 in each group). The most significant change was observed in the action potential duration, measured at half-amplitude (Fig. 6.4.E). These changes may cause changes in firing patterns.

Development of tonic firing patterns

In all immature neurons up to P11 (n = 32), depolarizing current pulses elicited initially only one action potential at pulse onset (Fig. 6.5.A).
Fig. 6.4. Development of action potentials.
A: Threshold potentials for action potential firing were slightly decreased in young neurons (< P10, numbers of neurons per age indicated below the bars). B: Comparison of typical action potentials recorded from P8 and P20 neurons. C: Action potential amplitude (mean ± S.E.) significantly increased in mature neurons (P20-21, n = 8), compared to young neurons (P5-9, n = 8). D: Action potential duration at half-amplitude (mean ± S.E.) significantly decreased in mature neurons (P20-21, n = 8), compared to young neurons (P5-9, n = 8).
Fig. 6.5. Development of tonic firing patterns.
A: Responses to increasing depolarizing current pulses (amplitude below traces) in a P9 neuron, held at -66 mV. Small current pulses evoked an early-onset single action potential. Larger current pulses evoked tonic firing of action potentials with steady firing frequency except at pulse onset. Note slow, large spike-AHPs. B: In a P20 neuron, increasing depolarizing current pulses at rest (-68 mV) evoked tonic firing after a delay. Larger current pulses shortened the delay to firing and elicited high-frequency tonic firing. Note fast after-spike AHPs. C: Firing frequency (1/last interspike interval) is plotted vs. current pulse amplitude. Young neurons (P5-9, holding potential around -70 mV) exhibit a lower threshold for train generation. Mature neurons (P19-21, resting potential around -70 mV) show a higher current threshold for train generation and steeper slopes in the firing frequency vs. current relationships.
The spike was followed by a slow AHP of large amplitude and long duration in contrast to mature neurons. This AHP may have prevented further firing. Increased current injection was necessary to evoke spike trains. Action potentials in immature neurons were sensitive to application of TTX (cf. Fig. 6.7.B, n = 11), as shown previously for mature MGBv neurons (Chapter 3, Tennigkeit et al. 1996). The immature tonic firing pattern disappeared gradually between P11 and P14. In mature neurons, the onset of tonic firing was usually preceded by a pronounced ramp-like delay and trains were evoked at threshold intensities (Fig. 6.5.B). The mature spike-AHPs have a fast time course and are Ca\(^{2+}\)-dependent (cf. Tennigkeit et al. 1996). Spike trains exhibited little frequency adaptation at all ages studied.

The frequency of tonic firing as a functions of injected current from potentials around -70 mV was studied in young (P5-9) and mature (P19-21) neurons. Young neurons exhibited far lower current thresholds for repetitive firing than mature neurons (Fig. 6.5.C). The slope of firing frequency/injected current was 0.11 ± 0.02 Hz/pA (n = 5) in young neurons and 0.16 ± 0.022 Hz/pA (n = 5) in mature neurons. Thus, young neurons required less current to reach similar firing frequencies as mature neurons, but more current was necessary to change the firing frequency in young than in mature neurons.

**HTS firing in young neurons**

In 55% of immature neurons (< P14, n = 44), strong depolarizing current pulses elicited doublet spikes, consisting of a large, short spike followed closely by a smaller, broad spike (Fig. 6.6.A). Mature neurons did not exhibit this firing pattern under control conditions, but after blockade of K\(^+\)-channels by 4-AP or TEA (Fig. 6.6.B, cf. Chapter 3). We tested the hypothesis that the doublets consist of a combination of a Na\(^+\)-action potential and an HTS.
Fig. 6.6. **HTSs during tonic firing in young neurons.**
A: Depolarizing d.c. injection evoked action potentials and spike doublets (one large spike coupled to a smaller, broad spike; asterisk). The smaller component of the spike doublets was blocked reversibly by removal of extracellular Ca$^{2+}$, leaving the large spikes intact. B: In a P19 neuron, similar doublets (asterisk) were evoked by depolarizing current pulse injection (40 pA) after application of 0.1 mM 4-AP. The smaller, broad spike of the doublet was also reversibly blocked by removal of extracellular Ca$^{2+}$. The large spikes remained intact with slowed repolarization.

Fig. 6.7. **Action potential and HTS firing patterns.** (next page)
A: In a P9 neuron, strong current pulse injection elicited spike doublets (asterisk) from a resting potential of -60 mV. After application of TTX, only the small, broad HTSs remained. Note higher threshold, but similar firing patterns as action potentials (first one short-onset spike with large AHP, spike trains after higher current injection). HTSs were blocked by application of Cd$^{2+}$ (50μM). B: In a P19 neuron, depolarizing pulses from rest (-69 mV) elicited firing after a delay. An increased current pulse elicited short onset tonic firing of action potentials. After application of TTX, similar current pulses elicited smaller voltage responses. Stronger current injection elicited HTSs, which were also delayed and evoked from a more depolarized potential than action potentials. The blockade of HTSs by application of Cd$^{2+}$ (50μM) was not overcome by increased current injection.
Removal of extracellular Ca\(^{2+}\) reversibly eliminated the smaller, broad spikes in young neurons (Fig. 6.6.A, \(n = 5\)) and the same spike type in mature neurons after 4-AP application (Fig. 6.6.B, \(n = 2\)). Application of Cd\(^{2+}\) (50 \(\mu\)M), a selective blocker of HTSs in MGBv neurons (Chapter 4), had the same effect in young (\(n = 2\)) and mature 4-AP treated neurons (\(n = 3\)).

In immature neurons, after TTX-blockade of action potentials (\(n = 11\)), broad, lower amplitude and Cd\(^{2+}\)-sensitive HTSs were evoked from a higher threshold (Fig. 6.7.A). Similar to action potentials, depolarization initially elicited single, fast-onset HTSs and trains after increased depolarization. In contrast, mature neurons fired action potential and HTS-trains after a delay to firing (Fig. 6.7.B).

We conclude that HTSs appear during tonic firing in immature neurons, but not in mature neurons. However, HTSs can participate in burst firing on the LTS in mature neurons (cf. Chapters 3 and 4).

**Development of the LTS burst response**

An obvious developmental feature was the absence of LTS burst responses in young MGBv neurons. Before P11, the LTS did not reach the firing threshold for action potentials (Fig. 6.8.A). On P11 and P12, only two neurons exhibited a burst of 3 spikes, 7 neurons had no spikes on the LTS and 7 neurons had only one spike (Fig. 6.8.B). This action potential was followed by a strong AHP that prevented a further voltage rise at this age. The LTS activation threshold was similar in neurons at all ages. Most mature neurons exhibited the typical LTS burst response of 3 or more spikes, followed by an HTS (Fig. 6.8.C). However, some neurons never elicit more than one or two spikes on the LTS (cf. Chapter 3, Tennigkeit et al. 1996). The average of the maximal number of spikes (action potentials and HTSs) evoked on an LTS as a function of age is shown in Fig. 6.8.D.
Fig. 6.8. Development of the LTS and burst firing.
A: In a young neuron (P8), small LTSs were evoked on the rebound from hyperpolarizing pulses, but did not elicit action potentials. B: In a P11 neuron, single action potentials were evoked on the LTS. Further voltage-rise is shunted by the spike-AHP. C: In mature neurons (P20), large LTSs were elicited from potentials below -75 mV. Bursts of action potentials and HTSs crowned the LTS, when elicited from below -80 mV. D: Plot of maximal number of spikes (mean ± S.E. per day, numbers of neurons studied above bars) vs. age shows no spikes on the LTS up to P10, then rise to an average of about 4 spikes /burst up to P15. Maximal number of spikes is unusually high on P21, due to absence of LTS-single-spike neurons in this group.
The neurons exhibit stable burst responses of about 4-5 spikes after P14. The value for P21 is higher, as there were no neurons with only one or two spikes in this group.

Development of resonance

The T-type Ca\(^{2+}\)-current plays an important role in the generation of thalamocortical synchronized oscillations. In the time-domain, it underlies the firing of spike bursts (Steriade et al. 1990), while in the frequency domain it endows thalamic neurons with resonant filter properties tuning them to particular frequencies (Hutcheon et al. 1994, Puil et al. 1994b). Interaction of this current with the intrinsic membrane properties is the mechanism for the generation of resonance around 1 Hz in MGBv (Chapter 5, Tennigkeit et al. 1994, 1997). In this study, resonance at such frequencies was observed in 20 out of 20 immature neurons (< P14, Fig. 6.9.A). However, the resonance was generally smaller and appeared at more negative values than in mature neurons (Fig. 6.9.B). The dependence of the resonance on the T-type Ca\(^{2+}\)-conductance was confirmed by blockade of the resonance by removal of Ca\(^{2+}\) from the extracellular medium (n = 5, not shown), as described previously in mature neurons (Chapter 5, Tennigkeit et al. 1994).

We conclude that resonance in immature neurons is only prominent at potentials, where the T-type Ca\(^{2+}\)-current is fully deinactivated (Coulter et al. 1989). At the depolarized resting potentials and spike thresholds of immature neurons, this resonant voltage range may be too hyperpolarized to have a major influence on burst generation.
A: ZAP current input elicited voltage responses at different holding potentials in a P9 neuron. Note increased low-frequency voltage response at -80 mV compared to other voltages. Plot of impedance magnitude vs. frequency from neuron in A reveals resonance at -80 mV as maximal voltage response around 0.8 Hz compared to lower or higher frequencies. Frequency responses at -60 and -70 mV did not exhibit resonance and displayed low-pass filter characteristics. B: In a P20 neuron, ZAP current input elicited increased low-frequency voltage response at -70 mV compared to other voltages. This is reflected in the frequency response as resonance around 0.8 Hz at -70 mV and low-pass filter characteristics at -60 and -80 mV.
6.3. **DISCUSSION**

*Morphological changes*

The observed increases in soma size and dendrite extension are similar to previous observations in MGBv and other thalamic nuclei (Coleman 1990, Perez-Velazquez and Carlen 1996, Scheibel and Scheibel 1978, Warren and Jones 1997). The expansion in membrane surface may partially account for the observed decrease in input resistance. The immature elaboration of the dendrites and expression of high-threshold Ca\(^{2+}\)-channels in the soma before dendrites (Jones et al. 1997) may have contributed to the observation of HTSs during tonic firing patterns in the soma (see below).

*Intrinsic membrane properties*

Subthreshold membrane rectification changed during the first two postnatal weeks. One of the most prominent signs in immature MGBv neurons was the presence of a depolarizing sag upon hyperpolarization, indicative of an \(I_H\), as observed previously in other young thalamic neurons and in mature MGBv after blockade of \(I_{KIR}\) (Pirchio et al. 1997, Tennigkeit et al. 1996, Warren and Jones 1997). However, as in our previous observations, the activation range of \(I_H\) was very negative (<-90 mV, cf. Fig. 6.2.A,C). Rises in internal Ca\(^{2+}\)-concentration may contribute to a modulation of \(I_H\) (Lüthi and McCormick 1998), as well as temperature dependence and sensitivity to internal cyclic AMP levels (Budde et al. 1997). A positive shift of the \(I_H\)-activation curve by 10 mV has been reported for an increase of 5°C (Watts et al. 1996), which could explain different activation ranges in mature MGBv neurons at different temperatures (Hu 1995, Tennigkeit et al. 1996). In this study, the activation of \(I_H\) was progressively occluded by the appearance of a fast-activating rectification
with a more positive activation range, due to an $I_{KIR}$, as observed previously (Fig. 6.2.C, cf. Chapter 3).

The changes in intrinsic membrane properties occurred up to the end of the second postnatal week, including increasingly hyperpolarized resting potentials, a decrease in input resistance and a shortening of time constants (Fig. 6.3.). Similar changes have been observed in other thalamic nuclei (Pirchio et al. 1997, Ramoa and McCormick 1994, Perez-Velazquez and Carlen 1996, Warren and Jones 1997). The decrease of the resting membrane potential has been attributed previously to an increased $\text{Na}^+$/K$^+$-pump activity (Fukuda and Prince 1992), increased K$^+$-currents (Spigelman et al. 1992) and a decrease in the Cl$^-$-reversal potential for GABA-activated currents (Zhang et al. 1991). The decrease in input resistance is usually attributed to the increased membrane area (cf. Fig. 6.1.) and a concomitant shortening of the membrane time constant suggests that specific membrane resistance decreases, assuming constant specific membrane capacitance. Such a conductance increase is consistent with an increased density of ion channels during maturation (cf. Huguenard et al. 1988, Spigelman et al. 1992).

**Action potentials and tonic firing patterns**

The firing threshold for action potentials was slightly depolarised in young neurons (Fig. 6.4.A), which has been attributed previously to an increase in Na$^+$-channel density during postnatal development (Huguenard et al. 1988). Action potentials from young neurons had a significantly lower amplitude and longer duration compared to those from mature neurons (Fig. 6.4.C,D). While the values for spike duration are higher than in other studies, presumably arising from experimentation at room temperature (cf. Thompson et al. 1985), the observed developmental changes are consistent with previous findings in thalamic
neurons (Pirchio et al. 1997, Ramoa and McCormick 1994, Perez-Velazquez and Carlen 1996, Warren and Jones 1997). The lower amplitude and the decreased rate of rise could be due to a lower density of Na$^+$-channels in the membrane (cf. Huguenard et al. 1988), whereas the increased duration may result from an increased Ca$^{2+}$-contribution to the falling phase of the action potential (cf. Chapter 3) and weak spike repolarizing mechanisms (Spigelman et al. 1992).

