The Hong Kong Polytechnic University

Department of Rehabilitation Sciences

Modulation of the thalamocortical projections on different layers of auditory cortex in guinea pigs

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A thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy

2008 Nov

CERTIFICATE OF ORIGINALITY

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_____ (Signed)

WANG Ningqian (Name of student)

Abstract of thesis entitled "Modulation of the thalamocortical projections on different layers of auditory cortex in guinea pigs" submitted by WANG Ningqian for the degree of Doctor of Philosophy at the Hong Kong Polytechnic University in Nov 2008

Abstract

The goal of this study was to understand the spontaneous neuronal activities and acoustic responses of neurons in the primary auditory cortex (AI), and the modulation of different divisions of the medial geniculate body (MGB) on different layers of the auditory cortex (AC) especially AI, through *in vivo* intracellular recordings and/or extracellular recordings in adult urethane-anesthetized guinea pigs.

One hundred and eighty nine neurons/units in AC, distributed among all six cortical layers, were recorded intracellularly and/or extrcellularly. Thirty-one of forty intracellular recorded neurons (77.50 %) and one hundred and thirty of one hundred and forty nine extracellular recorded units (87.25%) showed excitatory responses to a noise burst stimulus applied to the contralateral ear of the animals. The extracellularly recorded neurons showed synchronized spikes with the excitatory postsynaptic potential (EPSP), action potential (AP) and/or rhythmic oscillation of the intracellularly recorded neurons. The rhythmic oscillations evoked by the acoustic stimuli were predominantly in the spindle frequency band (14.77 ± 5.26 Hz) and had a long latency (75.37 ± 26.15 ms).

Acoustic responses appeared among the auditory cortical layers in a general sequence from the deep layers to the superficial layers. A number of neurons in

upper sublayer I and lower sublayer II responded first. Then layers IV, III, and VI responded, while finally layers I, II and V responded ($P < 0.05$). The direct, short latency projection from dorsal cochlear nucleus (DCN) to MGm and the projection from MGm to different layers of AC may explain why layer VI, upper sublayer I and lower sublayer II were activated first by the acoustic stimuli (Anderson et al., 2004; Anderson et al., 2006).

The neuronal responses in AI to electrical stimulation applied to different divisions of the MGB were recorded intracellularly and/or extracellularly. A stimulation electrode array containing three electrodes was implanted into the MGB, targeted to the medial and ventral divisions (MGm and MGv). The stimulation sites in different divisions of MGB and extracellular recording sites in AC were confirmed by electrical lesions. Intracellularly recorded neurons were labeled with Neurobiotin after physiological recordings.

Neurons in the AC showed spontaneous discharges, with occasional oscillatory activity. Similar to the acoustic stimuli, electrical stimulation of different MGB divisions evoked synchronized neuronal responses in AC layers.

Neurons in layer I of AI were predominantly modulated by electrical stimulation of the MGm. Electrical stimulation of different areas of the MGm had different effects on layer I neurons. Stimulation of some areas of the MGm increased the MP of layer I neurons, inhibited the spontaneous oscillation, and changed the neuronal activity. Stimulation of other areas of the MGm decreased the MP to tonic discharging or decreased the discharging rate of APs. This

two-direction modulatory effect that different areas of the MGm have on layer I may be involved in the maintenance of the state of layer I neuronal activity.

In auditory cortical layers II and III, more neurons responded to the electrical stimulation applied to MGm than MGv. Layers II and III responded to the thalamic electrical stimulation differently. Electrical stimulation of MGv induced long delayed oscillations or short delayed AP, followed by long lasting oscillations on layer II neurons. Similar long delayed oscillation was also observed on layer III neurons following electrical stimulation of MGv.

The sequence of thalamic stimulation-induced oscillations was the same as that of the acoustic response, from bottom to upper layers. The MGm stimulation induced EPSP/AP followed by rhythmic oscillation on layer III neurons had similar latencies to their acoustic responses while layer II neurons had shorter delayed response. The difference may be related to the activation of different projections from MGm to cortical layer III maybe via layer I when MGm was stimulated electrically (Andersen et al., 1980; Huang and Winer, 2000; Lee and Winer, 2008a) .

Acoustic responses could be inhibited or prolonged by thalamic electrical stimulation applied 20 or 50 ms before the acoustic stimuli. This is due to the lower excitabilities of the neurons, since thalamic electrical stimulation induced EPSP/AP or oscillaiton.

Spontaneous neuronal activity in layers I, II and III were always inhibited by the electrical stimulation of MGm. GABAergic neurons in cortical layers I and II

were likely involved in this process. However, spontaneous activity of some neurons in layers IV and V could be changed from tonic discharging to rhythmic oscillation by the electrical stimulation of MGm.

The present results indicate there is a functional segregation of the parallel pathways from the MGv and MGm to the AC. The thalamocortical projection from the MGv is the major pathway of auditory information, while the projection from the MGm is likely modulatory. The pathway from the MGm could regulate the general arousal of the auditory cortex. A fast feedforward modulation of the upper layers of the auditory cortex through the MGm pathway might enable the preparation of the auditory cortex to receive the auditory information forwarded from the MGv.

Relevant Publications

Journal papers:

GUO Yiping., SUN Xia., LI Chuan., **WANG Ningqian**., CHAN YingShing., HE Jufang. (2007) Corticothalamic synchronization leads to c-fos expression in the auditory thalamus. *Proc Natl Acad Sci U S A. 104(28):11802-7*

Conference papers:

WANG Ningqian., ZHANG Zhuo., CHAN YingShing., HE Jufang. (2007) Feed-forward modulation of the thalamocortical projections on different layers of the auditory cortex. *Society for Neuroscience 37th Annual Meeting San Diego, United States, November 3-7, 2007*

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I heartily thank my experimental guinea pigs that I used in this study. Their sacrifice is priceless in terms of the contribution to our understanding of the auditory thalamus and cortex, which has undoubtedly formed the foundation for future human studies.

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WANG Ningqian

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List of abbreviations

AC: auditory cortex
- AAF: anterior auditory cortex
- AI: primary auditory cortex
- AII: secondary auditory cortical field
- DC: dorsocaudal field
- S: small tonotopic cortical field
- DCB: dorsocaudal belt
- VCB: ventrocaudal belt
- DRB: dorsorostral belt
- VRB: ventrorostral belt

AP: action potential
AN: auditory nerve
BF: best frequency
CAF: central animal facilities
CN: cochlear nucleus
- DCN: dorsal cochlear nucleus

CNS: central neuron system
CT: corticothalamic
DAB: 3', 3'-diaminobenzidine
EPSP: excitatory postsynaptic potential
EEG: electroencephalography
GABA: γ-aminobutyric acid
IC: inferior colliculus
- CIC: central nucleus
- DCIC: dorsal nucleus
- ECIC: external cortices

i.p.: intraperitoneal
IPSP: inhibitory postsynaptic potential
ISI: inter-stimulus interval
LGB: lateral geniculate body
LTS: Low threshold calcium spikes
MGB: medial geniculate body
- MGd: dorsal nucleus
- MGm: medial nucleus
- MGcm: caudomedial nucleus
- MGrm: rostromedial nucleus
- MGs: shell nucleus
- MGv: ventral nucleus

O-DEP: onset depolarization
PAF: posterior auditory field
- VP: ventral posterior field
- DP: dorsal posterior field

PB: phosphate buffer
REM sleep: rapid-eye-movement sleep
SC: superior colliculus
s.c.: subcutaneous
S.D.: standard deviation
SOC: superior olivary complex
SPL: sound pressure level
SWS: slow-wave sleep
TRN: thalamic reticular nucleus
TC: thalamocortical
Vm: membrane potential

Chapter 1

Introduction

1.1 Background of the study

Auditory cortex (AC) is defined operatively as the final station of the auditory ascending pathway from the cochlear epithelium. It integrates, computes, decodes and processes the acoustic information carried along the thalamocortical (TC) pathways (Ehret, 1997).

