AN IN-VIVO INTRACELLULAR STUDY OF AUDITORY THALAMIC NEURONS

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THE HONG KONG POLYTECHNIC UNIVERSITY

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By

YU Yanqin

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CERTIFICATE OF ORIGINALITY

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it reproduces no material previously published or written nor material which has been accepted for the award of any other degree or diploma, except where due acknowledgement has been made in the text.

YU Yanqin

Abstract of thesis entitled

An in-vivo intracellular study of auditory thalamic neurons

Submitted by **YU Yanqin**

for the degree of ***Doctor of Philosophy***

at The Hong Kong Polytechnic University in **November 2003**

Abstract

The present study investigates the neuronal mechanism of the corticofugal modulation on the auditory thalamus, which transmits auditory information from the periphery to the cortex. In this study, the intrinsic electrophysiological properties and the auditory responses of the neurons in the medial geniculate body (MGB) were investigated through *in-vivo* intracellular recording from pentobarbital anesthetized guinea pigs, while the auditory cortex was electrically activated. The non-acoustically-driven firing rate was 45.8 ± 23.3 Hz (mean ± SD, n=8) at membrane potentials of -45 mV, 30.6 ± 19.4 Hz (n=14) at -50 mV, 18.0 ± 12.9 Hz (n=14) at -55 mV, and significantly decreased to 5.7 ± 7.4 Hz at -60 mV, and to 0.7 ± 1.5 Hz (n=10) at -65 mV (ANOVA, $p<0.001$). The discharge rate was calculated in the absence of acoustic stimuli over varied membrane potentials which were changed by intracellular injection of current or through automatic drifting. The maximum non-acoustically-driven rate was 160Hz. The auditory responsiveness of the MGB neurons was examined at membrane potentials over a range of –45 mV to –75 mV: the higher the membrane potential, the greater the responsiveness, and vice versa. For those neurons showed an excitatory postsynaptic potential (EPSP)

or spikes on the EPSP to the noise burst stimulus, the amplitude of the EPSP decreased as the membrane potential was hyperpolarized. For the neurons showed an inhibitory postsynaptic potential (IPSP), or a spike followed by an IPSP to the noise-burst stimulus, the amplitude of the IPSP decreased when the membrane potential was hyperpolarized. A putative non-low-threshold calcium spike (non-LTS) burst was observed in the present study. It showed significantly longer inter-spike intervals (11.6 ± 6.0 ms, $p<0.001$, *t*-test) than those associated with the putative low-threshold calcium spike (LTS) bursts (6.7 ± 2.4 ms, $p<0.001$, *t*-test).

Of 52 neurons responsive to sound, 29 showed excitatory auditory responses to acoustic stimuli. Ten of them showed burst-like responses to auditory stimuli, with excitatory postsynaptic potentials (EPSPs) exhibiting a mean amplitude and duration of 13.2+/-10.2 mV (range: 4-40 mV) and 184.0+/-126.6 ms (range: 20-420 ms), respectively. Fourteen showed a phasic response to an acoustic stimulus, with a mean amplitude and duration of the EPSPs of 10.2+/-5.5 mV and 158.6+/-95.4 ms. Five neurons showed a tonic response pattern. Fourteen neurons responded to auditory stimuli with an IPSP with a mean amplitude and duration of -10.1+/-2.5 mV and 350.7+/-273.6 ms, respectively. Neurons with EPSP patterns tended to have a sharp-tuning curve, a low response threshold and a short response latency. The neurons with an IPSP pattern and a tonic response pattern tended to show a non-tuning characteristic, or a broad/double-peaked tuning curve with a higher threshold. OFF neurons responded to auditory stimuli of different durations with different latencies, or deviations of latencies, in addition to different spike numbers. Most OFF and ON-OFF neurons showed membrane oscillations with a frequency

of about 5 Hz. The neurons changed their firing pattern to an LTS when the membrane potential was further hyperpolarized to < -75 mV. With an even lower membrane potential at < -85 mV, the neurons responded to the same noise-burst stimulus with an LTS burst.

Of 92 neurons that received corticofugal modulation on the membrane potential, 38 neurons received potentiation and 50 neurons received hyperpolarization. Corticofugal potentiation of the membrane potential (amplitude: 8.6±5.5 mV; duration: 125.5±75.4 ms) facilitated the auditory responses and spontaneous firing of the MGB neurons. This hyperpolarization of -11.3±4.9 mV in amplitude and 1023.0±635.8 ms in duration suppressed or even totally diminished the auditory responses and spontaneous firing of the MGB neurons.

Of 38 auditory excitatory neurons examined, 31 received corticofugal potentiation, four received corticofugal inhibition, and three received no effect. Of 33 auditory inhibitory neurons examined in the present study, 31 received corticofugal hyperpolarization on their membrane potential and two received no effect. The shapes of all of the EPSPs and IPSPs caused by the cortical stimulation were similar to those evoked by acoustic stimuli on each neuron.

All of the nine auditory excitatory neurons that located in the lemniscal MGB received a corticofugal depolarization on their membrane potentials. All of the eight auditory inhibitory neurons located in the non-lemniscal MGB received a corticofugal inhibitory modulation effect.

The present intracellular recording provides the first study of how the corticofugal projection gates the sensory information in the thalamus: that is

spatially selective depolarization and hyperpolarization of the lemniscal and non-lemniscal MGB neurons. Present results also suggest a possible segregation of the excitatory and inhibitory neurons. The fact that a similar shape of postsynaptic potential caused by both ascending and descending inputs indicates a neuronal endogenous characteristic irrespective of the physical locations of the synapses. The dependence of the temporal structure of the spikes/spike bursts on the stimulus may provide insight into the temporal coding of sound information in the auditory system. The response patterns of the OFF neurons suggests that spike timing could be another parameter used by the thalamic neurons to encode the stimulus information. The finding that most OFF and ON-OFF neurons showed membrane oscillations strengthens the idea that membrane oscillations might be more dominant in the non-lemniscal MGB than in the lemniscal MGB.

Relevant Publications

Full papers:

Yu YQ, Xiong Y, Chan YS, He J (2004). Corticofugal gating of auditory information in the thalamus: An *in-vivo* intracellular recording study. Journal of Neuroscience (In press)

He J, Yu YQ, Xiong Y, Hashikawa T, and Chan YS (2002). Modulatory effect of cortical activation on the lemniscal auditory thalamus of the guinea pig. J Neurophysiol 88: 1040-1050

Xiong Y, Yu YQ, Fujimoto K, Chan YS, He J (2003). An *in vivo* intracellular study of auditory thalamic neurons. Thalamus & Related Systems 2: 253-260 (co-1st author)

Yu YQ, Xiong Y, Chan YS, He J. *In-vivo* intracellular responses of the medial geniculate neurons to acoustic stimuli (Submitted)

Xiong Y, Yu YQ, Chan YS, He J. Effects of current-injection and cortical stimulation on auditory-responsive thalamic neurons: an *in-vivo* intracellular study. (Submitted)

Conference abstracts:

Yu YQ, Xiong Y, Chan YS, He J. Corticofugal gating of auditory information in the thalamus: An *in-vivo* intracellular electrophysiological study. Program No. 181.11. 2003 Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience, 2003. Online

Yu YQ, Chan YS, He J. Discharge pattern and corticofugal control of OFF/ON-OFF neurons in the guinea pig auditory thalamus: an *in-vivo* intracellular study. Program No. 354.4. 2002 Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience, 2002. Online.

Yu YQ, Xiong Y, Chan YS, and He J. Corticofugal modulation of the auditory information transmission in the auditory thalamus of the guinea pig: an *in vivo* intracellular electrophysiology study. In: Central Auditory Processing: Integration of Auditory and Non-Auditory Information. Ascona, Switzerland, p. 37, 2002.

Yu YQ, Xiong Y, Chan YS, He JF. Corticofugal control of the auditory information transmission in the auditory thalamus: an *in-vivo* intracellular electrophysiological study on the guinea pig. Neuroscience letters. 2002, Supplement 57: S10.

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Xiong Y, Yu YQ, Chan YS, He JF. *In-vivo* intracellular recording on the auditory thalamic neurons. Soc. Neurosci. Abstr. 2001, 27: 126

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List of Abbreviations

AI	primary auditory cortex
AAF	the anterior auditory field
BF	best frequency
EPSP	excitatory postsynaptic potential
IC	inferior colliculus
Icc	the central nucleus of the inferior colliculus
i.p.	intraperitoneally
IPSP	inhibitory postsynaptic potential
ISI	inter-spike-interval
LTC	low-threshold calcium
LTS	low-threshold calcium spike
MGB	medial geniculate body
vMGB	ventral division of medial geniculate body
dMGB	dorsal division of medial geniculate body
mMGB	medial division of medial geniculate body
sMGB	shell nucleus of medial geniculate body
cmMGB	caudomedial nucleus of medial geniculate body
rmMGB	rostromedial nucleus of medial geniculate body
non-LTS	non-low-threshold calcium spike
s.c.	subcutaneously
TRN	thalamic reticular nucleus

Chapter 1

Introduction

1.1 Background of the study

The MGB is the principal auditory thalamic nucleus, which in different species consists of several independently defined parts, each with different midbrain inputs and cortical targets, as well as different patterns of physiological representation.

Compared to the ascending thalamocortical projection, the MGB receives a much stronger reciprocal projection from the cortex (Andersen et al., 1980; Winer and Larue, 1987). Neurons in the deep layers of the auditory cortex project to the MGB or to the inferior colliculus (IC). In the cat, about half of all layer 6 pyramidal cells contribute to the corticogeniculate pathway. It has been estimated that each geniculate relay cell receives convergent input from at least 10 cortical cells, and most likely much more. The corticogeniculate axons influence their target cells in a topographic manner.

These corticofugal modulations consists of highly focused positive feedback to subcortical neurons "matched" in tuning to a particular acoustic parameter and of widespread negative feedback (lateral inhibition) to "unmatched" subcortical neurons. Corticothalamic fibers make excitatory synaptic contracts on the distal dendrites of thalamic principal cells (Liu et al., 1995a), as well as on inhibitory

local circuit cells (Golgi Type 2, inhibitory interneurons) of the main thalamic sensory nuclei and the thalamic reticular nucleus (TRN) (Yen et al., 1985). In addition, TRN sends inhibitory projections back to both principal cells and local circuit cells in the main thalamic nuclei. The presence of these local inhibitory neurons is species-specific and varies across the different sensory thalamic subdivisions. In the MGB of rats or guinea pigs, the relative frequency of local inhibitory neurons is 0-3%, while that in cats is 25-30%.

It has been suggested that the corticofugal projection provides a gating or gain-control mechanism in the transmission of information from the periphery to the cortex (Ryugo and Weinberger, 1976; Crick, 1984; Deschênes and Hu, 1990; Villa et al., 1991; Suga et al., 1997; Zhou and Jen, 2000).

Previous investigators have observed mostly inhibitory effects on the MGB neurons caused by activating the auditory cortex (Amato et al., 1969; Watanabe et al., 1966). Aitkin and Dunlop (1969) reported an antidromic effect and intrinsic inhibition in the MGB caused by cortical stimulation. Both corticofugal facilitation and inhibitory effects on the MGB neuronal responses to sound stimuli were demonstrated by cooling the primary auditory cortex (AI) (Ryogo and Weinberger, 1976; Villa et al., 1991). More recently, Suga and his colleagues have investigated the corticofugal modulatory effects on the thalamus and on the midbrain of the bat. They found that the corticofugal feedback could sharpen the frequency-tuning curves of thalamic neurons (Zhang et al., 1997), facilitate the time-domain processing of biosonar information (Yan and Suga, 1996), and rearrange the

frequency map of the IC of the midbrain (Yan and Suga, 1998; Zhang and Suga, 1997).

Intracellular recording by McCormick and von Krosigk (1992) showed that the delivery of two or more electrical stimuli to corticothalamic fibers at a high frequency causes a slow excitatory potential that lasted for seconds. These physiological results suggest that the corticofugal modulation has a long time constant. Since antidromic effects on thalamic neurons caused by AI activation have short latencies, these effects could be avoided by using a long time delay between electrical stimulation and the sound stimulus, leaving only long latency feedback effects.

In a previous study in our laboratory, we investigated the point-to-point effects of the feedback connections between AI to the ventral division of the MGB (vMGB) in the cat (He, 1997). Corticofugal modulation on the thalamic neuron was positively studied by electrical activation of AI and the modulatory effect on the onset responses to sound was studied by using the extracellular recording technique. In a recent extracellular study, He (2003a) has observed a mostly excitatory effect on the auditory response of the lemniscal MGB neurons and a mostly inhibitory effect on the ON responses of the non-lemniscal MGB neurons following cortical activation.

To understand the mechanism of the above results, the membrane potential of the thalamic neurons has to be observed, during the course of the presentations of acoustic stimulus to the ears and electrical stimulation in the cortex. *In vivo*

intracellular recording has to be adopted in this experiment to measure the membrane potential.

Recent anatomical study shows that the corticofugal projection to various divisions of the MGB, e.g., the medial, the dorsal, and the ventral divisions, are different. The corticothalamic and thalamocortical projections are not reciprocally overlapped and this reciprocal projecting system varies greatly from the ventral division to the medial division of the MGB. The corticofugal projection to the ventral division of the MGB is heavy, but that to the medial division of the MGB (mMGB) is light (He and Hashikawa, 1998). It is important to examine the physiological differences of the corticofugal modulatory effect on various divisions of the MGB by anatomical identification of the locations of the recorded neurons after *in vivo* intracellular physiological recording.

1.2 Aims of the study

The goal of this study was to understand the mechanism of the corticofugal projecting system in terms of modulating the auditory information transmission in the thalamus.

The first specific aim of this study was to explore the intrinsic electrophysiological properties of neurons in the auditory thalamus and their responses in pentobarbital anesthetized guinea pigs through intracellular recording. In a series studies to examine the corticofugal modulation on the thalamic neurons intracellularly, I also recorded neuronal responses to acoustic stimulus and gained

information about the intrinsic electrophysiological properties of these neurons. The present study investigated non-acoustically-driven firing, responses to auditory stimuli and to electrical current injection, and changes in neuronal responses to auditory stimuli while the membrane potential was manipulated through current injection.

The second specific aim of the present study was to examine the effect of the cortical activation on the auditory thalamus by *in vivo* intracellular recording. Both facilitatory and inhibitory effects on the response of the MGB neurons to auditory stimulus evoked by electrical activation of the primary auditory cortex have been observed in previous studies by *in vivo* extracellular recording. These effects were examined in this study by using the *in vivo* intracellular recording method to measure the membrane potential of the MGB neurons during the presentation of sound stimulus and/or cortical activation. The relationship between corticofugal modulation and the different firing patterns of the MGB neurons was also explored in this study.

The third specific aim of the present study was to examine the physiological differences of the corticofugal modulatory effect on neurons from various divisions of the MGB. A recent anatomical study showed a strong corticofugal projection to the ventral division and a very weak corticofugal projection to the mMGB (He and Hashikawa, 1998). It was speculated that the change of the membrane potential of the neurons in the mMGB in response to cortical activation and auditory stimulus would be different from that of the neurons in the ventral division of the MGB. The inhibitory input from the TRN, which would be activated by the corticofugal

projection, would be the major effect for the medial division, but the direct excitatory effect to be the major effect for the ventral division. The position of the MGB from which the recorded neuron came was identified to achieve this goal. Thus we know which kind of corticofugal modulation effect (facilitatory or inhibitory) is preferred in the different divisions of the MGB.

1.3 Significance of the study

The present study unraveled the physiological role of the corticofugal feedback system to the thalamus, which, it has been suggested, performs a gating or gain control function in the transmission of information from the periphery to the cortex, and to be greatly related to attentive perception. The present intracellular recording provides the first result regarding how the corticofugal projection gates the sensory information in the thalamus: that is spatially selective depolarization and hyperpolarization of the lemniscal and non-lemniscal MGB neurons. This study also suggests a possible segregation of the excitatory and inhibitory neurons. The result of a similar shape of postsynaptic potential caused by both ascending and descending inputs reflects a neuronal endogenous characteristic irrespective of the physical locations of the synapses. The dependence of the temporal structure of the spikes/spike bursts on the stimulus may provide insight into the temporal coding of sound information in the auditory system. The response patterns of the OFF neurons suggested that spike timing could be another parameter used by the thalamic neurons to encode the stimulus information. The finding that most OFF

and ON-OFF neurons showed membrane oscillations strengthens the idea that membrane oscillations might be more dominant in the non-lemniscal MGB than in the lemniscal MGB.

1.4 Outline of the thesis

Chapter 1 introduced the background and aims of this study. The significance of the present study was also mentioned in this chapter.

Chapter 2 provided a literature review about a background of basic anatomic and physiological knowledge of the organization of the thalamocortical pathway and the corticothalamical projection.

Chapter 3 describes non-acoustically-driven firing, responses to auditory stimuli and to electrical current injection, and changes in neuronal responses to auditory stimuli while the membrane potential was manipulated through current injection. The different patterns of the auditory responses of the MGB neurons and their tuning properties are also described.

Chapter 4 describes the most basic functions of the corticofugal modulation of the guinea pig: corticofugal facilitation and inhibition. It provides the first result regarding how the corticofugal projection gates the sensory information in the thalamus: that is spatially selective depolarization and hyperpolarization of the lemniscal and non-lemniscal MGB neurons.

Chapter 5 describes the auditory responses of the MGB neurons and the corticofugal modulation on the neurons in the auditory thalamus in relation to the

locations of the recorded neurons by anatomical confirmation after physiological study.

Chapter 6 summarized the findings, the conclusions and the functional implication of the present study.

Chapter 2

Literature review

The ascending primary auditory pathway consists of the cochlear nuclei, the superior olive, the IC, the medial geniculate body (MGB) and the auditory cortex (AC) (Rouiller and de Ribaupierre, 1985). The first relay of the primary auditory pathway is the cochlear nuclei in the brain stem, which receive the information from the auditory nerve. The second major relay in the brain stem is in the superior olivary complex: the majority of the auditory fibers here already cross the midline. Leaving this relay, the fibers carry the message up to the level of the mesencephalus: the inferior colliculus. A final relay, before the cortex, is the medial geniculate body. It's the principal auditory thalamic nucleus which plays an essential role in the processing of sound. The terminal of the primary auditory pathway is the primary auditory cortex.

The thalamus is at the crossroads of the brainstem, basal ganglia and telencephalic circuits. It is not to be regarded merely as a set of nuclei that relays afferent impulses en route to the cerebral cortex. Rather, it should be viewed as a unifying entity that operates as the ultimate gatemaster and can, in fact, conjure from the intrinsic properties of neurons in the resting and active states of the brain. Such states represent emerging properties of thalamic networks that, as gates, mark the entrance to the forebrain (Sherman and Guillery, 1996). The thalamus is actively implicated in shaping afferent signals through inhibitory processes, that it

participates in highly complex integrative functions and that it is crucial for shifting the functional mode of the brain in a continuous way between an adaptive behavioral state, open to the outside world, and a disconnected state when thalamic gates are closed (Steriade et al., 1997).

The function of the feedback corticothalamic loop is a matter of controversial hypotheses because investigations using reversible blockade of corticofugal activity have reported decreased, increased, mixed and no significant effects on thamlamic activity (Koch, 1987). The most likely reason of this discrepant results is the complexity of the thalamic networks: in an intact-brain animal, corticothalamic axons directly excite thalamocortical cells and indirectly inhibit them through excitation of the TRN and local-circuit GABAergic neurons. The importance of the thalamic modulation by corticofugal input is emphasized by the abundance of corticothalmic axons, about one order of magnitude larger than that of thalamocortical fibers (White and Hersch, 1982, Steriade et al., 1997). In this literature review, I take the auditory thalamus as an example to provide a background of basic anatomic and physiological knowledge of the organization of the thalamocortical pathway and the corticothalamical projection.

The auditory thalamus relays information from the inferior colliculus to the cortex. It includes the medial geniculate body and the lateral part of the posterior nucleus group (Imig and Morel, 1983). The MGB is the principal auditory thalamic nucleus, which in different species consists of several independently defined parts, each with different midbrain inputs and cortical targets, as well as different patterns of physiological representation.

When compared to the ascending geniculocortical projection, the MGB receives a much larger reciprocal projection from the cortex (Montero, 1991; Andersen et al., 1980; Winer et al., 2001). The corticofugal projection, which projects not only to the MGB but also to the IC and brainstem, has been suggested to provide a gating or gain control mechanism in the transmission of information from the periphery to the cortex (Ryugo and Weinberger, 1976; Deschênes and Hu, 1990; Villa et al., 1991; Montero, 1999). In terms of the number of cortical cells involved in the corticofugal projection, the corticocollicular projection is far weaker than the corticothalamic projection (Winer et al., 2001). In my study, I concentrate on the corticothalamic projection to the auditory thalamus.

2.1 Cytoarchitecture and cellular morphology of the MGB

The MGB consists of three divisions: vMGB, dMGB, and mMGB (Figure 1). The tripartite parcellation of the MGB was initially defined based upon cytoarchitecture and cellular morphology. A large number of subsequent studies have confirmed these initial findings in many species (cat: Morest and Winer, 1986; tree shrew: Oliver and Hall, 1978; rat: Clerici and Coleman, 1990; Clerici et al., 1990; LeDoux et al., 1987; mustache bat: Winer and Wenstrup, 1994a, b; rabbit: Caballero-Bleda et al., 1991; De Venecia et al., 1995; guinea pig: Strutz, 1987; monkey: Burton and Jones, 1976; Hashikawa et al., 1995; Molinari et al., 1995; human: Winer, 1984).

The three major divisions of the MGB that have been classically described are: (1) vMGB which mainly contains cells with tufted dendrite trees; (2) the dorsal division (dMGB, dorsal and caudo-dorsal part) which contains stellate cells with extensive dendritic arborss and bushy neuros with dendritic branches less tufted than those observed in vMGB; (3) the medial division which contains cells with the largest soma in the MGB (prominent magnocellular neurons) but also small tufted neurons, and more classical stellate cells. Each part projects to various auditory cortical fields.

The vMGB is the largest division of the MGB. It contains a densely packed aggregate of medium-sized and small neurons, with the former predominant. In the dMGB, the neurons have slightly smaller somata and are more dispersed, while the mMGB has a broad range of somatic sizes, including the largest cells in the MGB and a lower density of neurons than the other two divisions (Morest, 1965; Winer, 1992).

The MGB in the guinea pig is an ovoid about 2.7-3mm long in the rostrocaudal direction, with a mediolateral diameter of maximally 2.5mm and a vertical diameter of 2-2.5mm. The guinea pig MGB has been divided into four subnuclei (Redies and Brandner, 1991). The ventral nucleus of the MGB occupies a lateroventral position in the rostral two-thirds of the MGB and projects to two tonotopic fields (fields A and DC) of the auditory cortex. The cells in the vMGB are densely packed and not very deeply stained in neutral red preparations. The shell nucleus (sMGB) surrounds the vMGB like a continuous shell from dorsal, lateral and ventral. Neurons in the sMGB project to a non-tonotopic field situated

ventrocaudal belt and appear similarly cytoarchitectonically to those in the vMGB. But sMGB is not immediately apparent in Nissl material. Two more MGB subnuclei were identified. One is the caudomedial nucleus (cmMGB) which consists in Nissl preparations of the less densely packed and deeply stained large cells and projects in a sparce fashion to the entire, or nearly entire, auditory cortex. The other is the rostromedial nucleus (rmMGB) which lies medial to the vMGB in the rostral half of the MGB and projects to a small tonotopic cortical field (field s), which is not innervated by the vMGB. It can be distinguished from the vMGB by the less densely packed and deeper-stained cells.

2.2 The response property and tonotopical organization of the MGB

The neurons in vMGB have extremely uniform properties, which include high levels of spontaneous activity, sharp frequency tuning and tonotopic organization, and the shortest latency (8-15ms) responses to acoustic stimuli. The responses, which are trains of discharges, typical of thalamic relay neurons firing in the tonic mode, are invariably transient (on-responses) in anesthetized cats and do not habituate.

The neurons in dMGB are most divergent and their properties do not befit them for a role in the transfer of information that can serve as a basis for complex auditory discriminations. The neurons are relatively silent and a high percentage has extremely long (up to 100ms) latencies and inconsistent responses to auditory stimuli; some (15%) may not respond at all (Calford MB, 1983). When the neurons

do respond, the responses are less obviously transient than in the ventral nucleus, commonly labile or intermittent, usually habituate in the face of repetitive stimuli and may be novelty sensitive. So that when habituated, small fluctuations in stimulus intensity or frequency may restore discharges to a previous level. All response to a wide range of frequencies, cannot be tuned to a best frequency and therefore no obvious tonotopic representation of the auditory periphery is found in the dorsal nucleus.

The neurons of the mMGB are a mixed population. Half of the responsive neurons respond at short latency and are sharply tuned, with best frequency curves resembling those of neurons in the ventral nucleus; the remainder have long latencies, if they respond at all, are labile, tuned to a broad range of frequencies and resemble neurons in the dorsal nucleus. A greater proportion of the neurons than in other nuclei of the MGB display sustained discharges in response to acoustic stimuli. Some cells habituate to repetitive stimuli, while others do not (Clarey et al., 1992).

In the cat the vMGB is tonotopically organized with the low-frequency region located laterally, the middle-frequency region caudomedially, and the high-frequency region rostromedially (Aitkin and Webster, 1972; Clarey et al., 1992; Imig and Morel, 1984, 1985). In guinea pigs the tonotopic map runs rostrocaudally with high frequencies located rostrally and low frequencies caudally (Redies and Brandner, 1991; He et al., 2002). Cells in the non-lemniscal MGB typically show long latency, a bursting firing pattern, broadly tuned frequency response properties, and non-tonotopic organization. It has also been shown that some cells in the non-

lemniscal divisions respond not only to auditory stimuli but to other sensory modalities as well (Calford and Aitkin, 1983; He et al., 1997; He and Hashikawa, 1998; He and Hu, 2002; Hu, 1995; Kosaki et al., 1997; Rauschecker et al., 1997; Redies et al., 1989a; Redies and Brandner, 1991; Rouiller et al., 1989; Wepsic, 1966; Winer and Morest, 1983; Winer et al., 1992, 1999). Recently we found that cells with ON and OFF responses are also segregated in the MGB. The OFF and ON-OFF neurons tend to be located on the border region between the lemniscal and non-lemniscal nuclei or outside the lemniscal nucleus, respectively (He, 2001, 2002).

2.3 Structure and tonotopic organization of auditory cortex of the guinea pig

An important organization principle of the auditory cortex is tonotopy. A cell's best frequency is the frequency to which the cell has the lowest response threshold. Tonotopy means that cells are arranged in an orderly way according to their BF. Tonotopic organization has been used as a criterion to define functional subunits in the auditory cortex or other parts of the auditory system (Winer et al., 2001).

Most of the work on the functional subdivisions of the auditory cortex has been carried out in cats, primates, and bats. Redies and his colleague divided the auditory cortex of the guinea pig into several subdivisions (Redies et al., 1989a). A large tonotopic area is found in the anterior half f the auditory cortex. This area is named the anterior field (field A). Frequency tuning curves in field A are generally

narrow (Redies et al., 1989a). Responses to tone stimuli are strong, and latencies are short. Low best frequencies are represented rostrally, high best frequencies caudally. Caudal to the first field, there is a second, smaller tonotopic area. It lies in the dorsal half of the posterior auditory cortex and is therefore named the dorsocaudal field (field DC). The frequency specificity of the cell clusters in this area is as strong as in field A, but the tonotopy is discontinuous: In the dorsal half of field DC, high best frequencies are represented rostrally; the low frequencies are represented immediately caudal to the high frequencies, while the intermediate frequencies are missing. The primary auditory cortex of the guinea pig contains filed A and DC (Redies et al., 1989a). Ventrally in field DC, the frequency representation is more complete. A third tonotopic field was found rostral to field A. This field extends over a surface of less than 1 mm2 and was named the small field (field S). It contains a complete representation of the frequency range; high best frequencies are located rostrally, low frequencies caudally. The response latencies are slightly longer in field S than in fields A or DC, and the tuning curves are broader. A broad strip of nontonotopic cortex (auditory belt) surrounds fields A and DC caudally. This area was subdivided into the dorsocaudal and the ventrocaudal belt region (Redies et al., 1989a). In both areas, tuning curves are often broad, and response latencies are longer than in the tonotopic cortex. In the dorsocaudal belt, most multiunits react with a phasic on-response to pure tones; in the ventrocaudal belt, tonic responses occur more frequently. Another nontonotopic region is located in the anterior auditory cortex, rostral to the tonotopic fields, and was therefore

named the rostral belt. Tuning curves in this area are broad, latencies are short, and response thresholds are often high.

2.4 Afferent and efferent connections of the MGB

The MGB of virtually all mammals is a complex of several, cytoarchitectonically distinct nuclei, each of which to a large extent represents a separate channel through which auditory information flows to the cerebral cortex (Imig and Morel, 1984). The predominant input to the vMGB arises in the central nucleus of the ipsilateral inferior colliculus, with a weaker input from the same nucleus of the contralateral side. vMGB receives the most direct, oligosynaptic and therefore shortest latency pathway from the contralateral peripheral auditory apparatus, displays a high degree of topographic organization and projects to the primary auditory cortex (Calford and Aitkin, 1983; Imig and Morel, 1984).

The major inputs to dMGB arise in the ipsilateral pericentral nucleus of the inferior colliculus and in the nucleus sagulum. dMGB represents relays for less direct auditory input to the cerebral cortex, and the close coupling between the relay cells and the cochlea. The topographic organization is less evident, dMGB in the main, projects to auditory cortical area outside the primary auditory cortex.

The mMGB receives inputs from the external nucleus of the inferior colliculus, from scattered cells of the central nucleus (Calford and Aitkin, 1983) and from the deep layers of the superior colliculus (Graham, 1977). mMGB, receives ill-defined, mixed auditory, somatosensory, vestibular and possibly other afferents; its neurons

do not always respond to peripheral auditory stimuli or respond in varying ways and, in projecting to areas of cerebral cortex that extend well beyond the confines of the auditory areas. It is less restrictedly auditory in function than the other nuclei of the medial geniculate complex.

In the cat, the vMGB cells project primarily to AI and its mirror region, the anterior auditory field (AAF). The equivalent auditory fields in the guinea pig are the anterior (A) and the dorsocaudal (DC) fields (Redies et al., 1989b). The dMGB projects to areas of auditory cortex surrounding the primary cortex while the mMGB projects to all auditory cortices including its association cortex (Imig and Morel, 1983; Andersen et al., 1980; Winer et al., 1977). The mMGB of the non-lemniscal MGB, which is equivalent to the cmMGB of the guinea pig, also projects to and receives input from the amygdala and the basal ganglia (Wepsic and Sutin, 1964; LeDoux et al., 1990; Cruikshank et al., 1992; Shinonaga et al., 1994). The cortical projection of the vMGB is mainly to cortical layers IV and IIIb, while many mMGB cells project to layer I from where neurons in all layers receive input (Hashikawa et al., 1995; Jones and Burton, 1976; Mitani et al., 1984; Niimi et al., 1984; Winer, 1992).

The projection from the MGB to the tonotopic fields (the anterior field A, the dorsocaudal field DC, the small field S) of the auditory cortex and to the nontonotopic ventrocaudal belt in the auditory cortex of the guinea pig has well been investigated (Redies et al, 1989b). The anterior field A receives its main thalamic input from the vMGB. The projection is topographically organized. Roughly, the caudal part of the vMGB innervates the rostral part of field A and vice

versa. A second, weaker projection to field A originates in a magnocellular nucleus that is situated caudomedially in the MG and was therefore named the cmMGB. The dorsocaudal field DC receives input from the same nuclei as the anterior field, but the location of the labeled cells in the vMGB is different. There is only one continuous tonotopic map in the vMGB. This map is split when projected onto the cortex so that two adjacent tonotopic fields (A and DC) result. The cortical maps are rotated relative to the thalamic map in that rostral portions of the vMGB project to caudal parts of the tonotopic cortex and vice versa (Redies et al, 1989b).

2.5 An overview of the descending pathway from the auditory cortex

2.5.1 Corticocollicular projection

2.5.1.1 The cortico-collicular auditory system: anatomy

Neurons in the deep layers of the AC project to the MGB, the IC, or subcollicular auditory nuclei (Kelly and Wong, 1981; Saldana et al., 1996; Huffman and Henson, 1990). These corticofugal projections are tonotopically organized (Huffman and Henson, 1990; Malmierca et al., 1996). Corticothalamic fibers project only to the ipsilateral MGB and thalamic reticular nucleus (Huffman and Henson, 1990; Ojima, 1994). However, corticocollicular fibers bilaterally project to the IC. The ipsilateral projection is much more extensive and topographically organized than the contralateral projection (Saldana et al., 1996).

Therefore, ipsilateral corticofugal modulation is expected to be much larger than contralateral corticofugal modulation in the IC and MGB and to be frequency-dependent. Corticofugal projections are bilateral to the subcollicular nuclei: superior olivary complex and cochlear nucleus (Saldana et al., 1996). Corticofugal modulation is expected to take place even in the cochlea via olivocochlear neurons in the superior olivary complex. The central nucleus of the IC projects not only to the MGB and the superior colliculus, but also to medial olivocochlear neurons, which mostly project to contralateral cochlear outer hair cells. In general, olivocochlear neurons bilaterally project to the cochlea, although there are some differences in olivocochlear projections between species (Warr, 1997). Because the corticofugal system forms multiple feedback loops, the exploration of corticofugal functions is ongoing at different levels of the auditory system. An obvious critical experiment to be performed is the selective inactivation of individual feedback loops without injuring the ascending auditory system. Such an experiment, however, appears to be impossible because of anatomical complexity.

2.5.1.2 Corticofugal modulation of auditory signal processing in the inferior colliculus

Physiological data of corticofugal effects on the MGB and IC neurons have been controversial: (*i*) only or predominantly inhibitory (Massopust and Ordy, 1962; Watanabe et al., 1966; Amato et al., 1969; Sun et al., 1989; Sun et al., 1996; Jen et al., 1998). (*ii*) only or predominantly excitatory or facilitative (Andersen et al.,

1972; Orman and Humphrey,1981; Villa et al., 1991); or (*iii*) equally excitatory or inhibitory (Ryugo and Weinberger, 1976; Syka and Popelar, 1984; He, 1997; He et al., 2002; He, 2001). These data, regardless of the excitatory or inhibitory effect, indicate that one of the corticofugal functions can be nonspecific gain control. In the mustached bat, nonfocal inactivation of cortical auditory neurons, including neurons matched to recorded subcortical neurons, evokes a large reduction of the auditory responses of the subcortical neurons (Zhang and Suga, 1997; Yan and Suga, 1999). Matched means that electrically stimulated cortical neurons and recorded subcortical or cortical neurons are tuned to the same value of an acoustic parameter. Unmatched means that they are tuned to different values of an acoustic parameter. One of the corticofugal functions is amplification of the responses of subcortical neurons. Because there is a much larger number of corticofugal fibers than thalamocortical fibers, the corticofugal system should have much more elegant functions than simple gain control.

The massive corticofugal system, which is topographically as well-organized as the ascending auditory system (Games and Winer, 1988; Herbert et al., 1991; Huffman and Henson, 1990; Saldana et al., 1996; Winer et al., 1998), adjusts and improves ongoing subcortical auditory signal processing and reorganizes the subcortical frequency map (Gao and Suga, 1998, 2000; He, 1997; Jen and Zhang, 1999; Jen et al., 1998, 2001; Suga et al., 1997; Sun et al., 1989, 1996; Villa et al., 1991; Yan and Suga, 1996, 1998; Zhang and Suga, 1997, 2000; Zhang et al., 1997, 2000; Zhou and Jen, 2000). For example, cortical electrical stimulation synchronized with acoustic stimulation at low-repetition rate decreased auditory

responses and sharpened spatial selectivity and frequency tuning of corticofugally inhibited neurons in the central nucleus of the inferior colliculus (ICc) but produced the opposite effect on corticofugally facilitated collicular neurons (Jen and Zhang, 1999; Jen et al., 1998, 2001; Sun et al., 1989, 1996; Zhang et al., 1997, 2000; Zhou and Jen, 2000). This corticofugal modulation which typically vanished within 5~10 s after stimulation was called brief corticofugal modulation (Zhou and Jen, 2000). When cortical electrical stimulation synchronized with acoustic stimulation at the best frequency (BF) of single or clusters of cortical neurons was delivered at a s high-repetition rate, the BFs of collicular neurons were shifted toward the BFs of electrically stimulated cortical neurons. As a result, this corticofugal modulation increased the representation of the repetitive stimulus frequency in the ICc and reorganized the collicular frequency map for as long as 3 h (Gao and Suga, 1998, 2000; Suga et al., 1997, 1998; Yan and Suga, 1996, 1998; Zhang et al., 1997). This corticofugal modulation that persisted up to 3 h was called short-term corticofugal modulation (Zhou and Jen, 2000).

Corticofugal modulation of collicular auditory responses varies with stimulation conditions. While brief corticofugal modulation vanishes within 5~10 s after stimulation, short-term corticofugal modulation persists up to 40 min. However, neural mechanisms underlying the different time courses of brief and short-term corticofugal modulation remain to be studied. The neural circuits, synaptic mechanisms and neurotransmitters that mediate the corticofugal inhibition and facilitation of collicular responses also remain to be explored. Under high-repetition rate cortical electrical stimulation, studies in Suga's laboratory have

shown that cortical neurons mediate both highly focused positive feedback to subcortical neurons 'matched' in tuning to a particular acoustic parameter and widespread lateral inhibition to 'unmatched' subcortical neurons. This cortical feedback, which has been called 'egocentric selection', adjusts and improves subcortical signal processing and enhances the neural representation of frequent occurring signals in the central auditory system resulting in local reorganization of the frequency map in the ICc (Suga et al., 1997; Yan and Suga, 1996, 1998; Zhang and Suga, 1997; Zhang et al., 1997). It has been proposed that the corticofugal system may also be involved in subcortical information processing according to auditory experience based on associative learning and perhaps attention (Gao and Suga, 1998).