Using depolarizing current pulses from potentials around -70 mV, the tonic firing properties were compared in immature and mature neurons. Tonic firing patterns differed in several aspects. A prominent feature in young neurons is the fast onset of tonic firing (Fig. 6.5A), while mature neurons typically exhibit a delay to firing due to the presence of a subthreshold, slowly inactivating A-type K$^+$-current (Fig. 6.5B, cf. Tennigkeit et al. 1996). We presume that this current is not fully developed in immature neurons. Another unique feature in young neurons is the firing of single action potentials at low current inputs, while in mature neurons, spike trains are elicited after reaching the firing threshold (Fig. 6.5A,B). We attribute this single spike firing predominantly to the presence of large spike-AHPs, that prevent the generation of further action potentials. Consistent with this hypothesis, stronger current pulses could readily elicit spike trains. Large AHPs, both after spikes and spike-trains, are common findings in immature neurons (Pirchio et al. 1997, Ramoa and McCormick 1994, Warren and Jones 1997) and have been attributed to changes in the development of Ca$^{2+}$-activated K$^+$-currents (Perez-Velazquez and Carlen 1996, Spigelman et al. 1992).

Young neurons had a much lower current threshold for firing than mature neurons, which we attribute to the higher input resistance (note that curves in Fig. 6.5C were obtained from hyperpolarized starting potentials for comparison with mature resting potentials). Also, the resting membrane potential in immature neurons is closer to the firing threshold as in
mature neurons (cf. Fig. 6.3.A and 6.4.A). On the other hand, the function of firing frequency vs. current input exhibited a steeper slope in mature neurons, presumably due to the dramatic changes in the spike-AHP, as described above.

The development of intrinsic membrane properties may contribute to the observation of immature MGBv firing patterns *in vivo*, i.e. “on” responses of one or two spikes, but no trains or bursts (Pujol et al. 1970, Pujol 1972, cf. Pirchio et al. 1997). However, maturation of synaptic inputs and neurotransmitter receptor systems may play a role as well (Catania et al. 1994, Laurie et al. 1992, Turgeon and Albin 1994, Warren and Jones 1997).

*A role for high-threshold Ca\(^{2+}\)-spikes during development*

In about 50% of young neurons, HTSs contributed to tonic firing patterns upon strong depolarizing current injection. The HTSs were appended to action potentials as spike doublets (Fig. 6.6.). Such observations have not been reported previously. The favourable conditions of this study (room temperature and high-resistance seals) may contribute to these findings. The HTSs in young neurons exhibited similar properties as those observed previously in mature MGBv in regard to activation threshold and sensitivity to TTX, Ca\(^{2+}\) and Cd\(^{2+}\) (Chapters 3 and 4, Tennigkeit et al. 1996, 1998b). However, they also exhibited developmental features similar to the action potentials described above such as large AHPs of long duration, short delay to onset and single spike firing before train generation (Fig. 6.7.A). We attribute the presence of HTSs during tonic firing under control conditions to weaker K\(^{+}\)-currents in young neurons, as mature neurons exhibited similar firing patterns after application of the K\(^{-}\)-channel blockers 4-AP and TEA (Fig. 6.7.B, cf. Chapters 3 and 4). These findings are also consistent with previous observations, implying a control of dendritic HTS firing by persistent Na\(^{+}\) and K\(^{+}\)-currents (Chapter 4, Schwindt and Crill 1997). Also, the higher
resting potential, increased input resistance, the proximity of dendritic Ca\textsuperscript{2+}-channels due to short dendritic trees (cf. Fig. 6.1.) and the expression of Ca\textsuperscript{2+}-channels on somata before dendrites (Jones et al. 1997) may contribute to HTS firing in young neurons.

Contributions of HTSs to tonic firing in developing MGB\textsubscript{v} neurons may link electric activity to morphological changes and gene expression during MGB\textsubscript{v} development (see review by Spitzer et al. 1994).

_Burst firing on the LTS_

The absence of LTS burst firing is a prominent feature of immature MGB\textsubscript{v} neurons (Fig. 6.8.A). In very young neurons (<P11), the LTS did not couple to action potential firing at all. The main reason for this seems to be the developmental increase in the T-type Ca\textsuperscript{2+}-current (Pirchio et al. 1990, Ramoa and McCormick 1994). Contributing factors would also include the higher threshold for action potential generation in immature neurons (Fig. 6.4.A), an increased Ca\textsuperscript{2+}-activated K\textsuperscript{+}-current (Perez-Velazquez and Carlen 1996) and activation of subthreshold A-type K\textsuperscript{+}-currents (Huguenard et al. 1991, Pape et al. 1994, Tennigkeit et al. 1998). We confirmed previous observations of similar LTS activation ranges at all ages (Fig. 6.8., Pirchio et al. 1990 and 1997, Ramoa and McCormick 1994, Warren and Jones 1997). Action potentials of P11-13 neurons frequently prevented a further voltage rise due to their prominent AHPs (Fig. 6.8.B), while the LTS in mature neurons was powerful enough to reach threshold for HTSs (Fig. 6.8.C, cf. Chapters 3 and 4).

The LTS is a prerequisite for the generation of the burst firing mode during slow-wave sleep and absence seizures (see review Steriade et al. 1990). Previous _in vitro_ studies have linked the ability to burst with the onset of spindle waves (McCormick et al. 1995, Warren and Jones 1997). The observed age of onset of LTS bursting due to an increase in
LTS amplitude, the maturation of action potentials and membrane properties coincides with the onset of synchronized oscillations during deep sleep states in the developing rat EEG (Jouvet-Mounier et al. 1970, Mares et al. 1982).

**Development of frequency preference**

We used frequency-analysis methods (ZAP, cf. Puil et al. 1986, 1994b) to study the frequency preferences at different membrane potentials during postnatal development (Fig. 6.9.). Immature neurons exhibited similar features as mature neurons, i.e. low-pass filter characteristics at depolarized membrane potentials (near rest in young neurons, cf. Fig. 6.3.A) and a resonance around 1 Hz at hyperpolarized potentials (Fig. 6.9.A). While resonance was present in young neurons, it was often smaller and had its maximum at more hyperpolarized potentials compared to mature neurons. This is consistent with the observations of changes in the LTS (see above). Both features depend on the T-type Ca\(^{2+}\)-current, that requires strong hyperpolarization for complete deinactivation. Less deactivation is needed to observe a similar LTS response and resonance in mature than in immature neurons.

Insufficient T-current activation, or the activation of I_{KIR} which is not developed in immature neurons, may contribute to the reduction in resonance at more hyperpolarized potentials in mature neurons (Fig. 9B, Hutcheon et al. 1996, Puil et al. 1994b).

The developmental increase of the T-type Ca\(^{2+}\)-current is consistent with increased resonance and intrinsic oscillations (Lampl and Yarom 1997, Pirchio et al. 1997), which filter synaptic input and spike output (cf. Tennigkeit et al. 1997). Maturity of these features may be a prerequisite for synchronized sleep oscillations in MGBv.
Conclusion

Morphology, membrane properties, firing patterns and frequency preference undergo major changes during the first two postnatal weeks of rat MGBv development. The changes in firing patterns (early onset, single spike firing, HTSs during tonic firing, no burst firing) imply different balances of ionic currents during development. These changes may be necessary to integrate morphological changes, firing patterns and gene expression, depending on auditory activity. The conclusion of this developmental process around P13 may contribute to the onset of functional hearing and synchronized sleep oscillations.
7. Effects of metabotropic glutamate receptor activation

7.1. INTRODUCTION

Neurons in the MGBv receive excitatory auditory input from the inferior colliculus and from the primary auditory cortex (Andersen et al. 1980, Morel and Imig 1987, Winer and Larue 1987, see Jones 1985 for review). Both afferent sensory and corticothalamic inputs are glutamatergic (Hu et al. 1994, Bartlett and Smith 1995). However, only corticothalamic inputs activate metabotropic glutamate receptors (mGluRs), producing long-lasting excitatory responses (Bartlett and Smith 1995, He 1997). In other thalamic nuclei, these effects were mediated by postsynaptic mGluR 1 activation (Eaton and Salt 1996, Godwin et al. 1996a,b; Martin et al. 1992, Salt and Eaton 1996).

Activation of mGluRs can couple to a variety of effector mechanisms (see review by Pin and Duvoisin 1995). Synaptic stimulation and 1S,3R-ACPD-application can evoke postsynaptic currents associated with decreases of membrane conductance due to blockade of K+-currents (e.g. Charpak and Gähwiler 1991, Charpak et al. 1990, Guérineau et al. 1994, McCormick and von Krosigk 1992). Other mechanisms include the activation of a Na+/Ca2+-exchanger (Linden et al. 1994, Keele et al. 1997, Staub et al. 1992) and activation of Ca2+-dependent or -independent cation currents (Crépel et al. 1994, Guérineau et al. 1995, Mercuri et al. 1993, Zheng et al. 1995). In many instances, mGluR activation evokes multiple responses in a neuron (Crépel et al. 1994, Guérineau et al. 1995, Keele et al. 1997). In addition to these postsynaptic mechanisms, mGluRs have important roles in presynaptic modulation of transmission, synaptic plasticity and neuronal death (see reviews by Nicoletti et al. 1996, Pin and Duvoisin 1995).
Despite the widespread implications, the effects of mGluR-activation have not been studied in auditory thalamic neurons. In this chapter we used whole-cell patch-clamp recordings in the voltage- and current-clamp modes to describe, for the first time, the ionic mechanisms, G-protein- and voltage-dependence of mGluR-activated currents and their effects on firing patterns and frequency preference in MGBv.

7.2. RESULTS

Application of 1S,3R-ACPD depolarizes MGBv neurons

Activation of mGluRs by application of the selective agonist 1S,3R-ACPD depolarized all MGBv neurons studied (n = 67). Recovery from the depolarization elicited by an application of short duration (30-60 s) usually required 5-10 min (Fig. 7.1.A,B). The depolarization was not associated with a significant change in input conductance, measured from the voltage responses to hyperpolarizing current pulses from the original resting membrane potential (Fig. 7.1.A). We observed a small, but insignificant increase in input conductance of 5.4 ± 2.8 % (mean ± S.E., n = 12) under control conditions. The small increase was attributed to the activation of a slow afterhyperpolarization after tonic firing activity in some neurons, which was evoked concomitantly by the 1S,3R-ACPD-induced depolarization (cf. Fig. 7.4.A). In confirmation of this hypothesis, the conductance change was 0.6 ± 3.5 % on application of TTX (n = 7, cf. Fig. 7.7.A). The lack of a slope change in the V\f \text{-relationship near rest for the 1S,3R-ACPD-induced current} (see below) also implied that the depolarization was not associated with a change in input conductance. In two neurons, application of L-glutamate in the presence of the ionotropic glutamate receptor blockers CNQX (10 μM) and APV (50 μM) also elicited a reversible depolarization.
Fig. 7.1. 1S,3R-ACPD depolarizes MGBv neurons.
A: Application of 1S,3R-ACPD (50 μM for 30 sec) depolarized an MGBv neuron (Vrest = -64 mV). Voltage responses to current pulses before (1), during (2,3) and after (4) the effects of 1S,3R-ACPD application expanded below. Note similar voltage responses in 1,2 and 4 and tonic firing in 3. B: After application of TTX (300 nM) and Cd²⁺ (0.1 mM), a similar application depolarized another MGBv neuron. After recovery (~6 min), application of 1S,3R-ACPD for 7.5 min elicited a depolarization of similar amplitude. Note small desensitization of response (10-20%) after 8 min. C: Concentration-dependent depolarization evoked by 1S,3R-ACPD (conditions like B, first application). Bars represent the mean ± S.E. of the 1S,3R-ACPD-induced depolarization (ΔV in mV) measured at the peak response. Number of neurons tested for each concentration above bars.
In an initial series of experiments, the concentration-dependence of the 1S,3R-ACPD-evoked depolarization was established in 35 neurons in the presence of TTX (300 nM) and Cd\(^{2+}\) (100 \(\mu\)M) to block Na\(^{+}\) - and high-threshold Ca\(^{2+}\)-currents (cf. Chapters 3 and 4). Under these conditions, the depolarization evoked by 1S,3R-ACPD application (50 \(\mu\)M, 30 sec) was significantly reduced compared to control conditions (cf. Fig. 7.1.A and B, note different scale), probably due to blockade of an amplifying persistent Na\(^{+}\)-conductance (cf. Tennigkeit et al. 1998b). In the same neuron, 1S,3R-ACPD-application for 7.5 minutes did not increase the maximal amplitude of the depolarization and resulted in a small desensitization (10-20\% of the response. The neuron fully recovered within 10-20 min after the application, particularly after long applications (4-8 min, n = 6). Thus short 1S,3R-ACPD applications (50 \(\mu\)M, 30-60 s) reversibly elicited a maximal depolarizing response. The concentration-dependence of the 1S,3R-ACPD-evoked depolarization in the presence of TTX and Cd\(^{2+}\) is illustrated in Figure 7.1.C. Only one concentration was applied per neuron (n = 32), except in three cases. The concentration-dependence of the depolarization confirmed that applications of 1S,3R-ACPD at 50 \(\mu\)M elicited a maximal depolarizing response. Several responses of similar amplitude were elicited after sufficient recovery intervals (5-10 min, cf. Fig. 7.1.B). Similar procedures were used to investigate how the mGluR-antagonist, MCPG, ionic channel blockers and changes in extracellular ionic conditions altered the 1S,3R-ACPD response.