Auditory cortical areas could be identified to four groups based on their putative functional roles: tonotopic, nontonotopic, multisensory and limbic (Lee and Winer, 2008a). They are composed of basic microcircuits containing a few hundred cells arranged into a column (microcolumn) (Mountcastle, 1997; Buxhoeveden and Casanova, 2002). Almost all six cortical layers participate in the organization of the cortical columns and the interactions of the six cortical layers within and between auditory cortical columns play important roles in acoustic information processing (Mountcastle, 2003).

The TC inputs are redistributed across a massive corticacortical and corticothalamic neuronal network in the AC (Winer and Lee, 2007; Lee and Winer, 2008b, , 2008c). According to the thalamic origins and cortical laminar and areal terminations, the auditory TC system can be divided into two major parallel pathways (Winer and Morest, 1983; Huang and Winer, 2000; Jones,

2002): lemniscal and non-lemniscal TC pathways. The lemniscal (or primary) TC pathway, mainly arises from the ventral division (MGv) of medial geniculate body (MGB), projects to the middle layers (layers III-V, more than 85 % of the total axonal profiles) of the primary auditory cortex (AI) and other tonotopic auditory cortical areas (Mitani et al., 1985; Huang and Winer, 2000; Lee and Winer, 2008a). This pathway mediates the short-latency responses to acoustic stimuli carrying precise frequency, intensity, timing and high fidelity information to the AI (Ehret, 1997; Eggermont, 1998). The non-lemniscal (or secondary) TC pathway, mainly arises from the medial and dorsal divisions of MGB (MGm and MGd), projects mainly to the superficial layers of the non-primary auditory cortex (LeDoux et al., 1985; Mitani et al., 1985; Huang and Winer, 2000). A recent study using a more sensitive tracer indicates that the MGm projects to all auditory cortical areas as well as to other cortical areas (Lee and Winer, 2008a). The non-lemniscal TC pathway transmits broadly tuned (Calford and Aitkin, 1983), weaker, longer-latency, variable acoustic responses (Bordi and LeDoux, 1994a, , 1994b; Edeline et al., 1999) even multimodal (Bordi and LeDoux, 1994b) and limbic (Clasca et al., 1997) information to the auditory cortical areas.

Besides the TC pathways, AC receives other fiber input systems which are composed of neuronal fibers arising from and terminating within ipsilateral (corticocortical) and contralateral (commissural) neocortex especially within AC itself (Downman et al., 1960; Lee and Winer, 2008b, , 2008c). More than 75% of the commissural projections arise from homotopic topographically organized

nuclei on the contralateral hemisphere of the brain while layers III and V contain more than 95% commissural neurons (Lee and Winer, 2008b). Each auditory cortical area has intrinsic corticocortical inputs from itself (> 50% of the projection cells) and extrinsic corticocortical inputs from other auditory cortical functionally related areas (tonotopic to tonotopic, nontonotopic to nontonotopic, multisensory to multisensory, limbic to limbic) on the ipsilateral hemisphere of the brain. The intrinsic corticocortical input originates from layers II-VI while the extrinsic corticocortical input has unique area-specific origins (Lee and Winer, 2008c). The fulfilment of the AC function depends on the integration of the neuronal inputs from the primary, non-primary TC pathways, corticocortical and commissural pathways.

MGB is the most important nucleus in the auditory thalamus and the major thalamic source of acoustic inputs to AC (Winer et al., 1977), temporal polar cortex and amygdala (Shinonaga et al., 1994). Three major divisions are in MGB: MGv (involved in the lemniscal TC pathway), MGd and MGm (involved in the non-lemniscal TC pathway). Neurons in different divisions have different afferent inputs (Calford and Aitkin, 1983), morphological, physiological characteristics and areal and laminar terminations in the AC (Huang and Winer, 2000; Cruikshank et al., 2002; Rose and Metherate, 2005; Lee and Winer, 2008a). In this study, we are paying special attention to MGm which has been shown to possess some unique characteristics compared with MGv and MGd (Smith et al., 2006). MGm is poorly tonotopic organized (Rouiller et al., 1989) and has more

cell types than other divisions (Winer and Morest, 1983). After receiving little topographic neuronal inputs from the brainstem (Calford and Aitkin, 1983) and the dorsal cochlear nucleus (DCN) (Anderson et al., 2006), MGm neurons project to all auditory and some other cortical areas (Shinonaga et al., 1994; Lee and Winer, 2008a). Although the cortical laminar distribution of MGm axonal terminations involves an average of 5.4 layers while 4.6 and 4.7 layers for MGv and MGd, the largest TC axons arise from MGm neurons and terminate in cortical layer I (Huang and Winer, 2000). In layer I, most neurons are GABAergic and connected with the neuronal dendrites from the other five layers (Mitani et al., 1985; Winer and Larue, 1989; Huang and Winer, 2000). Some progress has been made using in vitro (Cruikshank et al., 2002; Rose and Metherate, 2005) and in vivo (Metherate and Ashe, 1993; Sukov and Barth, 2001) intracellular recordings, but little is known about the cellular and synaptic mechanisms by which the thalamic inputs are transmitted to and processed in AC and the modulatory effect of MGB on AC.

In the present study, we adopted the *in vivo* intracellular recording combined with multiple extracellular recording techniques to explore the modulation of different divisions of MGB (MGm and MGv) on different cortical layers in guinea pig AI. By comparing the different modulatory effects of MGm and MGv, we investigate the mechanisms by which the acoustic information is transmitted via the primary and non-primary TC pathways to and processed in the different cortical layers of AI.

1.2 Aims of the study

The aim of this study is to understand the mechanism of acoustic information processing in the AI and the modulation of MGv and MGm on the six layers of the AI in adult guinea pigs.

The first specific aim of the present study is to identify the acoustic information processing pathway along the six layers of AI by using *in vivo* multiple extracellular recording technique.

The second specific aim is to examine the modulatory effects of electrical stimulation of MGv and MGm on neuronal activities in different layers of the AI by using *in vivo* multiple extracellular recording techniques and *in vivo* intracellular recording techniques. The relationship between the lemniscal and non-lemniscal pathways at cortical level is also investigated.

1.3 Significance of the study

The present study provides understanding of the mechanism as to how the TC projection system (including the lemniscal and non-lemniscal TC pathways) carries acoustic information to the AI and how acoustic information is processed along the six cortical layers. It unravels the physiological role of the different TC projection pathways represented by the neuronal activities in different cortical layers of AI.

This study also investigates the mechanism of auditory information processing along all the layers of AC. Together with previous knowledge from extracellular recording results in our lab, we can provide more evidence about the modulatory function of TC system on AI and the interaction between the lemniscal and non-lemniscal pathways via the interactions among the auditory cortical layers or on the same cortical neurons.

1.4 Outline of the thesis

Chapter 1 introduces the background, objectives and significance of the present study.

Chapter 2 provides a literature review about the background of anatomical and physiological knowledge of organization of MGB, AC, TC and corticocortical projection system.

Chapter 3 describes the methodology employed in this thesis.

Chapter 4 demonstrates the auditory responses of the AI neurons and the thalamic electrical stimulation effects on the neurons in different cortical layers.

Chapter 5 summarizes the findings and conclusions of the present study.

Chapter 2

Literature review

This chapter explores the basic principles of anatomical and physiological properties and functions of auditory thalamus and AC. The first section describes the anatomical and physiological characteristics of the MGB and AC. The second section describes the TC projections from MGB to AC and the corticocortical projections between auditory cortical layers and areas. The third section describes the rhythmic neuronal activities in TC loop. After having established the neuronal input systems of AC originating from MGB and AC itself, this chapter moves on to analyze the function of these projections and their role in sensory information integration and processing.