2.5.2 Corticogeniculate projection

2.5.2.1 Corticofugal projection to the MGB

Thalamic nuclei receive reciprocal pathways from the same cortical areas to which they project. Corticothalamic neurons originate from layer VI as well as the lower part of layer V and are known to be glutaminergic and aspartergic. They are quite different from corticofugal neurons projecting to the brain stem or spinal cord. Neurons in the deep layers of the auditory cortex project to the MGB or to IC (Prieto and Winer, 1999; Rouiller and Welker, 2000; Winer et al., 2001; Winer et al., 1998). In the cat, about half of all layer 6 pyramidal cells contribute to the

corticogeniculate pathway. It has been estimated that each geniculate relay cell receives convergent input from at least 10 cortical cells and most likely much more. The corticogeniculate axons influence their target cells (both relay cells and interneurons) in a topographic manner (He, 1997).

In the cat, each auditory cortical area projects back reciprocally to the MGB: AI and AAF project to the vMGB, AII to dMGB, and all fields to mMGB (Andersen et al., 1980; Merzenich et al., 1982). The reciprocity of the thalamocortical and corticothalamic projections is not quantitatively rigid (Winer and Larue, 1987; He and Hashikawa, 1998). The corticothalamic projection covers a wider region in the thalamus than the region which projects to the same cortical field (Winer and Larue, 1987). In addition, the corticothalamic projection does not appear to be equally dense in all vMGB regions. Injection of a bidirectional tracer into the cortex labeled a large number of vMGB and dMGB cell bodies and corticothalamic terminals. In contrast the same injections retrogradely labeled many neurons in the mMGB but very few corticothalamic terminals (He and Hashikawa, 1998), hinting that the mMGB is likely to receive a relatively weaker facilitatory modulation from the cortex than other divisions.

Corticothalamic fibres make excitatory synaptic contracts on the distal dendrites of thalamic principal cells (Liu et al., 1995a), as well as on inhibitory local circuit cells (Golgi Type 2, inhibitory interneurons) of the main thalamic sensory nuclei and TRN (Yen et al, 1985). The ascending fibers of the thalamic relay neurons send collateral projections to the TRN before reaching the cortex (Jones, 1975; Crabtree, 1998). The TRN also receives feedback projections from

the cortex and projects back to the thalamus. The major sources of afferent input the TRN are the collaterals of thalamocortical and corticothalamic fibers, all of which pass through the TRN en route to and from the cerebral cortex. The collateral thalamocortical and corticothalamic inputs display a topographical distribution of their terminals in the TRN (Yen et al., 1985). The TRN neurons of all species are large and GABAergic (Houser et al., 1980; Oertel et al., 1983; Yen et al., 1985). The axons arising from the TRN neurons, after giving off one or two collaterals in the nucleus, only project back to the dorsal thalamus and terminate, but not to the cortex (Yen et al., 1985). Neurons in different areas of the TRN project to different regions of the thalamus and local circuit cells in the main thalamic nuclei (Cox et al., 1997; Crabtree, 1998, 1999; Liu et al., 1995b; Ohara et al., 1980; Rouiller et al., 1985; Shosaku and Sumitomo, 1983). But the presence of these local inhibitory neurons is species-specific and varies across the different sensory thalamic subdivisions. In the MGB of rat or guinea pig, the relative frequency of local inhibitory neurons is 0-3%, while that in cat is 25-30%.

2.5.2.2 Corticofugal modulation on the MGB

Previous investigators have observed mostly inhibitory effects on the MGB neurons caused by activating the auditory cortex (Amato et al., 1969; Watanabe et al., 1966). Aitkin and Dunlop (1969) reported an antidromic effect and intrinsic inhibition in the MGB caused by cortical stimulation. Both corticofugal facilitation and inhibitory effects on the MGB neuronal responses to sound stimuli were

demonstrated by cooling AI (Ryogo and Weinberger, 1976; Villa et al., 1991). More recently, Suga and his colleagues have investigated the corticofugal modulatory effects on the thalamus and on the midbrain of the bat. They found that the corticofugal feedback could sharpen the frequency-tuning curves of thalamic neurons (Zhang et al., 1997), facilitate the time-domain processing of biosonar information (Yan and Suga, 1996), and alternate the frequency map of the inferior colliculus of the midbrain (Yan and Suga, 1998; Zhang and Suga 1997). Corticothalamic modulatory effects on the MGB neurons are more often seen in the long time-scale responses such as the reverberatory response (Ryugo and Weinberger, 1976; Villa et al., 1991).

2.5.2.2.1 Corticofugal facilitatory effect on the lemniscal MGB

Corticofugal activation at the most effective facilitatory site usually increases the spontaneous firing (Villa et al., 1991) in cat vMGB neurons and enhances the responses to sound stimuli (He, 1997). In a minority of cat vMGB neurons it induces a small decrease in the spontaneous firing and the maximal responses to pure tones or noise-burst stimuli.

The areas of auditory cortex that provide corticofugal modulation of the MGB form patch-like patterns on the cortical topography. The size of patches ranges from 600 to 1,900 μm with an average of 1,130 μm (He, 1997). This is larger than the spread of the terminal projections of thalamocortical neurons in the cortex, but roughly the same size as terminal projections of the reciprocal corticothalamic

neurons in the MGB. We conjecture that a possible reason that such large functional patches were observed in AI is that they reflect the effects of the widely ramifying corticothalamic projections (He, 1997).

In the guinea pig, where, in contrast to the cat, interneurons only account for about 1% of the cell population (Arcelli et al., 1997), cortical activation caused both facilitatory and inhibitory effects on neuronal responses of auditory thalamic neurons but the great majority of the effects are excitatory. Compared to the cat, a higher percentage of vMGB neurons in the guinea pig are affected by corticofugal activation. Of the 303 vMGB samples recorded, 218 (71.9%) were corticofugally inhibited (He et al., 2002).

2.5.2.2.2 Corticofugal inhibitory effect on the non-lemniscal MGB

In contrast to vMGB, where activation of either the auditory field A or DC caused a mainly facilitatory effect on the neurons, activation of the same areas caused large inhibitory effects on neurons in the cmMGB and rmMGB, and smaller inhibitory effects on neurons in the sMGB (He, 1997; He et al., 2002).

Eighty-five percent (174/204) of the cells seen in the cmMGB and 73% (62/84) of the cells in the rmMGB showed an inhibitory effect. The responses of only 12% of the neurons in the rmMGB were inhibited more than 50%, while this proportion in the cmMGB was 36%. In the sMGB, 77% (83/108) of the samples showed inhibitory effects, but only 14% of the samples showed inhibitory effects of >50% (He, 2003).

Watanabe et al. (1966) reported corticofugal modulatory effects in only 20 MGB neurons out of 292. Of these, 6 showed a facilitatory effect and 14 were strongly inhibited by cortical activation. Referring to the data (Fig. 4 of Watanabe et al., 1966), the illustrated neuron was an ON-OFF neuron, which is only found in the non-lemniscal MGB or on the border of the lemniscal and non-lemniscal MGB, and showed a burst response, which could be found in both the lemniscal and non-lemniscal MGB, but more likely in the non-lemniscal MGB (He, 2001, He and Hu, 2002; Mooney et al., 1995). Therefore, it would be reasonable ton speculate that a great proportion of the inhibited neurons reported by Watanabe et al. (1966) were sampled from the non-lemniscal nuclei of the MGB. The data in our laboratory indicates that the inhibitory effect on the non-lemniscal MGB was generally widespread as we observed inhibition generated from all three cortical stimulation sites in many cases, though there were exceptional cases in which only one or two sites showed inhibitory effects (He, 2003). Besides cortical stimulation, cortical cooling can also affect corticofugal actions. Villa et al. (1991) reported that more neurons in the medial division of the MGB showed an increased spontaneous firing rate than other subdivisions, when the auditory cortex was cooled. It is also interesting to note that neurons with a tonic response showed more corticofugal inhibition than excitation (Ryugo and Weinberger, 1976). mMGB neurons show a tonic response pattern more than phasic ones.

2.5.2.2.3 Long time-constants of corticofugal facilitation on the lemniscal MGB

For MGB cells whose responses to acoustic stimuli are facilitated by cortical stimulation, the facilitation lasts for over 300 ms and has its maximal effect at 50–150 ms (He, 1997; McCormick and von Krosigk, 1992; He et al., 2002). The long effective facilitation period of the corticofugal modulation could be explained by the morphological structure of the corticofugal fibers, the synapses of which terminate on the distal parts of the dendrites and are mediated, in part, by NMDA receptor (Bartlett and Smith, 1999; Bartlett et al., 2000; Liu et al., 1995a; McCormick and von Krosigk, 1992).

The long facilitation period in the vMGB might also be related to the morphology of the terminals of corticothalamic projection. The vMGB receives only small-sized terminals from corticofugal projections, while the dMGB receives both small-sized and giant fingerlike terminals from the corticothalamic projection (Bajo et al., 1995; Rouiller and Welker, 1991).

2.5.2.2.4 Long time-constants of corticofugal inhibition on the non-lemniscal MGB

The corticofugal inhibitory effect lasts even longer, in some cases for over 1 s (Fujimoto et al., 2002). There are three possible pathways that might be involved in inhibiting the non-lemniscal MGB.

1. Via thalamic interneurons. Anatomical studies have revealed that there are very few interneurons in the dorsal thalamus of the guinea pig and rabbit: <1% (Arcelli et al., 1997; Spreafico et al., 1983, 1994). Therefore, corticofugal

activation of the thalamic interneurons is an unlikely candidate for the strong corticofugal inhibitory pathway. Another possible inhibition is from the presynaptic dendrites (PSDs) of the interneurons on the thalamic relay neurons (Ralston et al., 1988; Liu et al., 1995a; He, 1997; Pinault and Deschênes, 1988). However, in the cat, the PSDs account for only <6% of the total terminals, compared with an average of 35% inhibitory terminals in the cat, where interneurons account for 24–27% of the total population (Liu et al., 1995a; Rinvik et al., 1987). With such a small number of interneurons in the guinea pig thalamus, it is unlikely that the strong and nucleus-specific inhibition is mainly caused by the PSDs. The interneurons and PSDs might be accountable for the small inhibition in the vMGB (He, 1997).

2. Via IC GABAergic neurons. The auditory cortex projects to the IC and the projections are excitatory (Herbert et al., 1991; Ojima, 1994; Winer et al., 1998). In the cat, about 20% of the neurons in the central nucleus of the IC are GABAergic (Oliver et al., 1994). Among the tectothalamic projection IC neurons, GABAergic neurons account for 14–36% in the cat and 20–45% in the rat (Winer et al., 1996; Peruzzi et al., 1997). However, in a recent *in vivo* intracellular recording, we were still able to record a strong, long-lasting inhibition in the MGB after an electrical stimulation of the auditory cortex with a pulse train after the dissection of the tectothalamic fibers. This suggests that the strong inhibition was not mediated by the IC pathway (Fujimoto et al., 2002).

3. Via TRN neurons. As mentioned earlier, thalamocortical and corticothalamic pathways are not rigidly reciprocally connected (Winer and Larue,

1987; Winer et al., 2001). In a previous study, we found fewer corticofugal direct projections to the non-lemniscal MGB than to the lemniscal MGB (He and Hashikawa, 1998). Morphologically the GABAergic terminals form synapses on every part of the relay neurons, with a higher portion at the proximal and intermediate parts of the neuron than the distal parts (Liu et al., 1995a). However, the corticothalamic terminals have their main contacts on the distal dendrites, having an accumulative effect that could be counterbalanced by the strong GABAergic inhibition (Golshani et al., 2001).

The majority of the excitatory inputs to the TRN neurons are derived from the cerebral cortex (Liu and Jones, 1999), indicating that the corticofugal fibers to the TRN neurons have a stronger control over the TRN neurons' excitability. Recent studies by Crabtree and colleagues revealed that the interconnections between the TRN and the dorsal thalamus are cross-nucleus and crossmodality (Crabtree, 1999; Crabtree and Isaac, 2002; Crabtree et al., 1998). The TRN neurons also project to the contralateral TRN in the rat (Battaglia et al., 1994). The TRN neurons extend dendrites within the thin reticular sheet, which enable them to receive projections from a wide cortical region, and project to widespread areas in the ventroposterior nucleus of the thalamus (Liu et al., 1995b). This, together with the results of other studies, leads to the reasonable conclusion that the control of the thalamus via the TRN is widespread (Ohara et al., 1980; Liu et al., 1995b; Bourassa et al., 1995; Cox et al., 1997; Crabtree, 1998, 1999; Crabtree and Isaac, 2002). This long inhibition is likely to be caused by the prolonged $GABA_B$ inhibition from the TRN, which lasts for hundreds of milliseconds (Bartlett and Smith, 1999).Giant GABAergic

terminals of unknown origin have been found only in the non-lemniscal nuclei of the MGB, and not in the vMGB of the cat (Winer et al., 1999). This location in the non-lemniscal nuclei may in part help to explain our observation of stronger corticofugal inhibition in these regions of the guinea pig MGB (Fig. 5 of He, 2003). On the other hand, giant corticothalamic terminals were found only in the non-lemniscal MGB, though their physiological significance still requires investigation (Bajo et al., 1995; Ojima, 1994; Rouiller and Welker, 1991, 2000). In summary, activation of the corticothalamic fibers directly generates excitatory inputs to the MGB neurons including non-lemniscal neurons, and also activates the TRN neurons. These TRN neurons in turn inhibit the non-lemniscal neurons.

The guinea pig mMGB consists of large and deeply Nissl stained cells that project diffusely to the entire auditory cortex (Redies and Brandner, 1991), hinting at a power to adjust the total activity of the auditory cortex. Unlike the cortical projection of the vMGB, which mainly terminates in layers IV and IIIb, the mMGB projects to layer I of the cortex, from where cortical neurons in all layers may receive input (Jones and Burton, 1976; Mitani et al., 1984; Niimi et al., 1984). The cmMGB is involved in the integration of multisensory afferents (Wepsic, 1966; Winer and Morest, 1983; Edeline, 1990; Edeline and Weinberger, 1992). As described above, the activated primary auditory cortex strongly inhibits the cmMGB while strongly facilitating the vMGB, providing a possible explanation for the selective gating of the auditory information through the lemniscal MGB. This "switching off" of other unwanted sensory signals and the interference from the

limbic system would leave the auditory cortex prepared to process only the auditory signal.

2.5.2.2.5 Corticofugal modulation on different firing patterns

The corticofugal pathway modulates not only the number of spikes but also the temporal firing pattern (He, 2003). Cortical activation increases the first spike latency and broadens the firing pattern over the time domain. The mean latency of the first spike was increased from 11.2 ms without cortical stimulation to 17.1 ms when the cortex was activated by a single pulse, and to 16.0 ms with 30 pulses.

The temporal firing pattern is widely believed to encode information in signal transmission. Using an artificial neural network, Middlebrooks and colleagues (1994) demonstrated that a single neuron could more accurately distinguish a sound from a different direction when they use information regarding both spike number and spike timing than when they used only information regarding spike number. The neuron provides more information by using both the temporal and rate cues rather than by using the rate cue only (He, 1998; He et al., 1997; Middlebrooks et al., 1994).

Our recent data clearly shows that the corticofugal modulation changes the temporal firing pattern of thalamic relay neurons (Figs. 1 and 3 of He, 2003). If one applies the same method as Middlebrooks et al. (1994), it would not be difficult to predict that neurons with different temporal firing patterns may indicate different corticofugal modulation. It will be interesting to investigate whether neurons can

detect different acoustic stimuli more accurately while corticofugal modulation is activated. On the other hand, Ryugo and Weinberger (1976) demonstrated that the cooling of the cortex suppressed the long-latency rhythmic discharges of the MGB neurons. The changes in temporal patterns of the rhythmic and rebound discharges could be another perspective in encoding sensory information or changing mode of the transmission of the sensory information in the thalamus (Brosch et al., 2001; Cotillon and Edeline, 2000; Destexhe et al., 1999).

2.6 Function implication of the corticofugal modulation

The central auditory system creates many physiologically distinct types of neurons for auditory signal processing. Their response properties have been interpreted as being produced by divergent and convergent interactions between neurons in the ascending auditory system. Until recently, the contribution of the descending (corticofugal) system to the shaping (or even creation) of their response properties has hardly been considered. Recent findings indicate that the corticofugal system plays important roles in shaping or even creating the response properties of central auditory neurons and in reorganizing cochleotopic (frequency) and computational (e.g., echo-delay) maps (Crick, 1984; He, 1997, He et al., 2002; Jen and Zhou, 2003; Murphy and Sillito, 1987; Sillito et al., 1994; Suga et al., 2002; Villa et al., 1991). Therefore, the understanding of the neural mechanisms for auditory signal processing is incomplete without the exploration of the functional roles of the corticofugal system.

Corticofugal feedback projection to the thalamus has been suggested as performing a gain or gating control function in the transmission of information from the periphery to the cortex (Crick, 1984; Murphy and Sillito, 1987; Sillito et al., 1994; Villa et al., 1991). Recent studies in our and others laboratory have shown that the massive corticofugal system extensively adjusts and improves subcortical auditory signal processing in the frequency, amplitude, time, and spatial domains (He, 1997; Jen and Zhang, 1999; Jen et al., 1998; Suga et al., 1997; Villa et al., 1991; Yan and Suga, 1996, 1998; Zhang and Suga, 1997; Zhang et al., 1997). It was also reported the corticofugal system can bind of the different features of auditory signals. In the visual system, the corticofugal system evokes feature-linked synchronized discharges in the thalamic neurons. It also makes Stabilization of thalamic auditory responses via the thalamic reticular nucleus. The reticular nucleus takes a role as an adaptive filter. Corticofugal positive feedback has a high gain, so that ringing would be evoked if it is not incorporated with inhibition through the thalamic reticular nucleus. If the TRN does not operate properly, long-lasting discharges, perhaps responsible for tinnitus, would be produced. In addition, the corticofugal system probably mediates attentional modulation of auditory signal processing. In humans, visual attention reduces auditory nerve response and otoacoustic emissions evoked by a click (Suga et al., 2000). Suppose that there is a dynamic filter array to selectively gate/attend to the signals in our auditory system: (1) this filter array has to be placed at an as early a stage of the auditory pathway as possible according to the signal processing theory and (2) the gains of the filters are controlled based on the judgment of the central system. To find out what signal is

wanted and what should be filtered, the cortex must be involved. Therefore, the candidates for this filter array are limited to either/both corticothalamic and/or corticocollicular systems. Since this dynamic filtering process is likely a universal one for the visual and other sensory systems, the corticocolliculic system is unique to the auditory system, and may be specialized for other functions such as plasticity as suggested recently (Gao and Suga, 1998, 2000; Zhou and Jen, 2000) and sound localization (Semple and Kitzes, 1987; Shackleton et al., 2003; Yin and Kuwada, 1983), and the corticothalamic system is the ideal candidate for the filter array.

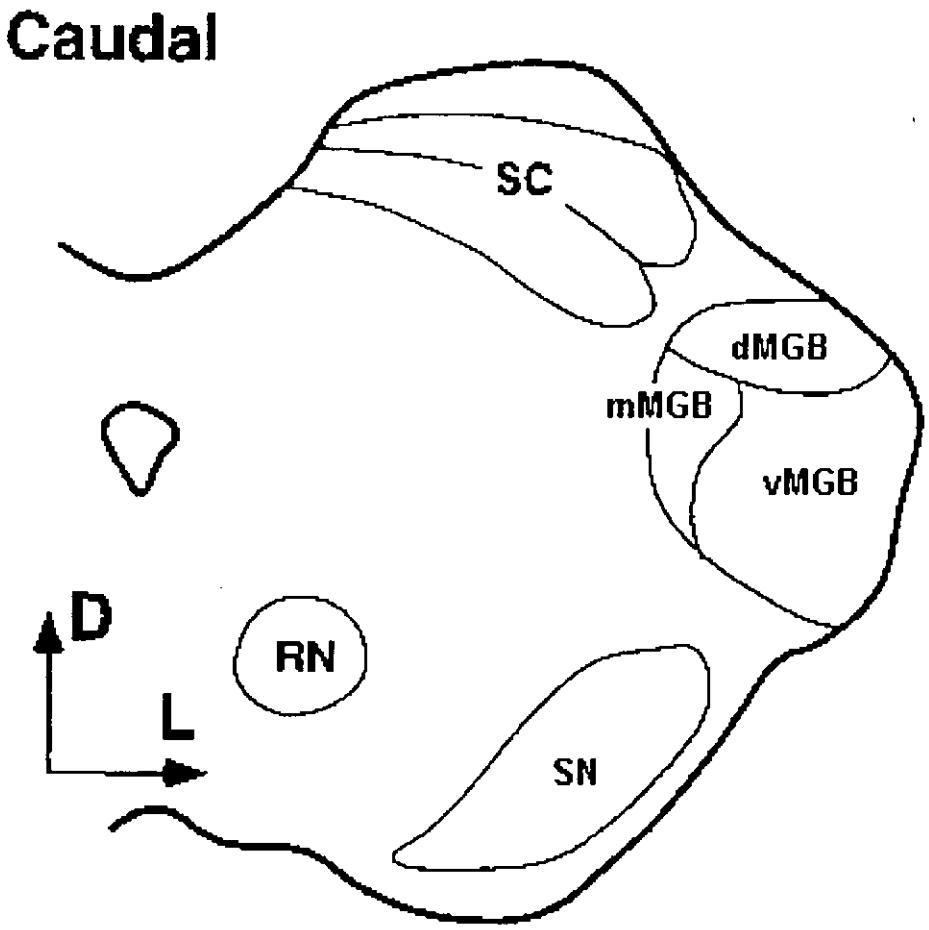

Figure 1 The Camera lucida drawings of different divisions of coronal sections through MGB. Calibration bar 1 mm. dMGB, mMGB, vMGB, dorsal, medial and ventral subdivisions of the medial geniculate body; SC, superior colliculus; SN, substantia nigra.

Chapter 3

Acoustic information processing in auditory thalamus of guinea pigs

3.1 Introduction

The auditory thalamus that relays information from the IC to the cortex includes the MGB and the lateral part of the posterior nucleus group (Imig and Morel, 1983). The tripartite parcellation of the MGB was initially defined based upon cytoarchitecture and cellular morphology. A large number of studies have confirmed these initial findings in many species (cat: Morest and Winer, 1986; tree shrew: Oliver and Hall, 1978; rat: Clerici and Coleman, 1990; Clerici et al., 1990; LeDoux et al., 1987; mustache bat: Winer and Wenstrup, 1994a, b; rabbit: Caballero-Bleda et al., 1991; De Venecia et al., 1995; guinea pig: Strutz, 1987; monkey: Burton and Jones, 1976; Hashikawa et al., 1995; Molinari et al., 1995; human: Winer, 1984;).

The guinea pig MGB has been divided into four subnuclei (Redies and Brandner, 1991). The ventral nucleus of the MGB occupies a lateroventral position in the rostral two-thirds of the MGB and projects to two tonotopic fields (fields A and DC) of the auditory cortex. The sMGB surrounds the vMGB like a continuous shell from dorsal, lateral and ventral. Neurons in the sMGB project to a non-tonotopic field situated ventrocaudal belt and appear similarly cytoarchitectonically to those in the vMGB. Two more MGB subnuclei were identified. One is the

cmMGB which consistms in Nissl preparations of large and deeply stained cells and projects in a sparce fashion to the entire, or nearly entire, auditory cortex. The other is the rmMGB which lies medial to the vMGB in the rostral half of the MGB and projects to a small tonotopic cortical field (field s), which is not innervated by the vMGB.

In the cat, and guinea pig, the lemniscal core of the MGB is the tonotopically organized ventral nucleus (Aitkin and Webster, 1971; Clarey et al., 1992). The non-lemniscal MGB consists of the medial and the dorsal nuclei in the cat (In guinea pigs it consists of the cmMGB and rmMGB). The neurons in the non-lemniscal MGB show long latency, bursty firing, broad or no frequency tuning, non-tonotopic organization, and multi-modal responses (de Ribaupierre and Toros, 1976; Calford and Webster, 1981; Calford, 1983; Winer and Morest, 1983; Imig and Morel, 1988; Hu, 1995; He and Hashikawa, 1998; He and Hu, 2002).

Most of our understanding of the MGB has been obtained with extracellular electrophysiological recordings. Intracellular recordings on the thalamic slice have provided insights into the synaptic mechanisms of neurons in the MGB (Hu et al., 1994; Hu, 1995; Li et al., 1996; Tennigkeit et al., 1996, 1998a; Bartlett and Smith, 2002). *In-vivo* intracellular recording in the IC has revealed a putative inhibitory mechanism for duration tuning in the bat, and complex interactions of excitatory and inhibitory input for binaural signal processing in the cat (Casseday et al., 1994; Kuwada et al., 1997). A recent *in vivo* intracellular study by Ojima and Murakami (2002) revealed that there are layer-specific differences in the auditory response characteristics of pyramidal neurons in the auditory cortex of cat. However, little is

known about the *in vivo* intracellular auditory response characteristics of the MGB neurons.

As compared to the ascending thalamocortical projection, the MGB receives a much stronger reciprocal projection from the cortex (Andersen et al., 1980; Winer and Larue, 1987). It has been suggested that corticofugal projection provides a gating or gain-control mechanism in the transmission of information from the periphery to the cortex (Ryugo and Weinberger, 1976; Crick, 1984; Deschênes and Hu, 1990; Villa et al., 1991; Suga et al., 1997; Zhou and Jen, 2000). Different techniques have revealed that the stimulated auditory cortex exerts facilitatory and/or inhibitory modulation on the thalamic relay neuron, either directly via stimulated neurons or indirectly polysynaptically (Watanabe et al., 1966; Ryugo and Weinberger, 1976; Villa et al., 1991; He, 1997; Suga et al., 1997; He et al., 2002).

During study of corticofugal modulation on the thalamic neurons intracellularly, I also recorded neuronal responses to acoustic stimulus. I recorded the responses of neurons in the MGB in relation to alterations in their membrane potentials. I studied the non-acoustically-driven firing, the responses to auditory stimuli and to electriccurrent injection, and the c hanges in neuronal responses to auditory stimuli while the membrane potential was manipulated through current injection. In this chapter, I also summarized the basic features of the neuronal responses to the auditory stimuli in the non-manipulated membrane potential as determined from *in vivo* intracellular recordings.

3.2 Materials and methods

3.2.1 Animal preparation

A total of 46 guinea pigs of both sexes, weighing 450–700 g with clean external ears, served as subjects. Anesthesia was initially induced with pentobarbital sodium (Nembutal; Abbott Labs, Irving, TX) (40 mg/kg, i.p.) and maintained at the same level as during surgery by supplemental doses of the same anesthetic (5-10 mg/kg/hr) during the surgical preparation and recording. An electrocorticogram from the left frontal cortex was used to monitor the anesthetic level of the animal. Atropine sulphate (0.05 mg/kg, s.c.) was given 15 minutes before anesthesia commenced and at regular intervals (0.01 mg/kg/hr, s.c.) during recording so as to minimize tracheal secretion. Body temperature was maintained between 37.5 and 38.5℃ by the use of a thermistor-controlled heating pad attached to the animal's abdomen. The animal was mounted in a stereotaxic device following the induction of anesthesia. A midline incision was made in the scalp, and craniotomies were performed to enable us to map the auditory cortex, to implant stimulation electrodes into the cortex, and to vertically access the MGB in the right hemisphere. The craniotomy opening was usually ~4.0×4.0 mm^2 where located above the auditory thalamus. The dura mater was removed above the auditory cortex and at a position vertically above the auditory thalamus. Before the left ear was freed from the ear bar, the head was fixed with two stainless steel bolts together with acrylic resin to an extended arm of the stereotaxic frame. These

ensured that the subject's head remained fixed to the stereotaxic device without misalignment.

For effective intracellular recording, it is not difficult to penetrate the neuron with the recording electrode, but it is more challenging to maintain stable recording for a prolonged period. Since breathing causes alternate positive and negative thoracic pressure changes, which transfer to the brain and cause movement of the brain, artificial ventilation was used and the animal's muscles were relaxed with gallamine triethiodide (50 mg/kg, i.p.). To minimize the pressure in the chest, bilateral pneumothorax was done and the trunk of the animal was suspended on a spinal frame. Intra-cranial pressure was reduced by release of the cerebrospinal fluid through an opening of the dura mater at the foramen magnum. The procedures were approved by the Animal Subjects Ethics Sub-Committee of The Hong Kong Polytechnic University.

3.2.2 Acoustic stimulus

Acoustic stimuli were generated digitally by a MALab system (Kaiser Instruments, Irvine, CA, USA), which was controlled by a Macintosh computer (Semple and Kitzes, 1993; He, 1997). Acoustic stimuli were delivered to the subject via a dynamic earphone (Bayer DT-48) mounted in a probe. The subject was placed in a double-walled soundproof room (NAP, Clayton, Australia). A tube on the probe conducted the acoustic stimuli to the contralateral ear. The pinnae are left intact. Prior to recording, tympanic sound pressure levels (expressed in dB SPL

in reference to 20 μPa root mean square) are calibrated over a wide frequency range under computer control by using a condenser microphone. Acoustic calibrations for both ears are stored in a computer file for use in controlling the attenuators used to obtain the desired SPLs. Repeated noise bursts (white noise, with a spectral band of 0-30 kHz) and pure tones with intervals of 1 s or longer and 5 ms rise/fall time were used to examine the neuronal responses.

For an MGB neuron, its best frequency and minimum threshold were determined by changing sound frequency and intensity. The minimum threshold is the minimum intensity of the sound which can evoke responses to BF pulses. The latency of the neuronal response was determined as the time lag between onset of the sound and the first spike. The latency-frequency functions were defined as the first spike latency in response to pure tones as a function of frequency at certain intensities: 80 dB, 60 dB, 40 dB, and 20 dB SPL. The latency shown here was the mean of the latencies of the first spikes over five repeated trials. The frequency response functions were defined as the responses to pure tones as a function of frequency at certain intensities. The number of spikes was summed over the onset responses to five repeats of pure-tone stimuli for each frequency.

ON-OFF responses are defined as responses with a time locked increase at both tone onset and offset. OFF responses are defined as responses that always occur following the offset of the stimulus. The durations of the acoustic stimuli were changed from 100 ms to 600 ms at least once for every OFF or ON-OFF neuron to distinguish the OFF neuron from the long-latency on response and ON-OFF response from on response with a rebound response.

3.2.3 Recording

I used a glass-pipette as the recording electrode which was filled with 1.0 M KCl. The resistance of the electrode was between 40 and 90 MΩ. The electrode was advanced vertically from the top of the brain by the stepping motor. After the electrode was lowered to 4-5 mm from the cortical surface, the cortical exposure was sealed by low-melting temperature paraffin. When the electrode was near or in the targeting area, it was then slowly advanced at 1 or 2 □m per step.

The MGB was stereotaxically accessed vertically from the top of the brain, according to a guinea pig brain atlas (Rapisarda and Bacchelli, 1977). The vertical coordinate of the electrode was determined with reference to the cortical surface at penetration. The electrode picked up the membrane potential showing negative value when it penetrated the membrane of a cell. After amplification, the membrane potential was recorded on a computer (AxoScope) which also simultaneously stored the auditory stimulus.

Membrane potentials were manipulated through the injection of either positive or negative current into the recording neuron. Positive current depolarized the neuron and negative current hyperpolarized it. The steady-state membrane potential stood for the baseline of the membrane potential when current was injected into the neuron. An extracellular DC potential was recorded after electrode withdrawal and was subtracted from the intracellularly measured membrane potential. Pure tones and noise bursts were used as acoustic stimuli. Neuronal responses to the acoustic

stimuli were recorded together with the responses to current injection. Low-threshold calcium spikes were elicited when the membrane potential was hyperpolarized to below -75 mV.

The time of spike occurrence relative to stimulus onset was also stored in the Macintosh computer used as the stimulus controller by the MALab software. The computer automatically created raster displays of the responses, together with frequency response functions (responses to pure tones plotted as a function of frequency).

3.3 Results

3.3.1 Spontaneous/non-acoustically-driven firing rate

Of 23 neurons tested, the non-acoustically-driven firings increased when the membrane potential was depolarized and decreased when it was hyperpolarized. As shown in Figure 1A, a neuron showed no spontaneous firings when its resting membrane potential was –63 mV, and a very few non-acoustically-driven firings when the membrane potential was between –60 and –56 mV. The non-acoustically-driven firings dramatically increased when the resting membrane potential was depolarized to above –55 mV as shown in the first row of Figure 1A.

The highest non-acoustically-driven firing rate (spontaneous firing rate) observed in the present study was 160 Hz (data not shown in the Figure 1) and the lowest was 0 Hz. The non-acoustically-driven firing rate depended on the

membrane potential: the lower the membrane potential the lower the non-acoustically-driven firing rate, and vice versa. This result was confirmed on all 23 neurons tested for this purpose in the present study. The non-acoustically-driven firing rate is shown as a function of the membrane potential for 17 neurons in Figure 1B (6 other neurons were excluded from statistics either because their resting membrane potential might have had a direct current bias or their spike amplitude was not large enough to overshoot to positive voltage). Available data points over five membrane potentials in Figure 1B were averaged and shown in Figure 1C. The non-acoustically-driven firing rate was 45.8 ± 23.3 Hz (mean ± SD, n=8) at a membrane potential of -45 mV, 30.6 ± 19.4 Hz (n=14) at -50 mV, 18.0 ± 12.9 Hz (n=14) at -55 mV, and significantly decreased to 5.7 ± 7.4 Hz at -60 mV and to 0.7 ± 1.5 Hz (n=10) at -65 mV (ANOVA, $p<0.001$). There was a large variation over different neurons at each membrane potential.

3.3.2 Membrane potential dependent auditory response

The acoustically-driven activity also changed as a function of the resting membrane potential. Figure 2 shows the responses of a neuron to acoustic stimulation at three different resting membrane potentials. The neuron fired at 6.5 spike/s, when the membrane potential was –55 mV, and decreased to 0.8 spike/s at –63 mV, and to null at –75 mV. Some neurons showed a large after-hyperpolarization following the non-acoustically-driven spikes (Figure 2) and others did not show after-hyperpolarization (Figure 1).

The auditory responsive neuron in Figure 2 responded repeated noise-burst stimuli, but not to pure tones (data not shown). The neuron showed a burst of 3 spikes when the resting membrane potential was –55 mV, the number of spikes in the burst decreased to an average of 2.5 spikes at –63 mV, and decreased further to <2 spikes at –75 mV. As it was hyperpolarized, the neuron showed a longer response latency from 21.8 ms at -55 mV, to 28.2 ms at -63 mV and 32.1 ms at -75 mV. The inter-spike-interval (ISI) of the auditory evoked spikes also increased from 12.4 ms at -55 mV to 13.1 ms at -63 mV and to 17.1 ms at -75 mV. The auditory responsiveness of the MGB neurons depended on the membrane potential in the range of –45 to –75 mV: the higher the membrane potential, the greater the responsiveness, and vice versa.

With a resting membrane potential above -70 mV, neurons with higher resting membrane potential responded with more spikes to auditory stimuli than those with a lower membrane potential. The neuron in Figure 3 responded with a train of 7-9 spikes in a resting membrane potential of -50 mV, however the number of spikes gradually decreased to two in -65 mV, one in -67 mV and to zero in -69 mV. A slow-rising excitatory postsynaptic potential could still be detected in the last two traces of right column of Figure 3A. The number of spikes from 37 trials of auditory responses was drawn as a function of the membrane potential for the neuron (Figure 3B). The resting membrane potential was drifted spontaneously during the course of recording.

The neuron in Figure 4A responded to an acoustic stimulus with a burst of spikes on a long-lasting EPSP when the resting membrane potential was -56 mV.

The same neuron changed to a single spike mode when its resting membrane potential drifted spontaneously to -59 mV and to an EPSP only when the resting membrane potential decreased further to -61 mV. I termed this type of neuron an auditory excitatory neuron.

The neuron in Figure 4A responded to the same stimulus with decreased amplitude of the EPSP as the membrane potential was hyperpolarized from -56 mV to -61 mV. The trend was confirmed for other neurons, as seen in the examples shown in Figure 4B.

Similar to dependence of the amplitude of EPSP on the steady-state membrane potential, the amplitude of the IPSP induced by the noise-burst stimulus decreased when the membrane potential was hyperpolarized. The results of five neurons are shown in Figure 5.

3.3.3 Excitatory auditory responses: EPSP responses

Twenty-nine neurons of 52 neurons showed excitatory responses to acoustic stimuli. Of them, 10 neurons showed burst-like responses to auditory stimuli. All of the neurons in Figure 6 responded with a burst-like response to the noise-burst stimulus. The neuron in Figure 6A showed a burst-like response to acoustic stimuli and burst spontaneous responses. The neurons in Figure 6B-D showed a similar auditory response of a prolonged EPSP with spikes on it, but with no spontaneous bursts.

Of all of the neurons examined, the highest firing rate in the bursts and sustained responses was 330 Hz (Figure 7). However, the firing included non-full-sized spikes, when the firing rate was higher than 240 Hz, and only became all full-sized spikes when the firing rate was 240 Hz or lower. For other neurons, I observed non-full-sized spikes when the membrane potential was higher than -47 mV. Among four neurons in Figure 6, three neurons responded with non-full-sized spikes to acoustic stimuli, as shown in the lower trace of Figure 6B, and in both traces in Figures 6C and 6D. I calculated the means of the amplitude and duration of the EPSPs for this type of neuron as 13.2+/-10.2 mV (range: 4-40 mV, n=9) and 184.0+/-126.6 ms (range: 20-420 ms), respectively.Fourteen neurons of 52 neurons examined responded to acoustic stimulus with an EPSP, as seen in the examples shown in Figure 8. Among them, some neurons showed a spike or a few onset spikes with the EPSP (Figure 8A, D, E) and others showed only EPSPs (Figure 8C and F) or EPSPs with spike(s) on them (Figure 8B). I examined the frequency tuning property of the neuron in Figure 8D and found that it was tuned to a low frequency of pure tones at 60 dB SPL. The neuron in Figure 8D showed an onset response, which was followed by a rebounded EPSP at about 120 ms after the first EPSP. A third EPSP could be detected in the first trace of Figure 8E. The neurons in Figure 8D-F showed rebounded EPSPs. The second EPSP in Figure 8D and F was about 200 ms and 240 ms after the onset of the acoustic stimulus.