**MGlur-antagonism and G-protein dependence of 1S,3R-ACPD effects**

Application of the mGluR antagonist MCPG (0.5 mM) reversibly reduced the depolarization induced by 1S,3R-ACPD to 36.8\% of control (Fig. 7.2.A).
Fig. 7.2. MCPG blocks 1S,3R-ACPD effects.
A: Bath-application of MCPG (0.5 mM) reversibly blocked the depolarization by 1S,3R-ACPD (50 μM). B: MCPG also blocked $I_{ACPD}$, evoked under voltage-clamp conditions (TTX and Cd$^{2+}$).
In the presence of TTX and Cd\textsuperscript{2+} in another neuron, MCPG reduced the depolarization to 44.4 % of control. Application of MCPG by itself evoked no changes in membrane potential or input resistance. Under voltage-clamp conditions (see Methods) application of MCPG (0.5 mM) reduced the 1S,3R-ACPD-evoked inward current (I\textsubscript{ACPD}) to 43.8 % of control (Fig. 7.2.B). As demonstrated previously in other thalamic neurons (Salt and Eaton 1995), these results demonstrate that MCPG antagonizes the 1S,3R-ACPD-evoked responses in MGBv neurons.

The involvement of G-proteins in the activation of I\textsubscript{ACPD} was studied by replacing GTP with the non-hydrolysable GTP-analogs, GTP\textgamma{}S and GDP\textbeta{}S (both at 0.3 mM), in the recording electrode. When GTP\textgamma{}S was applied internally, I\textsubscript{ACPD} was irreversibly activated upon application 1S,3R-ACPD (Fig. 7.3.A, n = 3). While the current was activated (up to 20 min), a second 1S,3R-ACPD application had no effect (Fig. 7.3.A). The magnitude of I\textsubscript{ACPD} was 148.3 ± 23.9 pA or 76.2 ± 28.4 % (n = 3) more than the average amplitude of I\textsubscript{ACPD} (84.2 ± 5.4 pA, n = 22, cf. Fig. 7.9.D). In contrast, I\textsubscript{ACPD} was reduced in the presence of internally applied GDP\textbeta{}S. This effect was enhanced after prolonged perfusion, in a manner similar to that observed with BAPTA in the recording electrode (see below). The maximal amplitude of I\textsubscript{ACPD} at 10 min after breaking through the cell membrane was 65.7 ± 3 pA or 22.0 ± 3.5 % less than the average I\textsubscript{ACPD} (Fig. 7.3.B, n = 3). When measured 20 to 30 min after breakthrough, the maximal amplitude of I\textsubscript{ACPD} was 32 ± 1.7 pA or 62.0 ± 2.1 % less than the average I\textsubscript{ACPD} (n = 3). These results imply an involvement of G-proteins in the activation of I\textsubscript{ACPD} and a role for GTP hydrolysis in the deactivation of I\textsubscript{ACPD}. 
Fig. 7.3. G-protein dependence of $I_{ACPD}$.
A: Inclusion of the non-hydrolysable GTP analog GTPγS in the recording electrode resulted in a large and irreversible $I_{ACPD}$ in the presence of TTX and Cd$^{2+}$. A second application of 1S,3R-ACPD 12 min later evoked no additional current. B: After inclusion of GDPβS in the recording electrode a small, reversible $I_{ACPD}$ was evoked 10 min after breakthrough. A second application of 1S,3R-ACPD 20 min later evoked an even smaller $I_{ACPD}$.
**Na⁺-dependence of 1S,3R-ACPD effects**

The 1S,3R-ACPD induced depolarization was not associated with a change in input conductance. Hence, we examined the possibility that activation of an inward current, rather than blockade of outward K⁺-currents mediated the depolarization (cf. McCormick and von Krosigk 1992). Indeed, a reduction of the extracellular Na⁺-concentration (from 150 mM to 26 mM, "low Na⁺") reversibly reduced the 1S,3R-ACPD-evoked depolarization to 10.7 and 14.1 % of control (Fig. 7.4.A, n = 2). In these neurons, the reduction of the extracellular Na⁺-concentration itself evoked a reversible hyperpolarization of 2 and 5 mV. The low Na⁺-conditions also reduced the rate of rise and amplitude of action potentials, but increased their duration (Fig. 7.4.B). The amplitude of the low-threshold Ca²⁺-spike (LTS) was also decreased and we observed a reduction of an inward rectification at very hyperpolarized potentials (Fig. 7.4.B). These effects were attributed to a reduction of the fast and persistent Na⁺-currents as well as a hyperpolarization-activated cation current (I₇), respectively. Because the reduction in these currents, particularly the persistent Na⁺-current, could have contributed to the blockade of the 1S,3R-ACPD-evoked depolarization, the effects of extracellular Na⁺-reduction on depolarization and IACPD were studied during TTX-application. Under these conditions, the change to low Na⁺ medium evoked a small hyperpolarization of 2.8 ± 1.1 mV (n = 5) or a steady-state outward current of 48 ± 21.8 pA (n = 5) under voltage-clamp conditions. The 1S,3R-ACPD-evoked depolarization was reversibly reduced by 70.4 ± 9.4 % of control (n = 4, not shown). Using voltage-ramp commands (see Methods) during TTX and Cd²⁺ application, the inward current IACPD had a V/I relationship which was parallel to the voltage-axis between -40 and -85 mV (cf. Figs. 7.5.-7.10.). Therefore, the action of 1S,3R-ACPD was not associated with a change in input conductance (n = 29).
Fig. 7.4. Low extracellular Na⁺ blocks depolarization.
A: Under control conditions, 1S,3R-ACPD (50 µM) depolarized an MGBv neuron from rest (-68 mV) to firing threshold. Hyperpolarization by negative d.c. injection back to rest reveals small increase in input conductance, as seen from downward voltage responses to continuous current pulses (-100 pA, 500 ms). This depolarization was reversibly reduced under low extracellular Na⁺-conditions. B: Low extracellular Na⁺-conditions evoked a small hyperpolarization (4 mV), compensated by d.c. injection. Firing patterns evoked by current pulses are similar, but action potentials show a decreased rate of rise and amplitude as well as an increased duration. Note reduction of LTSs despite decreased hyperpolarizing rectification.
Fig. 7.5. Blockade of $I_{ACPD}$ by low extracellular Na$^+$. 
A: Under voltage-clamp conditions, 1S,3R-ACPD (50 μM) application evoked an inward current (inset, $V_m$ = -65 mV). Current-responses to hyperpolarizing voltage-ramps (see methods for protocol) before and after application of 1S,3R-ACPD reveal $I_{ACPD}$ by subtraction. The $V/I$-relationship of $I_{ACPD}$ is mostly parallel to the $V$-axis with an inward slope below -90 mV, reversing at -109 mV. 
B: Low extracellular Na$^+$-conditions reduced $I_{ACPD}$. Note removal of slope at hyperpolarized potentials. 
C: Complete recovery was obtained.
During TTX and Cd\textsuperscript{2+} application, a reduction of the extracellular Na\textsuperscript{+}-concentration reversibly reduced $I_{\text{ACPD}}$ by 67.2 ± 10.3 % (Fig. 7.5., $n = 5$, $V_{m} = -70$ mV). These results implicate the activation of a Na\textsuperscript{+}-dependent current as a major mechanism for the 1S,3R-ACPD-evoked depolarization.

$Li^{+}$-dependence of 1S,3R-ACPD effects

To distinguish between possible activation of a Na\textsuperscript{+}-dependent current or a Na\textsuperscript{+}/Ca\textsuperscript{2+}-exchanger, 1S,3R-ACPD-effects were studied after replacement of 50 mM Na\textsuperscript{+} with Li\textsuperscript{+} in the ACSF (cf. Crépel et al. 1994, Keele et al. 1997). The partial replacement with 50 mM Li\textsuperscript{+} evoked a reversible depolarization of 4.8 ± 1.2 mV ($n = 4$) and a steady-state current of -60 pA (Fig. 7.6.B), attributed to partial inhibition of the Na\textsuperscript{+}/K\textsuperscript{+}-exchanger. During partial Li\textsuperscript{+}-replacement, the 1S,3R-ACPD-evoked depolarization was 8.3 ± 0.6 mV or 99.8 ± 12.1 % of control ($n = 4$, not shown). Under these conditions, $I_{\text{ACPD}}$ was 93% of control (Fig. 7.6.). The inward rectification below -90 mV, partially due to $I_{\text{H}}$, was reversibly reduced by Li\textsuperscript{+}, in accordance with results in cardiac cells (Maruoka et al. 1994). Our results indicate that Li\textsuperscript{+} can replace Na\textsuperscript{+} as a charge carrier for $I_{\text{ACPD}}$, and hence, a mechanism different from the activation of a Na\textsuperscript{+}/Ca\textsuperscript{2+}-exchanger (cf. Crépel et al. 1994, Keele et al. 1997).
Fig. 7.6. Extracellular Li\(^+\) and I\(_{\text{ACPD}}\).
A: Under voltage-clamp conditions, hyperpolarizing voltage-ramps evoked almost parallel current-responses before and after application of 1S,3R-ACPD, except at very hyperpolarized potentials (<-90 mV), obvious as slope change in subtracted I\(_{\text{ACPD}}\). B: Partial replacement of extracellular Na\(^+\) with Li\(^+\) (50 mM) changed I\(_{\text{ACPD}}\) only at potentials <-90 mV. C: Note partial recovery with similar hyperpolarizing rectification as A.
1S,3R-ACPD effects are sensitive to changes in intracellular, but not extracellular Ca$^{2+}$

Replacement of Ca$^{2+}$ with Mg$^{2+}$ in the ACSF did not significantly change the 1S,3R-ACPD evoked depolarization. On application of TTX and Cd$^{2+}$, the depolarization was 94.3 ± 4.6 % of control (n = 3, Fig. 7.7.A). Replacement of Ca$^{2+}$ with 10 mM Mg$^{2+}$ and coapplication of 1 mM EGTA in the ACSF has been shown previously to block a 1S,3R-ACPD-evoked inward current with a V/I-relationship similar to that described here (Keele et al. 1997). However, these conditions in the MGBv reversibly increased $I_{ACPD}$ by 18.7 % (n = 1, $V_m$= -70 mV, Fig. 7.7.B,C). These results, together with the fact that $I_{ACPD}$ could be evoked after blockade of high-threshold Ca$^{2+}$-currents by extracellular application of Cd$^{2+}$ (cf. Figs. 7.1.B, 7.5.-7.10.), provide evidence that voltage-dependent Ca$^{2+}$-currents are not required as a trigger for $I_{ACPD}$. However, this does not exclude the possibility of Ca$^{2+}$-dependent augmentation of $I_{ACPD}$ (see Discussion).

The inclusion of 10 mM BAPTA in the recording electrode to chelate intracellular Ca$^{2+}$ caused a time-dependent reduction of the 1S,3R-ACPD-evoked depolarization and $I_{ACPD}$. On application of TTX and Cd$^{2+}$, the 1S,3R-ACPD-evoked depolarization in two neurons was reduced by 49.4 and 48.1 % (cf. concentration-response relationship, Fig. 7.1.C) following a 12 or 30 min intracellular perfusion respectively. Under voltage-clamp conditions, $I_{ACPD}$ was 6.9 % greater than the average $I_{ACPD}$ (cf. Fig. 7.9.D) after 12 min in one neuron and was completely blocked after 30 min in another (not shown). The time-dependence of the BAPTA effect may relate to modulation of processes far from the electrode tip (see above Results on G-protein modulation). Thus, the possibilities exist, that a rise of the internal Ca$^{2+}$-concentration may activate the 1S,3R-ACPD-evoked depolarization and $I_{ACPD}$ or that Ca$^{2+}$-dependent phosphorylation may modulate $I_{ACPD}$. 
Fig. 7.7. Depolarization and $I_{ACPD}$ depend on intracellular, but not extracellular $Ca^{2+}$. 
A: After application of TTX (300 nM) and Cd$^{2+}$ (0.1 mM) to block $Na^+$ and high-threshold $Ca^{2+}$-spikes (control), 1S,3R-ACPD (50 μM) reversibly depolarized an MGBv neuron (Vm=-73 mV). Upward deflections are LTSs. Hyperpolarization by negative d.c. injection back to rest revealed no change in input conductance, indicated by voltage responses to continuous current pulses (-200 pA, 500 ms). Equimolar replacement of extracellular $Ca^{2+}$ with Mg$^{2+}$ did not change 1S,3R-ACPD-induced depolarization, but blocked low-threshold $Ca^{2+}$-spikes. B: Under voltage-clamp conditions, 1S,3R-ACPD (50 μM) application evoked an inward current (inset, Vm=-70 mV). Note parallel current-responses to hyperpolarizing voltage-ramps before and after application of 1S,3R-ACPD, resulting in $I_{ACPD}$ parallel to V-axis. C: Replacement of extracellular $Ca^{2+}$ with Mg$^{2+}$ (10 mM) and coapplication of EGTA (1 mM) did not change $I_{ACPD}$. 
Voltage-independent $I_{ACPD}$ is insensitive to changes in extracellular $K^+$ and $Ba^{2+}$, $Cs^+$

Because a combined activation of an inward current and blockade of an outward $K^+$-current could have resulted in the parallel V/I relationship of $I_{ACPD}$ between -40 and -85 mV (cf. Crépel et al. 1994, Shen and North 1992), we tested the sensitivity of $I_{ACPD}$ to changes in extracellular $K^+$-concentration and applications of the $K^+$-channel blockers $Ba^{2+}$ and $Cs^+$ in this voltage range. Changing extracellular $K^+$ from 5.25 to 2.5 mM reduced $I_{ACPD}$ slightly to 80.2 ± 7.6 % of control ($n = 3, V_m = -70$ mV, Fig. 7.8.A-C). The absolute values of $I_{ACPD}$ under control, $K^+$-channel blockade, reduced extracellular $K^+$- and $Na^+$-conditions are shown in Fig. 7.8.D. In 9 out of 20 neurons, $I_{ACPD}$ was voltage-independent below -40 mV (Fig. 7.9.A). Application of $Ba^{2+}$ (0.1-1 mM) did not significantly change $I_{ACPD}$ (97.7 ± 4.2 % of control; $n = 3, V_m = -70$ mV, Fig. 7.9.B). The current induced by $Ba^{2+}$ was linear and reversed close to the calculated $E_K$ (-85 mV), implying a blockade of leak $K^+$-currents, as described previously (cf. Fig. 2 in Crépel et al. 1994). Application of $Cs^+$ (2.5-3 mM) or coapplication of $Ba^{2+}$ and $Cs^+$ gave similar results, not affecting $I_{ACPD}$ significantly (107.7 ± 8.4 % of control; $n = 3, V_m = -70$ mV, Fig. 7.9.C). In summary, the results with application of $Ba^{2+}$, $Cs^+$ and reduced extracellular $K^+$ did not produce major changes in current amplitude and V/I relationship; we conclude, that the generation of the voltage-independent components of $I_{ACPD}$ between -40 and -85 mV does not primarily involve a blockade of $K^+$-currents.