2.1 Anatomy and physiology of MGB and AC

Several nuclei in the central neuron system (CNS) comprise the primary ascending auditory pathway including: the cochlear nucleus (CN), the superior olivary complex (SOC), the inferior colliculus (IC), the MGB and finally the AC (Rouiller and de Ribaupierre, 1985). As shown in Figure 1, the first relay station of the primary ascending auditory pathway is the CN in the brainstem, which receives the acoustic information from the auditory nerve (AN) connecting with the hair cells in the inner ear. The second relay station is the SOC where the

majority of the auditory fibers have already crossed the midline of the brain. Leaving the SOC, neuronal fibers carry the acoustic information up to the IC. The MGB in the thalamus is the final relay station and the terminal of the ascending primary auditory pathway is the AI, which has massive axonal projections back to auditory relay stations as well as other nuclei (see Figures 1 and 2).

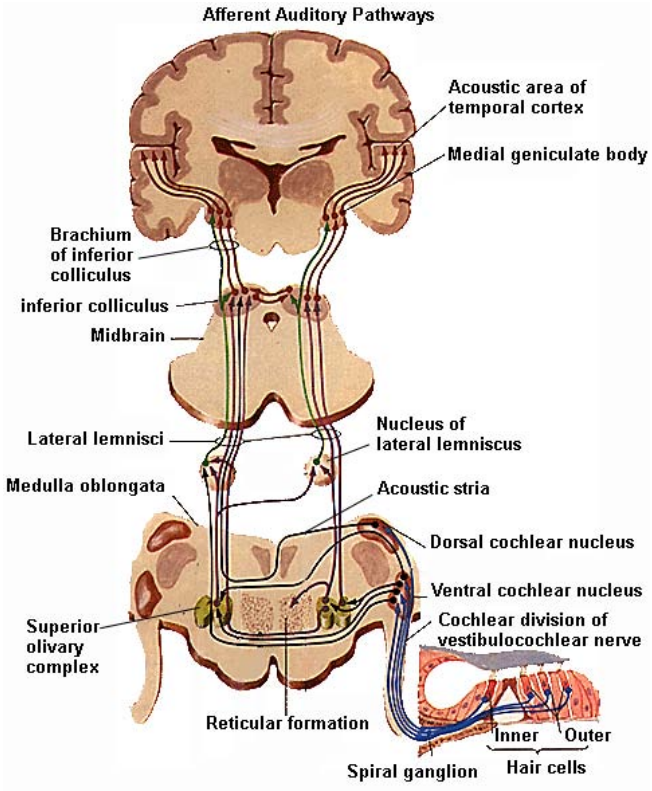

Figure 1. Schematic diagram of the ascending auditory pathways.

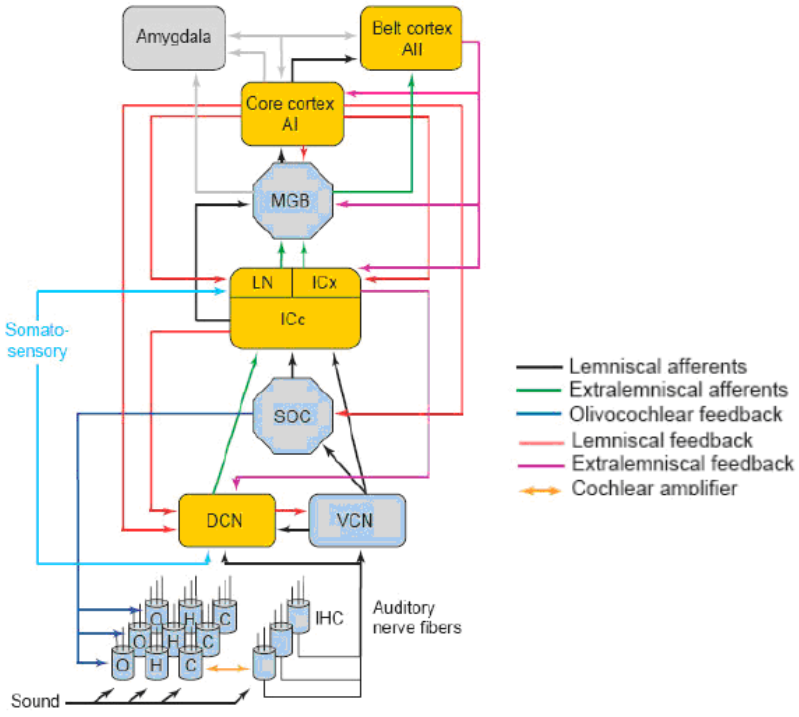

Figure 2. Schematic of the auditory pathways showing the ascending and descending projections among different neclui (Eggermont and Roberts, 2004).

Thalamus, at the crossroads of brainstem, basal ganglia and telencephalic circuits, is the major gateway for the flow of sensory information including inputs from both brainstem and basal nucleus toward the cerebral cortex. In the visual, auditory and somatosensory systems but not from olfaction, the cerebral cortex receives sensory signals from the thalamus. The MGB, between the lateral edge of the brainstem and the junction of the midbrain and the diencephalons in the auditory thalamus, is the final relay station before the auditory information arrives at the AC in all mammals and most vertebrates (Figure 3). The principal boundaries of MGB are: the anterior surface of superior colliculus (SC) (caudally), the lateral geniculate body (dorsally), the hippocampus (laterally), portions of the

intralaminar thalamic nuclei (ventrally), and the medial lemniscal and thalamofugal axons (anterolaterally). The MGB consists of several divisions (Winer and Morest, 1983): MGv, MGd and MGm, each with a unique pattern of midbrain afferents and cortical targets.

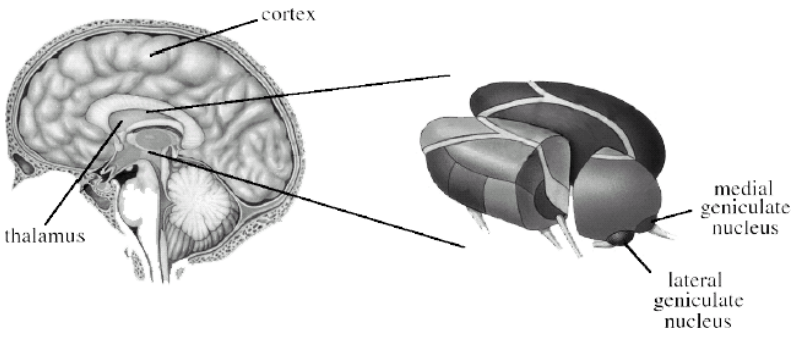

Figure 3. The location of thalamus and the magnification of thalamus showing MGB on the midline section through the human brain.

2.1.1 Structure and morphology of the MGB

TC neurons, or relay cells in thalamus, across auditory, visual and somatosensory systems share common principles with respect to the synaptic organization and topographic distributions (Jones, 2002). A large number of studies confirmed that MGB could be divided into three subdivisions (MGv, MGm and MGd) based on the dendritic morphology in many species [monkey: (Burton and Jones, 1976); tree shrew: (Oliver and Hall, 1978); cat: (Winer and Morest, 1983); human: (Winer, 1984); rat: (LeDoux et al., 1987); guinea pig: (Strutz, 1987; Clerici and Coleman, 1990); rabbit: (Caballero-Bleda et al., 1991); mustache bat: (Winer and Wenstrup, 1994a, , 1994b)]. For decades, cat MGB has been well studied and was served as the mammalian prototype. In cats, bushy and stellate neurons are most frequently found in the three MGB divisions: (1) MGv mainly contains bushy neurons with tufted dendrite trees; (2) Stellate neurons with extensive dendritic arbors and bushy neurons with less tufted dendritic branches are seen in MGd; and (3) MGm contains many cell types including the largest neurons in MGB (prominent magnocellular neurons), small tufted neurons, and more classical stellate neurons (Winer and Morest, 1983).

Many works on the identification of subdivisions of MGB have been done (Redies and Brandner, 1991; Edeline et al., 1999; Anderson et al., 2007). Recently Anderson et al. (2007) used cytochrome oxidase staining to identify the divisions of the MGB of guinea pig (Figure 4).