I calculated the means of the amplitude and duration of the EPSPs for this type of neuron as 10.2+/-5.5 mV (range: 3-24 mV, n=14) and 158.6+/-95.4 ms (range: 20-400 ms), respectively. Figure 9 shows the means of the amplitudes (left part)

and the durations (the right part) of the EPSPs of the above two subgroups: the burst-like responses and the phasic responses. Although they were not statistically significant, possibly due to the small sampling size and the large variation in the data, there were some differences between the two subgroups in the means of their amplitudes and durations of their EPSPs. The burst-like response subgroup showed a slightly larger amplitude and longer duration of the EPSP than did the phasic response group.

3.3.4 Excitatory auditory responses: tonic type

The neuron in Figure 10 showed an onset response, which was followed by a tonic response as the stimulus persisted. Five neurons were categorized under this type of neuron. There was a small gap of about 30 ms between the onset and the tonic responses on the neuron in Figure 10. The tonic response was sustained during the duration of the stimulus and prolonged when the duration of the stimulus was extended (Figure 10A). The neuron showed double peaks in the response-frequency functions of 80- and 60-dB SPL intensities (lower part of Figure 10B). Conversely, the neuron showed double valleys in the latency-frequency functions of 80- and 60-dB SPL (upper part of Figure 10B). Although the neuron responded to pure-tones of 60-dB SPL in 420 Hz and 620 Hz, as well as in 4.2 kHz, the spikes shown in the lowest row of Figure 10C were more likely to be spontaneous ones as each of them had a random latency. Thus, the neuron had a response threshold of 60 dB.

Of two neurons examined for frequency tuning property, one showed a double-peaked tuning characteristic (Figure 10), and the other showed a broad tuning to a low frequency with a high threshold of 60 dB.

3.3.5 Inhibitory postsynaptic potential responses to acoustic stimuli

All neurons in Figure 11 showed an IPSP to a noise-burst stimulus. The neurons in the left panel of Figure 11 (A-C) showed an onset spike before the IPSP, while the neurons in the right panel of Figure 11 (D-F) showed only an IPSP. Although all of the neurons showed different shapes of IPSPs, the IPSPs normally lasted for longer than 100 ms.

With varied membrane potentials, the neurons responded to the same acoustic stimulus with different IPSPs. The neuron in Figure 12 responded to a noise-burst stimulus with a larger IPSP when its resting membrane potential was higher: -18 mV at -55 mV, -17 mV at -57 mV, -9 mV at -63 mV, and -2 mV at -72 mV. The IPSP has a long time duration of 400-500 ms, judging from its inhibition to the spontaneous firing in Figure 12A-B. The neuron showed a spontaneous firing of about 50 Hz, when the resting membrane potential was -55 mV and 30 Hz at -57 mV. The spontaneous firing rate changed to 0 Hz when the resting membrane potential drifted to -63 mV. This spontaneous firing rate was kept at 0 Hz when the membrane potential was further hyperpolarized spontaneously.

Fourteen neurons responded to auditory stimuli with an IPSP. Their mean amplitude and duration of the IPSPs were calculated to be -10.1+/-2.5 mV (range: -

6 mV to -14 mV, n=14) and 350.7+/-273.6 ms (range: 100-1200 ms), as shown in Figure 13. The IPSPs were sampled at or near -60 mV when the neuron was examined at varied membrane potentials.

As shown in Figures 12, 11C and F, some inhibitory neurons showed a high spontaneous firing rate and the spontaneous firings were inhibited by the acoustic stimulus. Figure 14 shows a full time course of 4.8 sec of an inhibitory auditory response neuron, which had a spontaneous firing rate of > 30 Hz and was inhibited by the noise-burst stimulus for over 600 ms.

3.3.6 OFF and ON-OFF neurons

Seven OFF and two ON-OFF neurons of 52 neurons were recorded in the present study. Of them, three OFF neurons are shown in Figure 15. The neuron in Figure 15A responded to noise-burst stimuli of 100 ms, 200 ms, and 400 ms in duration. It showed better responses to stimuli with longer durations than to those of 100-ms duration. The neuron also showed a shorter latency of 50 ms to the offset of the stimulus of 400-ms in duration, compared with the latency of 125 ms to the stimulus of 200-ms duration.

The neuron in Figure 15B responded to noise-burst stimuli with durations of both 100 ms and 200 ms, with an OFF response to either an EPSP or an EPSP and a spike. The resting membrane potential of the neuron was -63 mV. This neuron responded with a varying latency of between 48 ms and 235 ms after the offset of the stimulus, when the duration of the stimulus was 100 ms. It responded to the

same stimulus of 200-ms in duration with a similar size of EPSPs, a similar probability of firing and a similar mean latency. The latency of the responses to different trials of a 200-ms stimulus was, however, more consistent at about 125 ms (range: 103 -142 ms) than that to a 100-ms stimulus (range: 48-235 ms).

The neuron in Figure 15C responded to noise-burst stimuli of varied durations and did not show selectivity of duration. It responded to the stimuli with an EPSP and single or double spikes.

An ON-OFF neuron is shown in Figure 16. It responded to the noise-burst stimuli of both 100 ms and 200 ms with an ON response of single or double spikes. The neuron also responded with an OFF response of either an EPSP or an EPSP with a spike on it.

All OFF neurons and one of two ON-OFF neurons showed some kind of membrane oscillation, as seen in an example shown in Figure 17. The OFF neuron showed a large after-hyperpolarization potential (AHP) following the OFF EPSP/spikes. The AHP lasted for about 200 ms and was followed in some cases by a second (first to fourth traces of Figure 17B) and a third AHP (first to third traces of Figure 17B). Converting to frequency, the membrane oscillation was about 5 Hz.

3.3.7 Normal spikes to low-threshold calcium spikes

With a resting membrane potential of above -70 mV, the thalamic neurons showed normal spikes, similar to those shown in Figure 4. The neuron in Figure 18 responded to a noise-burst stimulus with a normal spike when the membrane

potential was -66 mV. It responded to the same stimulus with an EPSP or a spike when the membrane potential was hyperpolarized to -74 mV. However, the neurons changed their firing pattern to an LTS when the membrane potential was further hyperpolarized to -77 mV. With an even lower membrane potential at -85 mV and -90 mV, the neuron responded to the same noise-burst stimulus with a spike burst. The lower the membrane potential, the more spikes burst.

Similarly, the neuron in Figure 19 responded to a noise-burst stimulus with a single normal spike when its steady-state membrane potential was -68 mV or above, responded to the same stimuli with only EPSPs when the potential was -72 mV, and responded with LTS spikes/spike-bursts when its membrane potential was further hyperpolarized to -78 mV or lower. The number of burst spikes in the LTS increased from 0-2 to 2-3 as the membrane potential was hyperpolarized from -78 mV to -92 mV.

Both of the neurons in Figures 18 and 19 had a no-spike membrane potential range or a silent membrane potential range around -75 mV. Some neurons do not have a silent membrane potential range, as shown in Figure 20. The neuron responded to a noise-burst stimulus with a spike when its membrane potential changed from -60 mV to -80 mV. It responded with an LTS burst when the membrane potential was further hyperpolarized to -90 mV.

It was also interesting to note that the depolarization before the LTC spikes or LTC spike burst, which evoked by the acoustic stimulus, was larger when the neurons were more hyperpolarized in the steady-state membrane potential (Figures 18, 19 and 20).

Two examples of auditory inhibitory neurons are shown in Figure 21. The neuron in Figure 21A showed an IPSP when the membrane potential was higher than -60 mV. The IPSP disappeared when the membrane potential was hyperpolarized to -65 mV, and changed to an EPSP when the membrane potential was further hyperpolarized to -80 mV. The neuron responded to the same stimulus with a slow EPSP and a spike after a build-up, which was likely to be an LTS spike, when the membrane potential was -85 mV and -90 mV. The neuron in Figure 21B responded to a noise-burst stimulus with an IPSP when its membrane potential was about -57 mV, but changed to an LTS spike/spike-burst while the steady-state membrane potential was hyperpolarized to -85 mV.

3.3.8 Two types of spike bursts

Compared with the frequently reported LTS bursts in the thalamus, the spike bursts in Figure 2 and 3 had a much longer ISI and were based on a higher membrane potential. Figure 22 shows a comparison between the two types of spike bursts. The spike trains in Figure 22A were evoked when the membrane potential was hyperpolarized into <-85 mV and were categorized as LTS bursts and those in Figure 22B had a longer ISI and rose from a higher membrane potential of about -53 mV. The ISIs between the first and second spikes and between the second and third spikes were sampled from 20 bursts for each neuron and are shown in Figure 22C. The mean over first and second ISIs of the LTS bursts in Figure 22C was 6.7

± 2.4 ms (n=40), which was significantly shorter than the mean ISI of the non-LTS bursts, 11.6 ± 6.0 ms (n=40, $p<0.001$, *t*-test).

The mean of the second ISI of the LTS bursts (7.1 ± 2.4 ms) was significantly longer than that of the first ISI (5.9 ± 1.2 ms, n=20, $p<0.01$, paired *t*-test). The mean of second ISI of the non-LTS bursts (13.1 ± 7.0 ms) was longer than that of the first ISI (10.1 ± 4.6 ms), but not statistically significant (n=20, $p>0.05$). The non-statistical significance might have resulted from the large deviation of the parameter.

3.3.9 Tuning characteristics of the neurons with different discharge patterns

3.3.9.1 Tuning characteristics of the EPSP neurons

Of seven neurons with excitatory potentials responding to noise-burst stimuli examined for their frequency selectivity, four showed a single peaked tuning curve with a threshold of below 40 dB SPL, three showed a tuning curve with a threshold higher than 40 dB SPL, and one was not tuned to any frequency.

Figure 23 shows a frequency-tuned neuron that responded to the noise-burst stimulus with an EPSP with spikes on it. The frequency response functions of varied intensities showed that the best frequency of this neuron was about 1 kHz (Figure 23B). The responsive latency tuning curves are also shown in Figure 23B. The neuron showing the shortest responsive latencies to intensities of both 60 dB and 80 was at 1 kHz. The shortest mean latency was 11.2 ms at 1 kHz of 80-dB

intensity. The shortest latency for 60 dB SPL was 13.1 ms, slightly longer than that of 80 dB SPL.

I sampled five to six points from each frequency response function and showed the detailed responses of their membrane potential to the pure-tone stimuli in Figure 23C. The neuron responded maximally to stimuli of 600 Hz and 1 kHz at 80 dB SPL with 4-5 spikes for each repeated trial. The neuron showed fewer spikes to stimuli of 2.0 kHz (2-4 spikes) and 200 Hz (1-2 spikes). The neuron responded to 100 Hz and 5 kHz at 80 dB SPL, but with uncertainty, showing a spike or spikes to some trials and no spike to others. The neuron responded to stimuli of 60 dB SPL with similar characteristics: 1) best to 600 Hz and 900 Hz with slightly fewer spikes than to the similar frequencies of 80 dB SPL, 2) fewer spikes to 420 Hz, 3) with uncertainty to 160 Hz and 2.8 kHz. However, it is obvious that the neuron responded to a narrower frequency range at 60 dB SPL than to the frequency range at 80 dB SPL. This range was further narrowed for stimuli at 40 dB SPL. The threshold of this neuron was 20 dB SPL, at which the neuron responded only to 1.0 kHz at its first trial.

The neuron in Figure 24 responded to the noise-burst stimulus with a single spike or a doublet, followed by an EPSP. The neurons showed a frequency-tuning characteristic with a BF of 560 Hz. The neuron has a response threshold of < 40 dB SPL at its best frequency. The neuron responded to the pure-tone stimuli of 80 dB SPL with a minimum latency of 13.4 ms. The shortest latency increased to 15.7 ms for the stimuli of 60 dB SPL, and further increased to 21.4 ms for the BF stimulus at 40 dB SPL.

The neuron in Figure 25 responded to the noise-burst stimulus with an EPSP with spikes on it. It responded to acoustic stimuli of low frequencies better than those of high frequencies. It responded to 4 kHz with a train of three spikes and a short response latency of 13.5±0.6 ms and to 1 kHz with one to two spikes and a shortest latency of 10.3±0.7 ms. The neuron also responded to other frequencies, with 2-3 spikes to 6 kHz (latency: 23.0±1.8 ms), and 0-1 spikes to 15 kHz (latency: 21.5 ms). The response-frequency function of Figure 25B showed the frequency tuning of the neuron, though the tuning was broad at 60 dB SPL. There was a trend that the neurons showed an increased first-spike latency when the frequency increased. Note that the burst of the lower trace of 6 kHz had a much longer ISI than that of the response to 4 kHz, even though both fired the same number of spikes (3). The ISIs between the first and second spikes and between the second and third spikes were 10.8 ms and 12.6 ms (n=2), respectively, for 6 kHz, while those for 4 kHz were 4.0±0.3 ms and 9.3±1.6 ms (n=5), respectively.

3.3.9.2 Tuning characteristics of the IPSP neurons

Of 8 neurons with inhibitory potentials responding to noise-burst stimuli examined for their frequency selectivity, 3 showed a high-threshold frequency selectivity, and the remainder were not tuned to specific frequencies. The example in Figure 26 shows a small EPSP before a large IPSP to the noise-burst stimulus (Figure 26A). The neuron showed a low frequency preference and a high response threshold of about 60 dB SPL. It responded to every stimulus of 400 Hz and 1.0

kHz at 80 dB SPL with either 1 or 2 spikes with a relatively uniform latency. The responses to 3.0-kHz and 7.0-kHz stimuli at 80 dB SPL showed varied latencies for different trials (Figure 26C). The minimum response latency to pure-tone stimuli was 13.3 ms when the intensity of the stimulus was 80 dB SPL. The response latency to 60-dB stimuli was 17.3 ms or larger.

3.4 Discussion

3.4.1 Non-acoustically-driven firing

The finding that the non-acoustically-driven firing rate depended on the membrane potential was not surprising. It has been reported that injection of a small positive current intracellularly would depolarize the membrane potential and cause an increase in the non-acoustically-driven firing rate (Smith and Populin, 2001; Sukov and Barth, 2001; Torterolo et al., 1995; White et al., 1994). The present study provides a quantitative assessment of the maximum non-acoustically-driven firing rate of the thalamic neurons and the relationship between the non-acoustically-driven firing rate and membrane potential.

3.4.2 Membrane potential dependent auditory responses

As the non-acoustically-driven firing rate, the auditory response, including the spike number and the amplitudes of the EPSP and IPSP, depended on the

membrane potential. The auditory response increased when the membrane potential was depolarized and decreased when the membrane potential was hyperpolarized. This is the first report to mention that the auditorily evoked EPSP and IPSP depend on the resting membrane potential. The result indicates that the responsiveness of the thalamic neuron can be controlled by adjusting the membrane potential. The present result of the membrane potential dependence of the responsiveness of the thalamic neurons would provide one basis for the mechanism of the corticothalamic modulation of thalamic neurons.

3.4.3 Two kinds of burst discharges

Burst responses have been frequently observed in the auditory thalamus and more often in the non-lemniscal MGB (Hu and Bourque, 1992; Strohmann et al., 1994; Hu, 1995; He and Hu, 2002). A commonly cited mechanism of thalamic neuronal bursting relies on voltage-dependent activation of an LTS or LTS bursts (Jahnsen and Llinás, 1984a; Steriade and Llinás, 1988; Deschênes and Hu, 1990; McCormick and Feeser, 1990; Hu, 1995; Turner et al., 1997). The result shows in Figure 22A was categorized as LTS bursts. The LTS bursts showed a shorter first ISI than the second ISI in agreement with previous findings (Figure 4, He and Hu, 2002; Hu and Bourque, 1992; Steriade et al., 1993b; Hu, 1995). The LTS bursts evoked by natural sound stimulation showed a relatively longer ISI than those recorded in the slice and evoked by current injection (3-4 ms, He and Hu, 2002 and

4-7 ms, Figure 22 of the present study *in vivo* versus 1-3 ms for the first and second ISI *in vitro*, Turner et al., 1997).

The burst responses of Figures 2, 22B and 25 were non-LTS and showed more spikes in the burst when the resting membrane potential was higher instead of lower. In general, the non-LTS bursts in Figures 2, 22B and 25 showed longer ISIs than that of the LTS bursts (Figure 22C). The characteristics of spike bursts in Figure 22B are similar to those recorded from neurons in the ventrobasal thalamus (Figure 10 of Turner et al., 1997). It was suggested that burst firing may have a stronger impact on the cortex (Swadlow and Gusev, 2001). It has been shown that neurons in the lemniscal and non-lemniscal MGB are involved in different signal processing providing a dual-pathway through the MGB (Merzenich et al., 1982; Imig and Morel, 1983; Jones, 1985; LeDoux et al., 1990; Edeline and Weinberger, 1992; Hu, 1995; Rauschecker et al., 1997; Kosaki et al., 1997; LeDoux, 2000; He, 2001; He and Hu, 2002). It would be interesting to determine whether there is any differences in spike burst generation in the lemniscal and non-lemniscal MGB in future study.

The inter-spike-interval of burst responses changed with the frequency of a pure-tone stimulus, even though the number of spikes could be similar. This result provides us with evidence supporting the theory that neurons code sensory information not only with the spike number, but also with the temporal structure of the spikes/spike-burst (Middlebrooks et al., 1994; He et al., 1997; He et al., 2002). The temporal structure and the firing rate of neuronal responses are dependent on each other. With intracellular recording, the temporal features of the spikes and

post-synaptic potentials should provide us valuable information to evaluate the temporal coding theory.

3.4.4 Firing patterns and maximum firing rate

I categorized the auditory response patterns into 1) excitatory potentials, 2) inhibitory potentials and 3) ON and OFF responses. I also classify the excitatory neurons into burst firing, phasic firing patterns, some with only EPSPs, and tonic firing. The inhibitory response neurons were further classified into spike and IPSP pattern, and only IPSP pattern.

With extracellular recording, previous investigators have categorized firing patterns in the cortex, the MGB and the IC into 1) sustained, 2) inhibitory, 3) phasic ON firing and 4) phasic OFF firing (Aitkin and Prain, 1974; Aitkin and Webster, 1972; Brugge and Merzenich, 1973; Suga, 1969; He, 2002). It is also known that the discharge patterns vary when the stimulus or the intensity of the stimulus changes (Aitkin and Prain, 1974; Aitkin and Webster, 1972; Brugge et al., 1970). The sustained firing pattern could be caused by either a long-lasting EPSP or an EPSP followed by a fast rebounded response that was sustained during the stimulus. The latter was classified as tonic response in the present study.

The present study is the first to report quantitatively on the amplitude and duration of the EPSPs evoked by auditory stimuli in the MGB. The mean amplitude of EPSPs is comparable to the amplitudes of EPSPs in the auditory cortex, where several mV to about 20 mV were observed (Ojima and Murakami, 2002). However;

while the amplitudes of EPSPs in the IC showed a relatively small value of below 10 mV for most neurons (Kuwada et al., 1997). It is surprising to note that the maximal amplitude of the EPSPs reached 40 mV in the present study.

A recent intracellular study on thalamic slice neurons has shown no differences in the intrinsic membrane features and the synaptic responses between the neurons in the lemniscal and non-lemniscal MGB (Bartlett and Smith, 1999). Yhese findings suggest different firing patterns are formed before the MGB or partially within the network among the inferior colliculus, the thalamus, and the cortex.

One-quarter of the neurons in the present study responded to an acoustic stimulus with an IPSP or a spike and IPSP. This IPSP pattern has also been observed in the IC (Kuwada et al., 1997), but not in the auditory cortex (Ojima and Murakami, 2002).

The acoustically-evoked IPSP depended on the resting membrane potential: the higher the resting membrane potential the larger the IPSP. The result raises the question of whether our general expectation that a neuron responds or is more ready to respond to stimulus when its membrane potential is higher is true or not.

In the present study, I examined the maximum firing rate for burst firing and found it to be 330 Hz, including non-full-sized spikes. The maximum rate for full-sized-spikes was 240 Hz. This rate may provide single-unit extracellular recordings with a reference on the maximum firing rate in the thalamus. The maximum firing rate in the thalamus is relatively low compared with that in the cochlear nucleus, from which a firing rate of over 1000 Hz was recorded (Wang and Sachs, 1994).

3.4.5 Tuning

In an early report of extacellular recording in the MGB, Adrian and colleagues (1966) showed burst responses of 4-5 spikes to the best frequency stimulus at the contralateral ear, similar to the result of the present intracellular recording (Figure 23). In the auditory cortex, the neurons responded to the best frequency with 3-4 spikes (Ojima and Murakami, 2002).

After a burst response to the pure-tone stimuli, an after-hyperpolarization potential was observed in Figure 10, although the same neuron responded to a noise-burst stimulus with spikes but no AHP. In the auditory cortex, the maximum AHP is often seen in the responses to the best frequency stimulus (Ojima and Murakami, 2002).

Neurons with a simple EPSP response to a noise-burst stimulus were more likely to be tuned to a characteristic frequency with a short latency and low response threshold, indicating a possible location in the lemniscal MGB. Neurons with a tonic response to the noise-burst stimulus showed multiple peaks in the tuning curve, a high response threshold, a long latency and double peaks in the latency-frequency function, all of which are characteristics indicating a possible location for the caudomedial nucleus of the MGB (equivalent to the medial division of the cat). Neurons responding with an IPSP to sound stimuli showed a higher response threshold and a broad-tuning property, which are more likely the features of the non-lemniscal MGB neurons.

3.4.6 OFF and ON-OFF responses

OFF neurons have been reported in the cochlear nucleus, the superior olivary complex, the inferior colliculus, the MGB and the auditory cortex (Kiang, 1965; Suga, 1964; Grinell, 1973; Grothe et al., 1992; Lesser et al., 1990; Neuweiler et al., 1971; Aitkin and Prain, 1974; He et al., 1997; He, 2001; 2002). Many OFF neurons are duration-selective and most duration-selective neurons in the IC are OFF neurons (He et al., 1997; He, 2002; Brand et al., 2000; Casseday et al., 1994; 2000; Ehrlich et al., 1997). The OFF neuron in Figure 15A was a long-duration selective neuron. In agreement with the previous finding, no short-duration-selective OFF neurons were found in the MGB in the present study (He, 2002).

The neuron in Figure 15A responded to acoustic stimuli of different durations with different spike timing in addition to spike numbers. The neuron in Figure 15B responded to stimuli of both 200-ms and 100-ms durations with a similar spike number and mean latency. The neuron, however, responded to the stimulus of 200 ms with a more consistant latency than to the stimulus of 100 ms, suggesting that the latency or the deviation of the latency could be another component to encode the stimulus information. This result provides us with evidence supporting the theory that neurons encode auditory information not only with a spike number, but also with the temporal feature of the spikes (Middlebrooks et al., 1994; He et al., 1997; He, 2002).

Stimulus-evoked oscillations of 5-15 Hz have been shown in the auditory thalamus with extracellular recording (Galambos et al., 1952; Aitkin et al., 1966; Cotillon and Edeline, 2000; Cotillon et al., 2000; Cotillon-Williams and Edeline, 2003). The membrane oscillation shown in the present study matches the oscillations of the previous extracellular recordings. In one of our recent studies, slow oscillation was found only in the non-lemniscal MGB (He, 2003b). OFF and ON-OFF neurons are located only in the non-lemniscal MGB or in the border region between the lemniscal and non-lemniscal MGB (He, 2001). Electrical stimulation of the posterior intralaminar nucleus of the thalamus, from which many OFF neurons have been recorded (He, 2001), triggered high-frequency cortical oscillations (Barth and MacDonald, 1996; Brett and Barth, 1997; Sukov and Barth, 2001). The present result, that all OFF neurons showed membrane oscillations of either stimulus-evoked or spontaneous slow oscillation (data not shown), once again strengthen the idea that the membrane oscillation might be more dominant in the non-lemniscal MGB than in the lemniscal MGB.

3.4.7 Low-threshold calcium spikes

Thalamic relay neurons have a high level of a1G calcium channel gene expression, which leads to LTS spikes or LTS bursts generated with a T-type Ca^{2+} current (Bal et al., 1995; Jahnsen and Llinás, 1984a, b; McCormick and Feeser, 1990; Talley et al., 1999; Coulter et al., 1989; Huguenard, 1996; McCormick and Bal, 1994; Ulrich and Huguenard, 1996; Sherman and Guillery, 1996). There is

another gene, HCN4, which is highly expressed in the thalamic neurons (Santoro et al., 2000) and leads to the H-current, a hyperpolarization-activated cationic current (Pape, 1996). The interaction between the H-current and the calcium channels is believed to facilitate the oscillation of the thalamic neurons (McCormick and Pape, 1990; Huguenard and McCormick, 1992; McCormick and Huguenard, 1992). This has also recently been observed in the MGB with *in-vivo* preparation (Cotillon et al., 2000; Cottillon-William and Edeline, 2003; He, 2003b).

The result in the present study in which LTS spikes appeared mostly when the steady-state membrane potential was below -70 mV agrees with previous reports (Steraide, 2001a). A single LTS could change to an LTS burst when the membrane potential was further hyperpolarized to below -80 mV in most of the neurons (Figure 18 and 19). Tonic firing at depolarized levels, at which the calcium current is inactivated, but burst firing at hyperpolarized levels, at which the calcium current is activated have also been previously described in other thalamic regions on *in-vivo* animals (Deschênes et al., 1984; Steriade and Deschênes, 1984; Mulle et al., 1985; Pare et al., 1987). With an *in-vivo* preparation, Timofeev and colleagues (2001) found that the amplitude of the depolarization before the LTC spikes had a negative correlation with the conditioning membrane potential. The results in Figures 18, 19 and 20 confirmed their finding.

An additional point to note in the present results was the silent window of the steady-state membrane potential, in which neurons responded only with an EPSP but no spike (Figure 18 and 19). The neurons responded to the acoustic stimulus with a normal spike when the membrane potential was higher than - 70 mV and

shifted to an LTS spike/spike burst when the membrane potential was hyperpolarized to below -75 mV. However, in some cases, the neurons responded with an EPSP only when the membrane potential was between -70 mV and -75 mV. Within this range of the membrane potential, the low-threshold calcium channel was not opened with the acoustically-evoked EPSP, which can be explained with the existing knowledge (Shepherd, 1984; Huguenard and McCormick, 1994). Topics regarding the correlation of the neuronal morphology and the silent window, and the physiological significance of the silent window remain to be further explored.

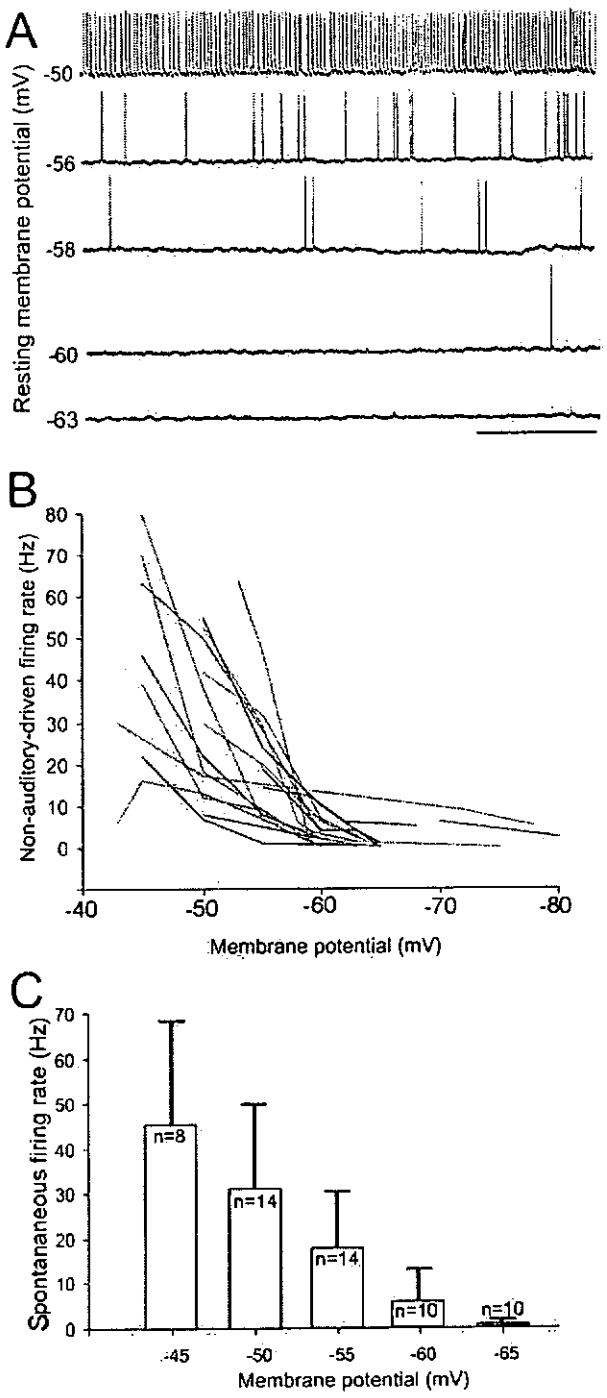

Figure 1 Spontanoeus firing as a function of resting membrane potential. A. Spontaneous neuronal firing at varied resting membrane potentials. Recording was carried out during natural drifting of the resting membrane potential. Time scale: 1 s. B. The relationships between the non-acoustically-driven firing rate and the resting membrane obtained from 17 typical neurons in the MGB. The membrane potential of some neurons was manipulated by injecting either negative or positive current into the neurons and is not specified in the figure. C. Averaged non-acoustically-driven firing rates at five sampled membrane potentials. Data were collected from the curves over the sampled membrane potential points in B.

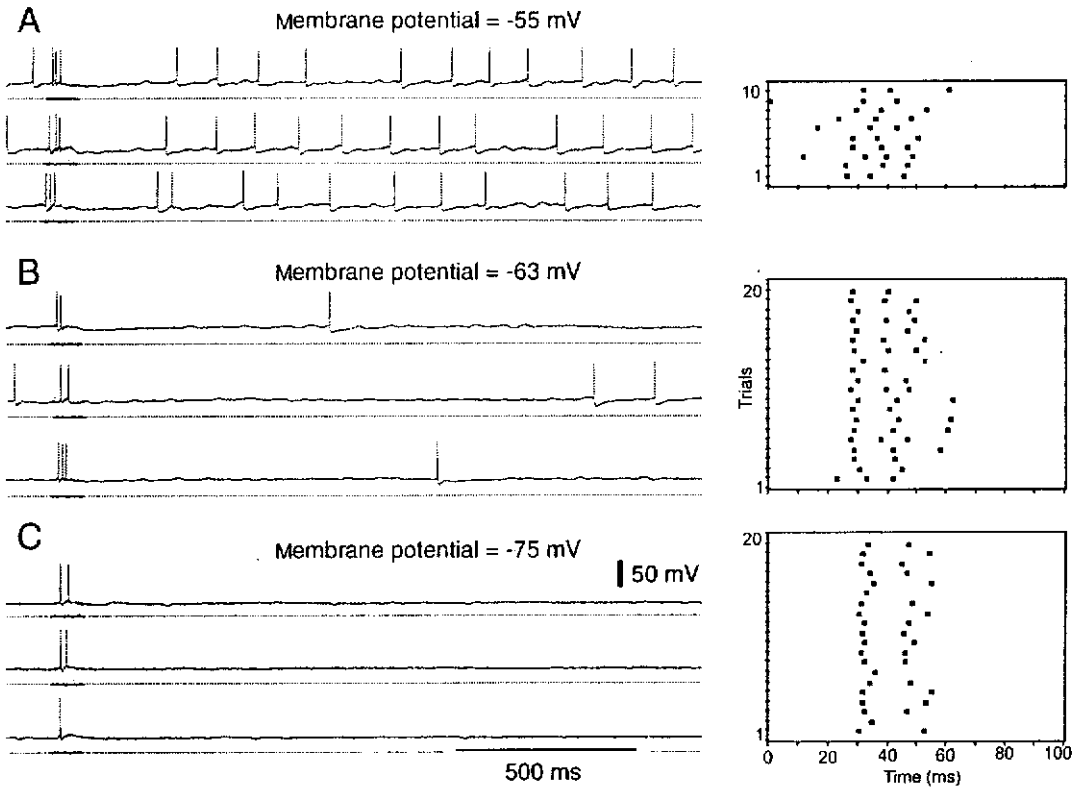

Figure 2 Neuronal responses as a function of membrane potential. The left panel shows the intracellular traces of the neuronal responses of an MGB neuron to noise-burst stimuli of 3 trials at varied membrane potential: A, -55 mV; B, -63 mV; C, -75 mV. Stimuli are shown under each response trace. Scale bars of time and voltage in C apply to all. The right panel shows the raster displays of the first 100 ms of the neuronal responses to repeated noise-burst stimuli of 10 trials (A) or 20 trials (B and C). Time scale in C applies to all.

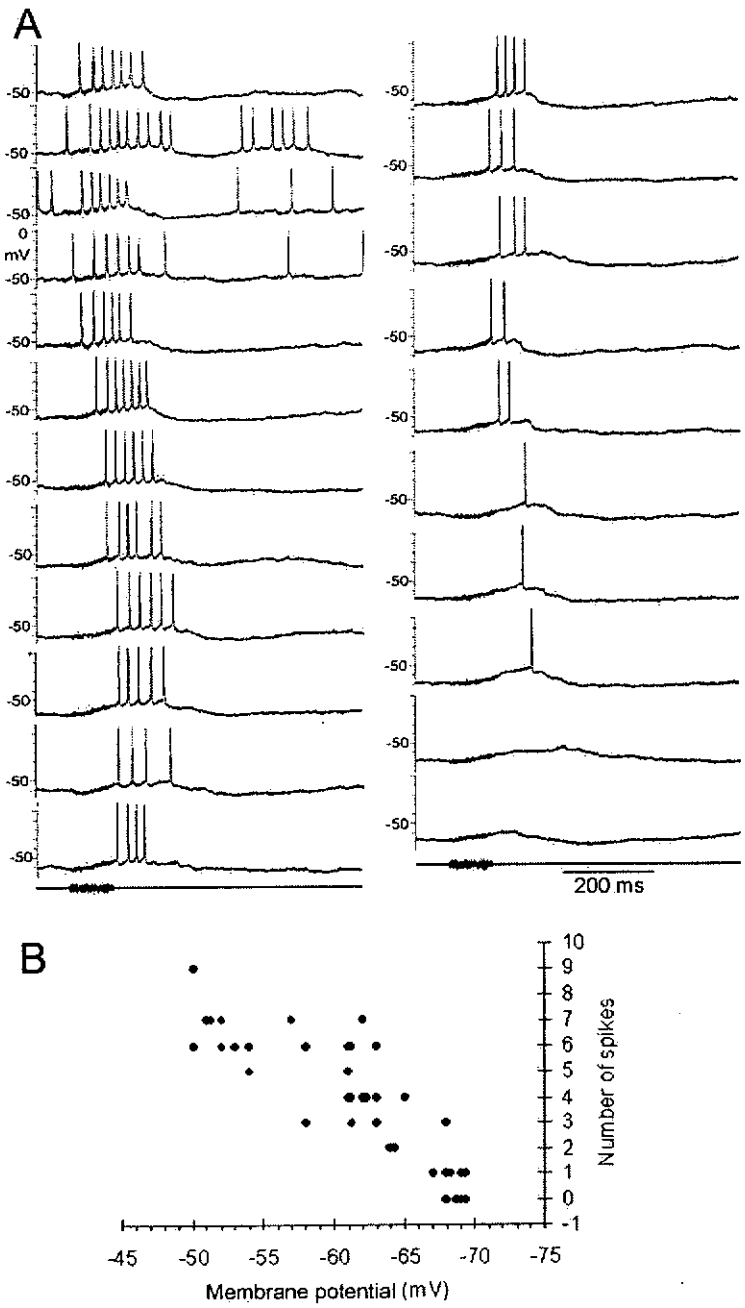

Figure 3 Relationship between the number of spikes in the auditory responses of an MGB neuron and its resting membrane potential. A. The MGB neuron responded to a noise-burst stimulus at varied resting membrane potentials. The membrane potential of the neuron was drifted spontaneously. The scale for the membrane potential is shown on the left to each trace. Auditory stimulus is shown below each column. Some noises, picked up by the recording electrode, were probably caused by the vibration of the acoustic probe which transmitted the vibration to the animal. B. The scattering graph shows the number of spikes in the response to the auditory stimulus verses the membrane potential.

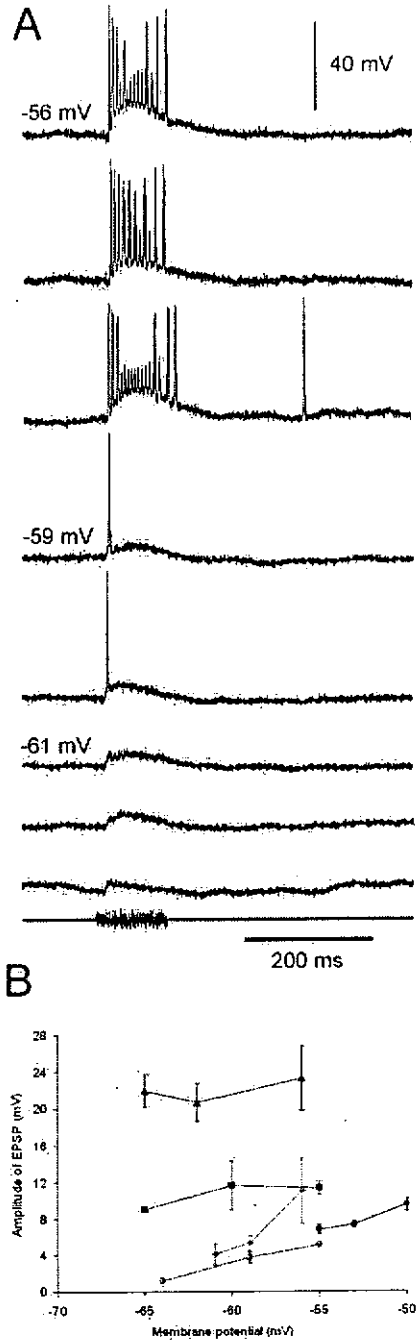

Figure 4 Amplitudes of EPSPs evoked by the noise-burst stimulus at varied membrane potentials for the MGB neurons. A. The neuron responded with an EPSP and spikes to an acoustic stimulus. The membrane potential of the neuron was manipulated by injecting a current into the recording electrode. The scale for the membrane potential is shown on the upper-right corner. An auditory stimulus is shown below the traces. B. Amplitudes of the EPSPs induced by the noise-burst stimulus at varied membrane potentials for five MGB neurons. The results were averaged over four trials. In some cases when four data points were not available, only 1-3 trials were averaged.