Application of $Ba^{2+}$ and $Cs^+$ blocks voltage-dependent components of $I_{ACPD}$

In 11 neurons, 1S,3R-ACPD application induced a slope in the V/I relationship of $I_{ACPD}$ below -85 mV (Fig. 7.10.A).
Fig. 7.8. $I_{ACPD}$ in low extracellular $K^+$.  
A: Under voltage-clamp conditions, 1S,3R-ACPD (50 µM) application evoked an inward current (inset, $V_m$ = -70 mV). Hyperpolarizing voltage-ramps before and after application of 1S,3R-ACPD evoked parallel current-responses. Note $I_{ACPD}$ parallel to $V$-axis. 
B: Low extracellular $K^+$-conditions shifted $V_{rest}$ to -77 mV and caused a small reduction in $I_{ACPD}$. 
C: Note also smaller recovery as A. Control current responses A-C are averages of 5 traces. 
D: Summary of maximal $I_{ACPD}$ (in pA, $V_m$ = -70 mV) under control and different extracellular ionic conditions. Error bars represent S.E., numbers of neurons per condition above.
Fig. 7.9. $I_{\text{ACPD}}$ is insensitive to Ba$^{2+}$ and Cs$^{+}$.

A: Under voltage-clamp conditions, hyperpolarizing voltage-ramps before and after application of 1S,3R-ACP (50 μM) resulted in parallel current-responses and $I_{\text{ACPD}}$ parallel to V-axis. B: Application of extracellular Ba$^{2+}$ (1 mM) induced a linear current, reversing at -81 mV, but did not change $I_{\text{ACPD}}$. C: Coapplication of extracellular Ba$^{2+}$ (1 mM) and Cs$^{+}$ (3 mM) also did not change $I_{\text{ACPD}}$. Control current responses A-C are averages of 5 traces.
In 6 of these neurons, $I_{ACPD}$ exhibited a reversal potential of $-101.2 \pm 1.7 \text{ mV}$. The blockade was occluded after application of extracellular $\text{Ba}^{2+}$ (0.1 mM, not shown) and after application of $\text{Cs}^{+}$ (2.5 mM, Fig. 7.10.A, $n = 3$) implying the blockade of an inward rectifier, like $I_{KIR}$. A reversible blockade of voltage-dependent responses below -85 mV also was observed under low $\text{Na}^{+}$ and $\text{Li}^{+}$-conditions (Figs. 7.5.B and 7.6.B, $n = 6$). This suggested reduction of a hyperpolarization-activated cation current ($I_{H}$) due to a negative shift in the reversal potential and $\text{Li}^{+}$-blockade (Maruoka et al. 1994), respectively. However, this current was not implicated in the voltage-dependent responses below -85 mV of $I_{ACPD}$ as it was not blocked by 1S,3R-ACPD and is insensitive to $\text{Ba}^{2+}$ (1 mM) in MGBv (Chapter 3).

After blockade of $\text{Na}^{+}$- and $\text{Ca}^{2+}$-spikes by TTX and Cd$^{2+}$, the application of large voltage ramps resulted in a linear slope for $I_{ACPD}$ at potentials positive to -40 mV, reversing at $-23.3 \pm 3.8 \text{ mV}$ ($n = 7$, Fig. 7.10.A). This response was blocked and a reversal potential was not observed on extracellular application of $\text{Cs}^{+}$ (2.5 mM, $n = 2$), implying a blockade of a voltage-dependent $K^{+}$-current (Fig. 7.10.B).

**Firing pattern changes and frequency response shifts by mGluR-activation**

Typically small depolarizing current pulses elicited LTS-burst firing in an MGBv neuron at rest (-75 mV, Fig. 7.11.A). During the 1S,3R-ACPD-evoked depolarization the evoked firing pattern changed to delayed tonic firing and immediate-onset tonic firing, as described previously ($n = 17$, cf. Chapter 3). Injection of negative d.c. brought the neuron back to the original resting membrane potential. However, the response was reduced to a single spike on the LTS, possibly due to dendritic depolarization and T-current deinactivation (see Discussion). A similar reduction of the burst response also was observed using
Fig. 7.10. Voltage-dependent components of $I_{ACPD}$.
A: Using large voltage-ramps, $I_{ACPD}$ is parallel between -40 and -85 mV and exhibits two voltage-dependent components above and below, resulting in reversal potentials of -108 and -23 mV (arrowheads). B: Both voltage-dependent components were blocked after application of Cs$^+$ (2.5 mM) and no reversal potentials are observed.
hyperpolarizing current pulses in 10 out of 17 neurons studied, full recovery of the burst response was observed within 15 min in 7 out of these 10 neurons. Under control conditions, the 1S,3R-ACPD-evoked depolarization reached threshold, eliciting spontaneous tonic firing of action potentials in 12 out of 15 neurons (cf. Figs. 7.1.A and 7.4.). In two neurons, 1S,3R-ACPD-evoked depolarization evoked bursts of action potentials and plateau potentials (not shown). In both neurons, application of TTX completely abolished the bursts and plateau potentials, leaving the 1S,3R-ACPD-evoked depolarization intact. Similar effects have been observed previously and may be due to Ca\(^{2+}\)-dependent augmentation of I\(_{ACPD}\), blockade of repolarizing K\(^+\)-currents, dendritic activation of high-threshold Ca\(^{2+}\)-spikes or a combination of these (cf. Klink and Alonso 1997, Tennigkeit et al. 1998b, Zheng et al. 1996).

The intrinsic membrane properties confer distinct voltage-dependent frequency preferences to MGBv neurons (Chapter 5, Tennigkeit et al. 1994, 1997). Normally, MGBv neurons exhibit lowpass filter characteristics at depolarized potentials. Close to rest, they exhibit a resonance near \(-1\) Hz (Fig. 7.11.B) due to interaction of the T-type Ca\(^{2+}\)-current with other intrinsic properties. During the 1S,3R-ACPD-evoked depolarization the frequency response reversibly shifted from resonant to non-resonant, but d.c. hyperpolarization reversed this shift (Fig. 7.11.B). Thus, activation of mGluRs shifts frequency responses due to its membrane depolarizing effects, but does not change the voltage-dependent currents, which underlie the impedance profile of MGBv neurons, or input conductance which can change the frequency responses at all membrane potentials (cf. Chapter 5).
Fig. 7.11. 1S,3R-ACPD-evoked firing pattern changes and frequency preference shifts.

A: An MGBv neuron (Vrest = -75 mV) depolarized by ~20 mV after bath-application of 1S,3R-ACPD (50 μM). Voltage-responses to depolarizing current pulses (100 pA, 100 ms) changed from LTS-burst (1) to delayed tonic (2) to fast-onset tonic firing (3) during depolarization. Hyperpolarization by injection of negative d.c. (-90 pA) to rest revealed only single spike response on the LTS (4). B: In an MGBv neuron at rest (-70 mV), the frequency-response curve exhibited a resonant hump at ~ 1 Hz. At a depolarized membrane potential (-60 mV, +d.c. injection), the frequency response shows low-pass filter characteristics. During application of 1S,3R-ACPD (50 μM), the membrane depolarized to -60 mV and shifted the frequency response to non-resonant. Negative d.c. injection to -70 mV reversed this shift. After recovery from 1S-3R-ACPD application, membrane potential and frequency responses were shifted back to control.
7.3. DISCUSSION

*Ionic mechanisms of $I_{ACPD}$*

The primary postsynaptic effect of mGluR activation in MGBv neurons is a depolarization due to activation of an inward current without a change in input conductance. This is evident from the voltage-independence of $I_{ACPD}$ between -40 and -85 mV, implicating activation of a cationic current (cf. Keele et al. 1997, Mercuri et al. 1993). We excluded the possibility that $I_{ACPD}$ results from $K^+$-channel blockade as we did not observe significant changes of $I_{ACPD}$ by lowering extracellular $K^+$-concentration or application of $K^+$-channel blockers (Crépel et al. 1994, McCormick and von Krosigk 1992). The observed dependence of $I_{ACPD}$ on extracellular Na$^+$ and the blockade of $I_{ACPD}$ by intracellular BAPTA is consistent with the activation of a Ca$^{2+}$-sensitive nonselective cationic current ("CAN" cf. Crépel et al. 1994, Shen and North 1992). However, such currents produce an increase in conductance and exhibit a reversal potential of -10 to +10 mV in the presence of extracellular $K^+$-channel blockers (Congar et al. 1997). We observed a reversal potential of $I_{ACPD}$ at -23.3 ± 3.8 mV, but no reversal potential was observed after application of extracellular Cs$^+$. Another possibility, the activation of a Na$^+/Ca^{2+}$-exchanger seems unlikely in view of the demonstration that Li$^+$ can replace Na$^+$ as a charge carrier for $I_{ACPD}$ (cf. Keele et al. 1997, Staub et al. 1992). The present results would implicate a Ca$^{2+}$-activated, voltage-independent Na$^+$-current with a shallow slope (due to high reversal potential) offset by the conductance increase as the main mechanism for $I_{ACPD}$.

We conclude, that the effector mechanism of $I_{ACPD}$ in MGBv is different from $K^+$-channel blockade reported in LGNd, implying diverse mGluR-effector coupling in thalamic sensory nuclei.
Voltage-dependent components of $I_{ACPD}$

A voltage-dependent component below -85 mV of $I_{ACPD}$ was observed in about 50% of MGBv neurons (cf. Fig. 7.10.A). The voltage-dependence and Ba$^{2+}$- and Cs$^{+}$-sensitivity of this component are consistent with a blockade of an inwardly rectifying current $I_{KIR}$ (Chapter 3, cf. Womble and Moises 1993). We did not observe the inward rectification and its modification by 1S,3R-ACPD application in all neurons, which may be explained by the requirement of $I_{KIR}$ or the intracellular pathway leading to $I_{KIR}$-blockade on soluble factors that wash out during whole-cell recording in some neurons. Also, diverse receptor-effector coupling could occur, as shown for several K$^-$-currents activated by muscarinic receptors or mGluRs in amygdala neurons (Womble and Moises 1994).

At potentials positive to -40 mV, $I_{ACPD}$ exhibited a changed slope in its V/I relationship, associated with a reversal potential of -23 mV. This could indicate an involvement of a voltage-dependent cation current (Haj-Dahmane and Andrade 1996). However, application of Cs$^+$ blocked this voltage-dependent component (cf. Fig. 7.9.A,B). Based on the results regarding a rise in interacellular Ca$^{2+}$, the voltage dependence and the Cs$^+$-sensitivity of the component, activation of a Ca$^{2+}$-activated K$^+$-current seems a more likely candidate for this voltage-dependent component positive to -40 mV (cf. Budde et al. 1992).

Receptor identity and intracellular signal transduction

The 1S,3R-ACPD-evoked depolarization and $I_{ACPD}$ in MGBv neurons may be mediated by an mGluR1 receptor subtype, based on its abundant presence in rat MGBv (Fotuhi et al. 1993, Martin et al. 1992, Salt and Eaton 1996) by analogy to mGluR1-
mediation of postsynaptic responses in other thalamic nuclei (Eaton and Salt 1996, Godwin et al. 1996a,b) and based on its sensitivity to MCPG (cf. Congar et al. 1997).

Using the GTP analogs GTPγS and GDPβS, an involvement of G-proteins in mGluR-mediated signal transduction was demonstrated for the first time in thalamic neurons. While G-protein activation would be expected for “metabotropic” receptors, 1S,3R-ACPD-activation of a cationic current has been reported without involvement of G-proteins, possibly via novel or membrane-delimited intracellular messenger systems (Guérineau et al. 1995). In MGBv neurons, intracellular application of GTPγS irreversibly activated $I_{ACPD}$, locking G-proteins in an activated position (cf. Ross 1989), whereas a similar application of GDPβS reduced $I_{ACPD}$. The effect was time-dependent, attributable to diffusion into the distal dendrites, where there are presumably abundant mGlurS and corticothalamic synapses (Godwin et al. 1996a, Jones and Powell 1969, Vidnyanszky et al. 1996). Also, with normal intracellular GTP, the depolarization and $I_{ACPD}$ outlasted the brief (30 sec) 1S,3R-ACPD-application for several minutes, in agreement with mediation by G-protein-activated intracellular messenger systems (cf. Pin and Duvoisin 1995).