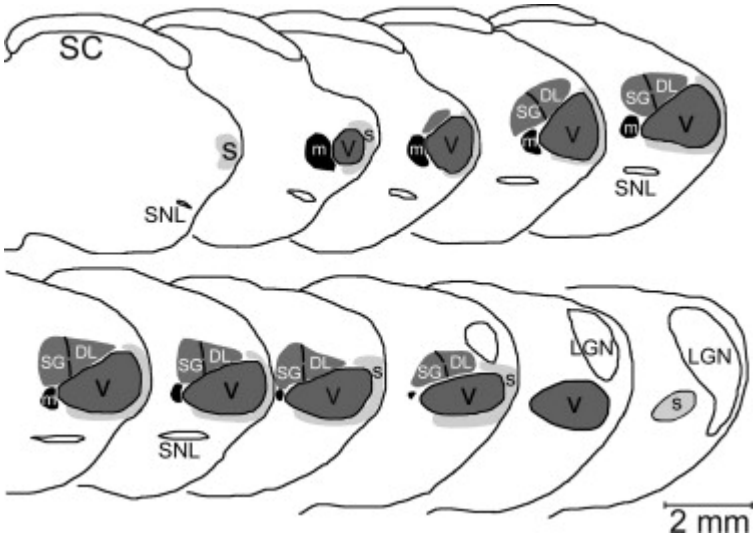

Figure 4. Line drawings of coronal section through the guinea pig thalamus showing the position of the MGB. Borders have been ascertained on the basis of staining for cytochrome oxidase and electrophysiological recordings. Sections: 100 μm thickness, taken every 200 μm. Scale bar = 2 mm. *Abbreviations*: SNL, substantia nigra pars lateralis; APT, anterior pretectal nucleus; DL, dorsolateral division of MGB; LGN, lateral geniculate nucleus; LP, lateral posterior nucleus; m, medial division of MGB; PIN, posterior intralaminar nucleus; Po, posterior thalamic nucleus; s, shell division of MGB; SC, superior colliculus; SG, supragenicuIate; v, ventral division of MGB (Anderson et al., 2007).

2.1.2 Physiological properties and tonotopic organizations of MGB

Neurons in MGv, the major origin of the lemniscal TC pathway, have extremely uniform properties: high levels of spontaneous neuronal activity, sharp frequency tuning, tonotopic organization, and short responding latency (8-12 ms) to the acoustic stimuli (Calford and Aitkin, 1983; Rodrigues-Dagaeff et al., 1989; Redies and Brandner, 1991; Edeline et al., 1999; Cetas et al., 2002; Zhang et al., 2008).

As an important origin of the non-lemniscal TC pathway, MGm has a large range of somatic sizes including the biggest cells in the MGB and a lower density of neurons than MGv and MGd (Winer and Morest, 1983). MGm has physiological response properties intermediate between the transient sharply tuned responses in MGv and the sustained broadly tuned responses in MGd (Rodrigues-Dagaeff et al., 1989; Rouiller and de Ribaupierre, 1989; Edeline et al., 1999; Anderson et al., 2004). Neurons in MGm mainly have high thresholds, broad frequency selectivity, and a tonic response pattern which may have a strong impact on the AC as well as amygdala (Zhang et al., 2008). Latency of MGm neurons responding to both pure tone and click acoustic stimuli encompasses the full range of latencies recorded from the MGv and MGd [Cat: (Aitkin and Webster, 1972; Calford and Webster, 1981; Rouiller et al., 1981; Rouiller and de Ribaupierre, 1989; Rouiller et al., 1989); Guinea pig: (Edeline et al., 1999)]. A greater proportion of very short latencies responses (< 6 ms) in MGm compared

with other MGB divisions (Rodrigues-Dagaeff et al., 1989; Rouiller et al., 1989) may be mediated by the direct projection from DCN to MGm (Anderson et al., 2006). About half MGm neurons have short response latency and are sharply tuned like those in MGv while the remainder have long latencies and are tuned to a broad range of frequencies similar to MGd neurons and some MGm neurons adapt to the repetitive stimuli, while others do not (Popper and Fay, 1992).

MGd neurons, having relatively low best frequencies (BF) (Zhang et al., 2008), have the most divergent physiological properties which do not befit them for a role during the transfer of acoustic information serving as a basis for complex auditory discrimination. A relative high percentage of MGd neurons have long (up to 100 ms) delayed and inconsistent acoustic responses; and about 15% of them may not respond at all (Calford and Aitkin, 1983). The auditory responses of MGd neurons are labile or intermittent, less obviously transient than MGv, and usually habituated to the repetitive stimuli. Small fluctuations in intensity or frequency of the stimulation may restore discharges to a previous level from the habituation. MGd neurons responded to a wide range of frequencies so no obvious tonotopic representation is found in MGd.

Tonotopic organization in the MGB is different in many species. In cats, MGv is tonotopically organized with the low frequencies located laterally, the middle frequencies caudomedially, and the high frequencies situated rostromedially (Calford and Aitkin, 1983; Imig and Morel, 1984, , 1985). However, high

frequencies are located in rostral MGv while low frequencies are located caudally in guinea pigs (Redies and Brandner, 1991; He, 2002).

Neurons in the non-lemniscal MGB (MGm and MGd, which are non-tonotopically organized) typically respond to the acoustic stimuli with long latencies, a bursting firing pattern, and are broadly tuned. Some cells in these divisions of the MGB respond not only to acoustic stimuli but also to other sensory modalities (Wepsic, 1966; Calford and Aitkin, 1983; Winer and Morest, 1983; Rouiller and de Ribaupierre, 1989; Redies et al., 1989a; Redies and Brandner, 1991; Winer et al., 1992; He, 1997; Kosaki et al., 1997; Rauschecker et al., 1997; He and Hashikawa, 1998; Winer et al., 1999; He and Hu, 2002).

2.1.3 Structure and tonotopic organization of AC

Most previous work on the functional organization of the AC has been carried out on cats, primates, rodents and bats. The number of auditory cortical fields changes in different species: there are three or four auditory fields in insectivores, four to seven in rodents, and six to more than eight in carnivores and primates (Morel and Kaas, 1992; Stiebler et al., 1997; Hackett et al., 1998).

An important organization principle of AC is tonotopy. The BF of a cortical neuron is the frequency of the acoustic stimuli to which the neuron has the lowest response threshold. Tonotopy means the cortical neurons are arranged in an ordered way according to their BFs. Tonotopic organization has been used as a criteria to define functional subdivisions in the AC as well as in other parts of the auditory system (Winer and Prieto, 2001).

Among all the auditory cortical fields, AI is the most important one defined anatomically by its distinct cytoarchitecture and connections with MGv. In both anesthetized and unanesthetized animal preparations, a large proportion of AI neurons are tuned to narrow ranges of frequency while a few neurons are more broadly tuned or exhibit multi-peaked tuning curves (Imig and Reale, 1980, , 1981; Redies et al., 1989b). Neurons with similar BFs occupy cortical bands orthogonal to the tonotopic gradient. In many species, at least one more tonotopically organized auditory field is found in addition to AI. Although audible frequency range is not fully represented as AI, the adjacent tonotopically organized auditory

cortical fields form mirror images of each other and share a range of frequencies at their border regions. The secondary auditory cortical field (AII), also organized tonotopically, lies ventrally to AI in a number of mammals. In addition to the direct projections between the primary sensory areas in different modalities (Budinger et al., 2006), at the borders of AII, neurons receive visual or somatosensory in addition to auditory inputs from thalamus (Berman, 1961a, , 1961b; Irvine and Huebner, 1979; Toldi et al., 1986; Clarey and Irvine, 1990a, , 1990b; Hofstetter and Ehret, 1992; Sukov and Barth, 2001).

According to the previous studies (Redies et al., 1989b; Anderson et al., 2006), the AC of guinea pig can be divided into several subdivisions (Figure 5):

(1) Field AI is in the anterior half of the AC and having narrow frequency tuning curves to the acoustic stimuli. AI neurons strongly respond to the pure tone stimuli with short latencies. It is highly tonotopically organized with the low BFs bands represented rostrally while the high BFs bands caudally.