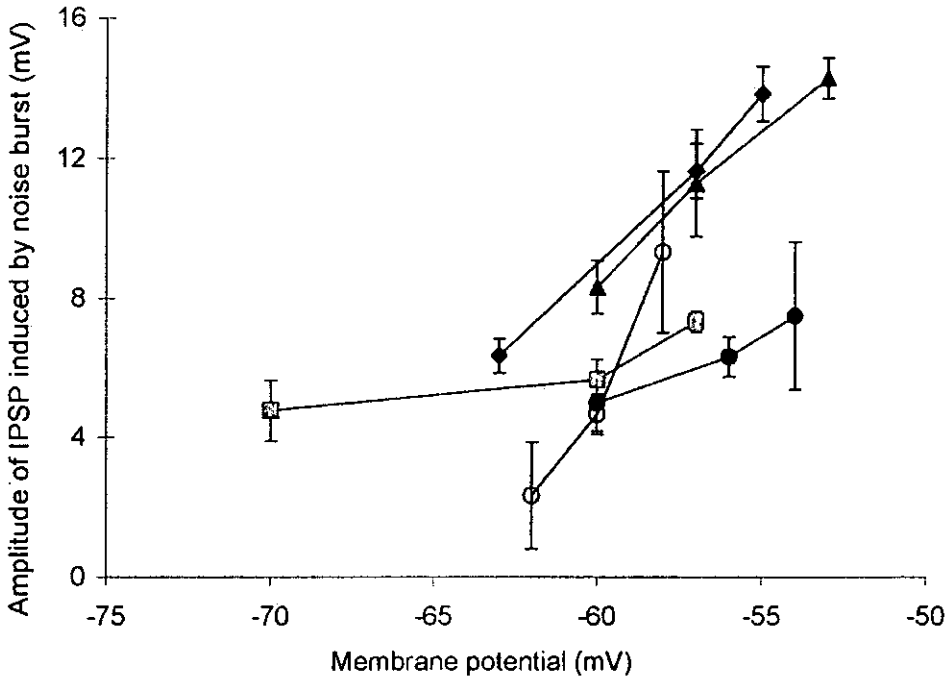

Figure 5 Amplitudes of the IPSPs induced by the noise-burst stimulus at varied membrane potentials for five MGB neurons.

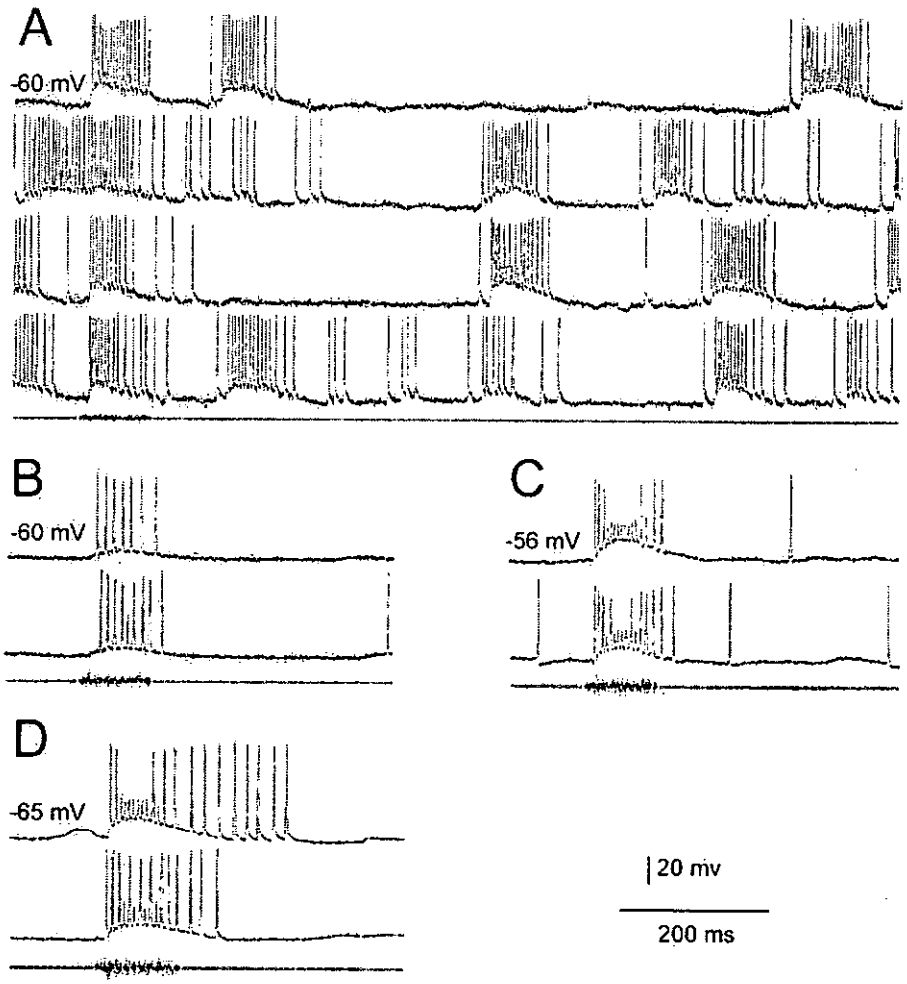

Figure 6 **Auditory responses of four MGB neurons.** A. Neuronal responses to four trials of noise-burst stimuli. B-D. Neuronal responses to two trials of noise-burst stimuli are displayed for each neuron. The resting membrane potentials for each neuron is shown on the left top corner. The stimulus signal shown below the last trace for each neuron applies to all repeats of responses. The inter-stimulus interval (ISI) was 1200 ms, and is only partially displayed. The noise-burst was set at the 60 dB re sound pressure level (SPL). Scale bars for time and voltage apply to all traces.

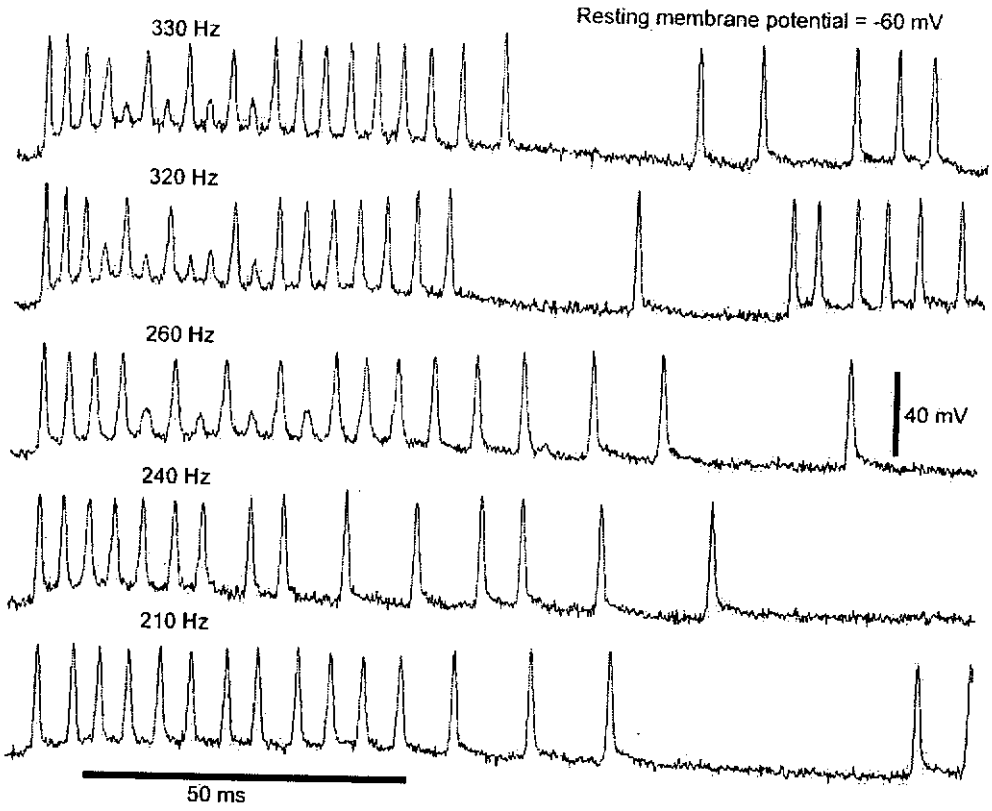

Figure 7 Spontaneous burst and auditory responses of a sustained responsive neuron. The neuron had the highest firing rate. Traces were sampled from five spike bursts. They are displayed in descending order by firing rate. The neuron showed non-full-sized spikes when the firing rate was higher than 240 Hz. The neuron had a resting membrane potential of -60 mV. Scales bars of voltage and time apply to all traces.

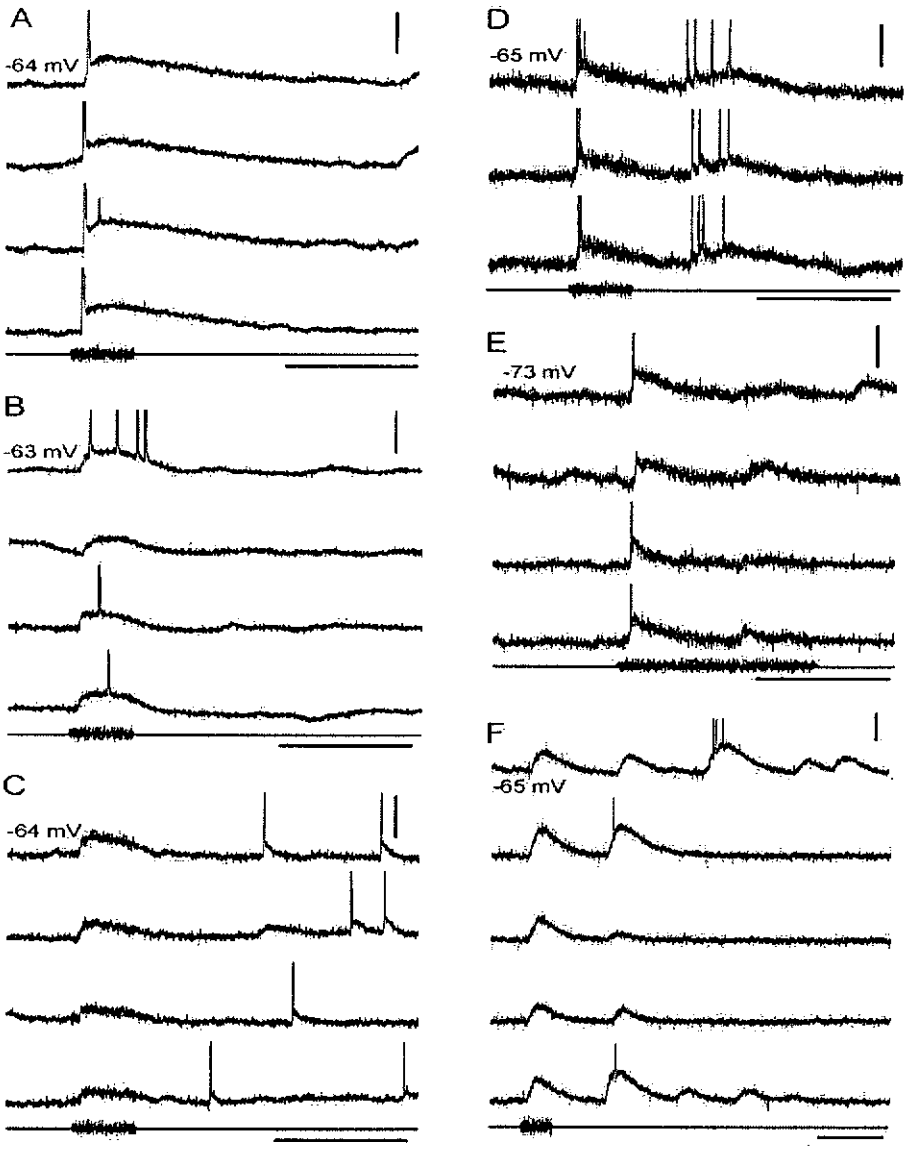

Figure 8 Neuronal responses of six neurons to repeated acoustic stimuli. A-C. Neurons responded to repeated noise-burst stimuli with an EPSP with/without spikes on it. D-F. Neurons responded to the stimulus with an EPSP, which was followed by rebounded EPSPs. Some neurons responded to the stimulus with spikes and EPSPs (A, B, D, E) and others with only EPSPs (C, F). Resting membrane potentials are indicated for each neuron. All spikes were cut short in the figure. Vertical bar: 20 mV, horizontal bar: 200 ms.

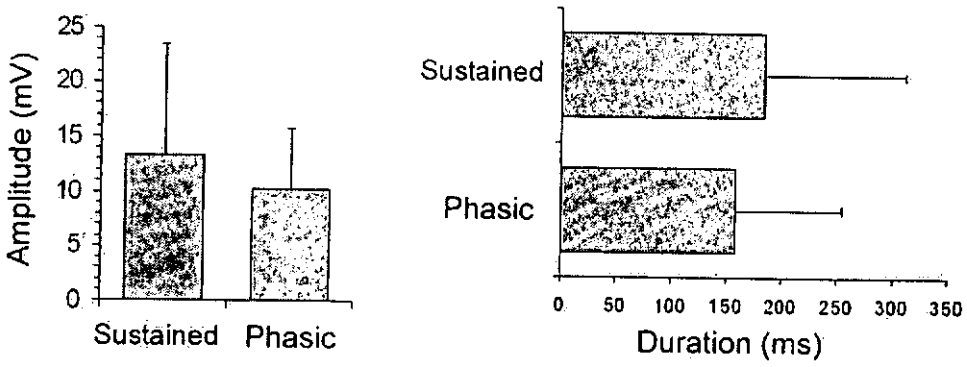

Figure 9 The means of amplitudes and durations of the EPSPs for two groups of neurons: the sustained response group and the phasic response group. The left bar graph shows the means of amplitude of the EPSPs and the right bar graph shows the means of duration of the EPSPs. Standard deviations are shown on the bars.

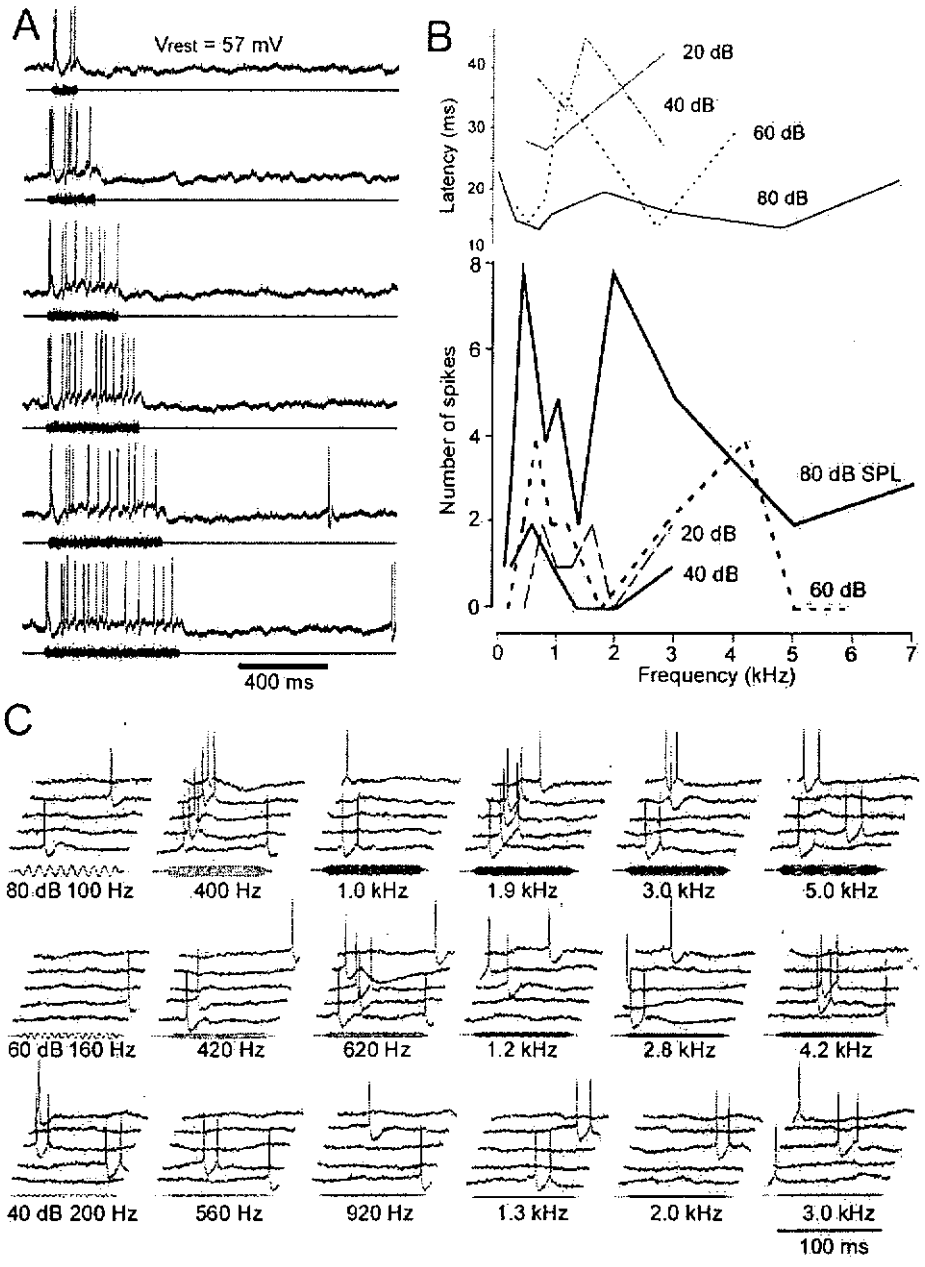

Figure 10 The tuning characteristics of a tonic response neuron. A. Responses to six noise-burst stimuli of varied durations at 80 dB SPL. B. Upper part: latency-frequency functions, defined as the first spike latency in the responses to pure tones as a function of frequency at certain intensities: 80 dB, 60 dB, 40 dB, and 20 dB SPL. The latency shown here was the mean of the latencies of the first spikes over five repeated trials. Low part: response-frequency functions, defined to be the responses to pure tones as a function of frequency at certain intensities. The number of spikes was summed over the onset responses to five repeats of pure-tone stimuli for each frequency. C. Neuronal responses to pure tones of varied frequencies and intensities. Five repeats of pure tones were presented for each frequency and intensity. Six frequencies were sampled for intensities of 80 dB, 60 dB, and 40 dB SPL from the frequency response functions in B.

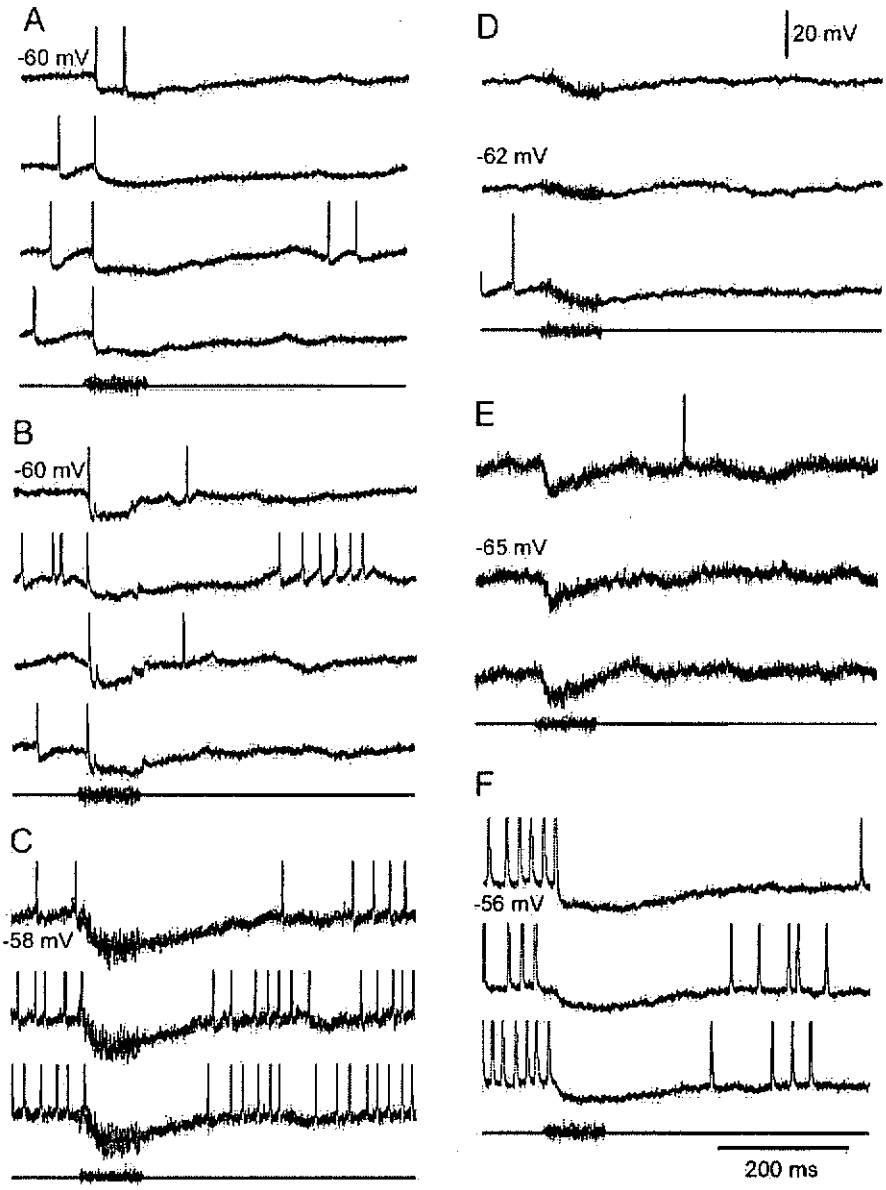

Figure 11 Auditory responses of six MGB neurons. Each neuron in (A-C) responded to noise-burst stimuli with a spike and an IPSP and each in (D-F) responded with an IPSP only. Four traces of neuronal responses to acoustic stimuli are shown for neurons (A) and (B), and three traces for (C-F). The auditory stimulus shown for each neuron applies to all traces. Some noises that were synchronous with the acoustic stimulus were detectable in the intracellular membrane potential recording traces in (C-D). They were probably caused by vibrations of the conducting tube that delivered sound to the subject. The scale bars for voltage and time apply to all traces in the figure.

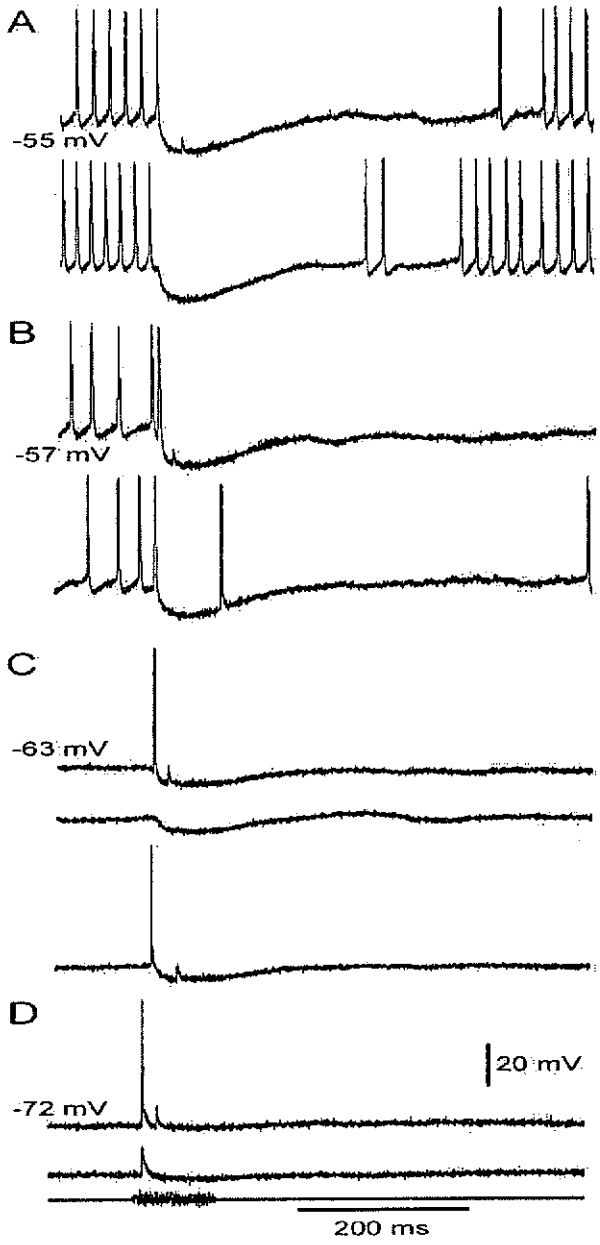

Figure 12 Responses of an MGB neuron to repeated noise-burst stimuli at varied resting membrane potentials. The membrane potential of this neuron drifted spontaneously during the course of recording and is indicated here: A, -55 mV, B, -57 mV, C, -63 mV, and D, -72 mV. The scale bars for voltage and time apply to all traces.

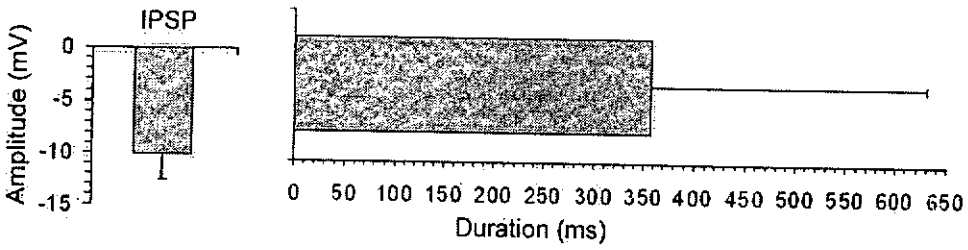

Figure 13 The mean amplitude and duration of the acoustically evoked IPSPs. The left bar graph shows the mean amplitude of IPSPs and the right bar graph shows the mean duration of IPSPs. Standard deviations are shown on the bars.

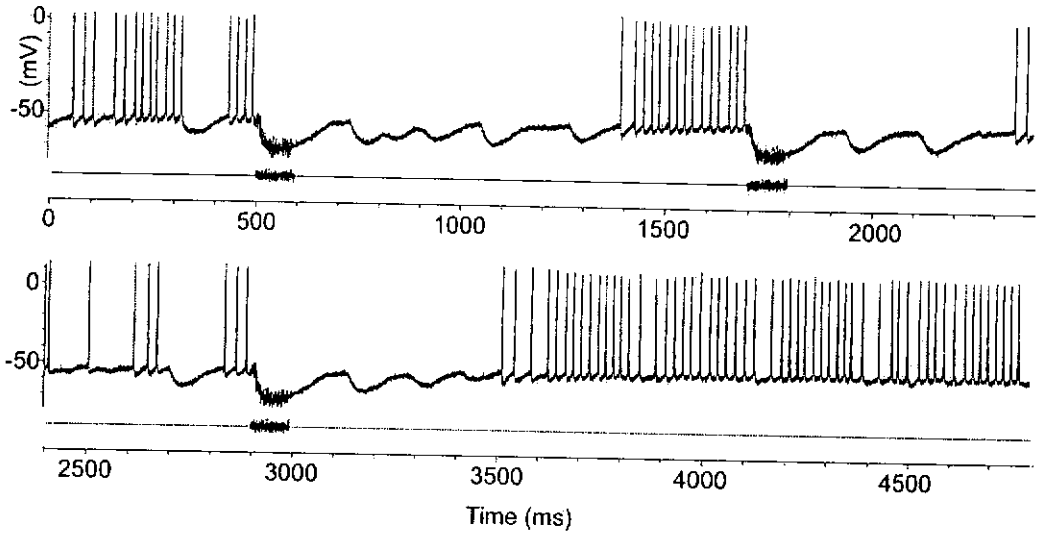

Figure 14 A time course of the spontaneous firing and auditory responses of an MGB neuron. Two traces are connected with each other and the acoustic stimulus signal was illustrated below the neuronal signal. Scales for the membrane potential are indicated on the left of the traces.

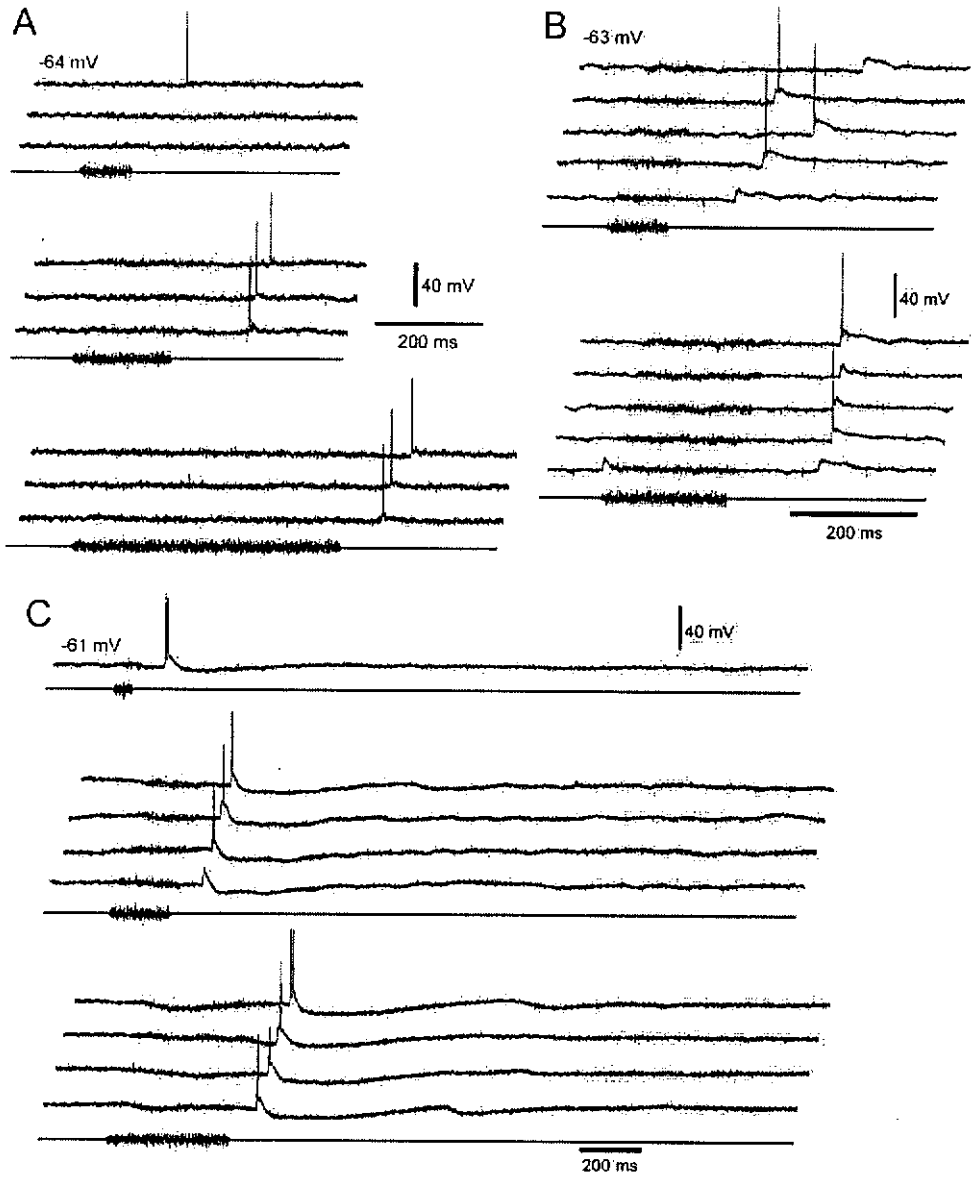

Figure 15 Three OFF neurons showed neuronal responses to noise-burst stimuli of varied durations. The neurons in (A and C) were tested with three durations of 100, 200 and 400 ms and that in (B) was tested with two durations of 200 and 400 ms. Resting membrane potentials and scale bars were indicated for each neuron.

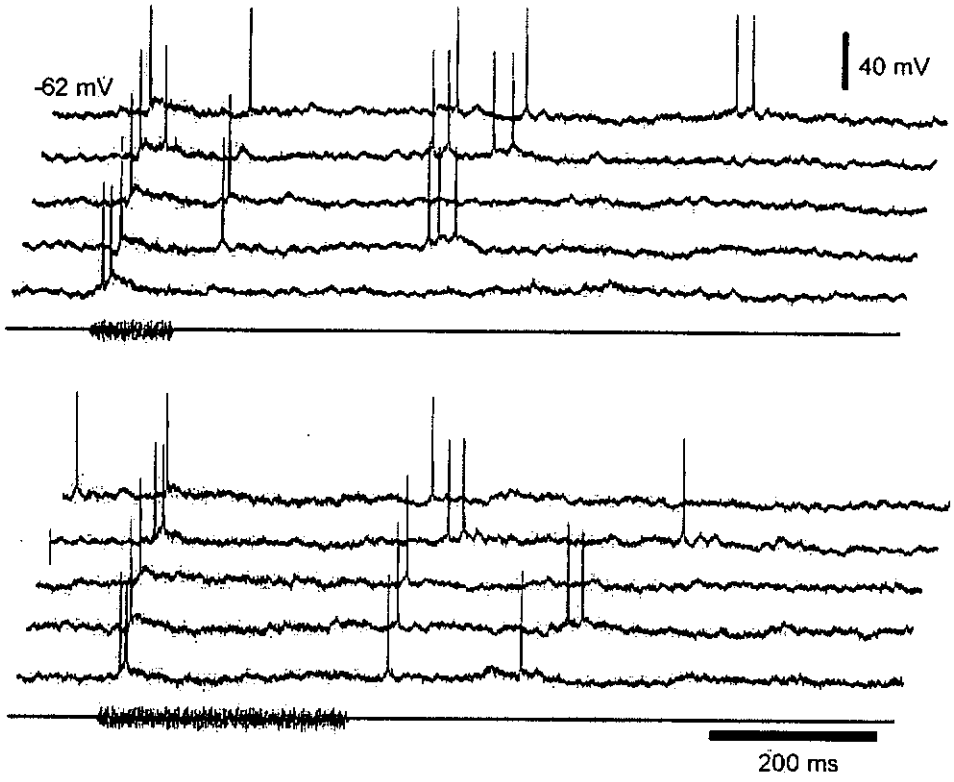

Figure 16 An ON-OFF neuron showed neuronal responses to noise-burst stimuli with two durations: 100 ms and 300 ms. Scale bars apply to all traces.

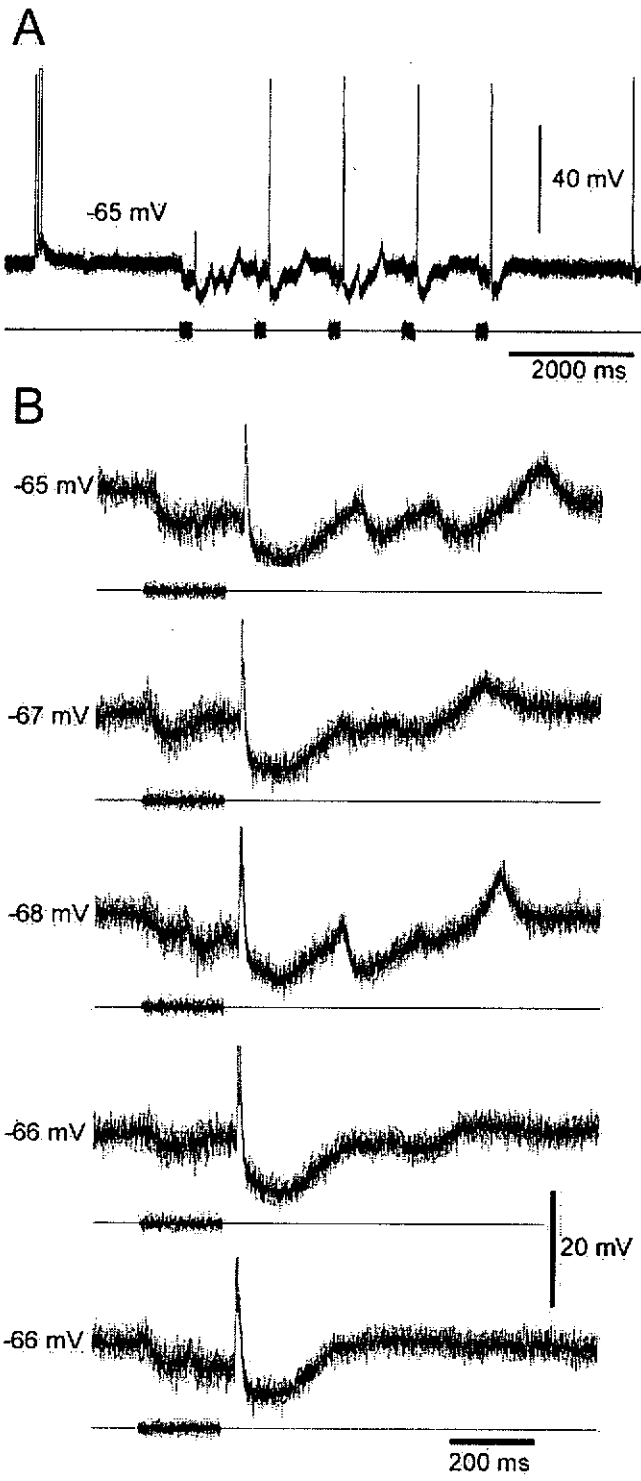

Figure 17 Membrane oscillation of an OFF neuron. (A) A long time period for the neuronal responses, including spontaneous firing and responses to noise-burst stimuli. (B) Time expended traces of the neuronal responses in (A). Acoustically evoked membrane oscillation is obvious after the stimulus offset (especially in the first to third traces of B). Scale bars are shown separately for (A) and (B).