Internal application of BAPTA produced a time-dependent reduction of $I_{ACPD}$ which may arise from several factors - delayed Ca$^{2+}$-chelation at sites distal from the electrode tip or variable modulation of $I_{ACPD}$ by Ca$^{2+}$ or Ca$^{2+}$-dependent phosphorylation. Intracellular Ca$^{2+}$ could be released from intercellular IP3-sensitive stores based on the common coupling of mGlur1 to IP3 receptors and the strong presence of colocalized mGlur1 and IP3 receptors in rat MGB (Fotuhi et al. 1993). A rise in intracellular Ca$^{2+}$, mediated by type mGlur 1 activation in thalamus has been implicated in pathological mechanisms like seizure generation or neurotoxicity (McDonald et al. 1993, Tizzano et al. 1995, see review by Nicoletti 1996).
1S,3R-ACPD-evoked changes in firing patterns and frequency preference

A depolarization was consistently induced by mGluR activation in MGBv neurons. This changed firing from the burst to tonic mode, as in the LGNd (Godwin et al. 1996b, McCormick and von Krosigk 1992). A depolarization due to raised extracellular $K^+$ or application of muscarinic agonists also produces such a shift in MGBv neurons (Hu 1995, Mooney et al. 1995). Both firing modes may have different functions for signal recognition and analysis in the visual system (see Discussion of Godwin et al. 1996b). In consideration of previous in vitro studies (cf. Fig. 3.3. in Chapter 3), the burst and tonic firing modes may comprise a whole spectrum of possible firing patterns, that also include single-spike firing on the LTS or delayed tonic firing. An interesting finding was the partial blockade of the LTS-burst response during the 1S,3R-ACPD-evoked depolarization despite hyperpolarizing d.c. injection in some neurons. Consistent with a mostly dendritic localization of the mGluRs in MGBv, a local dendritic depolarization by 1S,3R-ACPD would partially deactivate dendritic T-channels and reduce the burst response to a single spike on the LTS (cf. Tennigkeit et al. 1996). Strong dendritic depolarization may also set the stage for intraneuronal signalling via $Na^+$- and high-threshold $Ca^{2+}$-spikes, as observed previously (Chapter 4, Schwindt and Crill 1997). Also, mGluR activation could interact with other synaptic inputs, depending on stimulus patterns and previous activation (see Deschenes and Hu 1990, Salt and Eaton 1996, Scharfman et al. 1990).

A shift in frequency preference from resonance to non-resonant was also due to 1S,3R-ACPD-evoked depolarization (Fig. 7.11.). While mGluR activation in this study did not change the voltage-dependent impedance profile of MGBv neurons, the depolarizing shift
from resonant to non-resonant would change the filtering of synaptic input and frequency-dependent spike output (cf. Chapter 4).

Thus it appears that mGluR activation, interacting with the intrinsic membrane properties could confer mechanisms for plasticity and long-term modification of firing patterns and frequency responsiveness to thalamic auditory signal transmission.

**Physiological significance**

As described above, MGBv neurons do not simply relay firing patterns, but dynamically transform auditory information by means of their intrinsic membrane properties (cf. Tennigkeit et al. 1996, 1997, 1998b). This transformation is dependent on behavioral and attentional states, associated with the release of several neuromodulators, e.g. acetylcholine from the reticular formation of the brainstem and glutamate from the cortex (McCormick 1992b, Salt and Eaton 1996, Steriade and Llinás 1988). Interactions of brainstem and corticothalamic modulation may be mediated on the cellular level, specifically via G-protein mediated receptor crosstalk (cf. Ross 1989). Convergence of muscarinic and metabotropic glutamatergic receptors on the same effector systems has been reported in hippocampal neurons (Guérineau et al. 1994, 1995) and is probable for the leak K⁺-current blockade in the LGNd (McCormick 1992b). In rat MGBv, K⁺-current blockade has been proposed as a mechanism for a muscarinic depolarization (Mooney et al. 1995). As we observed here a different depolarizing mechanism for mGluR activation, corticothalamic and brainstem modulation of MGBv neurons would act independently. This independence of corticothalamic mGluR activation from behavioral brainstem modulation may be a requirement for signal identification in the auditory system during sleep (Tennigkeit et al. 1996).
The mGluR-mediated depolarization described here may be contributing to corticothalamic modulation of frequency-tuning in MGB as described previously (He 1997, Villa et al. 1991, Zhang et al. 1997). Also, these effects could play a role in thalamic gain control, reflect attentional state or enhance signal-background recognition, as proposed for the visual thalamus (Sherman and Koch 1986, Sillito et al. 1994, Singer 1977).
8. **GABAB-receptor activation changes membrane and filter properties**

8.1. **INTRODUCTION**

Neurons in the rat MGBv receive GABAergic inhibitory synaptic input from the inferior colliculus (Peruzzi et al. 1997) in addition to feedback from the thalamic reticular nucleus (Shosaku and Sumitomo 1983). A small number of GABAergic interneurons in the ventral partition (<1%; Winer and Larue 1988) may also contribute to this inhibition. Depending on the pattern of activity in these inputs (Kim et al. 1997), a combination of fast inhibitory postsynaptic potentials (IPSPs) mediated by GABA_A-receptors and slow IPSPs, mediated by GABA_B receptors, produce a long-lasting inhibition of auditory transmission in thalamocortical neurons.

A striking feature of the MGB is the strong expression of GABA_B receptors (Kaupmann et al. 1997) which have been implicated in the generation of oscillatory activity in thalamocortical neurons (Ulrich and Huguenard 1996b). After blockade of GABA_A receptors with bicuculline, for example, postsynaptic GABA_B-receptor activation induces synchronized oscillations at low frequency (<4 Hz) in *in vitro* preparations (von Krosigk et al. 1993; Williams et al. 1995). The oscillations resemble the epileptiform activity of absence seizures (Kim et al. 1997). Intrathalamic injections of the selective GABA_B-agonist, baclofen, evoke similar oscillations which are susceptible to blockade with GABA_B-antagonists (Clarke 1983, Liu et al. 1992). In many thalamocortical neurons, a GABA_B-receptor-induced hyperpolarization promotes oscillatory activity, due to a de-inactivation of the T-type Ca^{2+} current, which enables its interaction with the hyperpolarization-activated cation current, I_H (McCormick and Pape 1990b). In MGBv neurons, however, hyperpolarization does not readily induce spontaneous oscillations (Chapter 3). Nevertheless, these neurons should
support oscillatory activity with intrinsically amplified, low threshold Ca$^{2+}$ spikes (LTS) and bursts (Chapter 4), provided that periodic inputs straddle the LTS threshold. We have demonstrated previously a hyperpolarization-dependent resonance around 1-2 Hz in MGBv neurons (Chapter 5, Tennigkeit et al. 1994, 1997). Here, we used whole-cell patch-clamp recordings in the current- and voltage-clamp modes, combined with ZAP analysis methods (Chapter 2, Puil et al. 1986), to describe the effects of GABA$_B$-receptor activation on the membrane properties and frequency preferences of MGBv neurons.

8.2. RESULTS

_Baclofen application produces a hyperpolarization and increased input conductance_

Application of baclofen (1-20 min) reversibly hyperpolarized MGBv neurons and increased the input conductance, measured with hyperpolarizing current pulses (<15 mV) from rest (Fig. 8.1.A). The current/voltage (I/V) relationship had a reduced slope during baclofen application. The control and baclofen curves reversed at -79 ± 1 mV (n = 8), close to the calculated $E_K$ of -85 mV (Fig. 8.1.B). The hyperpolarization and conductance increased with the baclofen concentration. At 1 µM, the neurons hyperpolarized 1 mV and conductance increased by a factor of 1.15 relative to control (n = 2). Application of 5 µM baclofen evoked a hyperpolarization of 3.7 ± 0.9 (mean ± S.E., n = 3) and a conductance increase by a factor of 1.51 ± 0.31. At 10 µM, the hyperpolarization was 4.7 ± 0.4 (mean ± S.E., n = 9) and the conductance increased by a factor of 1.83 ± 0.16. The highest concentration, 50 µM, evoked a hyperpolarization of 8 mV in one neuron and increased conductance by a factor of 1.97 relative to control.
Fig. 8.1. Baclofen hyperpolarizes MGBv neurons and increases input conductance.

A: Application of baclofen (10 μM, 1 min) resulted in a hyperpolarization of 7 mV and decreased voltage responses to hyperpolarizing (downward) current pulses (-100 pA, 500 ms). After compensation of the baclofen-induced hyperpolarization with DC-injection, increased input conductance was apparent from the reduced voltage responses (see C). The LTS rebound bursts after hyperpolarizing current pulses (upward spikes, cf. C) are blocked. Complete recovery of the membrane potential, resistance and burst response was observed after 6 min. B: I/V plot of subthreshold voltage responses measured at the end of current pulses in C. Slope resistance was reduced by baclofen application (Bac), compared to control (Con) and recovery (Rec) curves. Note that curves crossed at -77 mV. C: Depolarizing and hyperpolarizing current pulses evoke tonic and rebound LTS burst firing respectively. Note presumed high-threshold Ca$^{2+}$-spike (HTS) appended to the burst of action potentials on the LTS. The increased conductance after application of baclofen (5 μM) completely shunted the voltage responses and prevented tonic and burst firing. This blockade was overcome by increasing the amplitude of current pulses; however, no HTS was evoked on the LTS. Same current pulses as in control evoked similar tonic and burst responses, including the HTS at 5 min after terminating the baclofen application. Membrane potentials were adjusted to the control resting potential.
During baclofen application, MGBv neurons maintained their voltage-dependent ability to discharge in the tonic and burst firing modes. However, larger depolarizing current pulses were required to evoke tonic firing from "resting potentials" that were reset to control values with DC-injection. Similarly, greater hyperpolarizing currents were required during baclofen application to elicit LTS-burst firing at the offset of the current pulses (Fig. 8.1.C). In such cases, the LTS always was slightly smaller than the control LTS. High-threshold Ca\textsuperscript{2+}-spikes (HTSs) that normally are appended to the LTS burst (Chapter 4) were not observed during baclofen application. In summary, baclofen produced inhibition by hyperpolarizing MGBv neurons and reduced the generation of action potentials by shunting current inputs.

*Baclofen antagonism by CGP 35348*

The baclofen-evoked hyperpolarization and increased membrane conductance were insensitive to blockade of action potential-dependent transmitter release by TTX (0.3 μM) and HTSs by Cd\textsuperscript{2+} (0.1 mM), implicating activation of postsynaptic GABA\textsubscript{B}-receptors (Fig. 8.2.A, n = 6). In the presence of TTX and Cd\textsuperscript{2+}, co-application of the specific GABA\textsubscript{B}-receptor antagonist, CGP 35348 (0.5 mM), reversibly blocked the baclofen-evoked hyperpolarization and increase in conductance (Fig. 8.2.B, n = 3). The antagonist reduced the baclofen-hyperpolarization from 4.7 ± 0.3 mV to 1 mV, and the conductance increase, from 81.3 ± 26.3 % to 11.6 ± 13.3 %. The antagonism of the baclofen-evoked responses by CGP 35348 was similar to observations of the suppression of long-lasting IPSPs in MGBv and the thalamic reticular nucleus (Peruzzi et al. 1997; Ulrich and Huguenard 1996a).
Fig. 8.2. Blockade of baclofen effects by CGP 35348 in an MGBv neuron.

A: After application of TTX and Cd\(^{2+}\), voltage responses to current pulses from rest (-10, -30, -50, -70 pA in all panels) evoked LTSs at the offset of current pulses. During application of baclofen (10 μM, 150 s) and with d.c. compensation for a 4 mV hyperpolarization, the voltage-responses decreased. Current pulses of increased amplitude (-90, -110 pA) in the neuron at potentials similar to control did not increase the amplitude of the baclofen-reduced LTS. Full recovery was obtained 5 min after end of baclofen application. B: Application of CGP 35348 did not change the resting potential or input conductance. Co-application of baclofen (10 μM, 150 s) did not evoke a hyperpolarization and did not reduce voltage responses or rebound LTSs. After washout of CGP 35348 for 10 min (right), baclofen elicited similar effects as in A.
By itself, CGP 35348 application had no effects on membrane potential or input conductance, implying an absence of agonist or constitutive activation of GABA<sub>B</sub>-receptors in the slice preparation.

*Baclofen evokes an inwardly rectifying, Ba<sup>2+</sup>-sensitive current*

To study the current evoked by baclofen, we applied slow voltage-ramps under voltage-clamp conditions in 6 neurons. The current was derived by subtraction of the voltage-current (V/I) relationships before and during application of baclofen. The baclofen current was outward, hyperpolarizing the neurons from rest but was inwardly rectifying at potentials negative to a reversal potential of -79 mV, i.e., close to E<sub>K</sub> (Fig. 8.3.A). The application of Ba<sup>2+</sup> (0.5 mM) completely abolished the baclofen current (Fig. 8.3.B, n = 3). The blockade by Ba<sup>2+</sup> and the reversal potentials for baclofen action derived from the I/V relationships in both current- and voltage-clamp studies imply that the hyperpolarization and conductance increase in MGBv neurons are caused by an inwardly rectifying K<sup>+</sup>-current. This differs from the GABA<sub>B</sub>-receptor activated linear K<sup>+</sup>-current activated by baclofen in thalamic reticular neurons but is similar to findings in the lateral geniculate nucleus (Crunelli et al. 1988, Ulrich and Huguenard 1996a).