(2) There is a second tonotopically organized area lies caudal to AI in the dorsal half of the posterior AC and therefore is named the dorsocaudal field (field DC). The field DC cells are tonotopically organized as strongly as those in AI, but the tonotopy is discontinuous in the dorsal half of field DC where the low BFs are represented immediately caudal to the high frequencies while the intermediate frequencies are missing.

(3) A third tonotopic auditory cortical field (S) is found rostral to the AI. This field extends over a surface of less than 1 mm^2 and the audible frequency range is

fully represented in it: high frequencies are located rostrally while low frequencies caudally. Field S neurons have longer auditory response latencies and broader tuning curves than those in field AI and DC.

Non-tonotopic auditory cortical fields surround the field DC caudally including (4) the dorsocaudal belt (DCB) and (5) the ventrocaudal belt (VCB) (Redies et al., 1989b). Neurons in both areas are broadly tuned and responded to acoustic stimuli with longer latencies than those in the tonotopic auditory cortical fields. Most DCB neurons reacted with an on-response to the pure tone stimuli while in the VCB tonic responses occur more frequently.

Another two non-tonotopic regions, located in the anterior AC rostral to the tonotopic fields, are termed as (6) the dorsorostral belt (DRB) and (7) the ventrorostral belt (VRB). Tuning curves of neurons in both areas are broad, response latencies are short and response thresholds are often high.

Recent work (Nishimura et al., 2007) using the optical imaging with voltage-sensitive dyes shows us that VRB could be divided into two areas, a ventrorostral field (VR) and a ventrocaudal field (VC). VR has similar properties as VRB while VC, which has novel properties, is identified as the mirror-symmetric tonotopic field to VR (Figure 6).

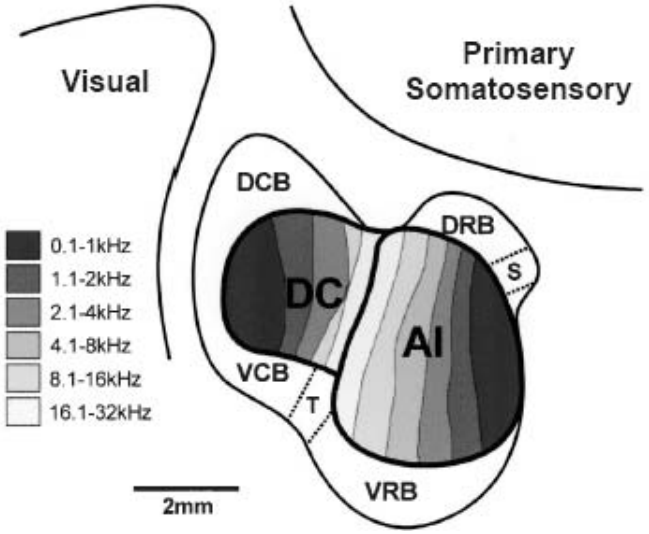

Figure 5. Position of auditory fields in relation to somatosensory and visual regions in guinea pig. An idealized representation of the frequency bands is indicated by the shaded bands in fields AI and DC (Wallace et al., 2000).

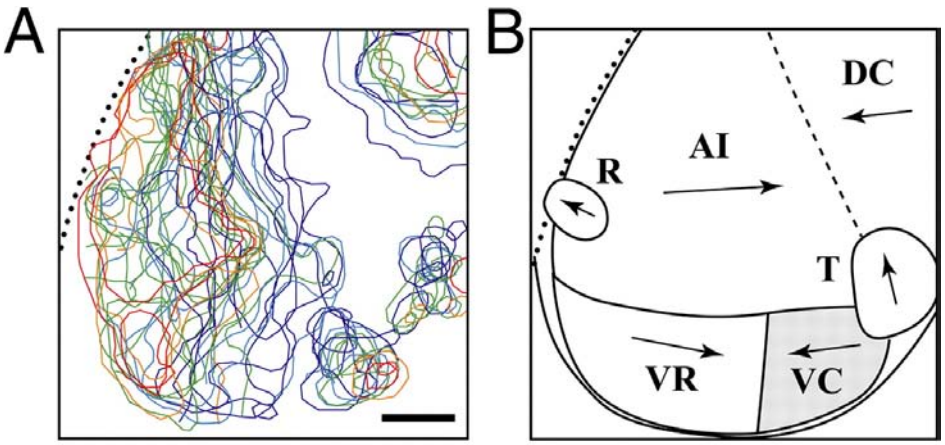

Figure 6. Consistency of response locations and tonotopy among animals: data derived from optical imaging. **A**: superposition of response contours evoked by pure tone from 8 animals. Responses to acoustic stimuli with 250 Hz (red), 500 Hz (orange), 1 kHz (green), 2 kHz (light blue), and 4 kHz (blue) tones were used for analyses. Data were not available for all tone frequencies. For superposition, the response contours from 1 animal was used as a standard; contours from other animals was shifted (< ± 1.5 mm; < ± 0.9 mm in most cases) and/or rotated (< ± 15°; < ± 4° in most cases) for contours in AI and DC to match those in the standard best (judged by eye). Note that the exact shape of the response contours

differed among animals, but the relative locations of the fields and the tonotopy in each field were consistent. Field T appeared to have more variability among animals. **B**: schematic summary of all fields. Field VC is newly identified here and is shaded. The arrow in each field depicts the frequency axis. We have not identified the ventral border of DC yet. Dotted lines in *A* and B mark the pseudosylvian sulcus. The scale bar in **A** (1 mm) also applies to **B** (Nishimura et al., 2007).

2.2 TC projections

2.2.1 Afferent projections to MGB

MGB contains several cytoarchitectonically distinct nuclei including MGv, MGm and MGd in virtually all mammals. MGB divisions, to a large extent, represent the parallel TC pathways through which the auditory information flows to the cortex (Imig and Morel, 1984; Huang and Winer, 2000; Lee et al., 2004; Lee and Winer, 2008a).

The predominant input to MGv arises in the ipsilateral central nucleus of the IC (CIC), and a weaker input arises from the same nucleus on the contralateral side of the brain. MGv, highly topotopically organized, receives the most direct, monosynaptic, shortest latency projections from the contralateral peripheral auditory apparatus and has efferent axonal projections to the AI (Calford and Aitkin, 1983; Imig and Morel, 1984).

The ipsilateral pericentral nucleus of the IC and the nucleus sagulum provide the major afferent inputs to MGd. Compared with MGv, MGd relays less direct auditory inputs to the cerebral cortex. The topotopic organization in MGd is much less evident than MGv and MGd mainly projects to the non-tonotopic auditory cortical areas arround the AI.

MGm receives afferent axonal projections from the external nucleus of IC (ECIC), scattered cells of CIC (Calford and Aitkin, 1983), the deep layers of SC (Graham, 1977; Morest and Winer, 1986), spinal cord (Jones and Burton, 1974;

LeDoux et al., 1987), vestibular nucleus (Roucoux-Hanus and Boisacq-Schepens, 1977), and DCN which mediated the short latency responses of MGm and other auditory nuclei (Henkel and Spangler, 1983; Anderson et al., 2006) including the ventral nucleus of the lateral lemniscus (Whitley and Henkel, 1984) and the cochlear nucleus complex (Strominger et al., 1977; Malmierca et al., 2002). Ill-defined, mixed auditory, somatosensory, vestibular and possibly other afferent inputs project to MGm as well. Neurons in MGm do not always respond to the acoustic stimuli or respond in various ways. MGm projects to cortical areas extending beyond the confines of AC. It has more complicated functions than other nuclei in MGB.

2.2.2 TC Projections

The axonal projection loops located between auditory thalamus and AC are illustrated in Figure 7 (Rouiller and Welker, 2000). The TC pathways arise from the different populations of thalamic neurons (different MGB divisions) and terminate in different areas and layers in cortex.