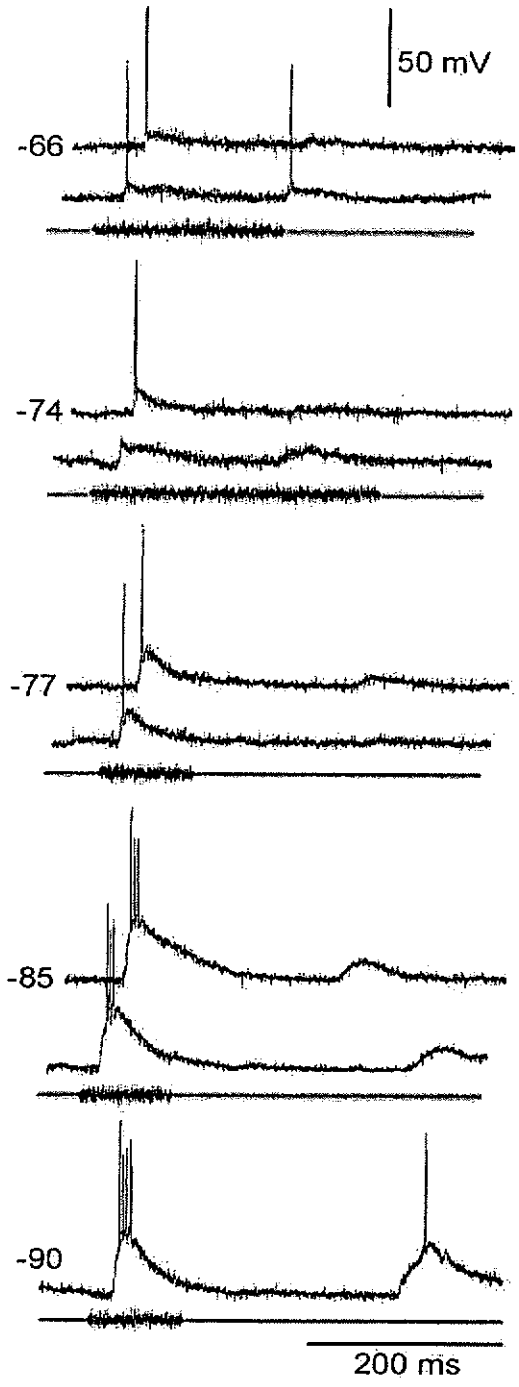

Figure 18 Changes in the discharge patterns of auditory responses in varied membrane potentials. The membrane potential was hyperpolarized by injecting a negative current through the recording electrode. The duration of the acoustic stimulus was changed to examine whether the neuron was an ON-OFF neuron.

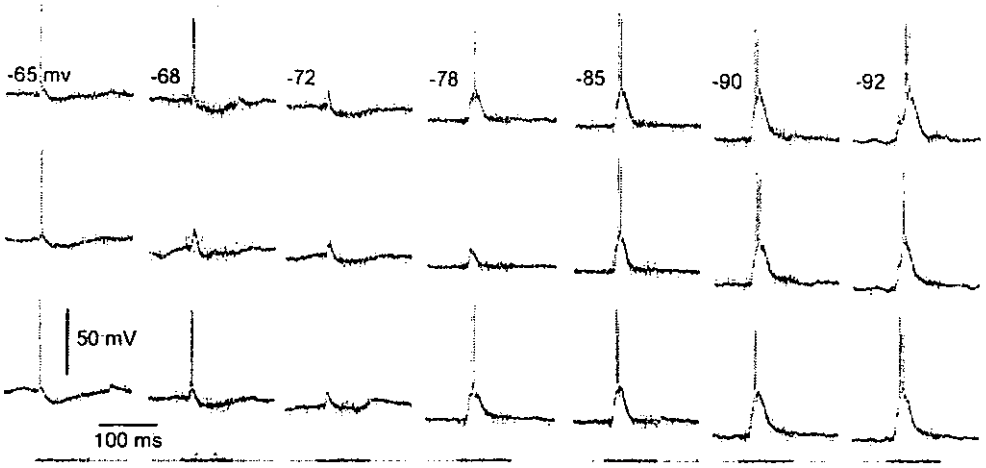

Figure 19 Neuronal responses to an auditory stimulus in varied membrane potentials. The membrane potentials are shown in the first row and the auditory stimuli are shown under the third row of the traces. The scale bars for the membrane potential and time apply to all traces.

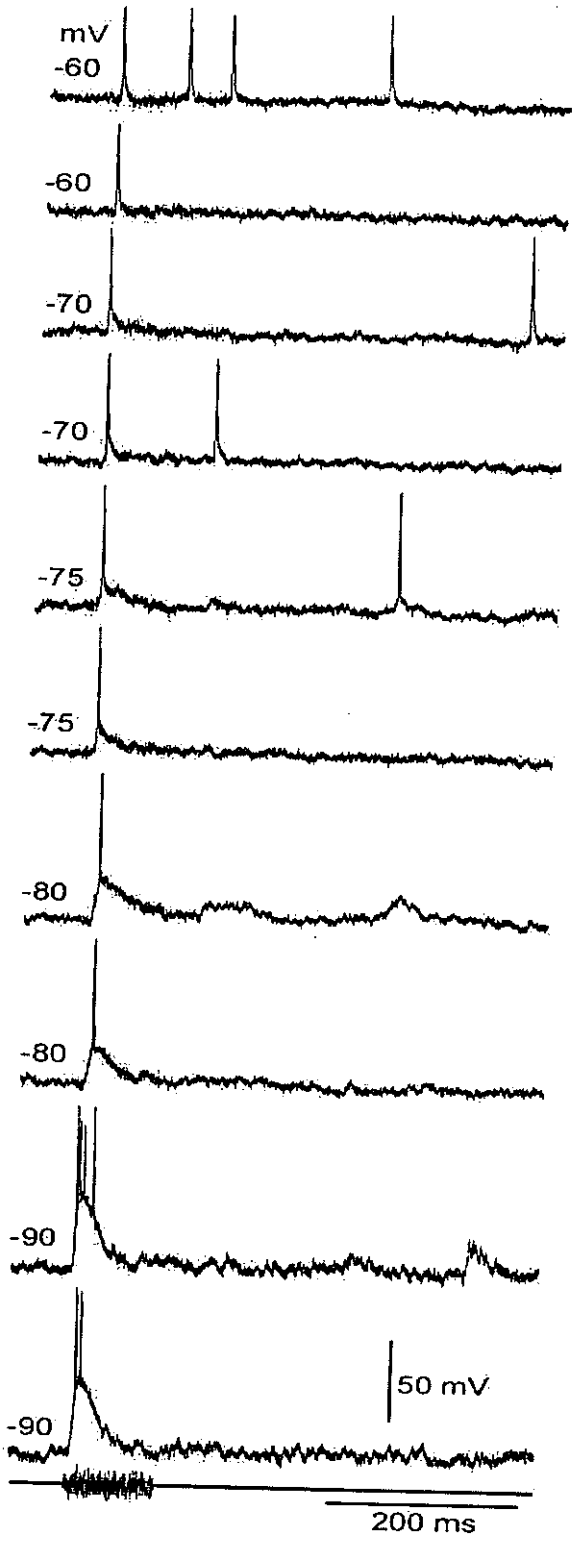

Figure 20 Neuronal responses to an auditory stimulus in varied membrane potentials. The membrane potentials are shown on the left of each trace and the auditory stimuli are shown under the traces. The scale bars for the membrane potential and time apply to all traces.

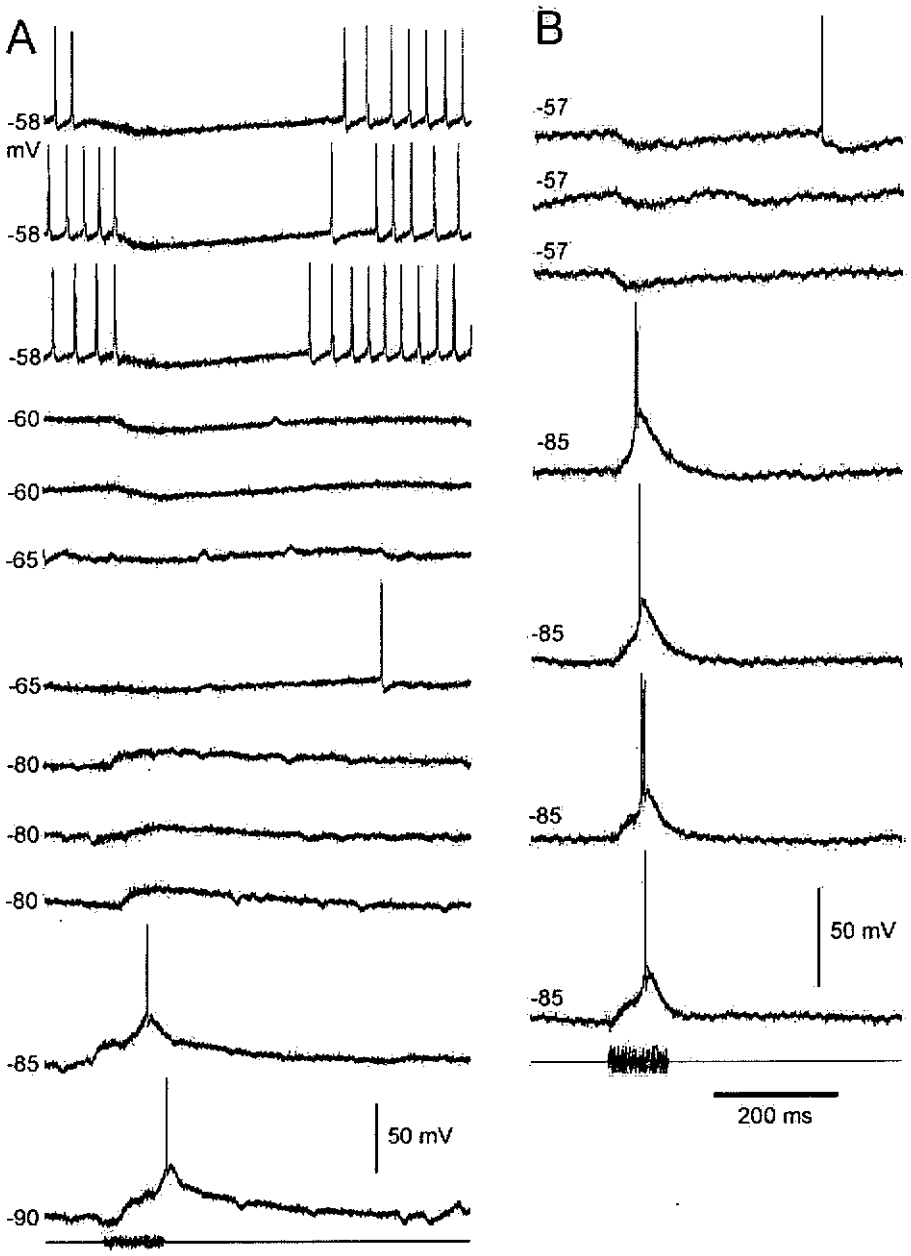

Figure 21 Neuronal responses to auditory stimulus in varied membrane potentials. The membrane potentials are shown on the left of each trace. Scale bars for the membrane potential apply separately to each neuron (A and B) and the scale bar for time applies to all traces.

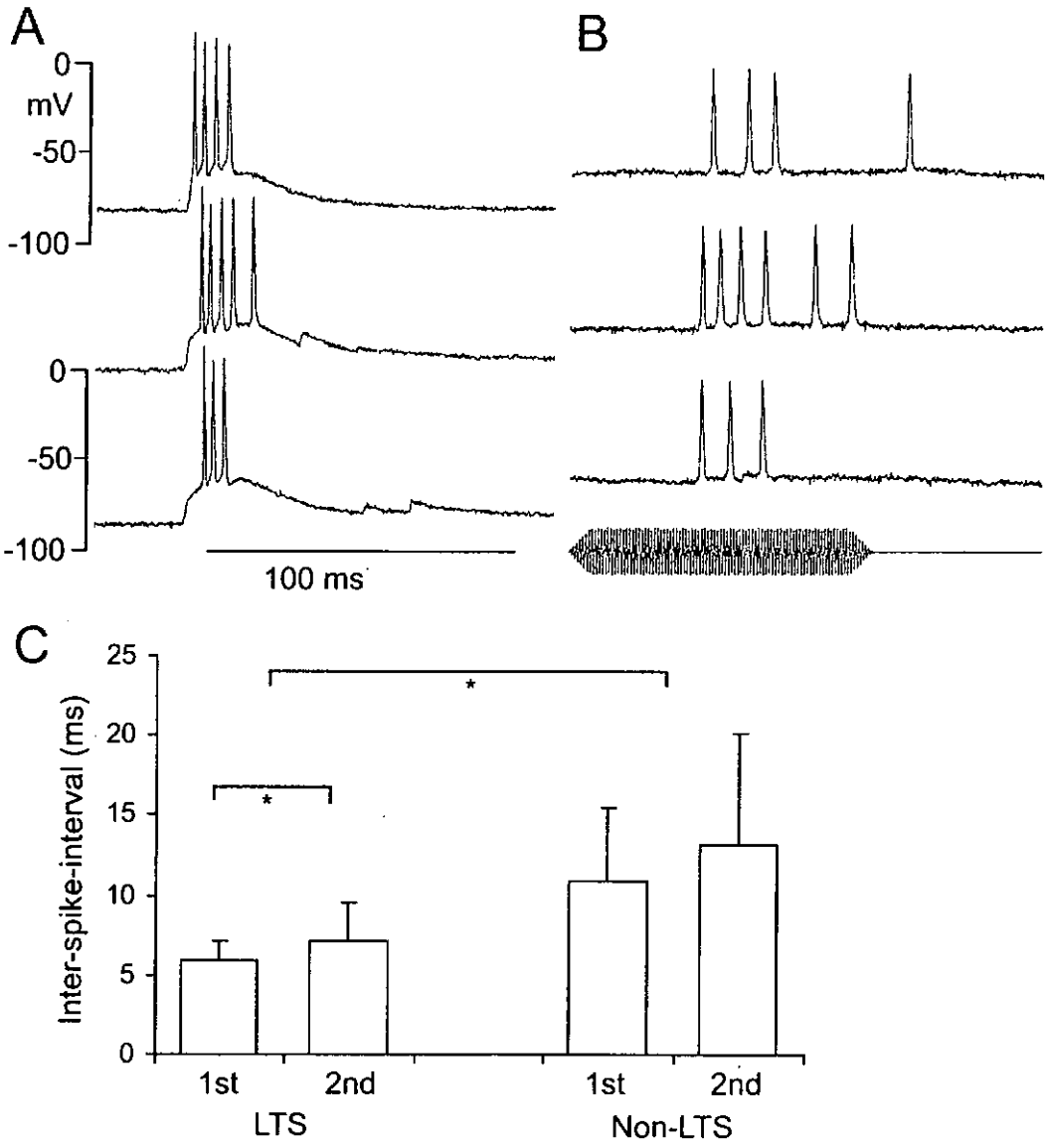

Figure 22 Spike bursts at varied membrane potentials. A. Non-acoustically-driven spike bursts at a hyperpolarized membrane potential of -85 mV. B. The neuron responded with a spike burst to pure-tone stimulus at a depolarized membrane potential of -53 mV. C. Mean inter-spike-intervals (ISI) of two neurons were calculated over 20 bursts. The first ISI means the interval between the first and second spikes and second ISI means that between the second and third spikes. LTS: low-threshold calcium spike. * $P < 0.01$.

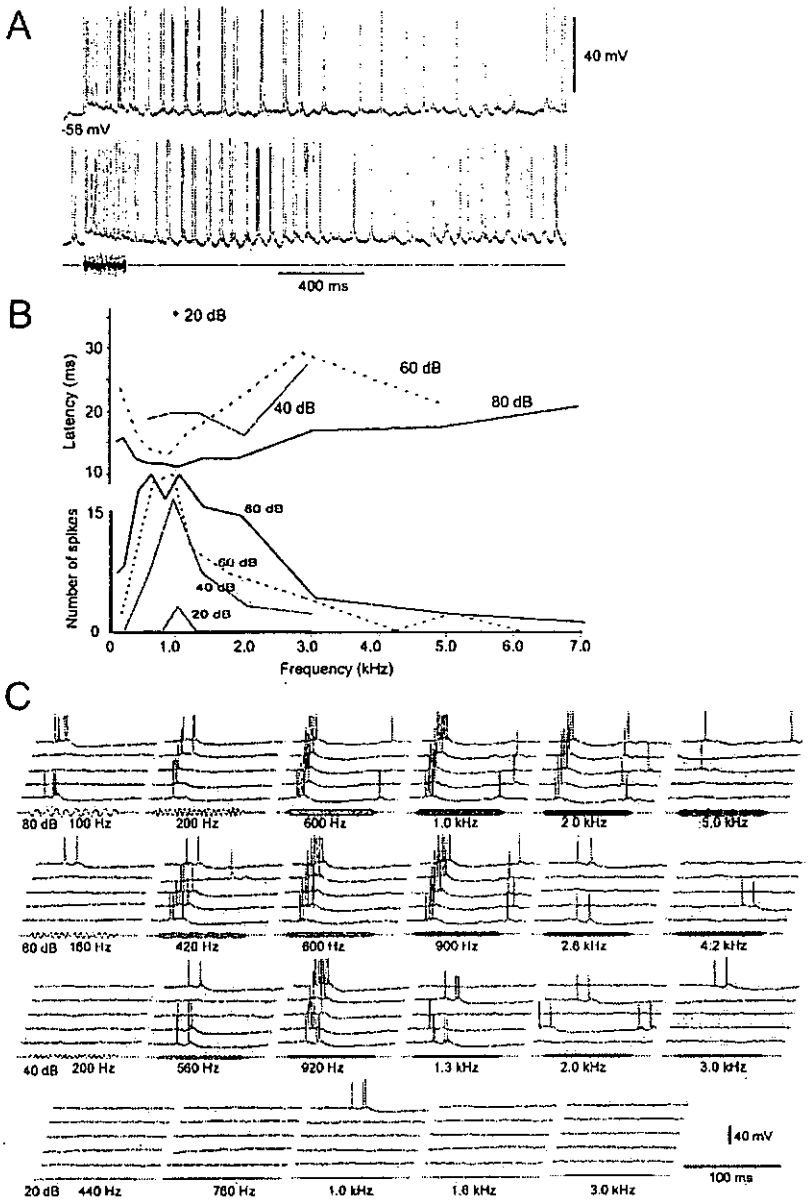

Figure 2 3 A f requency-tuned n euron r esponding t o a n oise-burst s timulus with a long-lasting EPSP. A. Responses to two repeats of noise-burst stimuli of 60 dB SPL. The inter-stimulus interval was 2.4 sec. B. Upper part: latency-frequency functions, defined as the first spike latency in response to pure tones as a function of frequency at certain intensities: 80 dB, 60 dB, 40 dB, and 20 dB SPL. The latency shown here was the mean of the latencies of the first spikes over five repeated trials. Low part: frequency response functions, defined as the responses to pure tones as a function of frequency at certain intensities. The number of spikes was summed over the onset responses to five repeats of pure-tone stimuli for each frequency. C. Neuronal responses to pure tones of varied frequencies and intensities. Five repeats of pure tones were presented for each frequency and intensity. Six frequencies were sampled from intensities of 80 dB, 60 dB, and 40 dB SPL and five frequencies from the intensity of 20 dB SPL from the frequency response functions in B. Scale bars apply to all.

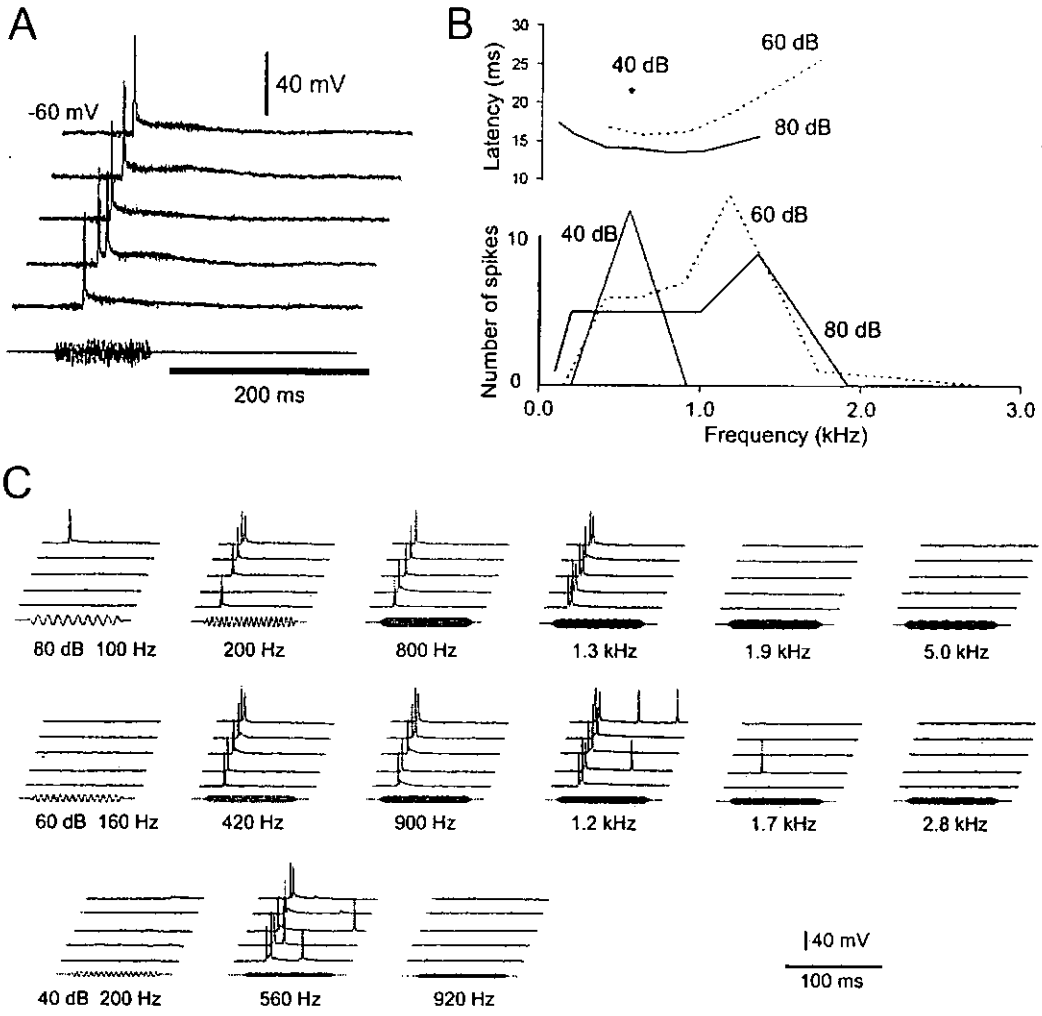

Figure 24 Neuronal responses to a noise-burst stimulus and tuning characteristics of an MGB neuron. A. Neuronal responses to a repeated noise-burst stimulus. B. Latency-frequency function and frequency response function. C. Neuronal responses to pure tones of varied frequencies and intensities. Conventions of Figure 23 apply here.

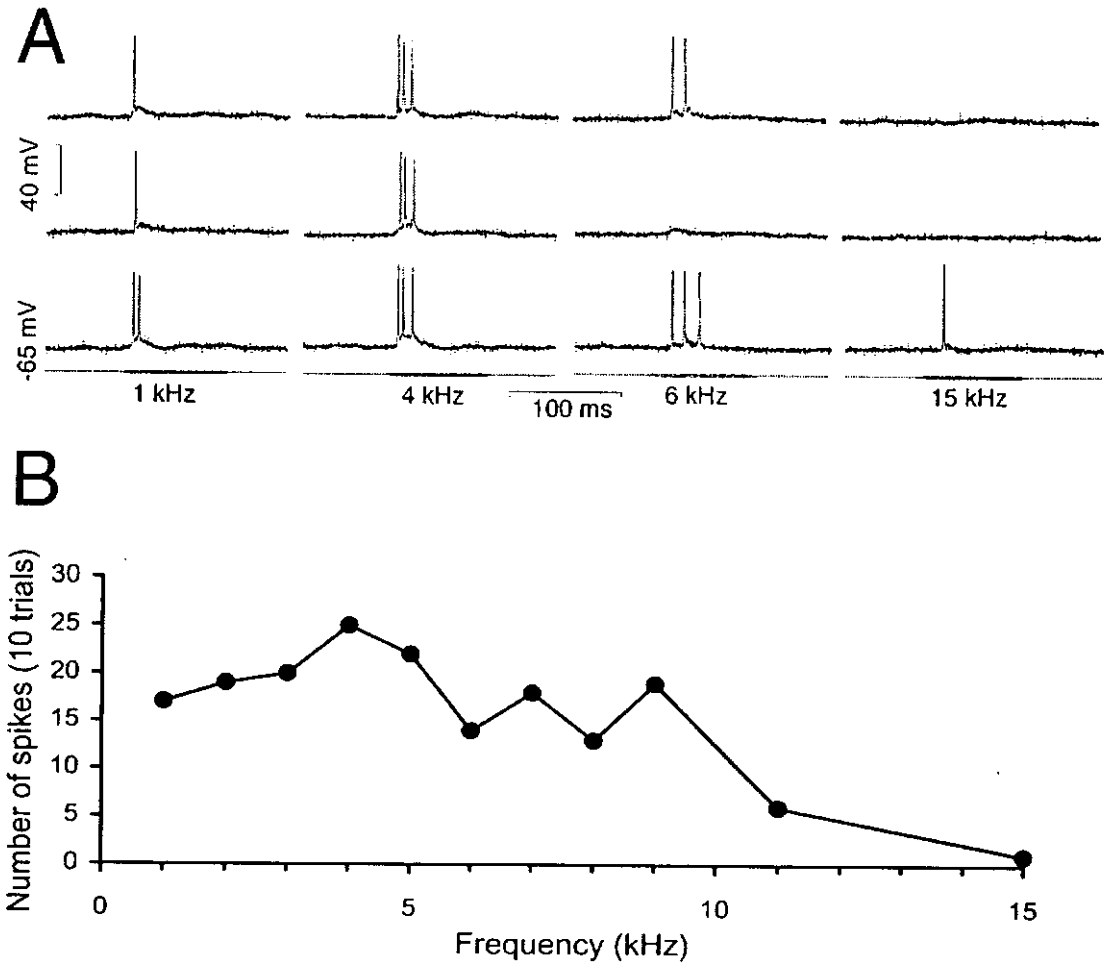

Figure 25 Frequency tuning of an MGB neuron. A. Neuronal responses to pure-tone of four different frequencies: 1 kHz, 4 kHz, 6 kHz, and 15 kHz. Three repeats, sampled from 10 repeats, are shown in the figure. The resting membrane potential was –65 mV. Scale bars apply to all. B. A response-frequency function at an intensity of 60 dB SPL and no current injection. The number of spikes was counted over 10 repeated pure-tone stimuli of various frequencies.

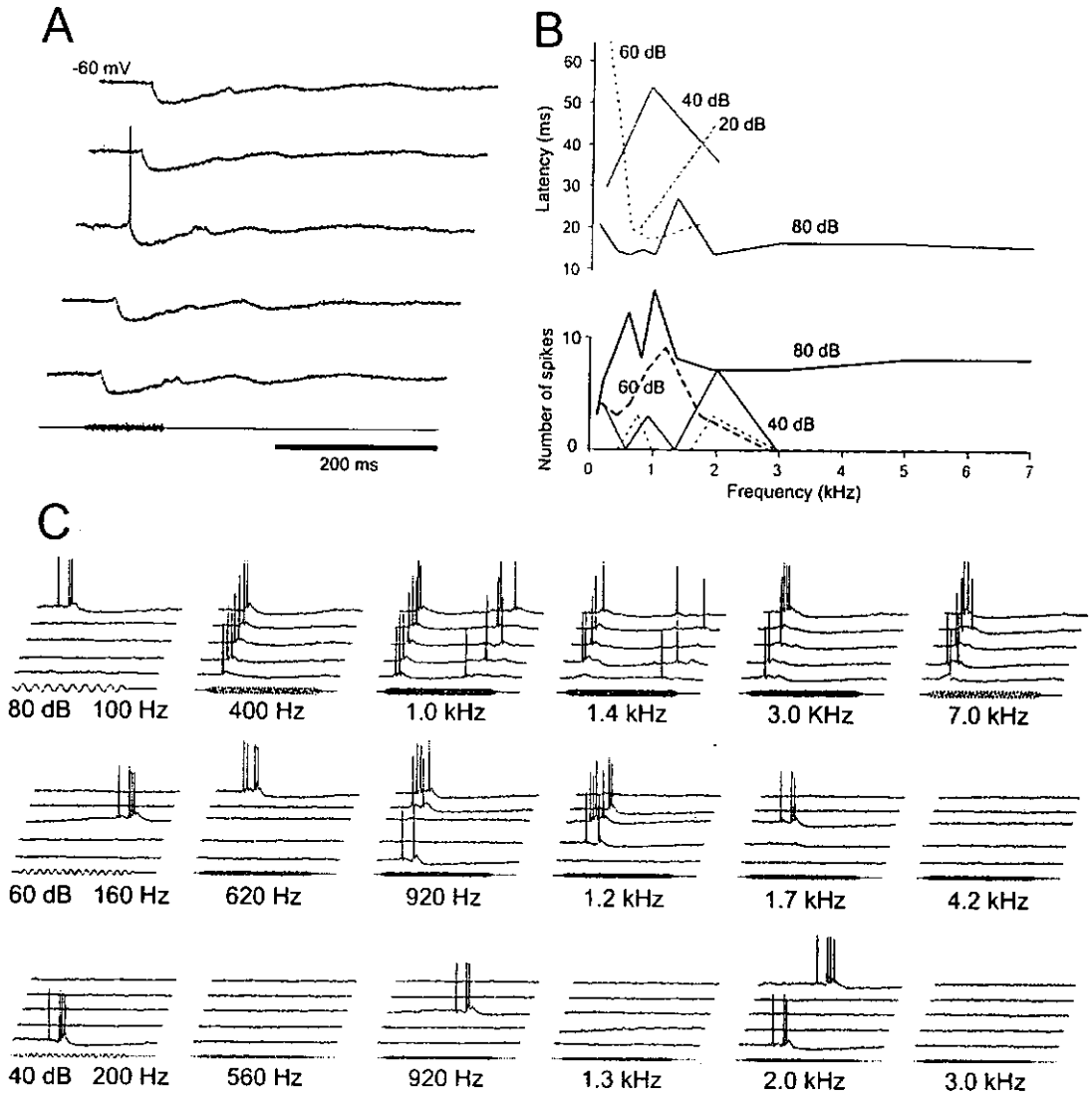

Figure 26 The tuning characteristics of an IPSP response neuron. A. Responses to five noise-burst stimuli of varied durations at 80 dB SPL. B. Upper part: latency-frequency functions, defined to be the first spike latency in the responses to pure tones as a function of frequency at certain intensities: 80 dB, 60 dB, 40 dB, and 20 dB SPL. The latency shown here was the mean of the latencies of the first spikes over five repeated trials. Low part: response-frequency functions, defined to be the responses to pure tones as a function of frequency at certain intensities. The number of spikes was summed over the onset responses to five repeats of pure-tone stimuli for each frequency. C. Neuronal responses to pure tones of varied frequencies and intensities. Five repeats of pure tones were presented for each frequency and intensity. Six frequencies were sampled for intensities of 80 dB, 60 dB and 40 dB SPL from the frequency response functions in B.

Chapter 4

Filtering of acoustic information on the thalamus by cortical feedback network

4.1 Introduction

The thalamus relays ascending information to the cortex, and in turn receives a much stronger reciprocal projection than the ascending one back from the cortex (Andersen et al., 1980; Liu et al., 1995a; Montero, 1991; Ojima, 1994). Neurons in the deep layers of the auditory cortex project to the MGB or to the IC. In the cat, about half of all layer 6 pyramidal cells contribute to the corticogeniculate pathway. It has been estimated that each geniculate relay cell receives convergent input from at least 10 cortical cells and most likely much more. The corticogeniculate axons influence their target cells (both relay cells and interneurons) in a topographic manner.

These corticofugal fibers are believed to exert a modulatory effect on the thalamus. These corticofugal modulations are based on highly focused positive feedback to subcortical neurons "matched" in tuning to a particular acoustic parameter and widespread negative feedback (lateral inhibition) to "unmatched" subcortical neurons. Corticothalamic fibers make excitatory synaptic contracts on the distal dendrites of thalamic principal cells (Liu et al., 1995a), as well as on inhibitory local circuit cells (Golgi Type 2, inhibitory interneurons) of the main thalamic sensory nuclei and TRN (Yen et al., 1985). In addition, TRN sends

inhibitory projections back to both principal cells and local circuit cells in the main thalamic nuclei. But the presence of these local inhibitory neurons is species-specific and varies across the different sensory thalamic subdivisions. In the MGB of rats or guinea pigs, the relative frequency of local inhibitory neurons is 0-3%, while that in cats is 25-30%.

It has been suggested cortical feedback to the thalamus provides a gating or gain-control mechanism in the transmission of information from the periphery to the cortex (Crick, 1984; Deschênes and Hu, 1990; He, 1997; Murphy and Sillito, 1987; Sherman and Koch, 1986; Yan and Suga, 1996). Earlier studies using the cooling technique by Ryugo and Weingberger (1976) and Villa et al. (1991) have demonstrated that the auditory cortex has two processes for modulating its thalamic relay nucleus, the MGB: facilitatory and inhibitory. Both processes have been observed in the MGB and IC while the cortex was activated by electrical stimulation (Amato et al., 1969; Chowdhury and Suga, 2000; He, 1997, 2003a; He et al., 2002; Jen et al., 1998; Jen et al., 2001; Jen and Zhang, 1999; Ma and Suga, 2001; Sakai and Suga, 2001, 2002; Suga et al., 1997; Sun et al., 1989, 1996; Watanabe et al., 1966; Xiao and Suga, 2002; Yan and Suga, 1996, 1998, 1999; Zhou and Jen, 2000). Electrical activation of the auditory cortex caused mainly strong facilitation and little inhibition on the lemniscal nucleus of the MGB and mainly inhibition on the non-lemniscal MGB (He, 1997, 2003a; He et al., 2002; Yan and Suga, 1996). In contrast with the lemniscal MGB where neurons showed short latency and sharp frequency tuning features, the non-lemniscal MGB showed long latency, a bursting firing pattern, broad/non-tuning properties, more OFF

responses, and non-tonotopic organization (Calford and Aitkin, 1983; Cruikshank et al., 1992; He, 2001, 2002; He and Hashikawa, 1998; He et al., 1997; He and Hu, 2002; Hu, 1995; Rauschecker et al., 1997; Winer, 1992; Winer and Morest, 1983). It was also found to be an integrative system involving multi-sensory afferents (LeDoux et al., 1990; Shinonaga et al., 1994; Wepsic, 1966).

Previous extracellular recording experiments have shown that the corticofugal modulation could sharpen the frequency tuning curves, stimulus duration tuning curves, and could gate the matched information and amplify the low-intensity sound, and control the plasticity in the MGB and IC (Chowdhury and Suga, 2000; He, 1997, 2003a; He et al., 2002; Jen et al., 2001; Jen and Zhang, 1999; Ma and Suga, 2001; Sakai and Suga, 2001, 2002; Suga et al., 1997; Sun et al., 1989, 1996; Xiao and Suga, 2002; Yan and Suga, 1996, 1998, 1999; Zhou and Jen, 2000). How do these functions on a single cellular level?

Studies of intracellular recording with slice preparation have revealed similar intrinsic membrane properties and different synaptic properties of the lemniscal and non-lemniscal MGB (Bartlett and Smith, 1999; Hu, 1995; Hu et al., 1994; Tennigkeit et al., 1996). It has also been revealed that corticofugal feedback can control the thalamic oscillation (Bal et al., 2000; Blumenfeld and McCormick, 2000; Golshani et al., 2001; Huntsman et al., 1999; Jacobsen et al., 2001) and that the corticofugal influence on the TRN is 2.4 times higher than that on the thalamic relay neurons (Golshani et al., 2001; Steriade, 2001b). However, a systemic linkage of the corticofugal projection is still to be investigated with the *in-vivo* preparation.

A direct *in-vivo* intracellular recording experiment becomes a most powerful means to address the above issues. In the present study, I use a simple animal model, the guinea pig with a very few thalamic interneurons (Arcelli et al., 1997; Spreafico et al., 1994) to uncover the most basic functions of the corticofugal modulation of the mammals: corticofugal facilitation and inhibition.

4.2 Materials and methods

4.2.1 Animal preparation for intracellular recording

Fifty-two guinea pigs served as subjects for the present intracellular recording study. Anesthesia was initially induced with pentobarbital sodium (Nembutal; Abbott Labs, Irving, TX) (40 mg/kg, i.p.) and maintained at the same level as during surgery by supplemental doses of the same anesthetic (5-10 mg/kg/hr) during the surgical preparation and recording. An electrocorticogram from the left frontal cortex was used to monitor the anesthetic level of the animal. Atropine sulphate (0.05 mg/kg, s.c.) was given 15 minutes before anesthesia commenced and at regular intervals (0.01 mg/kg/hr, s.c.) during recording so as to minimize tracheal secretion. Body temperature was maintained between 37.5 and 38.5℃ by the use of a thermistor-controlled heating pad attached to the animal's abdomen. The animal was mounted in a stereotaxic device following the induction of anesthesia. A midline incision was made in the scalp, and craniotomies were performed to enable us to map the auditory cortex, to implant stimulation electrodes into the cortex, and

to vertically access the MGB in the right hemisphere. The craniotomy opening was usually ~4.0×4.0 mm^2 where located above the auditory thalamus. The dura mater was removed above the auditory cortex and at a position vertically above the auditory thalamus. Before the left ear was freed from the ear bar, the head was fixed with two stainless steel bolts together with acrylic resin to an extended arm of the stereotaxic frame. These ensured that the subject's head remained fixed to the stereotaxic device without misalignment.

Since breathing causes alternate positive and negative thoracic pressure changes, which transfer to the brain and cause movement of the brain, artificial ventilation was used and the animal's muscles were relaxed with gallamine triethiodide (50 mg/kg, i.p.). To minimize the pressure in the chest, bilateral pneumothorax was done and the trunk of the animal was suspended on a spinal frame. Intra-cranial pressure was reduced by release of the cerebrospinal fluid through an opening of the dura mater at the foramen magnum.

I used a glass-pipette as the recording electrode which was filled with 1.0 M KCl. The resistance of the electrode was between 40 and 90 MΩ. The electrode was advanced vertically from the top of the brain by the stepping motor. After the electrode was lowered to 4-5 mm from the cortical surface, the cortical exposure was sealed by low-melting temperature paraffin. When the electrode was near or in the targeting area, it was then slowly advanced at 1 or 2 μm per step.