*Mediation of baclofen effects by G-proteins*

We considered the possibility that G-proteins may mediate the conductance increase and hyperpolarization, caused by baclofen. When the recording pipettes contained the non-hydrolyzable substrate for G-proteins, GTP<sub>γ</sub>S, MGBv neurons gradually hyperpolarized and increased in input conductance within the first minute after breakthrough (n = 6).
Fig. 8.3. Baclofen application elicits an outward current, sensitive to Ba\(^{2+}\)-blockade.
A: After application of TTX and Cd\(^{2+}\), slow hyperpolarizing ramp voltage-clamp protocols were applied. V/I relationships before and during application of baclofen (10 μM) show changes in slope conductance. The neuron hyperpolarized by 3 mV during the application. Below, subtraction of the V/I relationships revealed the inwardly rectifying current evoked by baclofen (I\(_{\text{Bac}}\)) which reversed near -79 mV. A linear fit of the I/V function above -80 mV (slope 2.86 pA/mV) is shown for comparison.
B: After application of Ba\(^{2+}\) (0.5 mM), baclofen elicited no additional current, apparent from the similar V/I relationships and their subtraction.
Several minutes later, the resting potentials were $-74.5 \pm 0.4$ mV ($n = 6$). This was significantly below control values ($-68.8 \pm 1.1$ mV, $p < 0.01$). The input conductances recorded during GTPγS applications were $22.8 \pm 3.6$ nS, i.e., significantly increased by 333% ($p < 0.01$) over control values ($5.3 \pm 0.4$ nS, cf. Chapter 3). Application of baclofen (10 μM) elicited no further effect during internal application of GTPγS ($n = 3$, not shown). Neurons recorded during GDPβS application had membrane potentials and input conductances similar to control neurons. However, they showed no response to baclofen (10 μM), applied 24-36 min after breakthrough ($n = 3$, data not shown). These results suggest that G-proteins mediate the baclofen-induced hyperpolarization and conductance increase in MGBv neurons.

Effects of baclofen on frequency responses

MGBv neurons normally exhibited a voltage-dependent resonance with an impedance maximum near 1 Hz, if the oscillating current input was applied within the activation range of the T-type Ca$^{2+}$-current. For example, the ZAP stimulus produced a resonant hump in the neuron of Figure 8.4A which was hyperpolarized to -73 mV with DC-injection. A spurious resonance remained at rest (-68 mV), whereas the neuron displayed lowpass filter characteristics when depolarized to -63 mV. Despite its hyperpolarizing effect, application of baclofen (5-10 μM) abolished a neuron’s ability to resonate. This resulted from a drastic reduction in impedance throughout the frequency range from 0.2 to 20 Hz, imposing lowpass filter characteristics of low quality at voltages between -80 mV and action potential threshold (~-50 mV, $n = 4$). In these neurons, the maximal impedance was reduced by ~50% at -63 mV and 30% at -73 mV.
Fig. 8.4. Baclofen reduces impedance and resonance in an MGBv neuron.
A: Under control conditions, the frequency response curves of an MGBv neuron showed lowpass filter characteristics at potentials depolarized by DC-injection (-63 mV), a small resonance near 1 Hz at the resting potential (-68 mV) and an increased resonance near 1 Hz at a hyperpolarized potential (-73 mV). B: Application of baclofen (5 μM) reversibly reduced the amplitude of the overall frequency response at all of the potentials. At rest and hyperpolarized potentials, resonance was almost completely blocked by baclofen, note similarity of the frequency response curves at all membrane potentials after baclofen application. C: Frequency response curves similar to control were observed 10 min after end of baclofen application.
We concluded that the activation of a shunting $K^+$ conductance rather than the resulting hyperpolarization was the dominant effect of $\geq 5 \, \mu M$ baclofen on the membrane dynamics in MGBv neurons.

8.3. DISCUSSION

We have observed that the application of a GABA$_B$-receptor agonist, baclofen, increased $K^+$-conductance which caused a hyperpolarization in MGBv neurons. Previous studies have shown such Ba$^{2+}$-sensitive actions of baclofen on neurons in other regions of the CNS (Misgeld et al. 1995). However, we additionally found that the increased conductance, not the hyperpolarization, dominated the inhibitory effects of baclofen. Despite compensating the baclofen-hyperpolarization, an increased input current was required to overcome the suppression of action potentials when the neuron was in the tonic firing mode (cf. Chapter 3). The shunt also diminished the amplitude and quality of LTS-bursts that normally characterize thalamic firing during slow-wave sleep. Hyperpolarizing prepulses of large amplitude were inadequate in compensating for the reduction in LTS amplitude. These effects occurred at baclofen concentrations that roughly correspond to maximal intrathecal therapeutic doses (e.g., Armstrong et al. 1997). Although the clinical relevance of the effects observed here are unknown, we would suggest that the activation of postsynaptic GABA$_B$-receptors can result in a forceful inhibition of MGBv neurons.

Baclofen application inhibited the HTS appearing on top of the LTS. To our knowledge, there are no reports that baclofen affects the T-type Ca$^{2+}$-current that produces the LTS. Baclofen causes a 10-20% reduction of high-threshold Ca$^{2+}$-currents in thalamic neurons (Guyon and Leresche 1995, Kammermeier and Jones 1997). However, it seems
unlikely that this action could account for the inhibition of Ca\textsuperscript{2+}-spikes under these circumstances. More likely, GABA\textsubscript{B}-receptors at dendritic locations (Kim et al. 1997) may influence membrane conductance and shunt both low and high threshold Ca\textsuperscript{2+}-currents, located on the dendrites (Destexhe et al. 1998; Jahnsen and Llinás 1984b). Whereas the hyperpolarizing effects of baclofen may have de-inactivated the T-current in the dendrites, thereby enabling LTS firing, the shunted LTS was probably of insufficient amplitude to reach threshold for the HTS. The activation of a 4-AP sensitive K\textsuperscript{+}-conductance, for example, has similar effects in MGBv neurons (Chapter 4). It seems likely, then, that synaptic activation of GABA\textsubscript{B}-receptors may shunt firing in MGBv neurons. This is consistent with results by Shosaku and Sumitomo (1983), who stimulated the thalamic reticular nucleus, suppressing auditory transmission for hundreds of milliseconds.

In view of the ability of baclofen to increase various K\textsuperscript{+} conductances (Misgeld et al. 1995), the baclofen current which we observed in MGBv neurons may represent combined effects on leak and rectifying conductances. First, baclofen application evoked an inwardly rectifying outward current in MGBv neurons, as in CA3 pyramidal cells (Gähwiler and Brown 1985; Sodickson and Bean 1996) and lateral geniculate neurons (Crunelli et al. 1988). Also, a markedly linear outward current at potentials positive to E\textsubscript{K} distinguished the baclofen current from inward rectifier currents, such as I\textsubscript{KIR}. As in the visual thalamus (McCormick 1992a) and hippocampus (Sodickson and Bean 1996), we observed that internal applications of GTP\gamma S occluded, and GDP\beta S blocked the effects of baclofen. These results implicate G-proteins in GABA\textsubscript{B}-receptor mediated effects.
Frequency responses and oscillations

Baclofen-activation of GABA$_B$-receptors appears to have different consequences for oscillatory behaviour in MGB neurons, compared to other thalamic neurons. The administration of baclofen in low micromolar concentrations resulted in little or no tendency for MGBv neurons to oscillate spontaneously, partly because of shunted LTS-firing. This contrasts with the effects of systemically administered baclofen which shifts the firing of ventrobasal thalamic neurons from multiple action potentials to oscillatory spike-burst discharges (Clarke 1983). Similarly, the hyperpolarization induced with $\gamma$-hydroxybutyrate due to activation of GABA$_B$-receptors leads to oscillations in lateral geniculate neurons (Williams et al. 1995). Synaptic or pharmacological activation of GABA$_B$-receptors can sustain periods of spontaneous oscillations in lateral geniculate body neurons which, after GABA$_A$-receptor blockade, resemble paroxysmal absence-type episodes (Destexhe et al. 1996; von Krosigk et al. 1993; Kim et al. 1997). This oscillatory behaviour involves the high-frequency spike-burst discharges of perigeniculate or thalamic reticular neurons that produce slow GABA$_B$-mediated IPSPs in relay neurons. Without GABA$_A$-receptor blockade, however, oscillations in the network between thalamic reticular and relay neurons may require a highly localized concentration of GABA$_B$-agonist because a persistently high, generalized concentration may shunt the prerequisite output of MGBv neurons.

Network connectivity is necessary for spontaneous normal or paroxysmal oscillations, but they additionally require intrinsic membrane properties. In thalamocortical neurons, a frequency selectivity is capable of enhancing oscillations in the network (Puil et al. 1994b). In MGBv neurons, this membrane resonance had a broad peak at low frequencies near ~1 Hz (at 22° C). The resonance is hyperpolarization-dependent, arising from an interaction of the
T-type Ca\(^{2+}\)-current with passive membrane properties (Chapter 5, cf. Hutcheon et al. 1994, Jahnsen and Karnup 1994, Puil et al. 1994b, Tennigkeit et al. 1994). In the present experiments, baclofen application hyperpolarized MGBv neurons but collapsed the impedance in a low frequency range, abolishing the resonant hump. Since the resonance normally occurs at hyperpolarized potentials that characterize states of sleep, the above observations may have more relevance for corresponding EEG patterns than for audition. However, GABA\(_B\)-receptor induced changes in resonance may have a role in disruption of auditory function, e.g. during absence epilepsy.
9. Isoflurane attenuates resonant responses in MGBv

9.1. INTRODUCTION

General anesthetics induce an unconscious state which anesthesiologists view as an insensitivity to stimuli from the external environment. Anesthetics decrease neuronal firing and transmission in many regions (Krnjevic and Puil 1997), disrupting the brain’s rhythmic activities that characterize conscious states (Moruzzi and Magoun 1949). The cortico-thalamocortical system has a crucial involvement in the responses to auditory, tactile, and visual stimuli as well as the rhythms during awareness and sleep (Barth and MacDonald 1996; Steriade et al. 1990, 1996). In humans, for example, anesthetics markedly attenuate the middle latency response of auditory evoked potentials (Galambos et al. 1981, Madler et al. 1991) and the auditory steady-state responses (“ASSR”) to rapidly presented stimuli (40/s; Plourde 1993, 1996; Plourde and Villemure 1996). These potentials arise mainly from neuron circuits of the primary auditory cortex and medial geniculate body of the thalamus.

The effects of anesthetics on neurons of the thalamus have attracted recent interest (Angel and LeBeau 1992, Ries and Puil 1993, Sugiyama et al. 1992, see also Steriade et al. 1996) because anesthetics may disorganize the temporary relationship between the sensory responses and coherent oscillations or reduce the background synchronous activity implicated in awareness and cognition (Plourde 1993, Plourde and Villemure 1996). Normally, a transfer of sensory information to the cortex results from synaptic stimuli interacting with intrinsic membrane properties of thalamocortical neurons. In thalamic slices, this integrative activity produces a frequency selectivity, identified with alternating current inputs as resonance (cf. Chapter 5, Hutcheon et al. 1994, Puil et al. 1994b, Ströhmann et al. 1994, 1995), or viewed overtly as voltage oscillations in a subthreshold range (McCormick and
This frequency selectivity, which is likely subject to modulation during behavioral and anesthetic states (cf. Angel 1993, McCormick 1992b), acts as a filter in the conversion of synaptic inputs to output firing. We used whole-cell patch-clamp recordings in the current-clamp mode, combined with ZAP analysis (Chapter 2) to investigate the effects of a volatile anesthetic, isoflurane, on the frequency preferences and firing patterns in MGBv neurons. Understanding anesthetic effects on resonance and firing modes is important in the physiology of the auditory system where neurons sharply modify their pattern of synaptically evoked discharge depending on the type of anesthesia (Zurita et al. 1994).

9.2. RESULTS

The results described here, were obtained from measurements on a total of 14 medial geniculate neurons. We applied only one concentration of IFL to each neuron, all in different slices, except in the case of 4 neurons (cf. Fig. 9.2.).

Isoflurane blockade of resonant responses and frequency selective firing

We assessed the effects of isoflurane on the frequency responses in 11 neurons at membrane voltages that reflect wakefulness and sleep (Steriade et al. 1990). First, to simulate the tonic firing mode, we used DC to depolarize the neurons to \( \sim -60 \text{ mV} \) and then injected subthreshold as well as threshold swept-sinewave current stimuli. The smaller stimulus displaced the membrane potential by 8-10 mV whereas the larger stimulus evoked firing at low frequencies (Fig. 9.1.A). The upper frequency response curves in Figure 9.1.A (right), corresponding to the subthreshold and threshold responses, show low-frequency preference typical for depolarized MGBv neurons under control conditions. Application of isoflurane (IFL; 1 %) reduced the overall voltage responses to subthreshold and threshold
swept-sinewave currents, abolished the action potentials and reduced the impedance magnitude below 10 Hz (Fig. 9.1.B). Although firing returned on increasing the current magnitude, the impedance profile remained reduced. The impedance did not change at frequencies above 10 Hz, implying that IFL did not greatly affect membrane capacitance (cf. Puil and Gimbarzevsky 1987).

We then tested the ZAP-responses of neurons in the phasic (burst) firing mode near rest, or hyperpolarized beyond ~-70 mV with DC-injection (Figs. 9.1.B and 9.2.B,C). A ZAP-analysis with a subthreshold swept-sinewave-current revealed a broad resonant hump that peaked at ~1 Hz (Fig. 9.1.B). At large current amplitudes, LTSs and action potentials contributed to the voltage responses when the input swept through the resonant frequencies (centered near 1 Hz). This frequency-selective firing was confirmed using sinewave current inputs, shown in Figure 9.1.C. Application of IFL (1 %) to neurons in this hyperpolarized state reduced the low-frequency bulge in the voltage response and blocked firing (Fig. 9.1.B). Despite this marked reduction in impedance, some frequency preference remained in the same frequency range while the neuron was in this hyperpolarized state. The suppression of firing was surmountable by increasing the amplitude of the swept-sinewave-current which re-established frequency selective firing, independent of the input waveform (Fig. 9.1.B,C).