The organization of the projections from the MGB to AC is quite similar in different mammals (Radtke-Schuller, 2004; Radtke-Schuller et al., 2004). In cat, MGv neurons project to AI and its mirror region, the anterior auditory field (AAF) (Imig and Morel, 1984) wihch in guinea pig correspond to fields AI and DC. MGd projects to the auditory cortical areas surrounding the AI while MGm projects to all AC areas including its association cortex (Winer et al., 1977; Niimi and Matsuoka, 1979; Andersen et al., 1980; Imig and Morel, 1983; He et al., 2002; Lee and Winer, 2008a) and even to the somotosensory cortex, prefrontal cortex, amygdala and basal ganglia (Wepsic and Sutin, 1964; Russchen, 1982; LeDoux et al., 1990; Shinonaga et al., 1994; Cruikshank et al., 2002; Kimura et al., 2003).

The TC pathway from MGv to the middle layers of AI is also called the lemniscal TC pathway (or the primary TC pathway) and the non-lemniscal TC pathways (or the secondary TC pathway) are defined as the TC projections from MGd, MGm, peripeduncular nucleus and other non-primary thalamic nuclei to AC (Ryugo and Killackey, 1974; Caviness and Frost, 1980; Herkenham, 1980; Arnault and Roger, 1990; Clerici and Coleman, 1990; LeDoux et al., 1991a;

LeDoux et al., 1991b; Linke and Schwegler, 2000). Besides the prominent target in the AC, each MGB division projects to other cortical areas in many species [guinea pig: (Redies et al., 1989a); rat: (Clerici and Coleman, 1990); cat: (Huang and Winer, 2000)]. Although the TC projections to the tonotopically organized AI and AAF arise mainly from the tonotopic parts of MGv, in cat AI and AAF also receive significant non-tonotopic projections (also called heterotopic projections) from thalamus (Lee et al., 2004) playing an important role in cortical plasticity and signal presentation. The thalamic inputs provided by MGm neurons to all auditory cortical fields are multisensory and capable of long-term potentiation (Gerren and Weinberger, 1983).

TC projections to both the tonotopic fields including AI, DC, field S, and to the non-tonotopic VCB in AC of guinea pig has been well investigated by Redies and colleagues (Redies et al., 1989a). AI receives thalamic tonotopically organized inputs mainly from MGv. Roughly, the caudal part of MGv projects to the rostral part of AI and the rostral part of MGv to the caudal part of AI. A weaker thalamic projection to AI originates in a magnocellular nucleus located caudomedially in MGB (MGcm). The field DC receives thalamic input from the different neurons in the same nuclei as project to the AI. The continuous tonotopic map in MGv is parallel when it projects onto the cortex so that two adjacent tonotopic fields (AI and DC) result.

TC projections differ in their cortical laminar organization in different species. In cat, (1) MGv and parts of MGd mainly projected to the cortical layers III-IV

with little to layer I; (2) MGd projects mainly to layer I and little to layers III-IV; and (3) MGm, whose axonal trunks stretch laterally for a long distance in the cortical layer I, has the lowest density of labeling in layers I, III-IV, and VI (Niimi et al., 1984; Mitani et al., 1985; Huang and Winer, 2000; Lee and Winer, 2008a). Average 4.6, 4.7 and 5.4 layers of AC receive projections from MGv, MGd and MGm respectively (Huang and Winer, 2000). In monkey, (1) MGv has projections mainly to layer IV and deep sub-layer III (layer III_b); (2) MGd projects largely in layer III_b; and (3) MGm has few axon terminals, some terminate in the middle cortical layers while others in layer I (Hashikawa et al., 1995). In rabbit, some TC axons projecting to the cortical layer I also terminate in layers II-V. These extensive connections between auditory thalamus and AC indicate that concomitant activation across 1,500 μm-wide zones and perhaps more than one mode of TC activation, the lemniscal TC activation, exist (Cetas et al., 1999).

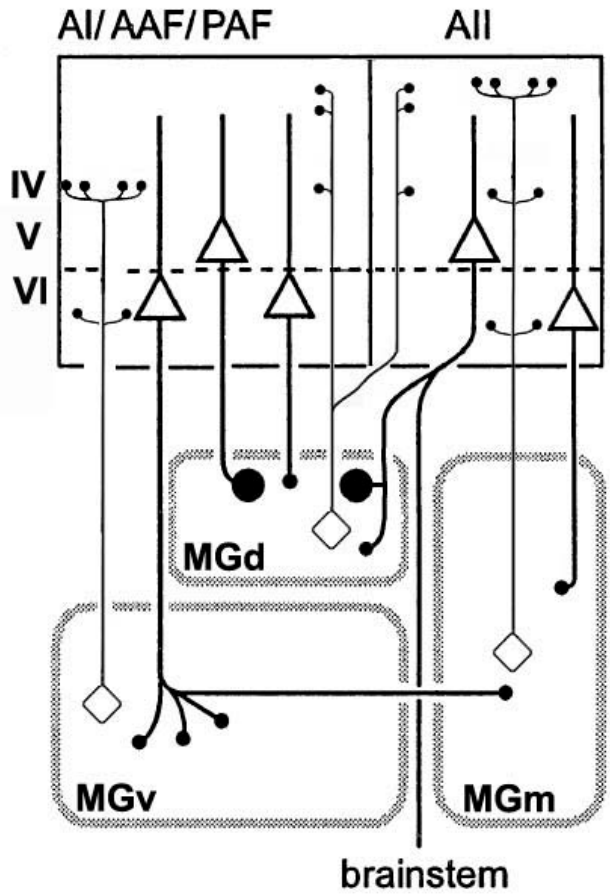

Figure 7. Schematic representation of cortico-thalamo-cortical loops for the tonotopically organized auditory cortical areas AI, AAF, PAF and AII in cat. *Open triangle*: corticothalamic (CT) neurons. *Open diamond*: TC neurons. *Filled circle (small and large)*: axon terminals. The cortical layers IV, V, and VI are indicated (Rouiller and Welker, 2000; Winer and Prieto, 2001).

2.3 Intrinsic connections in AC

There are complicated intrinsic interlaminar and intralaminar connections among the six layers of AC as shown in Figure 8 (Mitani and Shimokouchi, 1985; Mitani et al., 1985). Both the commissural system (Lee and Winer, 2008b) and the corticocortical system (Lee and Winer, 2008c) compose the intrinsic connections within AC.

A primary flow of information travels from the middle layers (III and IV) to the supragranular layers (I and II) and then back to the infragranular layers (V and VI) when AC is processing the acoustic signals (Matsubara and Phillips, 1988; Ojima et al., 1991; Wallace et al., 1991; Lee and Winer, 2008b, , 2008c). Barbour and Callaway (2008) found that AI layer II/III pyramidal neurons received strong excitation primarily from layers II-IV. They have substantial axonal arbors in layer IV, and connect to layer IV excitatory neurons. Most or all of these layer IV excitatory neurons project out of the local cortical circuit. Layer IV of AI integrates thalamic and strong layer IV recurrent excitatory input with relatively direct feedback from layer II/III and provides direct cortical output (Barbour and Callaway, 2008). In general, AI layer IV neurons are receiving strong monosynaptic projections from MGB (mainly from MGv). The numerous local axon collaterals of layer III neurons connect their immediate vicinity in layers I, II, IV and V. Besides their intrinsic local connections, the layer I horizontal cells receive monosynaptic inputs from the slow conducting fibers of MGm neurons

and project to layer II. The pyramidal neurons in layer II receive inputs principally from layers I and III and distribute their axonal terminals into layers V and VI. The main axonal branches of layer VI pyramidal cells project back to MGB and their local collaterals project to the deeper cortical layers. The layer V pyramidal cells project their axonal terminals to both MGB and IC.

In cats, more than 50% of the corticocortical projection neurons in each auditory cortical area recieve intrinsic inputs (that arise from the cortical area itself) (Lee and Winer, 2008c). Neurons in auditory cortical areas also have highly topographic and clustered extrinsic projections to other functionally related cortical areas (tonotopic to tonotopic, nontonotopic to nontonotopic, limbic-related to limbic-related, multisensory to multisensory). The intrinsic corticocortical inputs arise from all layers except layer I while the extrinsic inputs have area-specific origins. Rather than a simple serial or hierarchical corticocortical projection pattern, modest converging projections in each cortical area may contribute to the complexity of physiological responses in AC.