4.2.2 Acoustic stimuli

Acoustic stimuli were generated digitally by a MALab system (Kaiser Instruments, Irvine, CA, USA), which was controlled by a Macintosh computer (Semple and Kitzes, 1993; He, 1997). Acoustic stimuli were delivered to the subject via a dynamic earphone (Bayer DT-48) mounted in a probe. The subject was placed in a double-walled soundproof room (NAP, Clayton, Australia). Repeated noise bursts and pure tones with intervals of one second or longer and 5 ms rise/fall time were used to examine the neuronal responses.

4.2.3 Electrical stimulation

We have performed several mapping studies of the auditory cortex prior to the present study and we found that the stimulation site made only quantitative differences of the corticofugal modulation on the thalamic neurons (He, 2003a; He et al., 2002). In the present study, I omitted the mapping procedure to save time for intracellular recording. An electrode array consisting of three parallel electrodes was then implanted into the auditory cortex (the anterior and dorsocaudal auditory fields). I used electrical current pulse trains of 0.1-ms width, 200-Hz frequency, and 20 pulses to activate the already mapped auditory cortex in most cases (Edeline et al., 1994; He, 1997; He et al., 2002). The number of the pulses was examined as a parameter in the present study. Electrical currents of 50-200 μ A were applied to the auditory cortex, ipsi-laterally to the recording thalamus through either a mono- or bi-polar low impedance electrode array. Following a delay interval of 100 ms, a

sound stimulus was delivered to the contra-lateral ear of the recording hemisphere after the end of the cortical stimulation (He, 1997, 2003a).

4.2.4 Data acquisition and analysis

The electrode recorded the membrane potential showing a negative value when it penetrated the membrane of a cell. After amplification, the membrane potential with artifact of electrical stimulation as well as the auditory stimulus was stored on the computer with the aid of commercial software (AxoScope, Axon). No manipulations of membrane potentials were made on the data presented here. The amplitudes of EPSP and IPSP were calculated to be the change of the membrane potential caused by cortical stimulation. The durations of EPSP and IPSP caused by cortical stimulation were calculated to be the lasting of the EPSP from the last electrical stimulation pulse.

4.3 Results

The present results were collected from 92 neurons in 52 animals.

4.3.1 Corticofugal modulation on the membrane potential

Ninety-two neurons that received corticofugal modulation on the membrane potential have been studied in the present study. Figure 1 shows two typical

examples of the corticofugal modulation on the membrane potential: one was potentiation and one hyperpolarization. The membrane potential of the neuron in Figure 1 A w as d epolarized f or 1 3 m V a nd t he spontaneous f iring r ate i ncreased from <1 Hz to 21 Hz on average (counted over 1 s period, lower panel of Figure 1A). Thirty-eight neurons received corticofugal potentiation in their membrane potentials. The membrane potential of the neuron in Figure 1B was hyperpolarized for -20 mV by cortical stimulation (lower panel). The spontaneous firing was almost diminished by the corticofugal hyperpolarization from 3 Hz. The hyperpolarized membrane potential lasted during the successive pulse trains of the stimulation. Fifty neurons received corticofugal hyperpolarization in their membrane potential.

4.3.2 Corticofugal facilitation of the thalamic response to auditory stimuli

With a resting membrane potential above -70 mV, the corticofugal potentiation of the membrane potential facilitated auditory responses of the MGB neurons. Three examples of corticofugal facilitation of the thalamic response to auditory stimuli are shown in Figure 2. The auditory responsive neuron in Figure 2A responded to a repeated noise-burst stimulus with 1 or 2 spikes in the control condition when the cortex was not stimulated (Figure 2A-a). The neuron received a corticofugal potentiation of about 10 mV in its membrane potential. It responded to the same acoustic stimulus as the control condition with more spikes (2-5) when the cortex was activated by electrical stimulation (Figure 2A-b). The neuron had a

frequency selectivity of low frequencies at 1-4 kHz was presumably located in the lemniscal MGB (Figure 2A-c).

The neuron in Figure 2B responded with 1-2 spikes to a noise-burst stimulus in the control condition, and increased its responses to 4-6 spikes/spikelets, when the cortex was activated. The neuron in Figure 2C had a lower membrane potential of -65 mV. The neuronal response to the same pure-tone stimulus increased from 2-4 spikes/spikelets to 6 spikes/spikelets after the cortex was activated.

Figure 3A-B shows the summated potentiations (EPSPs) of two neurons evoked by cortical stimulation: neuron A showed a potentiation of 13 mV and lasted for 113 ms and neuron B showed a potentiation of 9 mV and lasted for 128 ms. The mean compound EPSP of the 30 neurons that received corticofugal potentiantion of their membrane potential was 8.6±5.5 mV (range: 2-24 mV) and lasted for 125.5±75.4 ms (range: 27-400 ms).

4.3.3 Cortical activation hyperpolarizes the membrane potential of the thalamic neurons

Results of corticofugal inhibition presented in the present study were obtained while the membrane potential were between -55 mV and -67 mV. The three neurons shown in Figure 4 received a hyperpolarization in their membrane potentials when their auditory cortexes were electrically activated. The neuron in Figure 4A showed a very strong hyperpolarization of about -20 mV from the cortical activation (see right column, lower panel of Figure 4A), which totally

inhibited the auditory ON-response seen in the control condition without cortical activation (see left column, lower panel of Figure 4A). In Figure 4B, the spikes of ON-response seen in the control condition were inhibited by cortical activation which hyperpolarized the neuron about –6 mV from its resting membrane potential of -68 mV. The neuron, however, still responded with an EPSP followed by an IPSP to the same auditory stimulus. Figure 4C shows an MGB neuron which responded to noise-burst stimuli with 1-2 spikes (left panel). The cortical activation hyperpolarized the membrane potential about -10 mV, which inhibited the neuronal responses to the auditory stimuli from spikes to EPSPs (right panel).

Corticofugal inhibition was more dominant than corticofugal facilitation and lasted for a longer time period. Corticofugal inhibition could totally switch off the ON auditory responses.

4.3.4 Long corticofugal inhibitory effect

All neurons in Figure 5 received an inhibitory effect when the auditory cortex was electrically activated. All inhibitions lasted for long-time periods over 400 ms. The neuron in Figure 5A showed a resting potential of -57 mV and received an accumulated hyperpolarization, (or compound IPSP) of about –8 mV. The neuron in Figure 5B had a resting membrane potential of -55 mV and received an compound IPSP of –19 mV, while that in Figure 5C had -55 mV and –7 mV respectively and the neuron in Figure 5D had -57 mV and –20 mV respectively. All

the inhibitory effects on the neurons in Figure 5 had a long-lasting effect: A, 590 ms; B, 750 ms; C, 1150 ms; and, D, 440 ms.

It was interesting to note that the neuron in Figure 5D showed an compound IPSP and initially lasted for only 115 ms. The inhibitory effect, however, bounced back and back again, making the total inhibitory effect longer than 400 ms. Overall 33 neurons that showed an obvious inhibitory effect were averaged for the initial compound IPSP duration, as well as, the total inhibitory effect induced by the electrical cortical stimulation (Figure 5E). Only those neurons that showed a rebounded inhibitory response were accounted for in the later statistics. The mean compound IPSP was -11.3±4.9 mV (n=33, range: 3-21 mV) and the mean compound IPSP duration and the total inhibitory duration were 210±210.1 ms (n=33, range: 90-1220 ms) and 1023.0±635.8 ms (n=16, range: 300-3200 ms) respectively.

4.3.5 Number of stimulation pulses verse corticofugal effect

In the present study, I have tested the parameter of the number of electrical stimulation pulses on 12 neurons, two of which are shown in Figure 6A and B. I have changed the pulse number from 1 to 20 and examined the corticofugal modulatory effect on the thalamic neurons. Both neurons shown in Figure 6A and B received inhibitory effects from the cortical stimulation. Changes of the number of the stimulation pulses caused only quantitative differences in their evoked compound IPSPs in thalamic neurons (Figure 6C). The mean amplitude of the

IPSPs caused by electrical cortical stimulation increased from 7.7±6.3 mV to 9.4±7.1 mV and to 10.7±4.8 mV, when the number of stimulation pulses increased from 1 to 5 and to 10 or 20 (n = 7). The duration of IPSP also increased from 107.5±34.9 ms to 150.7±23.8 ms and to 224.3±78.8 ms, respectively. The durations of EPSP and IPSP caused by cortical stimulation were calculated to be the lasting of the EPSP from the last electrical stimulation pulse. It would be difficult to accurately calculate the effect of the number of pulses on the duration of the inhibition. The duration, however, had only same quantitative differences caused by the parameter of the number of stimulation pulses.

Of the 12 neurons examined, five showed corticofugal facilitation. The mean amplitude of the EPSP increased from 7.0±3.7 mV, to 8.9±5.2 mV, and to 11.5±2.9 mV, when the number of stimulation pulses increased from 1 to 5 and to 10 or 20. The duration of the EPSP increased from 59.0±29.6 ms to 141.0±48.3 ms and to 220.0±21.6 ms, when the number of pulses increased from 1 to 5 and to 10 or 20.

4.3.6 Corticofugal modulation effect of neurons of different discharge patterns

4.3.6.1 Corticofugal potentiation of auditory excitatory neurons

Of 38 auditory excitatory neurons examined in the present study, 31 received corticofugal potentiation, four received corticofugal inhibition, and three received no effect. Three neurons in Figure 7 showed an EPSP and a spike or a few spikes to an acoustic stimulus, and received a depolarization on the membrane potentials

when the auditory cortex was electrically stimulated with a pulse or a train of pulses. The corticofugal depolarization on neuron A was 11 mV; neuron B, 7 mV; and neuron C, 9 mV.

The neuron in Figure 8 showed a similar auditory response as those in Figure 7A with an EPSP and a spike. The neuron received a depolarization from cortical stimulation (Figure 8B). The neuron showed a frequency tuning feature at a frequency of between 1 and 4 kHz (Figure 8C).

In summary, the auditory excitatory neurons showed a higher level of responsiveness with a higher membrane potential, and vice versa when its membrane potential was above -65 mV. Most of these neurons received an excitatory corticofugal modulation on their membrane potentials (one more example is shown in Figure 9). The neuron in Figure 9 responded with a train of pulses (from 5 to 8) to a noise-burst stimulus when the resting membrane potential was -60 mV. It decreased the amplitude in the EPSP and spike number to only one when the resting membrane potential was spontaneously decreased to -65 mV. The neurons received an excitatory corticofugal modulation for over 15 mV in their membrane potential, with a latency of about 20 ms (Figure 9C).

4.3.6.2 Corticofugal hyperpolarization of auditory inhibitory neurons

Of 33 auditory inhibitory neurons examined in the present study, 31 received corticofugal hyperpolarization on their membrane potential and two received no effect. Three examples are shown in Figure 10. Neuron A had a resting membrane

potential of -62 mV and responded to an acoustic stimulus with an IPSP of -6 mV; neuron B had a resting membrane potential of -65 mV and responded with an IPSP of -14 mV; and neuron C had a resting membrane potential of -56 mV and responded with an IPSP of -18 mV. All of the neurons received a hyperpolarization when the auditory cortex was activated with an electrical pulse train.

It is interesting to note that the IPSPs caused by the cortical stimulation were similar to those evoked by acoustic stimuli for each neuron. Neuron A received a relatively slow hyperpolization from cortical stimulation, while neuron B received a fast hyperpolarization from cortical stimulation, with a similar shape of IPSP caused by the acoustic stimulus in the left panel. The neuron in C showed an intermediate time constant of hyperpolarization from cortical stimulation.

The neuron in Figure 11 responded to a noise-burst stimulus with a spike or a short EPSP, which was followed by a prolonged IPSP. As mentioned in Chapter 3, this type of neuron was categorized as an auditory inhibitory neuron. The neuron received a corticofugal hyperpolarization, as shown in the right panel of Figure 11. The corticofugal hyperpolarization inhibited the onset spike, leaving only an EPSP in the first and second traces and nothing in the third trace. The IPSP that followed showed no change in shape.

4.3.6.3 Corticofugal inhibition of the membrane potential induced an LTS response to an acoustic stimulus

A normal spiked neuron with a resting membrane potential of -62 mV, which received a strong corticofugal hyperpolarization of > 15 mV, responded with an LTS burst to an auditory stimulus (Figure 12). The neuron showed normal spontaneous spikes at 200-300 ms after the cortical stimulation.

4.4 Discussion

Anatomically, the corticothalamic fibers terminate on the distal parts of the dendrites of relay neurons and have a cumulative effect on the neurons (Deschênes and Hu, 1990; McCormick and von Krosigk, 1992; Liu et al., 1995a, b; He, 1997). Extracellular studies suggested that the corticothalamic fibers change the responsiveness of thalamic neurons rather than directly evoking them to fire (He, 1997, 2003a; He et al., 2002). The present result of the membrane potential dependence of the responsiveness of the thalamic neurons would provide one basis for the mechanism of the corticothalamic modulation of thalamic neurons (results in Chapter 3). The exact mechanism by which corticofugal fibers exert modulatory effects on the thalamic relay neurons is being addressed currently in this study with intracellular recording of the responses to an auditory stimulus while the corticofugal pathway is being directly manipulated.

4.4.1 Facilitatory effect

Previous extracellular studies have indicated that specific cortical stimulation (stimulation site functionally matched with the recording site in the thalamus and the inferior collicuclus) resulted mainly in facilitatory effects in the cat and bat (Gao and Suga, 1998; He, 1997; He et al., 2002; Zhang et al., 1997; Zhang and Suga, 1997; Zhou and Jen, 2000). Of 20 corticofugal modulatory neurons obtained by Watanabe et al. (1966), 6 neurons had facilitatory effects, with a maximum increase of 57% in the number of spikes. A similar phenomenon was also observed in the cat vMGB, where the interneurons accounted for one-fourth of the total population (He, 1997; Villa et al., 1991). Compared to the cat, the corticofugal modulation on the guinea pig's thalamus has a much broader effective area, supposedly resulting from the fewer interneurons in the guinea pig's thalamus (He et al., 2002). Intracellular recording results in Figures 2 and 3 demonstrate how the corticofugal facilitation was applied to the thalamic neurons and the subsequent changes of the auditory responses. The result shown in Figure 2 is comparable to the extracellular result shown in Figure 11 of He, 1997.

The present results clearly indicate that cortical activation could potentiate the membrane potential by 8.6 mV. The corticofugal potentiation facilitated the neuronal responses to acoustic stimuli of the thalamic neurons. The corticofugal potentiation lasted for an average period of 125.5 ms (range: 27-400 ms). This period is comparable to the time constant of corticofugal facilitation on the thalami of the cat and the guinea pig - a few hundreds milliseconds (He, 1997; He et al., 2002).

Morphologically it is known that about half of the synapses on the thalamic relay neuron are RS terminals (small profiles with rounded vesicles, defined by Guillery, 1969; and Ralston et al., 1988) (Jones and Powell, 1969a, b; Liu et al., 1995a, b). The majority of the RS terminals appear to derive from corticothalamic fibers (Jones and Powell, 1969a). This dense synaptic input into the thalamic relay neurons is clearly excitatory activating r-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl-D-aspartate (NMDA), and metabotropic glutamate receptors (mGluR) (Bartlett and Smith, 1999; Deschênes and Hu, 1990; McCormick and von Krosigk, 1992; Tennigkeit et al., 1999). The mGluR is coupled to G proteins, and has its action through the inositol trisphosphate second messenger pathway and lasts for time periods up to a few hundred of milliseconds (McCormick and von Krosigk, 1992; Tennigkeit et al., 1999). The corticothalamic terminals have their main contact on the distal dendrites thereby delivering an accumulative effect, which again suggests having a slow effect on the thalamic neurons and maintaining the corticofugal modulation for a long period (Bartlett et al., 2000; Liu et al., 1995a).

Most auditory-excitatory neurons receive an excitatory input from the cortex. The auditory-excitatory neurons with a prolonged EPSP are likely to be located in the lemniscal MGB, as these neurons have shown good frequency-tuning characteristics and short responsive latencies as mentioned in Chapter 3.

In the present study, I found that the shapes of the EPSPs caused by the acoustic stimulus and cortical stimulation were similar (Figures 7, 8, and 9). The RS terminals have their main contact on the distal dendrites, while the ascending

afferent terminals end mainly on the proximal and intermediate dendrites (Bartlett et al., 2000; Liu et al., 1995a). Their results and the present result together suggest that the shape and persistence of the EPSP depend on the intrinsic characteristics of the neuron, not on the physical locations of the synapses from which the neuron receives input.

4.4.2 Corticofugal inhibitory effect on thalamic neurons

Activation of the auditory cortex produced more inhibition than facilitation on the thalamic neurons. The average compound IPSP on the thalamic neurons caused by the cortical stimulation was 11.3 mV, which was larger than the mean corticothalamic EPSP. The compound IPSP lasted for a long duration of 210.8 ± 210.1ms. Total inhibition on the thalamic neurons by the cortical stimulation including the rebounded inhibition lasted even longer for 1023.0±635.8 ms.

In a recent extracellular study, He (2003a) has observed a mostly inhibitory effect on the ON responses of the non-lemniscal MGB neurons following cortical activation. In some cases, the ON response was switched off by the cortical stimulation (Figures 1 and 2 of He, 2003a). The corticofugal inhibition was widespread (Figures 5 and 6 of He, 2003a). Of 20 corticofugal modulatory neurons obtained by Watanabe et al. (1966), 6 showed a facilitatory effect and 14 were strongly inhibited by cortical activation.

He showed that corticofugal inhibition lasted for over 100 ms (Figure 4 of He, 2003a). A slice intracellular study showed that the neurons in the dorsal division of

the non-lemniscal MGB responded to thalamic radiation stimulation with a long duration IPSP for over 200 ms, suggesting that the stimulation activated the TRN fibers which inhibit the MGB neurons through $GABA_B$ receptors (Figure 8 of Bartlett and Smith, 1999).

The majority of the excitatory inputs to the TRN neurons are derived from the cerebral cortex (Liu and Jones, 1999), indicating that the corticofugal fibers to the TRN neurons control the TRN neurons' excitability (Golshani et al., 2001; Steriade, 2001b). The TRN neurons extend dendrites within the thin reticular sheet, which enable them to receive projections from a wide cortical region, and project to widespread areas in the ventroposterior nucleus of the thalamus (Liu et al., 1995b). The TRN neurons project diversely to the thalamus (Bourassa and Deschênes, 1995; Jones, 1975; Shosaku and Sumitomo, 1983; Simm et al., 1990; Pinault et al., 1997). Recent physiological results indicate that the TRN terminals have a very strong inhibitory effect on the thalamic relay neurons (Bartlett et al., 2000; Golshani et al., 2001). It is known that the $GABA_B$ receptor has a time constant of a few hundred of milliseconds (Bartlett and Smith, 1999; Kim et al., 1997; Tennigkeit et al., 1998; Ulrich and Huguenard, 1996). In summary, activation of the cortex directly generates excitatory input to the lemniscal MGB neurons. This also activates the TRN neurons through corticothalamic fibers, which in turn inhibit the non-lemniscal MGB neurons in a time period of over a few hundred of milliseconds.

Though both the cortex and thalamus have an intrinsic capacity to generate oscillations of varied frequencies, the corticothalamic loop is an ideal oscillator (Bal et al., 2000; Golshani and Jones, 1999; Steriade, 2001a; Steriade et al., 1993a,

b; von Krosigk et al., 1993). The oscillatory inhibitory wave in the membrane potential in Figure 5D might be caused by recurrent inhibition of the corticothalamic loops. After the thalamus has received an impact (partly facilitation and partly inhibition) from the cortex, the thalamus sends back an excitation to the TRN with the cortex or directly and the echo in turn inhibits the thalamus. A question arises here when the recurrent inhibition is caused by thalamo-cortico-TRN-thalamic loop or through thalamo-TRN-thalamic loop. A recent study by Golshani and colleagues (2001) showed that the amplitude of excitatory postsynaptic conductances (EPSCs) evoked in TRN neurons by minimal stimulation of corticothalamic fibers were 2.4 times larger than that in the relay neurons. The GluR4-receptor subunits labeled at corticothalamic synapses on TRN neurons outnumbered those on relay cells by 3.7 times (Golshani et al., 2001). Although the question needs further investigation, these results would favor the recurrent inhibition might be caused by the thalamo-cortico-TRN-thalamic loop. The oscillatory corticothalamic loop serves another role to lengthen the corticofugal inhibitory effect from a mean of 210.8 ms to a mean of 1023.0 ms (range: 300-3200 ms).

As mentioned in the Chapter 3, many neurons showed an inhibitory response to an acoustic stimulus in the present study. The auditory IPSP could be generated 1) before reaching the thalamus, 2) in the intrinsic circuit of the thalamus, and 3) from the feedback of the thalamic reticular nucleus. A similar acoustically evoked inhibition has been reported in the cat IC (Kuwada et al., 1997). Their results

indicate that the auditory IPSP recorded in the present study could have partially resulted before reaching the thalamus.

A substantial proportion (20-30%) of the retrogradely labeled IC neurons after an injection in the MGB are GABAergic, and are supposedly inhibitory (Winer et al., 1996; Peruzzi et al., 1997). This inhibitory circuitry could simply change the firing pattern from facilitatory in the IC to inhibitory in the MGB.

Since in the present study I used a simple animal model of guinea pigs, in which GABAergic neurons accounted for only <1% in the MGB (Arcelli et al., 1997; Spreafico et al., 1983; 1994; Winer and Larue, 1996), a strong inhibitory response is unlikely to be generated within the intrinsic circuit of the MGB. Another possible inhibition is from the pre-synaptic dendrites (PSDs) of the interneurons on the thalamic relay neurons (Ralston et al., 1988; Liu et al., 1995a). The PSDs account for only < 6% of the total number of terminals in the cat's thalamus (Liu et al., 1995a). With such a small population of interneurons in the guinea pig's auditory thalamus, the PSDs are unlikely to cause such a strong inhibition on their own.

Although a more thorough investigation is necessary, the inhibitory neuron is likely to be located in the non-lemniscal MGB judging from the frequency tuning characteristics of such neurons (results in Chapter 3). In the present study, I found that the inhibitory neurons received an inhibitory effect from the auditory cortex. The logic can be now linked together: auditory inhibitory neurons are located in the non-lemniscal MGB (results in Chapter 3), neurons in the non-lemniscal MGB

receive corticofugal inhibition (He, 2003a), and auditory-inhibitory neurons therefore receive corticofugal inhibition (results in this chapter).

An interesting result found in the present study was that the inhibitions caused by both the auditory stimulus and the cortical stimulation have a similar waveform. This result implies that the two inhibitions were possibly from the same source, the TRN. In other words, some of the auditory inhibitions observed in the present study might be caused by the recurrent inhibition of the TRN.

The TRN receives inputs from both the thalamus and the cortex (Jones, 1975), and has a very strong inhibitory effect on the thalamic relay neurons (Bartlett et al., 2000; Golshani et al., 2001). An auditory stimulus evoked a spike or an EPSP on some neurons before a prolonged IPSP (left panel of Figure 11), but cortical stimulation caused a prolonged IPSP (right panel of Figure 11). This result further supports our speculation that the TRN is the source of the inhibition.

4.4.3 Functional remarks

Together with previous knowledge from extracellular recording, the corticofugal excitation amplifies the matched ascending auditory information via the lemniscal MGB to the cortex with a varied time period ranging from tens of millisecond to a few hundred of millisecond. The corticofugal projection also switches off some thalamic neurons, presumably the non-lemniscal MGB, with a long-lasting inhibition of a few hundred of milliseconds to seconds. Since the non-lemniscal MGB is involved in multi-sensory integration and in amygdala and basal

ganglia interaction, switching off the non-lemniscal MGB might functionally prepare the auditory cortex for only auditory information processing. A strong and long-lasting inhibition means an effective switching with a limited corticofugal effect. The long inhibition is probably caused by the recurrent inhibition of either the thalamo-cortico-reticulo-thalamic or the thalamo-reticulo-thamaic loop.

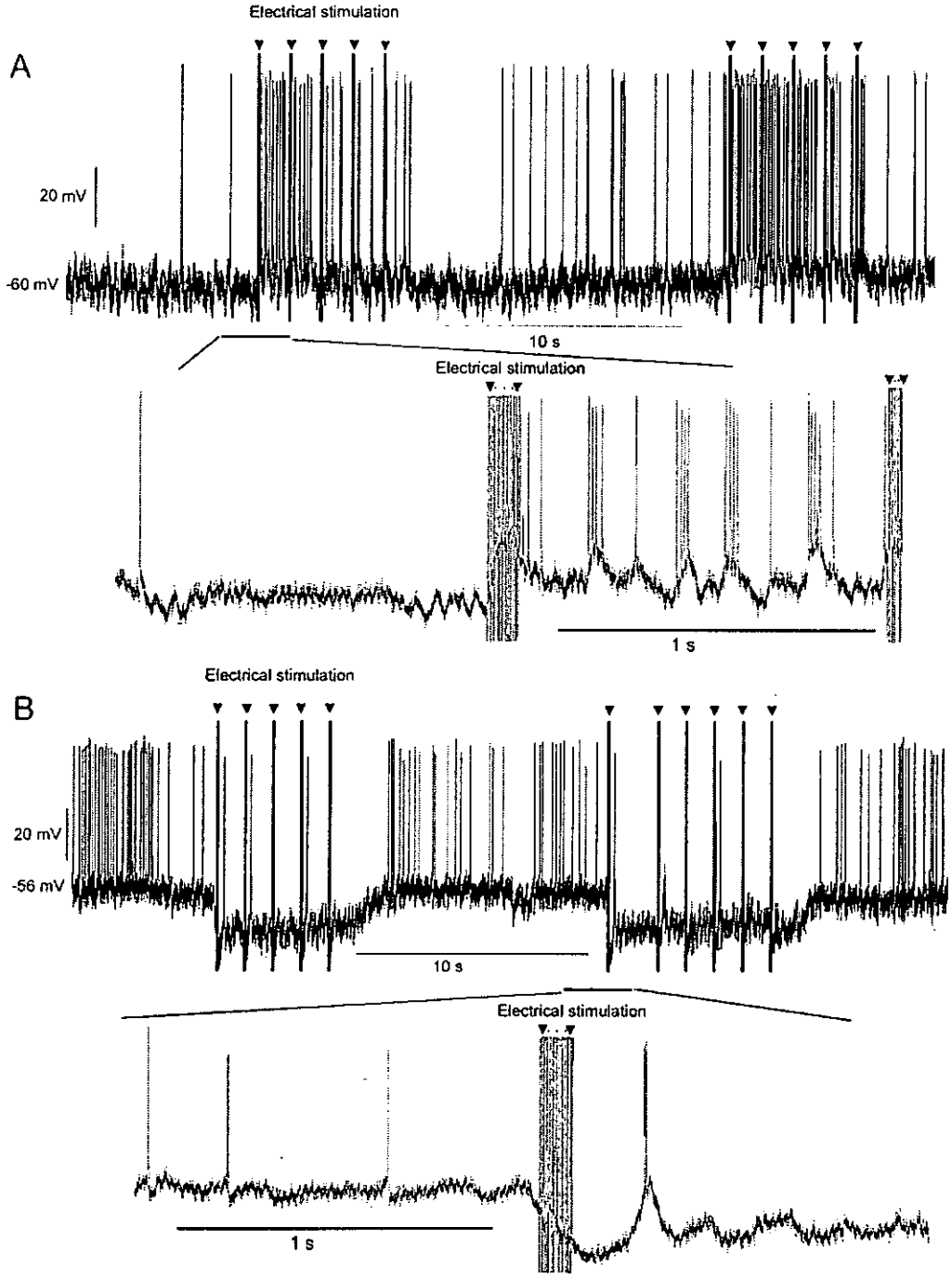

Figure 1 Electrical stimulations of the cortex modulate the spontaneous firings of two MGB neurons. A. Cortical stimulation depolarized the membrane potential and increased the spontaneous firing rate of the MGB neuron. B. Cortical activation hyperpolarized the membrane potential and decreased the spontaneous firing rate of another MGB neuron. Arrowhead indicates the artifact of a pulse train of the electrical stimulation, which consisted of 20 0.1-ms-width and bi-phase pulses at 200 Hz. The stimulation current was 100 μA. Resting membrane potentials are indicated on the left to the intracellular recording traces.

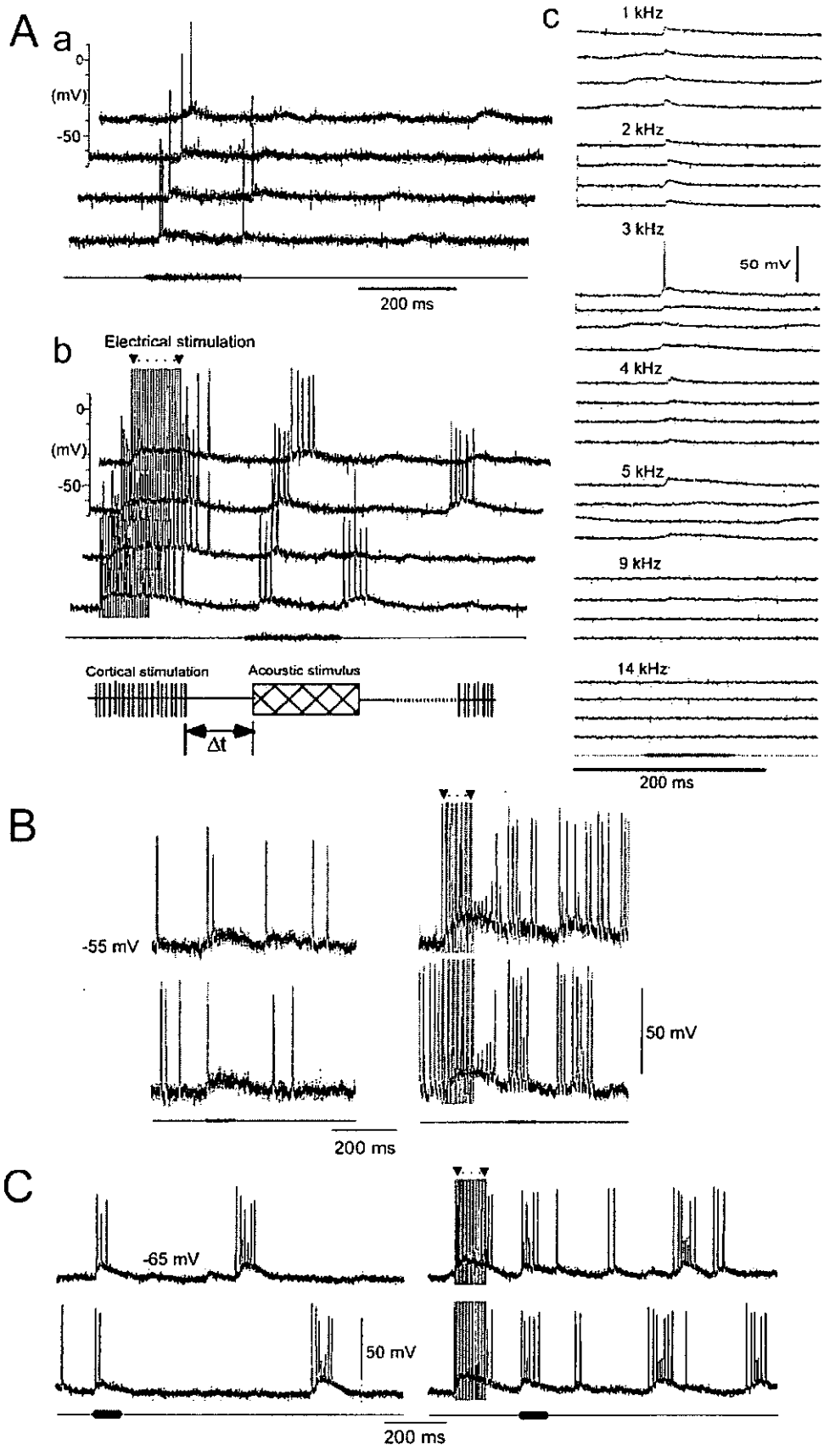
A
a
0
(mV)
-50
200 ms
b
Electrical stimulation
0
(mV)
-50
Cortical stimulation
Acoustic stimulus
Δt
c
1 kHz
2 kHz
3 kHz
50 mV
4 kHz
5 kHz
9 kHz
14 kHz
200 ms
B
-55 mV
50 mV
200 ms
C
-65 mV
50 mV
200 ms

Figure 2 Cortical activation facilitates the auditory response of three MGB neurons (A, B, and C). (A-a). The neuron responded to acoustic stimulus of noise burst with an ON response of either 1 or 2 spikes followed by a rebound. Four repeats of the responses are overlapped with each other. The resting membrane potential was -64 mV. Scale bars of the membrane potential and time are shown on the left of the second trace and below the traces - they apply to all traces. (b). The neurons responded to the same acoustic stimulus of that in (a) with more spikes, after the cortical activation. Cortical stimulation consisted of 20 0.1-ms-width and bi-phase pulses in 200 Hz. The experimental paradigm is shown below the traces. The time scale bar in (a) applies to (b). The resting membrane potential was -66mV, similar to that in (a). (c). Neuronal responses to pure-tone stimuli of varied frequencies. Scale bars of both the membrane potential and time apply to all traces. The timing of the auditory stimulus shown below the traces, applies to all traces. The conventions here apply to the following parts of the figure B, C. The left and the right panels show auditory responses without and with cortical stimulation,. Arrowhead indicates the artifact of a pulse train of the electrical stimulation, which consisted of 20 0.1-ms-width and bi-phase pulses at 200 Hz (A, C) or 10 0.1-ms-width and bi-phase pulses at 100 Hz (A). The neuron in (B) had the resting membrane potential of -55 mV and that in (C) of -65 mV. The vertical and horizontal scale bars are 50 mV and 200 ms respectively (both B and C).

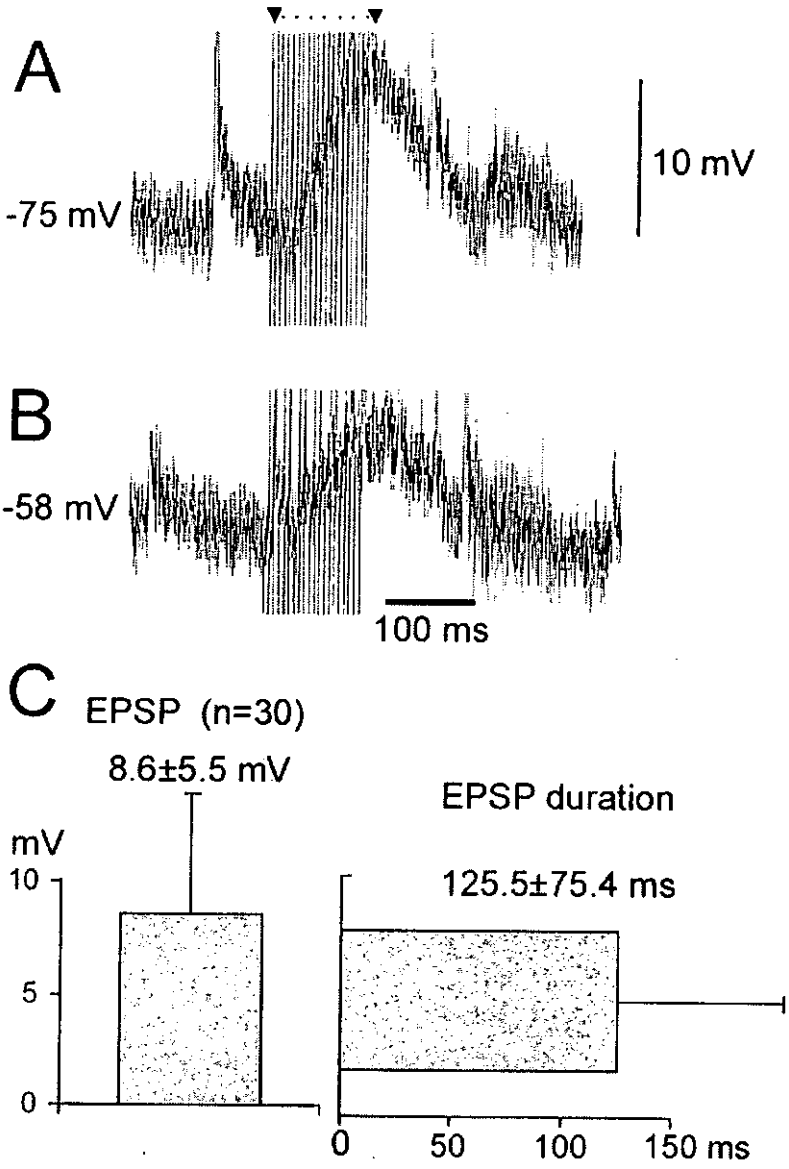

Figure 3 The cortical stimulation results in a membrane potentiation and averageEPSP on the thalamic neurons. The neurons in (A) and (B) had a resting membrane potential of -75 mV and -58 mV respectively. The scale bar of the membrane potential applies to both neurons. Arrowhead indicates the artifact of a pulse train of the electrical stimulation, which consisted of 20 0.1-ms-width. The statistics of the average EPSPs of 30 neurons is shown in (C). The left vertical bar graph shows the mean amplitude and standard deviation of the compound EPSP of the neurons. The right horizontal bar shows the mean and deviation of the lasting duration of the EPSP of the neurons.