**Effects of isoflurane on the impedance amplitude profiles**

Application of IFL (0.5-3 %) reversibly decreased the amplitude of frequency responses at membrane voltages between ~ -100 mV and action potential threshold (~ -50 mV, n = 11). Normally, MGBv neurons at depolarized potentials exhibited lowpass filter characteristics. In a concentration-dependent manner, IFL reduced the amplitude of the frequency responses (<10 Hz) of neurons in this membrane potential range.
Fig. 9.1. IFL reduces impedance and frequency selective firing in an MGBv neuron.
A: At a depolarized membrane potential (-60 mV), swept-sinewave-current injection (0.1 to 20 Hz) evoked voltage responses that were largest at low frequencies (<1 Hz). An increase in current amplitude caused firing at these low frequencies (truncated action potentials). Application of IFL (1%) reduced the voltage responses at low frequencies (<10 Hz; cf. frequency response curves at right) and suppressed firing which required an increased current amplitude. Note that the impedance magnitude was not affected by the changed current amplitude and contamination of the voltage response by action potential firing. B: At a hyperpolarized membrane potential (-70 mV), the swept-sinewave-current (0.1 to 20 Hz) evoked a resonance in the voltage response at ~1 Hz. An increase in the current amplitude produced firing in the resonant frequency range (~1 Hz, cf. frequency response curves at right). During IFL application, the impedance reduction was greatest at the resonant frequencies. C: Sinewave current inputs (same amplitudes as swept-sinewave-current inputs in B) at -70 mV elicited action potential firing at the resonant frequency, not above or below. Note the different time-scales and truncated action potentials. On application of isoflurane (1%), a larger amplitude input (same as large swept-sinewave-current input in B), also elicited frequency selective firing. Scale bars: A, B: voltage, 40 mV; current, 200 pA. C: voltage, 20 mV; current, 200 pA.
During application of 2 % IFL, the maximum in the frequency response of a DC-depolarized neuron was reduced to ~ 50 % of control (Fig. 9.2.A, n = 8). The effects of 2 % IFL on the frequency responses were relatively greater in neurons at potentials near rest where they displayed a resonance; in the example of Figure 9.2.B, the maximal amplitude in the frequency response was reduced to ~ 40 % of control. The amplitude of the resonance peak near ~ 1 Hz was increased at DC-hyperpolarized potentials, in neurons under control conditions (Fig. 9.2.C). At concentrations of 0.5-3 %, IFL produced a marked depression of this resonant response. Application of IFL at 2 % to a neuron in this hyperpolarized state, reduced the amplitude of the maximal frequency response to about 30 % of control and eliminated the voltage-dependent resonant responses (n = 8). Administration of IFL at 3 % produced the same effect (n = 2). As a result, higher IFL concentrations abolished the differences in the frequency-responses observed at different membrane potentials, imposing low-pass characteristics of low quality at all membrane voltages.

*Effects of isoflurane on subthreshold responses to current pulses and firing*

The frequency response curves near zero frequency indicated that IFL increased input conductance. As the observed changes in frequency preference depend on an altered interaction of the resting conductance and T-type Ca\(^{2+}\) current activation, we examined the effects of IFL on the voltage responses to depolarizing and hyperpolarizing current pulse inputs in 14 neurons.

*Subthreshold responses.* We observed that application of IFL hyperpolarized MGBv neurons and increased input conductance, measured with small hyperpolarizing current pulses that displaced the membrane potential from rest (~ -68 mV) by <10 mV.
Fig. 9.2. Isoflurane, in a concentration dependent manner, decreases impedance and resonance in an MGBv neuron.

A: Application of IFL in concentrations of 0.5, 1, and 2% reversibly reduced the amplitude of the overall frequency response when the neuron was depolarized by DC-injection to -62 mV. B: At rest (-67 mV), the control frequency response had a resonant hump centered near 1 Hz. The overall magnitude of the frequency response curve was reduced by serially increasing concentrations of IFL. C: The control frequency response curve exhibited a pronounced resonance near 0.8 Hz when the neuron was hyperpolarized to -72 mV. An increase in the concentration of IFL produced a gradual reduction in the impedance magnitude < 10 Hz. Note an almost complete blockade of resonance and similarity of the frequency response curves at the three voltages on application of 2% IFL.
These changes were concentration-dependent. At 0.5 %, IFL (n = 3) elicited a hyperpolarization of 1-2 mV and increased conductance by 8.8, 9.5 and 43.5 %. At 1 %, isoflurane hyperpolarized 3 neurons by 3-4 mV, and despite DC-compensation, increased conductance by 22.5, 38.6 and 61.8 % (cf. Fig. 9.1.). Application of 2 % IFL increased conductance by 76.6 ± 13.5 % (mean ± SE, n = 6; Fig. 9.3.A,B). A hyperpolarization of 4-6 mV, requiring DC-compensation, accompanied this conductance increase.

**Tonic firing mode.** Isoflurane reversibly reduced the subthreshold voltage responses and tonic firing evoked by square pulse current injections into neurons in the DC-depolarized state. As with the increased swept-sinewave current-input, square pulses of larger amplitude elicited tonic firing during IFL application (Fig. 9.3.A).

**Burst firing mode.** When the neurons were at rest, IFL application (2 %) suppressed the LTS-burst firing at the offset of hyperpolarizing pulses (Fig. 9.3.B). Larger amplitude pulses that hyperpolarized the neurons to ~ -110 mV were only partly effective in reversing the blockade of the rebound burst response (not shown). In a DC-hyperpolarized state, LTS-burst firing evoked by depolarizing current steps was reversibly blocked by application of 2 % IFL despite injection of current pulses of much greater amplitude (Fig. 9.3.C).

In summary, the blockade of the LTS-burst response during isoflurane application seemed attributable to an increased membrane conductance and a reduction in the evoked LTS.
Fig. 9.3. Isoflurane suppresses tonic and burst firing by increasing input conductance in an MGBv neuron.

A: An application of 2% IFL eliminated the tonic firing of action potentials evoked by depolarizing current steps at a depolarized potential (-63 mV). Current pulses of a greater amplitude evoked tonic firing, overcoming the blockade. An increased input conductance due to IFL application is apparent in the subthreshold steady-state I/V relationship, determined near the end of current pulses of 500 ms duration (see B). B: Application of 2% IFL reduced voltage responses and eliminated the rebound burst responses at the offset of current pulses that hyperpolarized the neuron from rest (-68 mV). Depolarizing DC-current was applied to compensate for a 4 mV hyperpolarization induced by IFL. C: Application of 2% IFL reversibly blocked LTS-burst firing evoked by depolarizing current steps in the neuron held at -73 mV, despite current pulses of much greater amplitude. Calibration bars apply to all records.
9.3. DISCUSSION

Our major finding is that isoflurane reversibly attenuated membrane impedance, in particular, a low-frequency resonance in MGBv neurons. At the lowest concentration (0.5 %) which corresponds to ~ 1 % at 37 °C (see Chapter 2), isoflurane reduced the resonant hump and increased steady-state input conductance. At higher concentrations, isoflurane eliminated the resonance, creating a flat bandpass function between 0.1 and 10 Hz, and markedly increased input conductance. In MGBv neurons of the intact animal, the effects on resonance and conductance would alter the transformation, or prevent relay, of signals to the primary auditory cortex. For example, a depression of transmission through the ventrolateral and lateral geniculate thalamus occurs during administration of halothane or pentobarbital in cats (Marshall and Murray 1980).

The flattened frequency responses of MGBv neurons likely relate to isoflurane’s effects on the membrane properties that normally generate resonance at potentials near and below rest. In thalamic neurons of mammals, but not birds (Ströhmann et al. 1994), a resonant hump in the voltage domain arises from the interaction of a low threshold, T-type Ca\(^{2+}\)-current with the membrane leak current and capacitance (mediodorsal neurons, Hutcheon et al. 1994; Puil et al. 1994b; lateral geniculate nucleus neurons, Jahnsen and Karnup 1994; MGBv neurons, Tennigkeit et al. 1994). An increase in input capacitance, possibly due to an increased membrane fluidity, occurs in peripheral sensory neurons during isoflurane anesthesia (Puil and Gimbarzevsky 1987). We did not observe any evidence that IFL application may have increased input capacitance.

A depression of the T-current may contribute to the observed changes in resonance. Isoflurane and other volatile anesthetics, or barbiturates, depress low and high threshold Ca\(^{2+}\)- currents in various neurons (ffrench-Mullen et al. 1993, Gross and Macdonald 1988; Krnjevic
and Puil 1988, Puil et al. 1994a, Study 1994, Takenoshita and Steinbach 1991). We found that isoflurane had the greatest effect at hyperpolarized potentials where resonance was largest, i.e., at potentials where a large pool of T-type Ca\textsuperscript{2+}-channels is available for activation (Coulter et al. 1989). While both activation and inactivation of the T-current contribute to the development of the resonant hump, the slower inactivation parameter dominates the low frequency peak in thalamocortical neurons (Hutcheon et al. 1994). Pentobarbital and the induction agent, propofol, enhance the apparent steady-state inactivation and accelerate the rate of inactivation, decreasing Ca\textsuperscript{2+}-currents (ffrench-Mullen et al. 1993, Gross and Macdonald 1988, Gundersen et al. 1988, Olcese et al. 1994). Isoflurane may have similar actions in suppressing the T-current (Study 1994, Takenoshita and Steinbach 1991) but this may not be the main explanation for our observations on resonance, particularly at the higher IFL-concentrations.

Isoflurane application greatly increased membrane conductance, producing a shunt that likely reduced resonant responses. This occurred when isoflurane shunted firing of action potentials elicited by current pulses, as observed in ventrobasal thalamic neurons (Ries and Puil 1993). In both types of neurons, the effects of IFL on the firing of action potentials also were surmountable by greatly increasing the amplitudes of the input current. In MGB\textsubscript{v} neurons, larger amplitude swept-sinewave-currents produced the same frequency preference, compared to controls. An increase in leak current accompanied the depression of the T-current by isoflurane in ventrobasal thalamic neurons (Ries and Puil 1993), also shown for the depression of Ca\textsuperscript{2+}-currents in other neurons (neocortical, Puil et al. 1994a; hippocampal, Study 1994). This raises some uncertainty about the amount of reduction in the T-current due to an isoflurane-induced decrease in Ca\textsuperscript{2+}-channel activity, as opposed to the increase in leak conductance. Under the present conditions, the concomitant effects of isoflurane on
resonance, action potential firing, and low threshold Ca$^{2+}$-spikes, imply that an increased conductance, probably for K$^+$ (cf. halothane, Nicoll and Madison 1982, Sugiyama et al. 1992; IFL, Berg-Johnsen and Langmoen 1990, Ries and Puil 1993), produced a large part of the reduction in resonance.

The decreased tendency for the membrane potential to oscillate and the flat bandpass filter function in MGBv neurons under isoflurane anesthesia has consequences for the centripetal transfer of auditory information. Normally, the subthreshold frequency preference would couple synaptic inputs with preferred dynamic profiles and/or repetition rates to firing, filtering the output to the cortex. The in vitro slice conditions used here exclude depolarizing cortical and brainstem inputs and allow neuronal expression of a slow delta-like (1-4 Hz) rhythm (cf. McCormick and Pape 1990b). Under these conditions, resonance at 1-2 Hz tunes thalamocortical neurons to activity at such frequencies from other structures and to bursts within the same frequency range. Isoflurane anesthesia would disrupt such synchronizing mechanisms in MGBv neurons, similar to the disruption of oscillations in other thalamic neurons on administration of a barbiturate in vivo (Fig. 11 in Curró Dossi et al. 1992). This would corrupt or prevent the transfer of auditory information. In addition, the increased conductance, hyperpolarization, and decreased LTS bursts observed in thalamic neurons (cf. Results; Ries and Puil 1993), may contribute to the electroencephalographic burst-suppression patterns during deep isoflurane anesthesia (Ogawa et al. 1992, Steriade et al. 1994).
10. General Discussion

Auditory thalamic coding and corticothalamic oscillations depend on an interaction of synaptic and intrinsic membrane properties. In addition, neuromodulation from brainstem nuclei and from within the auditory system change the MGBv responses. An understanding of signal transformation in MGBv requires knowledge of the intrinsic membrane properties and neuromodulatory effects, which were characterized for the first time in MGBv neurons. Their importance for signal transformation was suggested by the similarity of firing patterns observed in this study and in vivo (Chapter 3, Aitkin and Prain 1974, Creutzfeldt et al. 1980). However, many important variables of MGBv signal transformation, such as factors underlying synaptic dynamics and neuromodulation remain unknown. In analogy to the detailed studies in the corticothalamic system, which have led to an understanding of the generation of the oscillatory rhythms during sleep and pathological states (McCormick 1992b, Steriade et al. 1990, von Krosigk et al. 1993), the knowledge of intrinsic membrane properties, network connectivity, synaptic and neuromodulatory inputs in the whole auditory tecto-thalamo-cortical system is a prerequisite for understanding higher auditory coding. The functional significance and interdependencies of these membrane and synaptic properties for network dynamics can then be assessed with mathematical models (cf. Hutcheon et al. 1994, McCormick and Huguenard 1992).

Mechanisms for the generation of MGBv output patterns

Several factors of the intrinsic membrane properties contribute to the spike output in MGBv neurons. The voltage-dependence of the firing patterns arises from the activation and interaction of different currents (cf. Chapters 3 and 4). The membrane voltage in vivo is controlled by the synaptic input and neuromodulatory effects. Longer lasting neuromodulation
due to the metabotropic effects of auditory neurotransmitters was characterized for the first time in MGBv in this study and suggests an intrinsic modulation of signal transformation (Chapters 7 and 8, cf. Katz and Frost 1996). Excitation and inhibition in MGBv signal transformation depends not only on membrane voltage changes, but also on conductance changes. In this study, reduced voltage changes to current injection and flattened frequency responses were caused by an increase in conductance following GABA_B receptor activation or application of the general anesthetic, isoflurane (Chapters 8 and 9). Another feature of the currents identified in this study is their time dependence, expressed as activation, inactivation and deinactivation kinetics on various timescales, which are essential for the generation of specific firing patterns (Chapters 3 and 4). These kinetics also endow the MGBv neurons with frequency filter properties (Chapter 5). Neuromodulation of these kinetics plays an important role in network dynamics, e.g. I_H changes modify oscillatory burst firing in the LGNd (McCormick and Pape 1990b). Finally, the functional consequences of precise localization of ion channels and receptors on the somatodendritic membrane become increasingly apparent. These spatial arrangements imply different signal integration within single neurons (see below, Chapters 4 and 7).