Most commissural projections (> 75%) are homotopic and from corresponding topographically organized cortical areas while the heterotopic projections (> 1mm beyond the main homotopic projection) constitute about 25% of the commissural projections. More than 95% of the commissural neurons are clustered in cortical layers III and V. Some areas have almost entirely commissural connections from layer III (temporal cortex and AII) while others predominantly from layer V (AAF) or from layers III and V (the dorsal auditory

zone). The commissural system is essentially homotopically connected since the divergence was less than 3% in each cortical area. Consistent with TC and corticocortical systems, the commissural cells have a topographic distribution.

In addition to the interlaminar connections within auditory cortical functional columns, the following hierarchical organizations between functional columns are also established in the AC (Rouiller et al., 1990; Rouiller et al., 1991): AI and AAF, interconnected by strong lateral connections, occupy the lowest functional level, and the AII, VP (ventral posterior) and PAF each occupies a successively higher functional level. Only the highest functional level seems to be connected with the limbic system directly and involved in some advanced functions such as memory and emotional processes. The intrinsic connections in cat AI are in a patchy distribution fashion. Neurons in AAF, DP (dorsal posterior), VP and V (ventral) project to the ipsilateral AI and so do the contralateral AI, AAF and D (dorsal). In AI, DP and VP, neurons are connected with the bilateral AAF and contralateral AI. The layer III containes the greatest concentration of corticocortical cell bodies, while a somewhat lower concentration in the layer V (Thomas and Lopez, 2003).

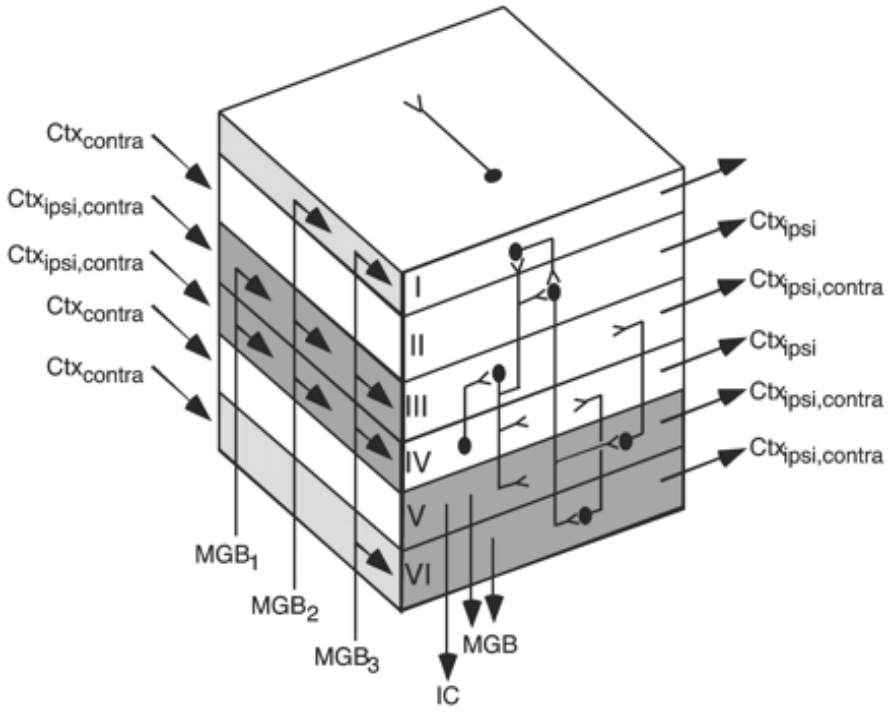

Figure 8. Intrinsic connections of AI in cat.
Left face shows thalamic and corticocortical inputs and *right face* shows interlaminar connections as well as thalamic, collicular, and corticocortical outputs. Lemniscal thalamic inputs (MGB_1) end only in layers III and IV, while nonlemniscal inputs also activate layer I (MGB_2) or layers I and VI (MGB_3) (MGB_1 includes MGv, as well as the dorsal, dorsal superficial and suprageniculate nuclei; MGB_2 represents the deep dorsal and caudal dorsal nuclei; and MGB_3 is MGm). Corticocortical projections from the ipsilateral hemisphere (Ctxipsi) terminate in the middle layers, but commissural inputs (Ctxcontra) are widely distributed among layers II-VI. Within the column, layer IV and lower layer III receive the major lemniscal thalamic input, initiating a flow of information into the supragranular layers and then down to the infragranular layers. Layers II/III also extends long-range lateral projections to form horizontal connections with other cortical columns (symbolized by neuronal projection on top of cube). Feedback to the auditory thalamus (MGB) originates primarily in layer VI but also in layer V, and projections to the inferior colliculus (IC) emerge from layer V. Major corticocortical projections to both the ipsilateral and contralateral hemispheres emerge from layers II and III, but layers IV-VI also provide some corticocortical outputs (Mitani et al., 1985; Huang and Winer, 2000).

2.4 Rhythmic oscillations in auditory thalamus and AC

2.4.1 Spontaneous rhythmic oscillations in auditory thalamus and AC

Previous electrophysiological studies on thalamus and neocortex during different states of vigilance revealed a variety of electrical fast and slow rhythmic oscillations which indicates that the cerebral cortex is constantly active even during sleep (Steriade et al., 1991; Steriade et al., 1993a; Steriade et al., 1994; Contreras and Steriade, 1995; Amzica and Steriade, 1995a, , 1995b; Contreras and Steriade, 1996; Steriade and Amzica, 1996; Timofeev and Steriade, 1996; Steriade et al., 1996a; Timofeev and Steriade, 1997). Three slow rhythmic oscillations (Spindle: 7-15 Hz, see Figure 9, 10; Delta: 1-4 Hz and Slow oscillation: 0.3-1.0 Hz, see Figure 10) could be seen during the slow wave sleep (SWS), and two fast rhythmic oscillations (Beta: 15-30 Hz; Gamma: 30-60 Hz, see Figure 10) were discovered during the active states of waking and rapid-eye-movement (REM) sleep. Neuronal activities measured using electroencephalography (EEG) recording shift from the low amplitude, high frequency rhythmic oscillations to the large amplitude slow ones when the state of the brain changes from wakefulness to sleep. These rhythmic oscillations may play an important role in highly integrative brain functions, e.g., consciousness, attentive perception, plasticity and memory.

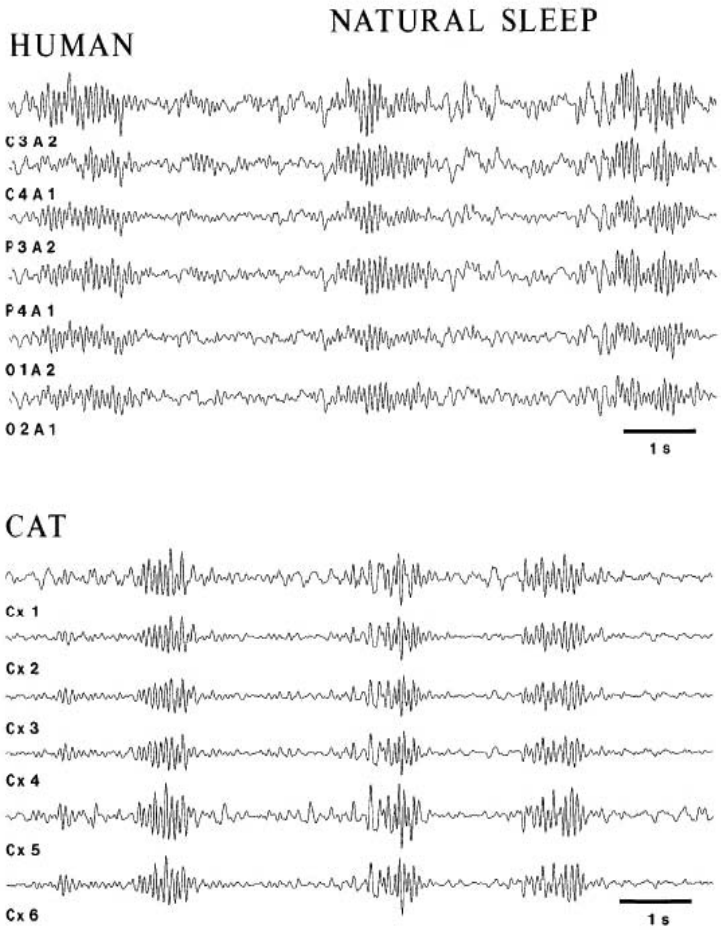

Figure 9. Cortical spindle waves during natural sleep of humans and cats (Contreras et al., 1997).