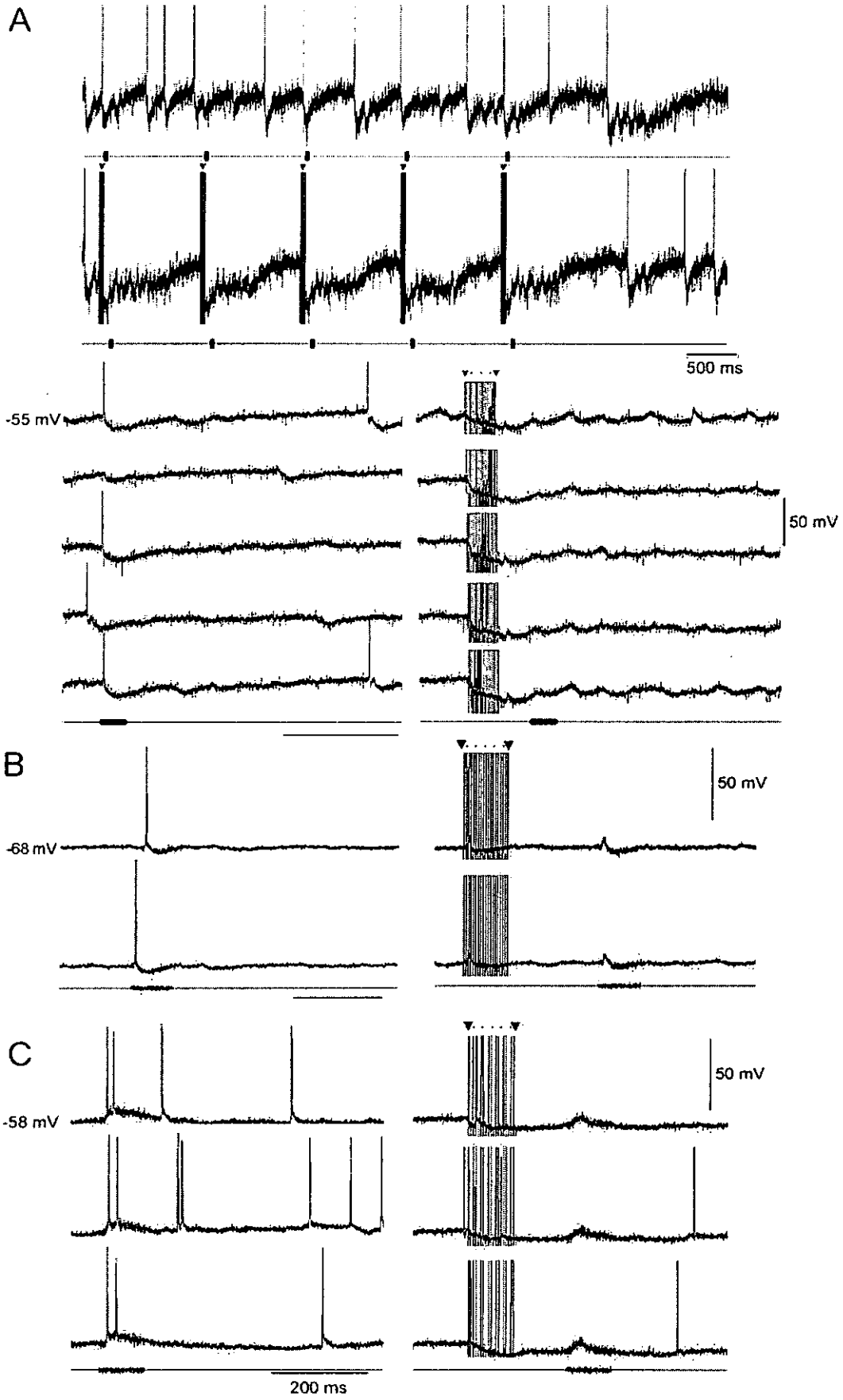
A
500 ms
-55 mV
50 mV
B
50 mV
-68 mV
C
50 mV
-58 mV
200 ms

Figure 4 Cortical stimulation inhibits the neuronal responses to auditory stimulus of three MGB neurons. A. The upper part of the figure shows the time period of the neuronal responses to auditory stimuli and to those that were each preceded with an electrical stimulation. The electrical stimulation consisted of a pulse train of 20 pulses - the artifact of each is indicated with an arrowhead. The auditory stimulus signal is shown below the neuronal response signal. The lower part of the figure shows the expanded traces for the recording. The left panel shows the neuronal responses to five repeats of a pure-tone stimulus and the right panel shows the neuronal responses to the same stimuli, which were preceded by an electrical stimulation of the cortex. B, C. Responses to a repeated noise-burst stimulus of two MGB neurons at a resting membrane potential of -68 mV (B) and -58 mV (C), without cortical stimulation (left panel) and with cortical stimulation (right panel). Scale bars for time in the lower part of A, B, and C were 200 ms.

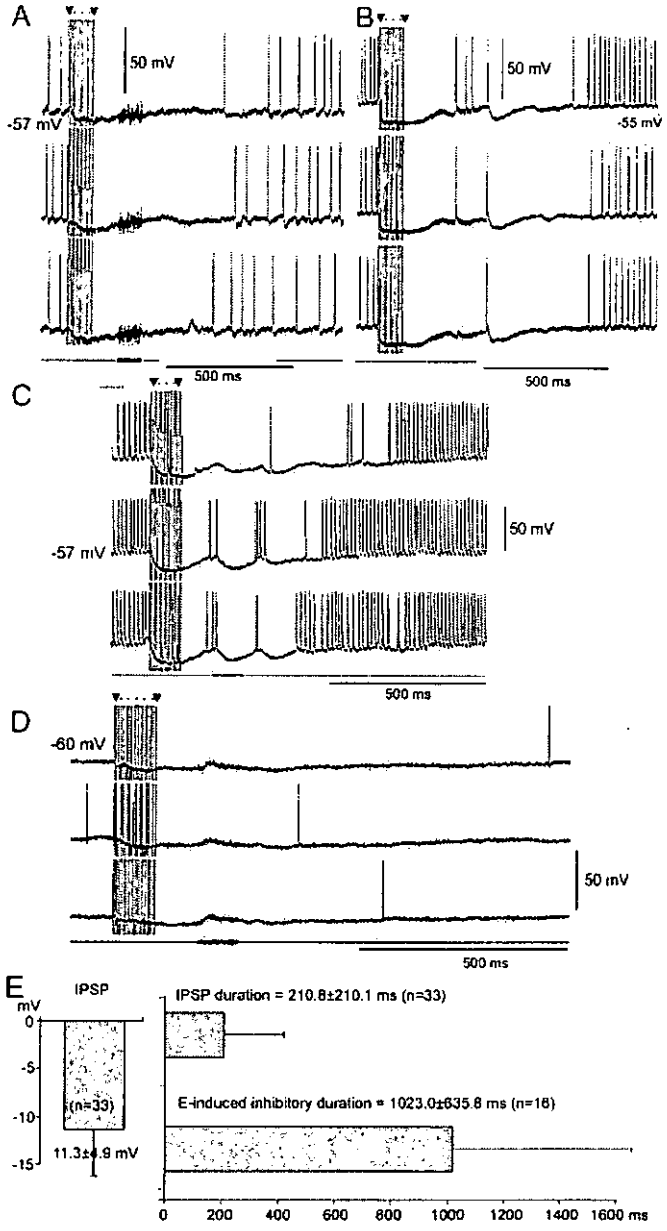

Figure 5 Corticofugal hyperpolarization on the thalamic neurons has a long duration. Three trials of neuronal responses to electrical and auditory stimuli are shown for each of the neurons with resting membrane potentials of -57 mV (A), -55 mV (B), -55 mV (C), and -57 mV (D) respectively. A standard pulse train consisting of 20 pulses at 200 Hz was applied to the cortex (the artifact of each is indicated with an arrowhead) and thesummated hyperpolarization, i.e., compound IPSP was calculated on 33 neurons. The mean and standard deviation of the compound IPSP amplitudes are shown in the left vertical bar graph of (E). The mean and standard deviation of the first IPSP duration are shown in the upper bar and those of the duration of the total inhibitory effect caused by the electrical stimulation of the cortex are shown in the lower bar of the right section of (E).

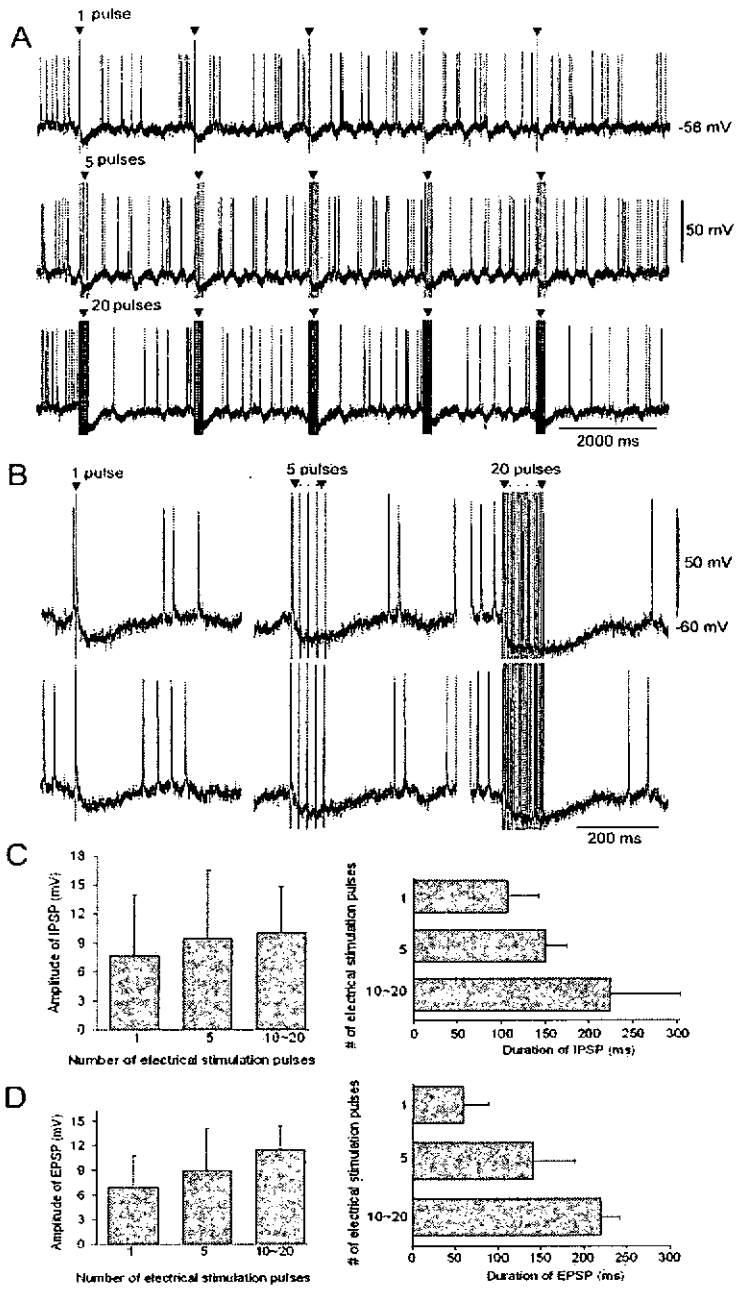

Figure 6 Cortical stimulations of varied pulse number result in an compound IPSP and EPSP. Electrical stimulations consisted of 1, 5, and 20 pulses at varied frequencies. The artifact of each pulse train is indicated with arrowhead. Electrical stimulations were repeated for five times on each trace in (A). The resting membrane potential of neuron was -58 mV. Electrical stimulations were repeated two times for the neuron in (B), which had a resting membrane potential of –60 mV. C. The bar graphs show the means of the amplitudes (left panel, n=7) and durations (right panel) of the IPSP caused by cortical stimulation of varied numbers of pulses. D. The bar graphs show the means of the amplitudes (left panel, n=5) and durations (right panel) of the EPSP caused by cortical stimulation of varied numbers of pulses. The durations here, also included the time period of the pulse-train and the accumulative effects by all pulses.

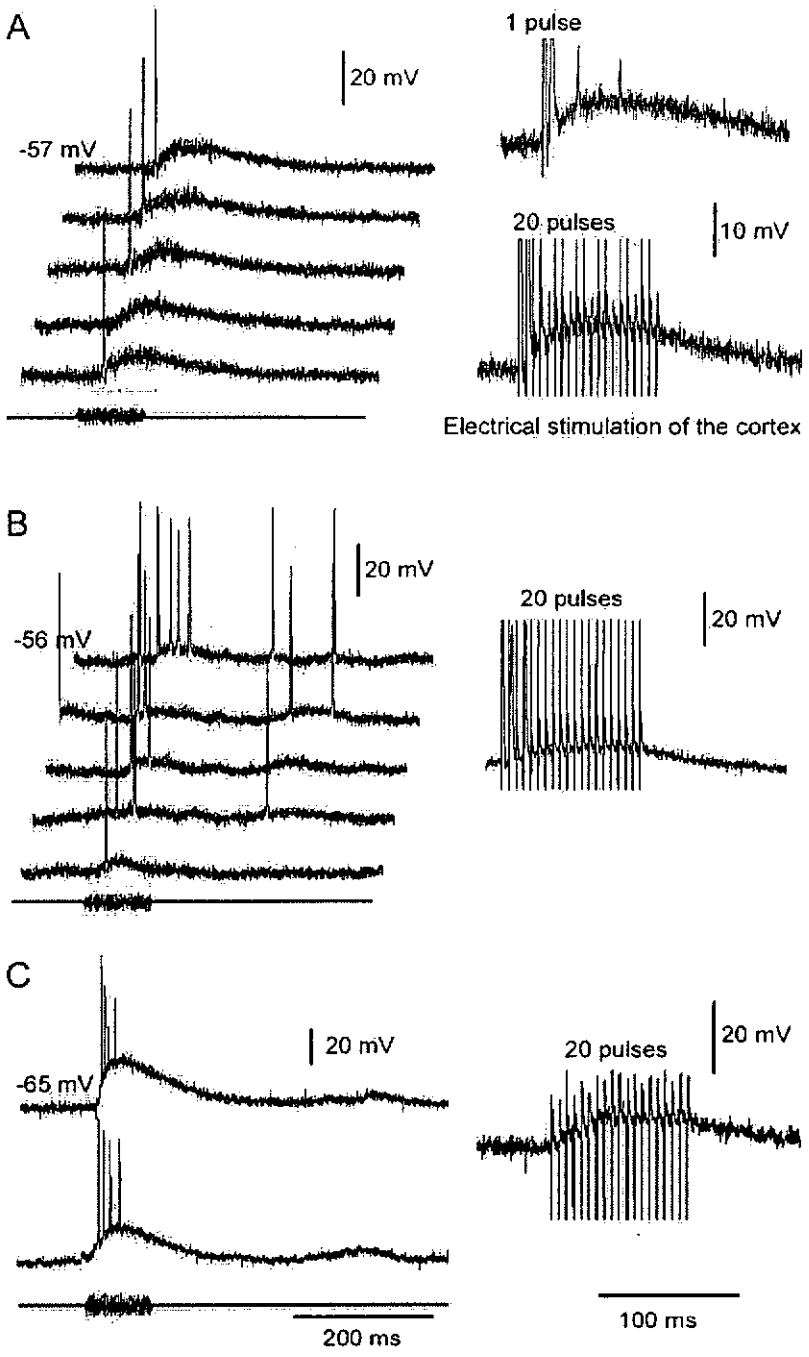

Figure 7 Cortical stimulation potentiates the membrane potential of three MGB neurons with auditory EPSP responses. The left and the right panels show auditory responses without and with cortical stimulation, respectively. The neuron in (A) had a resting membrane potential of -55 mV; that in (B), -65 mV; and that in (C), -65 mV. The neuron in (A) was examined with 1 electrical pulse and 20 electrical pulses in the auditory cortex. The stimulation current was 100 μA.

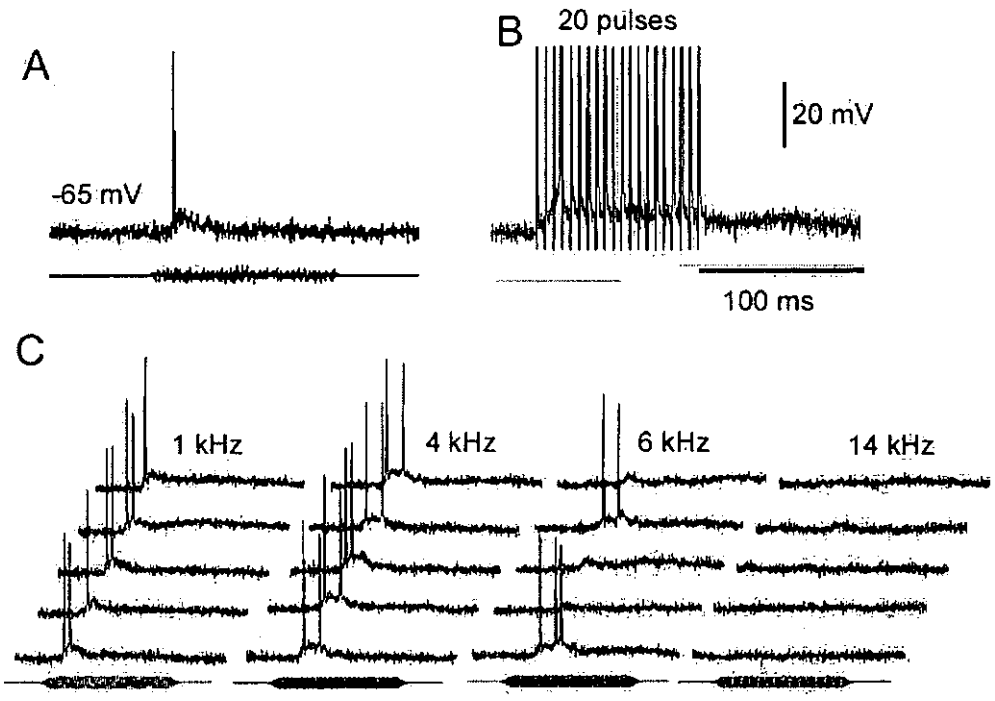

Figure 8 An example of a low-frequency tuned, corticofugally-potentiated neuron. A. The MGB neuron responded to acoustic stimuli with a spike and an EPSP. B. The membrane potential was depolarized by cortical stimulation. Scale bars apply to both (A) and (B). C. Five trials of responses to pure-tone stimuli at 60 dB SPL are shown at each of the frequencies: 1 kHz, 4 kHz, 6 kHz, and 14 kHz.

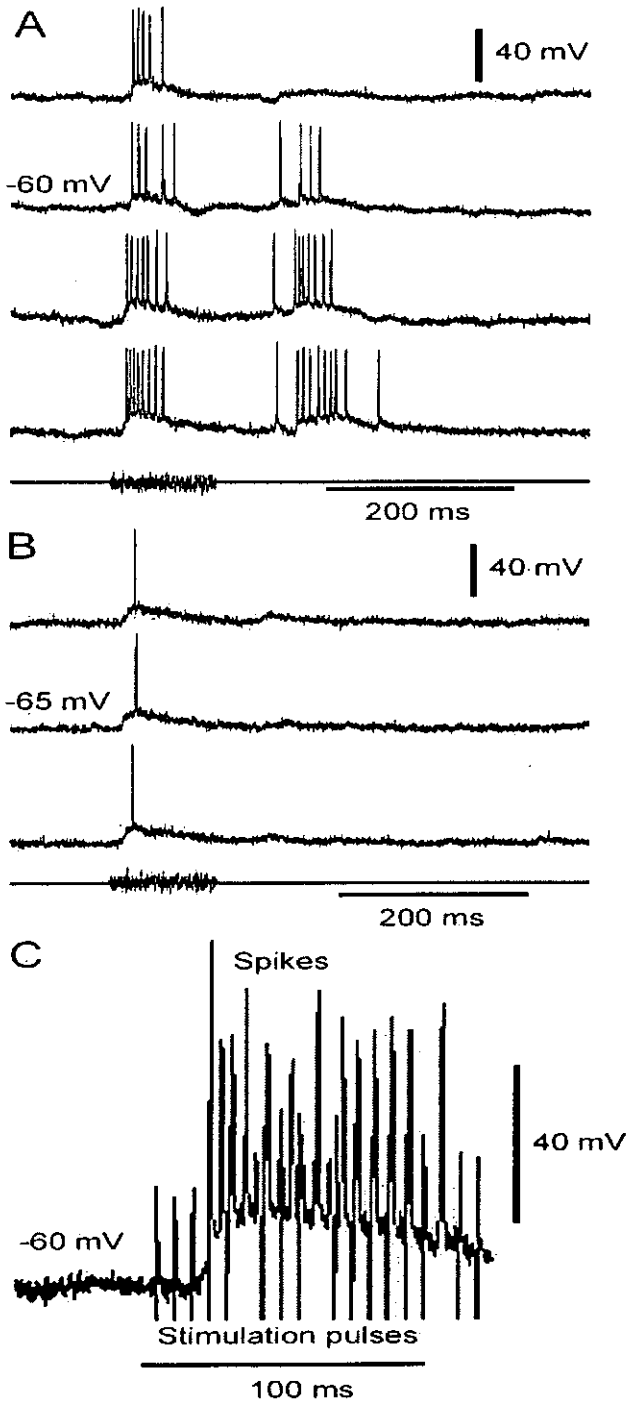

Figure 9 Responses of an MGB neuron to auditory stimuli with varied membrane potential under cortical stimulation. A. Responses torepeated noise-burst stimuli, when the resting membrane potential was -60 mV. B. Responses to the same stimulus, when the membrane potential was hyperpolarized by injecting a negative current through the recording electrode to -65 mV. C. The neuronal response to an electrical stimulation of a pulse-train of the auditory cortex. Scale bars are separately indicated.

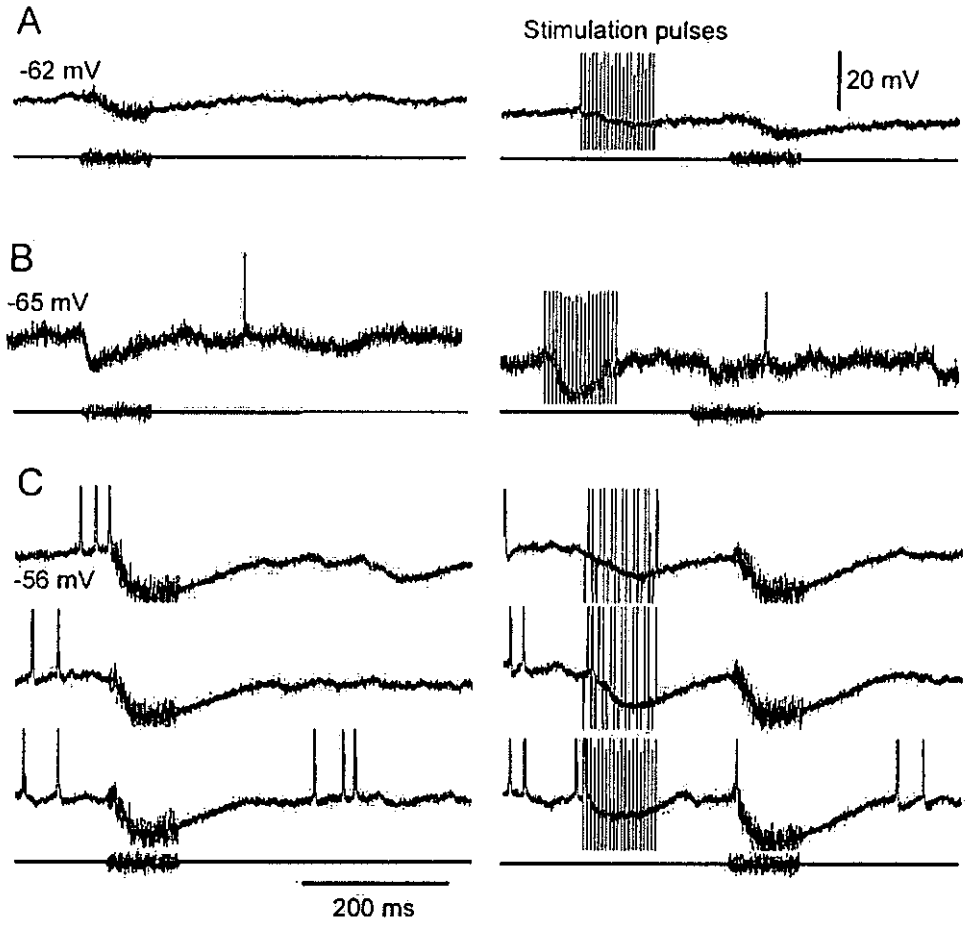

Figure 10 Neuronal responses to acoustic stimulus and cortical stimulation. The left panel shows neuronal responses to a noise-burst stimulus of three MGB neurons (A, B, and C) and the right panel shows the neuronal responses of corresponding neurons to a cortical stimulation followed by a noise-burst stimulus.

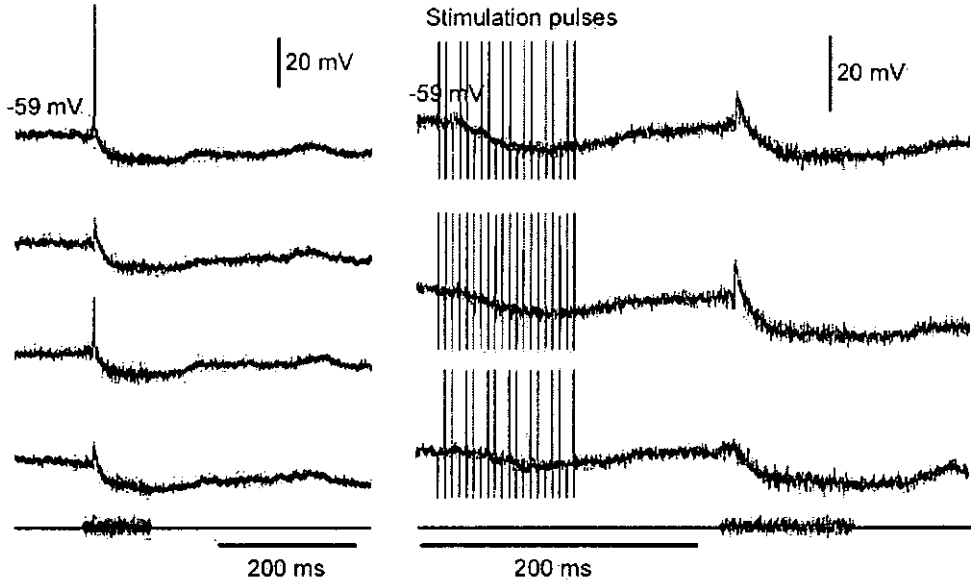

Figure 11 Neuronal responses to an acoustic stimulus and cortical stimulation. The left panel shows the neuronal responses to repeated noise-burst stimulus and the right panel shows the neuronal responses to a cortical stimulation followed by a noise-burst stimulus.

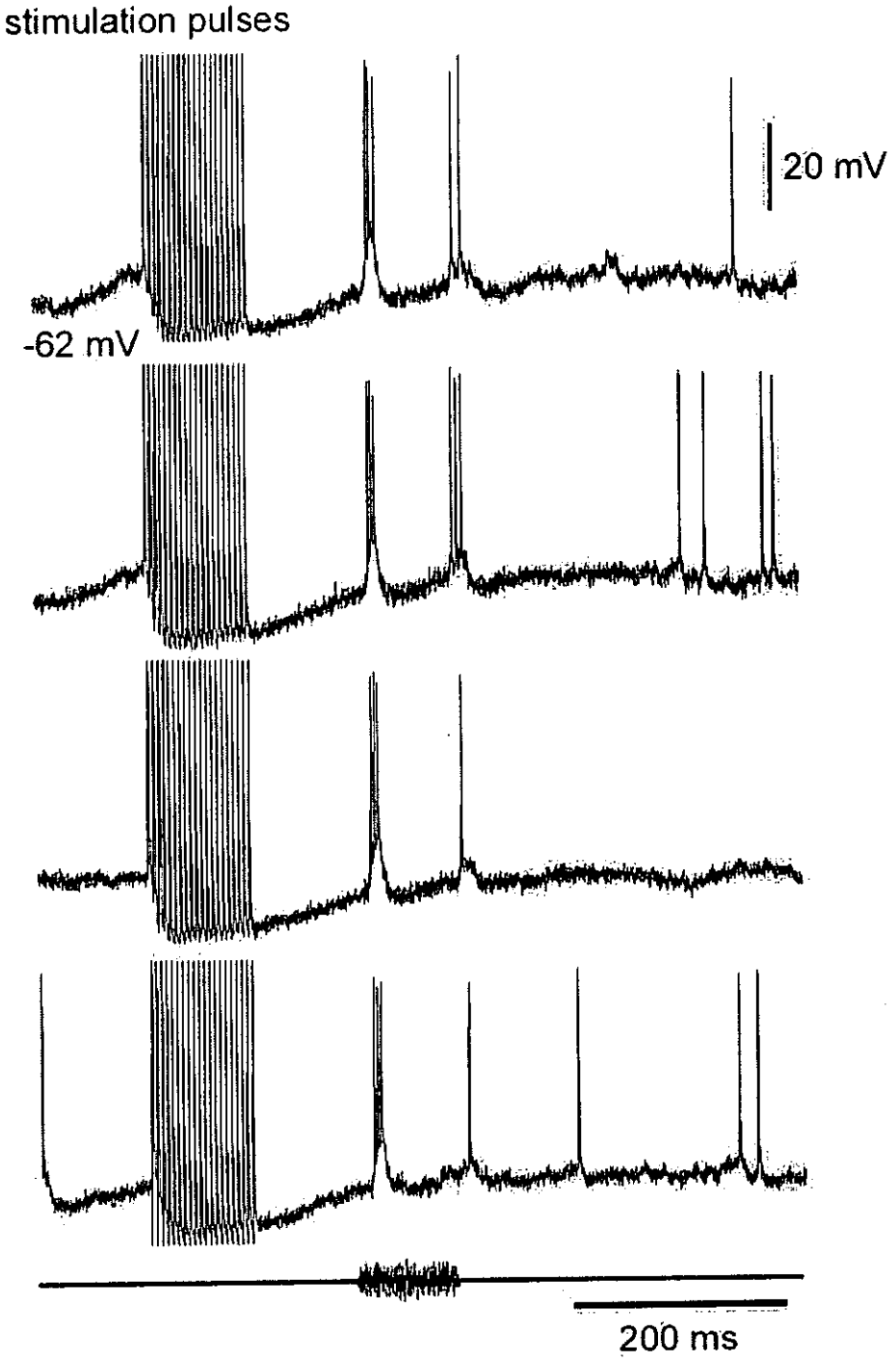

Figure 12 Neuronal responses to electrical stimulation and an auditory stimulus. The resting membrane potential was -62 mV and the scale bars apply to all traces.

Chapter 5

Anatomical confirmation of the recorded thalamic neurons

5.1 Introduction

The auditory thalamus that relays information from the inferior colliculus to the cortex includes the MGB and the lateral part of the posterior nucleus group (Imig and Morel, 1983). Based upon cytoarchitecture and cellular morphology, the MGB has been divided into three subdivisions: (1) the ventral division; (2) the dorsal division (dMGB, dorsal and caudo-dorsal part); and (3) the medial division which contains cells with the largest soma in the MGB (prominent magnocellular neurons, mMGB). Each division projects to various auditory cortical fields. Reciprocally, the MGB receives corticofugal inputs from the auditory cortex. A large number of studies have confirmed these initial findings in many species (cat: Morest and Winer, 1986; tree shrew: Oliver and Hall, 1978; rat: Clerici and Coleman, 1990; Clerici et al., 1990; LeDoux et al., 1987; mustache bat: Winer and Wenstrup, 1994a, b; rabbit: Caballero-Bleda et al., 1991; De Venecia et al., 1995; guinea pig: Strutz, 1987; monkey: Burton and Jones, 1976; Hashikawa et al., 1995; Molinari et al., 1995; human: Winer, 1984;).

The guinea pig MGB has been divided into four subnuclei (Redies and Brandner, 1991). The ventral nucleus of the MGB occupies a lateroventral position in the rostral two-thirds of the MGB and projects to two tonotopic fields (fields A

and DC) of the auditory cortex. The shell nucleus surrounds the vMGB like a continuous shell from dorsal, lateral and ventral. Neurons in the sMGB project to a non-tonotopic field situated ventrocaudal belt and appear similarly cytoarchitectonically to those in the vMGB. Two more MGB subnuclei were identified. One is the cmMGB which consists in Nissl preparations of large and deeply stained cells and projects in a sparce fashion to the entire, or nearly entire, auditory cortex. The other is the rmMGB which lies medial to the vMGB in the rostral half of the MGB and projects to a small tonotopic cortical field (field s), which is not innervated by the vMGB.

In the cat, and the guinea pig, the lemniscal core of the MGB is the tonotopically organized ventral nucleus (Aitkin and Webster, 1971; Clarey et al., 1992). The non-lemniscal MGB consists of the medial and the dorsal nuclei in the cat (In guinea pigs it consists of the cmMGB and rmMGB). The neurons in the non-lemniscal MGB show long latency, bursty firing, broad or no frequency tuning, non-tonotopic organization, and multi-modal responses (de Ribaupierre and Toros, 1976; Calford and Webster, 1981; Calford, 1983; Winer and Morest, 1983; Imig and Morel, 1988; Hu, 1995; He and Hashikawa, 1998; He and Hu, 2002).

Most of our understanding of the MGB has been obtained with extracellular electrophysiological recordings. Intracellular recordings on the thalamic slice have provided insights into the synaptic mechanisms of relay neurons in the MGB (Hu et al., 1994; Hu 1995; Li et al., 1996; Tennigkeit et al., 1996, 1998; Bartlett and Smith, 2002). *In-vivo* intracellular recording in the inferior colliculus has revealed a putative inhibitory mechanism for duration tuning in the bat, and complex

interactions of excitatory and inhibitory input for binaural signal processing in cats (Casseday et al., 1994; Kuwada et al., 1997). A recent *in-vivo* intracellular study by Ojima and Murakami (2002) revealed that there are layer-specific differences in the auditory response characteristics of pyramidal neurons in the auditory cortex. However, little is known about the *in-vivo* intracellular response characteristic of the MGB neurons to natural sound stimuli.

As compared to the ascending thalamocortical projection, the MGB receives a much stronger reciprocal projection from the cortex (Andersen et al., 1980; Winer and Larue, 1987). It has been suggested that this corticofugal projection provides a gating or gain-control mechanism in the transmission of information from the periphery to the cortex (Ryugo and Weinberger, 1976; Crick, 1984; Deschênes and Hu, 1990; Villa et al., 1991; Suga et al., 1997). Different techniques have revealed that the stimulation in auditory cortex exerts facilitatory and/or inhibitory modulation on the thalamic relay neuron, either directly via stimulated neurons or indirectly polysynaptically (Watanabe et al., 1966; Ryugo and Weinberger, 1976; Villa et al., 1991; He, 1997; Suga et al., 1997; He et al., 2002).

Previous extracellular studies have indicated that specific cortical stimulation (stimulation site functionally matched with the recording site in the thalamus and the inferior collicuclus) resulted mainly in facilitatory effects in the cat and bat (Gao and Suga, 1998; He, 1997; He et al., 2002; Zhang et al., 1997; Zhang and Suga, 1997; Zhou and Jen, 2000). In a recent extracellular study, He (2003a) has observed a mostly excitatory effect on the auditory response of the lemniscal MGB neurons and a mostly inhibitory effect on the ON responses of the non-lemniscal

MGB neurons following cortical activation. Recent anatomical study shows that the corticofugal projection to various divisions of the MGB, e.g., the medial, the dorsal, and the ventral divisions are different. The corticothalamic and thalamocortical projections are not reciprocally overlapped and this reciprocal projecting system varies greatly from the ventral division to the medial division of the MGB. The corticofugal projection to the ventral division of the MGB is heavy, but that to the mMGB is light (He and Hashikawa, 1998). It is important to examine the physiological differences of the corticofugal modulatory effect on various divisions of the MGB by anatomical identification of the locations of the recorded neurons after *in vivo* intracellular physiological recording.

In a series studies to examine the corticofugal modulation on the thalamic neurons intracellularly, I could also recorded neuronal responses to acoustic stimulus. The present study aimed to investigate the auditory responses of the MGB neurons and the corticofugal modulation on the relay neurons in the auditory thalamus in relation to the locations of the recorded neurons by anatomical confirmation after physiological study.

5.2 Materials and methods

Nineteen guinea pigs for intracellular recording and anatomical confirmation were prepared as mentioned before. Acoustic stimuli and electrical stimulation were delivered to the animals same as described in Chapter 4.

The electrode recorded up the membrane potential showing a negative value when it penetrated the membrane of a cell. After amplification, the membrane potential with artifact of electrical stimulation as well as the auditory stimulus was stored on the computer with the aid of commercial software (AxoScope, Axon). The first spike latency was determined by calculating the time elapsed between the onset of the acoustic stimulus and the onset of alteration of the membrane potential.

For the 19 subjects, I filled the recording pipette with neurobiotin (Neurobiotin™, Vector, 1-2% in 1M KCl) and injected the tracer into 1-4 neurons in each subject after physiological recording. Neurobiotin was delivered into the neuron by passing rectangular depolarizing current pulses (150 ms, 3.3 Hz, 2 nA) for 1-4 mins. After 1-5 hours, the subjects were deeply anesthetized with sodium pentobarbital and perfused transcardially with 0.9% saline followed by a mixture of 4% paraformaldehyde in a 0.1M phosphate buffer (pH 7.3). The brains were removed and post-fixed in 4% papraformaldehyde overnight and moved to 0.1M phosphate buffer containing 30% sucrose. The thalami were cut transversally using a freezing microtome at a thickness of 90 μm. Sections were collected in 0.01 M potassium phosphate-buffered saline (KPBS, pH 7.4) and then incubated in 0.1% peroxidase-conjugated avidin-D (Vector) in KPBS with 0.5% Triton X-100 for 4-6 hr at room temperature. After detection of peroxidase activity with 3', 3'-diaminobenzidine (DAB), section were examined under microscope and photographed. Those sections containing labeled neurons were mounted on gelatin-coated slides and counterstained with neutral red (1%, Sigma).

5.3 Results

5.3.1 Different latencies of the auditory responses of the neurons in the lemniscal and non-lemniscal MGB

Of twenty neurons stained by Neurobotin, nine neurons were confirmed as being located in the ventral MGB and eleven neurons were located in the non-lemniscal MGB.

The first spike latencies of the auditory responses of the neurons in the lemniscal MGB were 13.11 ± 7.27 ms (n=9, range: 5~23 ms) at their resting membrane potential and those of the neurons in the non-lemniscal MGB were 25.43 $\pm$ 12.37 ms (n=7, range: 10~50 ms). The neurons in the non-lemniscal MGB responded to a same acoustic stimulus with significantly longer latencies than the neurons in the lemniscal MGB ($P<0.05$, t-test).

5.3.2 Excitatory auditory responses in the lemniscal MGB

All of the nine neurons in the vMGB showed excitatory responses to acoustic stimuli. Among them, three neurons showed burst-like responses to auditory stimuli. Figure 2 showed two examples. The neuron in Figure 2A showed a burst-like response to a noise-burst stimulus and a burst spontaneous firing pattern. The

neurons in Figure 2B responded with a burst-like response to the noise-burst stimulus.