The role of membrane properties in oscillations

Thalamocortical oscillations are a hallmark of brain activity and characterise behavioral states, e.g. deep sleep (Steriade and McCarley 1990, Steriade et al. 1990). Synaptic and intrinsic membrane properties govern frequency and synchronization of thalamic oscillations. Intrinsic rhythmic LTS burst firing has been implicated in the generation of delta waves (Steriade et al. 1990). While the kinetics of the T-type Ca^{2+}-current determine the oscillation frequency, the resonance produced by this current has been proposed to contribute to its thalamocortical
synchronization (Chapter 5, Puil et al. 1994). While synchronized oscillations are thought to inhibit reliable information transfer (McCormick and Feeser 1990), oscillatory synchronization of neuronal responses may be a signature of coherent neuronal ensembles and required for feature integration in thalamocortical processing (Singer 1993, Steriade et al. 1996). On the other hand, oscillations could provide a temporal context for synaptic inputs (Buzsaki 1997). Disruption or „oversynchronization“ of oscillations in the thalamocortical system can lead to insensitivity to sensory stimuli (as in the anesthetic state, Chapter 9, cf. Steriade et al. 1994) or pathological states such as absence epilepsy (Steriade et al. 1991, von Krosigk et al. 1993).

Methodological considerations

The methods used in this study (Chapter 2) are of importance for the interpretation of the results, comparisons of other studies utilizing different methods and extrapolation to the functional system in vivo.

The in vitro slice preparation was suitable to study the intrinsic membrane properties of single MGBv neurons in isolation. The patch-clamp technique provided a very good signal to noise ratio due to a high access resistance. We recognized that the exchange of pipette solution and intracellular solution may have changed membrane properties (e.g. I_H in MGBv) that depend on intracellular Ca^{2+} buffering, phosphorylation states or soluble second messenger molecules. However, the systematic changes of extra- and intracellular milieu allowed for elucidation of ionic mechanisms and reversal potentials, for comparison with calculations (Chapters 7 and 8).

The experiments were performed at room temperature, which would affect current kinetics and Ca^{2+}-dynamics (cf. Thompson et al. 1985). These effects did not play a major role, as experiments at higher temperature (32-34°C) exhibited similar firing patterns and frequency
preferences (Fig. 5.1). Although the neurons were not visualized before establishing the patch-clamp recording, we assume, based on action potential amplitudes and thresholds (cf. Johnston et al. 1996), that we recorded from or near the soma (cf. Discussion Chapter 3). However, some recorded events may have originated in the dendrites, which probably exhibit different membrane properties and play an active role in thalamic signal transformation (cf. Discussion Chapters 4 and 7, see below).

The experimental conditions of this study (Chapter 2) may have prevented the occurrence of overt oscillations, as observed in vivo (cf. Chapter 1). While delta oscillations have been observed in MGBv slice preparations (McCormick and Prince 1988), the experimentation at room temperature may be responsible for our findings. Spindle and gamma oscillations require intact connections to TRN or cortex (Steriade et al. 1991, 1996, von Krosigk et al. 1993), which were severed in this study. With intact connectivity, spindle oscillations can also be evoked and studied in vitro (Bal et al. 1995, Warren and Jones 1997).

Variation of MGBv membrane properties

This study confirms the homogeneity of MGBv relay neurons in terms of membrane properties, which has been postulated previously based on cellular morphology and auditory responses (Ehret and Romand 1997, Morest 1964). The similarity of membrane currents and neuromodulatory mechanisms (cf. Chapters 3-8) resulted in similar firing patterns, frequency preferences and neuromodulation in vitro, except for small variations regarding the number of action potentials and the presence of an HTS on the LTS, which were attributed to varied balances of intrinsic currents (Chapters 3 and 4). The extraordinary firing patterns observed in immature MGBv neurons during the first two postnatal weeks were also attributed to different
balances of MGBv currents (Chapter 6). Thus similar, yet complex and dynamically interacting intrinsic membrane properties can result in a large variation of signal transformation.

**Comparison of MGBv and other thalamic neurons**

The question, what differentiates MGBv neurons from other thalamic neurons, is of interest as different membrane properties may yield clues for specific encoding of auditory features. Previous studies concluded that all thalamic neurons, including neurons of the TRN, have very similar properties (Jahnsen and Llinás 1984a). More detailed studies of membrane properties in several thalamic nuclei disproved such a priori statements on several issues. For example, the different T-current and neuromodulation in the TRN compared to sensory thalamic relay nuclei are responsible for the unique firing patterns and the role of the TRN in spindle oscillations (Bal and McCormick 1993, Huguenard and Prince 1982, Warren and Jones 1997). Within MGB, different firing latencies in MGBv and MGBd have been attributed to different intrinsic membrane properties, like the presence or absence of $I_H$ (Hu 1995). Comparing the MGBv and LGNd, the number of interneurons (1% versus 20% in the rat, Winer and Larue 1996), the presence and absence of afferent GABAergic input (Peruzzi et al. 1997) and different neuromodulatory mechanisms (Tebecis 1974, McCormick 1992b, McCormick and Prince 1987, 1988) would suggest differences in signal transformation. However, more detailed studies are necessary to investigate the functional consequences of the different mechanisms for signal transduction in sensory thalamic nuclei.
High-threshold spikes in MGBv

A novel and prominent finding in our studies was the presence of HTS firing, either as part of the powerful LTS burst response or upon depolarization (Chapter 4). Similar observations were made earlier in other thalamic nuclei, however, in some of these studies the HTSs may have been interpreted as Na\(^+\)-action potentials based on their paradoxical sensitivity to TTX due to I\(_{\text{Nap}}\)-modulation (Chapter 4, Jahnsen and Llinás 1984b, Schwindt and Crill 1997). The high threshold for evoking HTSs could indicate a requirement for strong depolarization, e.g., by synchronous excitatory input, mGluR activation or blockade of K\(^+\)-conductances (cf. Chapter 4). On the other hand, the local threshold may be different from our somatic measurements (Fig. 4.3.), if the HTSs originates in distal dendrites (Schwindt and Crill 1997). Indeed, increasing experimental evidence indicates a dendritic origin of HTSs (Jahnsen and Llinás 1984b). Firing of HTSs in vivo would be difficult to distinguish from action potential firing in extracellular studies, thus requiring intracellular recordings in behaving animals (Kamondi et al. 1998). An HTS appearance under anesthesia would not be expected due to membrane hyperpolarization and shunting (Chapter 9). While the function of HTSs in auditory signal transfer in MGBv neurons requires further studies, dendritic high-threshold Ca\(^{2+}\)-currents have been implicated in the modulation of corticothalamic gamma feedback oscillations (Pedroarena and Llinás 1997) and short-term plasticity phenomena like augmenting responses in other thalamic neurons (Steriade and Timofeev 1997).

Putative dendritic firing patterns

Spatiotemporal signal processing takes on a new meaning in somatodendritic signalling within single neurons (cf. Discussion Chapter 4, Johnston et al. 1996). Advances in imaging and patch-clamp technology enabled the systematic study of dendritic membrane properties and the
contributions of active dendrites to signalling: Recent results revealed action potential propagation into the dendrites (Stuart and Sakman 1994), active propagation of spikes from the denrites to the soma (Schwindt and Crill 1997) and completely independent action potential generation in soma and dendrites (Kamondi et al. 1998). The amplitude of dendritic spikes is controlled by dendritic membrane properties (Hoffman et al. 1997) and neuromodulation (Tsubokawa and Ross 1997). In thalamic neurons, imaging studies demonstrate strong calcium signalling in the dendrites (Munsch et al. 1997, Zhou et al. 1997). Furthermore, combined experimental and modelling studies provide strong evidence for a primarily dendritic location of T-Type Ca\(^{2+}\)-channels (Destexhe et al. 1996, 1998), consistent with an inability to voltage-clamp the LTS (Coulter et al. 1989, Ries and Puil 1993).

In MGBv neurons, such a spatially different distribution of ion channels or receptors would predict membrane potential gradients within the somatodendritic compartments and could indicate the generation of different spikes in soma and dendrites (cf. Discussion Chapter 4, Schwindt and Crill 1997). This may be facilitated by spatially selective neuromodulatory inputs, reported for mGluRs on distal dendrites opposite corticothalamic, but not sensory afferents (cf. Godwin et al. 1996a). Also, highly synchronous synaptic input may be required for dendritic spike generation (Kamondi et al. 1998). As a consequence, integration of synaptic input and intrinsic membrane properties in the dendrites may differ dramatically from somatic signalling. Thus, dendritic burst and tonic firing modes could be subject to quick changes, depending on synaptic input, rather than longer-lasting neuromodulatory states (e.g. wakefulness or deep sleep). In this regard, phasic burst responses during wakefulness have been interpreted as a mechanism for saliency coding in sensory signalling (Crick 1984, Destexhe et al. 1998, Guido and Weyand 1995, Sherman and Guillery 1996).
Extrinsic and intrinsic neuromodulation

Behavioral state-dependence of thalamic firing patterns is dependent on neuromodulatory activity in brainstem nuclei (Hu et al. 1989a, Steriade and McCarley 1990, Steriade et al. 1993, Williams et al. 1994). The transmitters involved in mediating these states (acetylcholine, serotonin, noradrenaline) usually evoke longer-lasting effects due to involvement of G-protein mediated signal transduction (McCormick 1992b, McCormick and Prince 1987, 1988, Mooney et al. 1995). State-dependent neuromodulation affects thalamic neurons differently, e.g. cholinergic neuromodulation has been shown to differentially modulate firing patterns in thalamic relay cells, interneurons and thalamic reticular cells (Hu et al. 1989 a,b; Pape and McCormick 1995).

In contrast, we identified mechanisms in this study, that would enable auditory input to change its own transformation, which was suggested previously as a function of corticothalamic feedback either directly (mGluR, Chapter 7) or via the TRN (GABA<sub>B</sub>, Chapter 8). The activation of mGluRs by cortical feedback provides a strong depolarization, possibly enabling HTSs or at least inactivating the T-current and shifting firing modes (cf. Godwin et al. 1996a,b, McCormick and von Krosigk 1992). On the other hand, activation of GABA<sub>B</sub> receptors can set the stage for burst firing by deinactivation of the T-Type Ca<sup>2+</sup>-current (Crunelli and Leresche 1991). However, tonic GABA<sub>B</sub> receptor activation may shunt MGBv firing, similar to the action of general anesthetics (Chapters 8 and 9).

Two important aspects of intrinsic auditory neuromodulation remain to be identified in MGBv: First, the requirements for activation of mGluRs, i.e. specific network dynamics such as synchronized or burst synaptic input (Kim et al. 1997) or a cooperative transmitter action at the receptors (Destexhe and Sejnowski 1995). Second, the functional significance of selective
corticothalamic input for mGluR activation but not for GABA_B-receptor activation (Hu et al. 1994, Peruzzi et al. 1997, Bartlett and Smith 1995).

**Outlook-future studies**

The identification of intrinsic membrane properties and their modulation by neuromodulators and drugs in MGBv in this study greatly enhances our understanding of signal transformation in MGBv. However, many further studies are required for an understanding of signal transformation in the MGBv:

On the level of intrinsic membrane properties, the role of dendritic membrane properties and spikes for intracellular communication and their influence on the neuronal output to the cortex requires further study (see above).

As a next step, the interaction of synaptic input with the intrinsic membrane properties in the generation of firing patterns and oscillations should be investigated. Identification of the specific tectothalamic and corticothalamic modulation of the signal transfer through MGBv (cf. Chapter 7 and 8) would yield insight into intrinsic auditory modulation and feedback, e.g. the stimulus-dependent activation of mGluRs or GABA_B-receptors (McCormick and von Krosigk 1992, Kim et al. 1997).

Another interesting subject to study is the role of interneurons in MGBv. This seems especially promising in light of the great phylogenetical variation of the number of interneurons in MGBv, which may indicate differences in auditory coding (Winer and Larue 1996). With the advent of infrared-videomicroscopy, enabling visualization of the neuron before establishing a patch-clamp recording, preselection of interneurons would overcome experimental problems arising from their scarcity.
In vivo, systematic studies of responses to auditory stimulation, while monitoring behavioral states, are urgently needed. This seems a prerequisite, in light of our isoflurane results (Chapter 9) and the large response differences between awake and anesthetized animals (cf. Chapter 1). While the argument, responses would only differ “quantitatively, not fundamentally” has been put forward in IC studies (Ehret and Romand 1997), this is clearly not a valid conclusion for the MGB (Aitkin et al. 1966). Ideally, studies on the systems level would include recordings from behaving animals, optimally combined with discrimination or learning paradigms, to further elucidate the role of the MGBv in auditory coding (cf. Covey et al. 1996).

Such studies should be extended to investigations of the mechanisms of thalamic neuromodulators on MGBv neurons and their release during behavioral states. Studies on cat and guinea pig have revealed species differences, which preclude an easy transfer of results to other species (cf. McCormick and Prince 1987, 1988). Histaminergic, purinergic and peptidergic effects and mechanisms have not been studied at all in MGBv.

Future studies seem especially fruitful, when findings from different experimental and theoretical levels, ranging from cellular to behavioral and modelling studies, are integrated.
11. References