Six neurons responded to the acoustic stimulus with an EPSP, as seen in the examples shown in the left panel of Figure 3. Among them, three neurons showed a spike or a few onset spikes with the EPSP (Figure 3A, B) and the rest showed only EPSPs (example in Figure 3C).

Figure 4 shows a multi-polar neuron located in the ventral division of the MGB. This neuron responded with 1-2 onset spikes with the EPSP to a noise-burst stimulus. This neuron showed a membrane oscillation followed the noise burst stimuli of different durations, as seen in Figure 4A. There are prolonged after-hyperpolarization potentials following the EPSP/spikes. The AHP lasted for about 300 ms and was followed in some cases by a second (second trace of Figure 4A) and a third (first, fourth and sixth traces of Figure 4A), even a fourth AHP (third and fifth traces of Figure 4A). Converting to frequency, the membrane oscillation was about 4-5 Hz. This neuron was an auditory excitatory neuron showed a narrow tuning to a low frequency of 0.9 kHz with a low threshold of 20dB. The membrane potential of this neuron was depolarized by the cortical stimulation of a 5-pulse train which resulted in a greater EPSP and spikes to the same noise-burst stimulus (Figure 4B).

5.3.3 Auditory responses in the non-lemniscal MGB

Of the eleven neurons located in the non-lemniscal MGB, eight responded to the acoustic stimuli with an IPSP, two did not respond to the acoustic stimuli, and one showed a tonic response to a noise-burst stimulus.

Of the eight neurons showed an IPSP to auditory stimulus (for short, I call auditory inhibitory neurons hereafter), five showed an onset spike before the IPSP. Three of the eight auditory inhibitory neurons showed only an IPSP to a noise-burst stimulus (examples as in Figure 5A, B). It is interesting to note that the neuron in Figure 5B showed a large IPSP followed by 1-2 small IPSPs to the noise-burst stimulus. This neuron showed a broad tuning to a low frequency with a high threshold of 40 dB. Although the three neurons showed different shapes in their IPSPs, the IPSPs normally lasted longer than 100 ms.

One neuron showed an onset response, which was followed by a tonic response as the stimulus persisted. There was a small gap of about 50 ms between the onset and the tonic responses of this neuron. The tonic response was sustained over the duration of the stimulus. The neuron showed a broad tuning to a low frequency of about 1 kHz with a high threshold of 40 dB. This neuron was anatomically located in the cmMGB.

5.3.4 Corticofugal facilitation and inhibition

Of 9 neurons confirmed as being located in the vMGB, eight received corticofugal depolarization on their membrane potentials and one received no corticofugal effect. Figure 3 shows the physiologies of corticofugal modulation, the

morphology of neurobiotin labeling, and the anatomical locations in the thalamus with neutral red counterstaining of three representative neurons.

The neuron in Figure 3A responded to a noise-burst stimulus with two spikes and a sustained EPSP and was depolarized by the cortical stimulation of a 5-pulse train. The multipolar neuron was located in the vMGB.

The neuron in Figure 3B responded to a noise burst stimulus with a spike and a short-lasted EPSP. The cortical stimulation caused a small EPSP and resulted in a doublet in its response to the same noise-burst stimulus. The neuron was identified as being in the vMGB.

The third neuron in Figure 3C responded with an EPSP to the noise-burst stimulus and was depolarized by the cortical stimulation of a 10-pulse train which resulted in a greater EPSP to the same noise-burst stimulus. The neuron was located in the vMGB.

All of the 11 neurons located in the non-lemniscal MGB received corticofugal hyperpolarization on their membrane potentials. The two representative neurons shown in Figure 5 were located in the cmMGB. Figure 5 shows their physiology of corticofugal modulation, their morphology of neurobiotin labeling, and their anatomical locations in the thalamus with neutral red counterstaining.

The neuron in Figure 5A showed a small inhibitory response to the noise-burst stimulus and was inhibited by the cortical stimulation of a 5-pulse train. This neuron was located in the cmMGB. This neuron received an inhibitory effect when the auditory cortex was electrically activated. The inhibition lasted for a long-time period up to 1000 ms (Figure 6). The neuron in Figure 6 (same as the neuron in

Figure 5A) showed a resting potential of -57 mV and a high frequency spontaneous activity before and after electrical stimulation of the auditory cortex. It received ansummated hyperpolarization, (or compound IPSP) of about –8 mV.

The neuron in Figure 5B showed an IPSP to the noise-burst stimulus. The neuron received a hyperpolarization from the cortical stimulation of a 5-pulse train. The neuron was again located in the cmMGB.

The neuron in Figure 7 showed an IPSP to a noise-burst stimulus with a resting membrane potential of -62 mV. It received a strong corticofugal hyperpolarization of > 8 mV, responded with rebounded LTS bursts to auditory stimuli (Figure 7). The neuron showed normal spontaneous spikes at 300-400 ms after the cortical stimulation. This neuron was located in the cmMGB.

5.4 Discussion

5.4.1 Auditory responses of the MGB neurons in various subdivisions

All of the 9 anatomically confirmed the vMGB neurons were auditory-excitatory neurons. This kind of neuron usually showed good frequency-tuning characteristics and a short responsive latency. As mentioned in Chapter 3, neurons with a simple EPSP response to a noise-burst stimulus were more likely to be tuned to a characteristic frequency with a short latency and low response threshold. The present study confirmed their locations in the lemniscal MGB.

Eight out of 11 neurons in the non-lemniscal MGB responded to an acoustic stimulus with an IPSP or a spike and IPSP. The IPSP pattern was also observed in the inferior colliculus (Kuwada et al., 1997), but not in the auditory cortex (Ojima and Murakami, 2002).

The present study found that those neurons responding with an IPSP to sound stimuli, usually showed a higher response threshold and a broad-tuning property, which was more likely to be in the non-lemniscal MGB. The present study confirms that all of the auditory inhibitory neurons are located in the non-lemniscal MGB.

Chapter 3 showed that neurons with a tonic response to a noise-burst stimulus showed multiple peaks in the tuning curve, a high response threshold, a long latency, and double peaks in the latency-frequency function, all of which are characteristics indicating a possible location for the caudomedial nucleus of the MGB (equivalent to the medial division of the cat). The present study found that one neuron with a tonic auditory response was located in the cmMGB supporting the above hypothesis.

Redies and Brandner (1991) reported that the neurons in the ventral nucleus of the MGB respond vigorously to both pure tone and noise-burst stimuli. They show mostly narrow frequency tuning curves and short response latencies. The responses to pure tones of the neurons located dorsally, laterally and ventrally to the vMGB were often less vigorous than those in the vMGB. Many neurons had broad frequency tuning curves, and longer response latencies of greater than 12 ms. The present result that the response latencies of the neurons in the lemniscal MGB were

about half of those of the neurons in the non-lemniscal MGB confirmed the previous extracellular recording (He, 2002).

A recent intracellular study on thalamic slice neurons showed no differences in the intrinsic membrane features and the synaptic responses between the neurons in the lemniscal and non-lemniscal MGB (Bartlett and Smith, 1999). Different firing patterns observed in the present study were supposedly produced before reaching the MGB or partially within the network among the inferior colliculus, the thalamus, and the cortex.

The neuron in Figure 4 was identified as being located in the border of the vMGB and cmMGB. It responded to acoustic stimuli of different durations with different spike numbers. The neuron showed a membrane oscillation of 4-5 Hz. Stimulus-evoked oscillations of 5-15 Hz have been shown in the auditory thalamus with extracellular recording (Galambos et al., 1952; Aitkin et al., 1966; Cotillon and Edeline, 2000; Cotillon et al., 2000; Cotillon-Williams and Edeline, 2003). The membrane oscillation shown in the present study matches the oscillations of the previous extracellular recordings. OFF and ON-OFF neurons are located only in the non-lemniscal MGB, or in the border region between the lemniscal and non-lemniscal MGB (He, 2001). All OFF neurons in the present study showed membrane oscillations of either a stimulus-evoked or a spontaneous oscillation. Not only OFF neurons, ON neurons in the border region between the lemniscal and non-lemniscal MGB also exhibited acoustic-triggered oscillation.

The results in this chapter and Chapter 3, in which LTS spikes appeared mostly when the steady-state membrane potential was below -70 mV, agree with

previous reports (Steraide, 2001a). Normal firing at depolarized levels, at which the calcium current is inactivated, but burst firing at hyperpolarized levels, at which the calcium current is activated, have also been previously described in other thalamic regions in *in-vivo* animals (Deschênes et al., 1984; Steriade and Deschênes, 1984; Mulle et al., 1985; Pare et al., 1987). The neuron shown in Figure 7 added evidence from the auditory thalamus.

5.4.2 Locations of the MGB neurons of corticofugal facilitation and inhibition

5.4.2.1 Corticofugal facilitatory effect

Most auditory-excitatory neurons receive an excitatory input from the cortex. In this study, all of the 10 neurons in the vMGB were depolarized when the auditory cortex was electrically stimulated.

Morphologically, it is known that about half of the synapses on the thalamic relay neuron are RS terminals (small profiles with rounded vesicles, defined by Guillery, 1969; and Ralston et al., 1988; Jones and Powell, 1969a, b; Liu et al., 1995a, b). The majority of the RS terminals appear to derive from corticothalamic fibers (Jones and Powell, 1969a). This dense synaptic input into the thalamic relay neurons is clearly excitatory activating r-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl-D-aspartate (NMDA), and metabotropic glutamate receptors (mGluR) (Bartlett and Smith, 1999; Deschênes and Hu, 1990; McCormick and von Krosigk, 1992; Tennigkeit et al., 1999). The

mGluR is coupled to G proteins, and has its action through the inositol trisphosphate second messenger pathway and lasts for time period up to a few hundred of milliseconds (McCormick and von Krosigk, 1992; Tennigkeit et al., 1999). The corticothalamic terminals have their main contact on the distal dendrites, thereby delivering an accumulative effect, which again suggests having a slow effect on the thalamic neurons and maintaining the corticofugal modulation for a long period (Bartlett et al., 2000; Liu et al., 1995a).

Previous extracellular studies have indicated that specific cortical stimulation (the stimulation site functionally matched with the recording site in the thalamus and the inferior collicuclus) resulted mainly in facilitatory effects in the cat and bat (Gao and Suga, 1998; He, 1997; He et al., 2002; Zhang et al., 1997; Zhang and Suga, 1997). Of 20 corticofugal modulatory neurons obtained by Watanabe et al. (1966), 6 neurons had facilitatory effects, with a maximum increase of 57% in the number of spikes. A similar phenomenon was observed in the cat vMGB, where the interneurons accounted for one-fourth of the total population (He, 1997; Villa et al., 1991). Compared to the cat, the corticofugal modulation on the guinea pig's thalamus has a much broader effective area, supposedly resulting from the fewer interneurons in the guinea pig's thalamus (He et al., 2002). The present intracellular recording results demonstrate how the corticofugal facilitation was applied to the thalamic neurons and the subsequent changes of the auditory responses.

The present result that all auditory-excitatory neurons in the lemniscal auditory thalamus received prolonged excitatory inputs from the auditory cortex again confirmed the finding previous extracellular studies and the speculation in

Chapter 4 that specific cortical stimulation (the stimulation site functionally matched with the recording site in the thalamus and the inferior collicuclus) resulted mainly in facilitatory effects (Gao and Suga, 1998; He, 1997; He et al., 2002; Zhang et al., 1997; Zhang and Suga, 1997).

5.4.2.2 Corticofugal inhibitory effect

In a recent extracellular study, He (2003a) has observed a mostly inhibitory effect on the ON responses of the non-lemniscal MGB neurons following cortical activation. In some cases, the ON response was switched off by the cortical stimulation (Figures 1 and 2 of He, 2003a). The corticofugal inhibition was widespread (Figures 5 and 6 of He, 2003a). He also showed that the corticofugal inhibition lasted for over 100 ms (Figure 4 of He, 2003a). A slice intracellular study showed that the neurons in the dorsal division of the non-lemniscal MGB responded to thalamic radiation stimulation with a long duration IPSP for over 200 ms, suggesting that the stimulation activated the TRN fibers which inhibit the MGB neurons through $GABA_B$ receptors (Figure 8 of Bartlett and Smith, 1999).

The TRN receives inputs from both the thalamus and the cortex (Jones, 1975), and has a very strong inhibitory effect on the thalamic relay neurons (Bartlett et al., 2000; Golshani et al., 2001). The majority of the excitatory inputs to the TRN neurons are derived from the cerebral cortex (Liu and Jones, 1999), indicating that the corticofugal fibers to the TRN neurons control the TRN neurons' excitability (Golshani et al., 2001; Steriade, 2001b). The TRN neurons extend dendrites within

the thin reticular sheet, which enable them to receive projections from a wide cortical region, and project to widespread areas in the ventroposterior nucleus of the thalamus (Liu et al., 1995b). The TRN neurons project diversely to the thalamus (Bourassa and Deschênes, 1995; Jones, 1975; Shosaku and Sumitomo, 1983; Simm et al., 1990; Pinault et al., 1997). Recent physiological results indicate that the TRN terminals have a very strong inhibitory effect on the thalamic relay neurons (Bartlett et al., 2000; Golshani et al., 2001). It is known that the $GABA_B$ receptor has a time constant of several hundred of milliseconds (Bartlett and Smith, 1999; Kim et al., 1997; Tennigkeit et al., 1998; Ulrich and Huguenard, 1996).

In summary, activation of the cortex directly generates excitatory input to the lemniscal MGB neurons. This also activates the TRN neurons through corticothalamic fibers, which in turn inhibit the non-lemniscal MGB neurons in a time period of over a few hundred of milliseconds. In conclusion, auditory inhibitory neurons are located in the non-lemniscal MGB, and receive corticofugal inhibition.

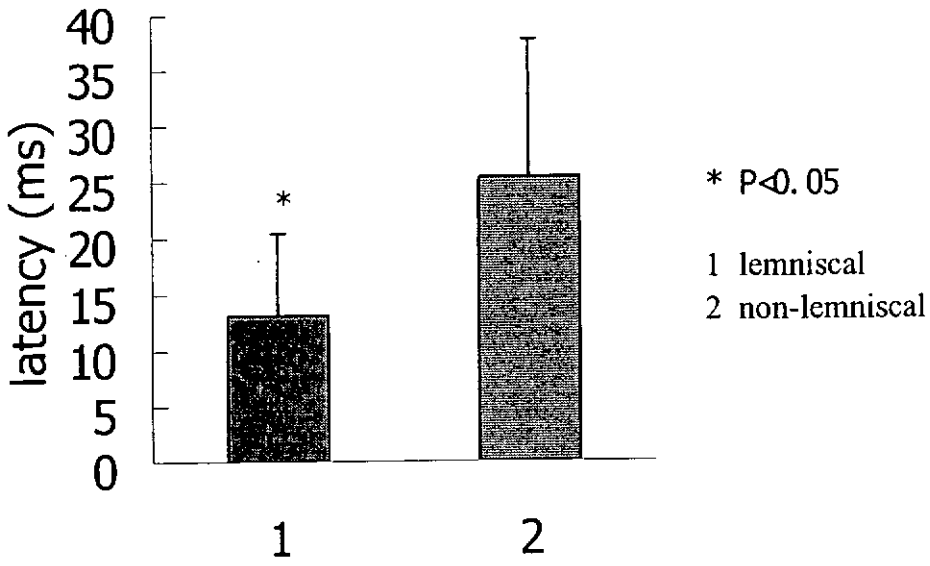

Figure 1 The mean fisrt spike latencies of the auditory responses for two groups of neurons: the neurons in the lemniscal MGB and non-lemniscal MGB. Half of a standard deviations are shown on the bars.

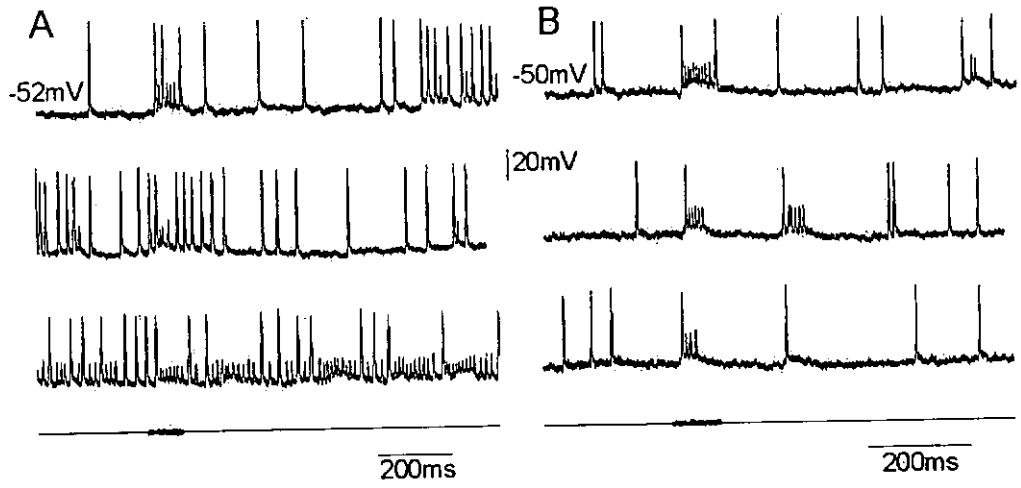

Figure 2 Auditory excitatory responses of two MGB neurons (A, B). Neuronal responses to three repeats of noise-burst stimuli are displayed for each neuron. Resting membrane potentials for each neuron are shown on the left top corner. The stimulus signal shown below the last trace for each neuron applies to all repeats of responses. The noise-burst was set at the 60 dB re sound pressure level (SPL). Scale bars for time and voltage apply to all traces.

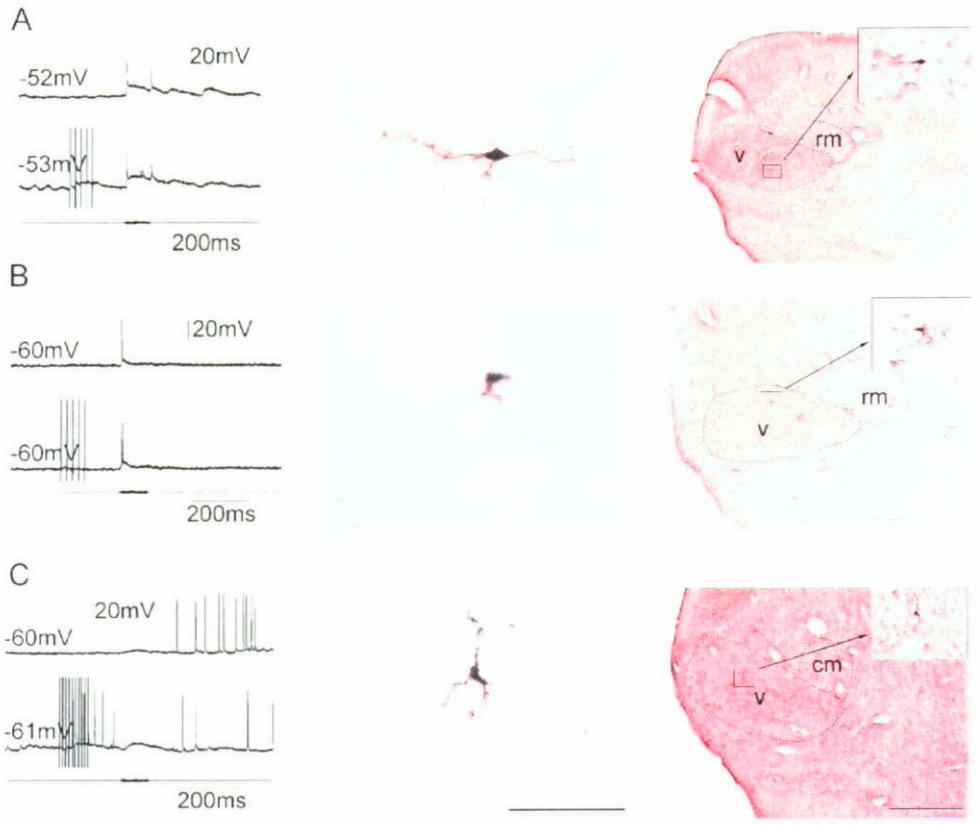

Figure 3 Anatomical locations of three corticofugal facilitatory MGB neurons. The left panel shows the neuronal responses to the noise-burst stimulus and the combination of a cortical electrical stimulation and the noise-burst stimulus. The middle panel shows the labeled neurons stained with neurobiotin. The right panel shows a lower magnification of the section after it was counterstained with neutral red. Based on the neuronal architecture, we could parcel the MGB into varied nuclei. The labeled neurons were highlighted on either the right or left top corner of each right-panel photograph. The neurons in A-C responded to auditory stimulus with an EPSP and spike(s) and a depolarization to the electrical stimulation in the auditory cortex. v, ventral nucleus; cm, caudomedial nucleus; rm, rostromedial nucleus of the MGB. Scale bars: 100 μm in the middle panel; 1000 μm in the right panel.

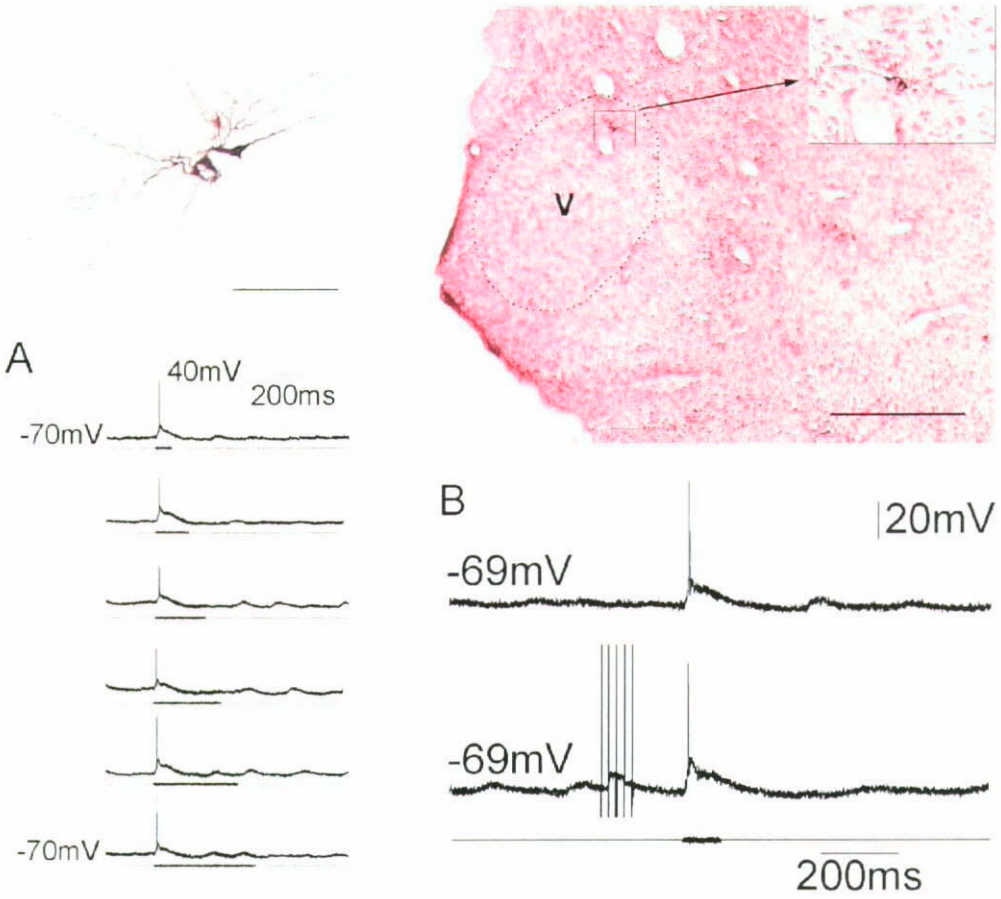

Figure 4 Anatomical location of an MGB neuron. The left panel shows the labeled neuron stained with neurobiotin and the neuronal responses to the noise-burst stimulus of varied durations (from 100 ms to 600 ms, Figure A). The right panel shows the combination of a cortical electrical stimulation and a lower magnification of the section after it was counterstained with neutral red. Based on the neuronal architecture, we could parcel the MGB into various nuclei. The labeled neuron was highlighted on either the right or left top corner of the right-panel photograph. v, ventral nucleus. Scale bars: 100 μm in the left panel; 1000 μm in the right panel.

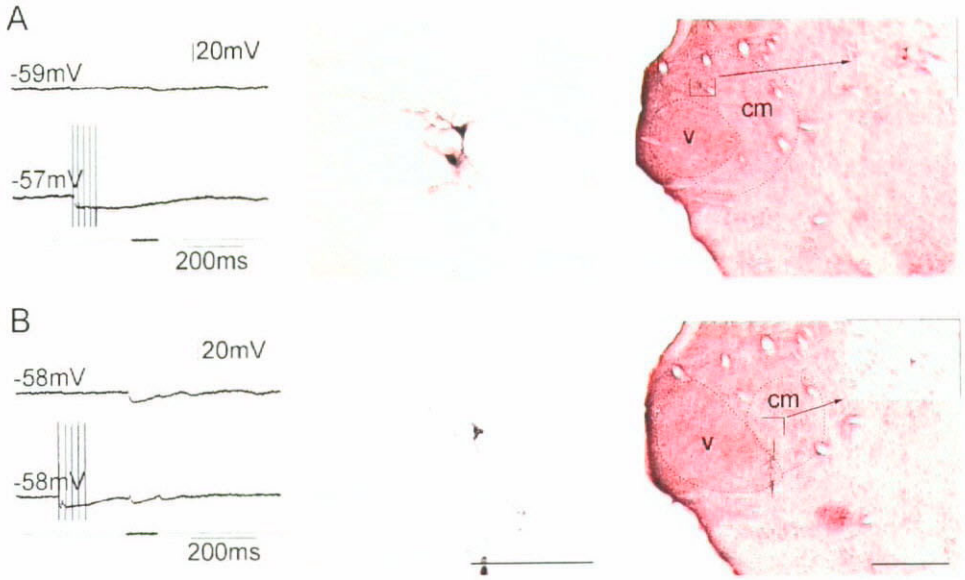

Figure 5 Anatomical locations of two corticofugal inhibitory MGB neurons. The left panel shows the neuronal responses to the noise-burst stimulus and the combination of a cortical electrical stimulation and the noise-burst stimulus. The middle panel shows the labeled neurons stained with neurobiotin. The right panel shows a lower magnification of the section after it was counterstained with neutral red. Based on the neuronal architecture, we could parcel the MGB into various nuclei. The labeled neurons were highlighted on either the right or left top corner of each right-panel photograph. The neurons in A and B responded with an IPSP to auditory stimulus and were inhibited by the electrical cortical stimulation. v, ventral nucleus; cm, caudomedial nucleus; rm, rostromedial nucleus of the MGB. Scale bars: 100 μm in the middle panel; 1000 μm in the right panel.

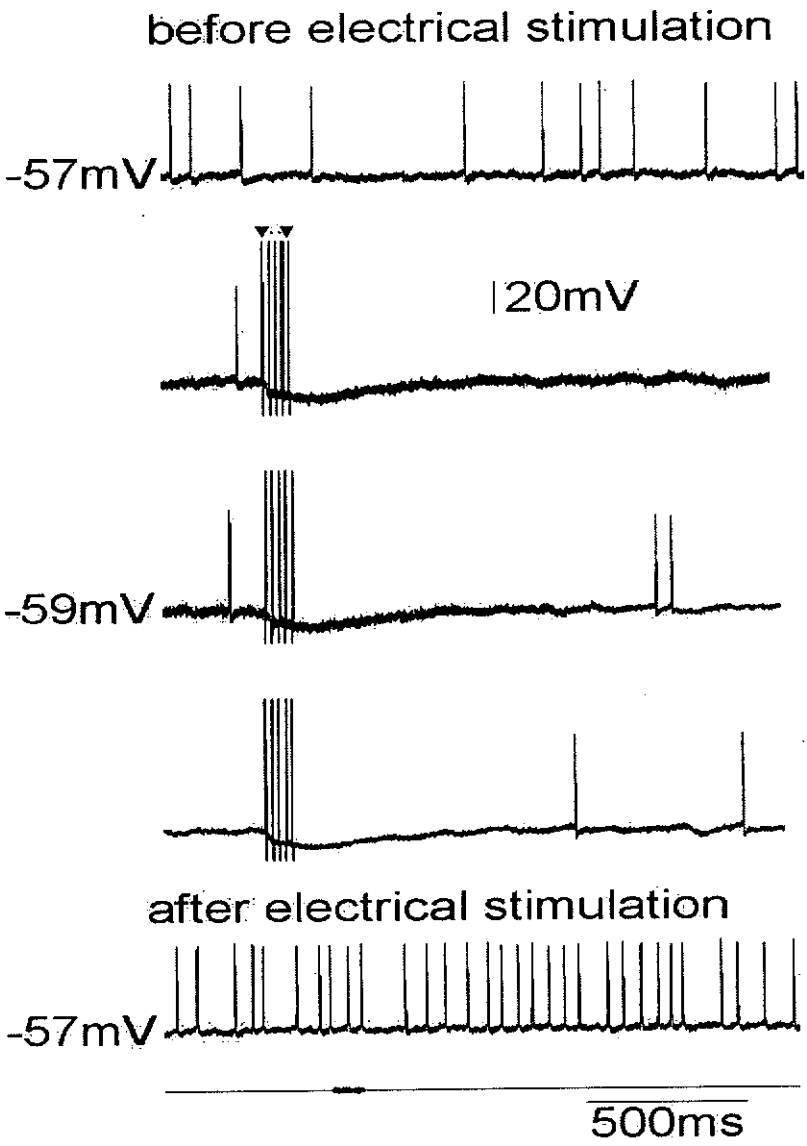

Figure 6 Corticofugal hyperpolarization on the thalamic neuron (the same neuron as in Figure 5A) has a long duration. Five trials of neuronal responses to electrical and auditory stimuli are shown for the neuron located in the non-lemniscal MGB with resting membrane potentials of -57 mV. A pulse train consisting of 5 pulses at 50 Hz was applied to the cortex (the artifact is indicated with arrowheads) and thesummated hyperpolarization, i.e., compound IPSP was calculated to be up to 1000 ms.

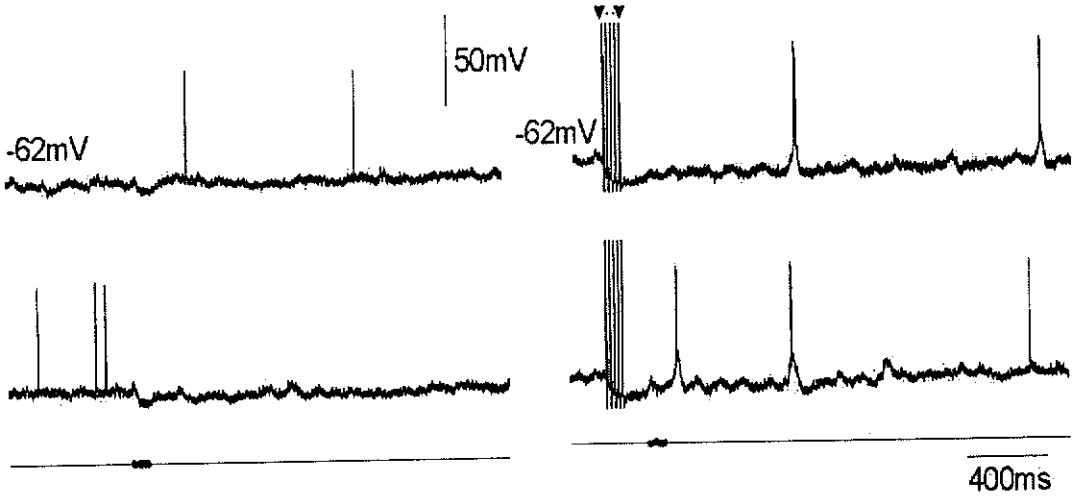

Figure 7 Neuronal responses of an MGB neuron to an auditory stimulus with or without electrical stimulation. Arrowheads indicate the artifact of a pulse train of the electrical stimulation, which consisted of 5 0.1-ms-width and bi-phase pulses at 50 Hz. The resting membrane potential was -62 mV and the scale bars apply to all traces.

Chapter 6

Summary of findings and conclusions

6.1 Summary of finding

The present study investigates the neuronal mechanism of the corticofugal modulation on the auditory thalamus, which transmits auditory information from the periphery to the cortex. In this study, the intrinsic electrophysiological properties and the auditory responses of the neurons in the MGB were investigated through *in-vivo* intracellular recording from pentobarbital anesthetized guinea pigs, while the auditory cortex was electrically stimulated. The auditory responses of the MGB neurons and the corticofugal modulation on the neurons in the auditory thalamus were also investigated in relation to the locations of the recorded neurons by anatomical confirmation after physiological study.

The discharge rate was calculated in the absence of acoustic stimuli at different membrane potentials that were changed by intracellular injection of current, or through automatic drifting. The non-acoustically-driven firing rate was examined at membrane potentials over a range of –45 mV to –65 mV: the higher the membrane potential, the greater the rate, and vice versa. The maximum non-acoustically-driven firing rate observed in the present study was 160 Hz. The auditory responsiveness of the MGB neurons was examined at membrane potentials

over a range of –45 mV to –75 mV: the higher the membrane potential, the greater the responsiveness, and vice versa.

A putative non-LTS burst was observed in the present study. It showed significantly longer inter-spike intervals than those associated with the putative LTS bursts. Many neurons changed their firing pattern to an LTS when the membrane potential was further hyperpolarized to < -75 mV. With an even lower membrane potential at < -85 mV, the neurons responded to the same noise-burst stimulus with an LTS burst.

Many excitatory auditory neurons showed burst-like responses to auditory stimuli with EPSPs. Some showed a phasic response to an acoustic stimulus, with a smaller mean amplitude and shorter duration of the EPSPs than those of burst-like excitatory neurons. Neurons with EPSP patterns tended to have a sharp-tuning curve, a low response threshold and a short response latency. These neurons responded to the same stimulus with a decreased amplitude of the EPSP as the membrane potential was hyperpolarized. The other excitatory auditory neurons showed a tonic response pattern. Many neurons responded to auditory stimuli with an IPSP with a mean amplitude and duration of -10.1+/-2.5 mV and 350.7+/-273.6 ms. The amplitude of the IPSP induced by the noise-burst stimulus decreased when the membrane potential was hyperpolarized. The neurons with an IPSP pattern and a tonic response pattern tended to show a non-tuning characteristic, or a broad/double-peaked tuning curve with a higher threshold. OFF neurons responded to auditory stimuli of different durations with different latencies or deviations of

latencies in addition to different spike numbers. Most OFF and ON-OFF neurons showed membrane oscillations with a frequency of about 5 Hz.

Corticofugal potentiation of the membrane potential facilitated the auditory responses and spontaneous firing of the MGB neurons. Corticofugal hyperpolarization decreased or even totally diminished the auditory responses and spontaneous firing of the MGB neurons. The shapes of all of the EPSPs and IPSPs produced by cortical stimulation were similar to those evoked by acoustic stimuli on each neuron. Most corticofugal facilitatory neurons were confirmed as being located in the lemniscal MGB and most corticofugal inhibitory neurons were located in the non-lemniscal MGB by reconstruction of the neurobiotin labeled neurons and anatomical identification of the locations of the neurons in the MGB by counterstain of neutral red. These results suggest a possible segregation of the excitatory and inhibitory neurons. Most auditory-excitatory neurons receive an excitatory input from the cortex. Most auditory-inhibitory neurons in the non-lemniscal MGB receive corticofugal inhibition.

6.2 Conclusions

The present intracellular recording unveiled as the first time the physiological mechanism of the corticothalamic modulation system at the neuronal level. The corticofugal projection gates the sensory information in the thalamus through spatially selective depolarization and hyperpolarization of the lemniscal and non-lemniscal MGB neurons. The present results also suggest a possible segregation of

the excitatory and inhibitory neurons. The fact that a similar shape of postsynaptic potential caused by both ascending and descending inputs indicates a neuronal endogenous characteristic irrespective of the physical locations of the synapses. The dependence of the temporal structure of the spikes/spike bursts on the stimulus may provide insight into the temporal coding of sound information in the auditory system. The response patterns of the OFF neurons suggest that spike timing could be another parameter used by the thalamic neurons to encode the stimulus information. The finding that most OFF and ON-OFF neurons showed membrane oscillations strengthens the idea that membrane oscillations might be more dominant in the non-lemniscal MGB than in the lemniscal MGB.

6.3 Functional implication of this study

This study unraveled the synaptic mechanisms by which cortical activation influences activity in the MGB and the physiological role of the corticofugal feedback system to the thalamus. The corticofugal projection gates the sensory information in the thalamus through spatially selective depolarization and hyperpolarization of the lemniscal and non-lemniscal MGB neurons. Overall facilitative or inhibitory corticofugal modulation indicates the gain control function.

In the visual system, a cortical stimulation can also generate both EPSPs and IPSPs in thalamic relay neurons (Dubin and Cleland, 1977; Ahlsen et al., 1982; Deschenes and Hu, 1990). The resultant effect on visual relay cell discharge may depend upon topograghic considerations: stimulation at a visuotopically-defined

locus in the visual cortex of a cat is followed by both excitatory and inhibitory effects on relay cells situated along retinotopically corresponding projection columns in the dorsal lateral geniculate nucleus (Tsumoto et al., 1978). The inhibitory effect extends into adjacent, non-retinotopically corresponding projection columns, probably because of divergence of corticothalamic fibers beyond the corresponding column and because of the similar divergence of axons of inhibitory perigeniculate nucleus cells, which are likely to be the major source of this inhibition (Murphy and Sillito, 1996). Excitation of a topographically related group of thalamic cells and inhibition of adjacent, non-topographically related cells is also seen in the ventral posterior nucleus after focal stimulation of the somatosensory cortex (Rapisarda et al., 1992).

Like the visual and somatosensory systems, a general function of the corticothalamic projection is to selectively gate/attend to the signals in our auditory system. The gains of the filters are controlled based on the judgment of the central system. The cortex serves to find out what signal is wanted and what should be filtered. This judgment is dynamic and changing across the time.

According to the signal theory, it is preferable to have unwanted signal removed as at early stage as possible. To remove the unwanted auditory signal, it would be better to remove the noise before it reaches the cortex. The corticothalamic feedback serves as the best location for the executive role of the dynamic filter.

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