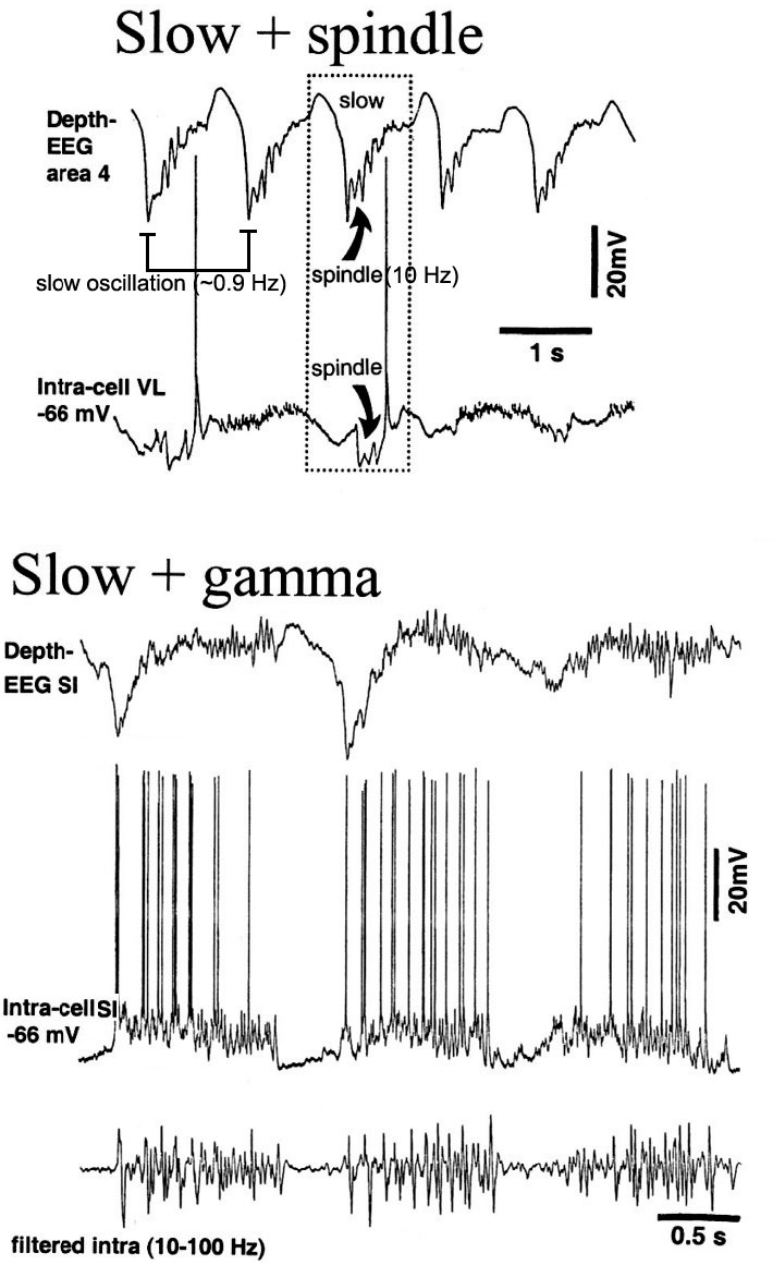

Figure 10. Coalescence of slow oscillation with spindle and gamma rhythms recorded from cortical and thalamic neurons in cats intracellularly. The spindle (upper) and gamma rhythms (bottom) happened during the depolarizing phase of the slow oscillations (Steriade, 2006).

SWS, characterized by the synchronized low frequency rhythmic oscillations less than 15 Hz, used to be thought as a mark for the global inhibition of the cerebral cortex. This view has been changed by recent studies using intracellular

recordings in naturally sleeping animals. Unexpectedly high levels of spontaneous neuronal activities were discovered during SWS indicating that the intracortical dialog is maintained and even increased. These rhythmic neuronal activities serve important cerebral functions such as the consolidation of memory traces acquired during wakefulness even when the thalamic gates for outside signals are closed (Timofeev and Steriade, 1996; Steriade and Timofeev, 2003).

The synchronized fast rhythmic oscillations within the beta/gamma frequency band (20-60 Hz) replace the slow rhythms during the brain active states of wakefulness and REM sleep (Steriade et al., 1991; Steriade et al., 1996a; Steriade et al., 1996b). Fast rhythmic oscillations are also found on the depolarizing components of the slow (< 1 Hz) oscillation (Steriade and Amzica, 1996; Steriade et al., 1996a). The fast rhythmic oscillations, having distinct spatial and temporal features compared with the low frequency oscillations, are synchronized among cortical areas and with related thalamic nuclei. These results demonstrate that different degrees of synchronization happen among neurons during various functional states. Spontaneous brain rhythmic oscillations may lead to responsiveness increase and plastic changes in the strength of synaptic connections among neurons, through which information is stored (Steriade and Timofeev, 2003).

The origin of the spontaneous rhythmic oscillations has been well studied by many neuroscientists (Steriade et al., 1993b; Steriade et al., 1994; McCormick and Bal, 1997). In general, spindle oscillation is generated largely through a cyclical

interaction between the TC neurons in MGB and neurons in the thalamic reticular nucleus (TRN). During the process of spindle generation, both the intrinsic membrane properties and interconnections of TC and TRN neurons are involved. Neurotransmitters, released from the brainstem, hypothalamus, basal forebrain, and cerebral cortex, induce a depolarization of TC and TRN neurons and enhance the excitability in many cortical pyramidal cells. Thus the sleep rhythms are suppressed and a state conducive to sensory processing and cognition is induced (McCormick and Bal, 1997).

2.4.2 Evoked rhythmic oscillations in auditory thalamus and auditory cortex

Neuronal firing rate, both at single cell and map level, used to be considered as the unique code for the transmission of information within the CNS since the early sixties. That is, the level of action potentials (APs) firing to a stimulus represents the efficiency of information processing. It is widely accepted now that neuronal firing rate is not the only factor encoding the information in the CNS. Temporal coding can mean that the onset of the APs and/or the interspike intervals give an accurate representation of a stimulus at the single cell level. As to small cell assemblies, it can be expressed by the short-time scale coordinations of neuronal discharges. At the large populations of cells level, synchronization of neuronal populations responding to different parameters of a stimulus can be involved in the neural code. Neuronal rhythmic oscillations can synchronize large cell populations and can be triggered by acoustic stimuli which suggests that the rhythmic oscillations may play an important role in temporal coding and integration of multiple sensations (Kreiter and Singer, 1996; Engel et al., 2001). In this section, we focus on the evoked rhythmic oscillations in the auditory TC system.

Using multiunit or single unit recordings, long latency oscillations of about 10 Hz were observed in the AC evoked by tones (Sally and Kelly, 1988; Maldonado and Gerstein, 1996; Cotillon and Edeline, 2000) or trains of clicks (Eggermont, 1992) under various anesthetic conditions (urethane, ketamine, pentobarbital).

The rhythmic oscillations of 8-12 Hz were found in the auditory thalamus (Bordi and LeDoux, 1994a; Cotillon and Edeline, 2000) especially in the auditory sector of TRN (Shosaku and Sumitomo, 1983; Cotillon and Edeline, 2000). Both stimulus-locked and non stimulus-locked oscillations were detected under anesthesia and the stimulus-locked ones were prominent. Figure 11 provides examples of multiunit recordings showing oscillations under two kinds of anesthetics.

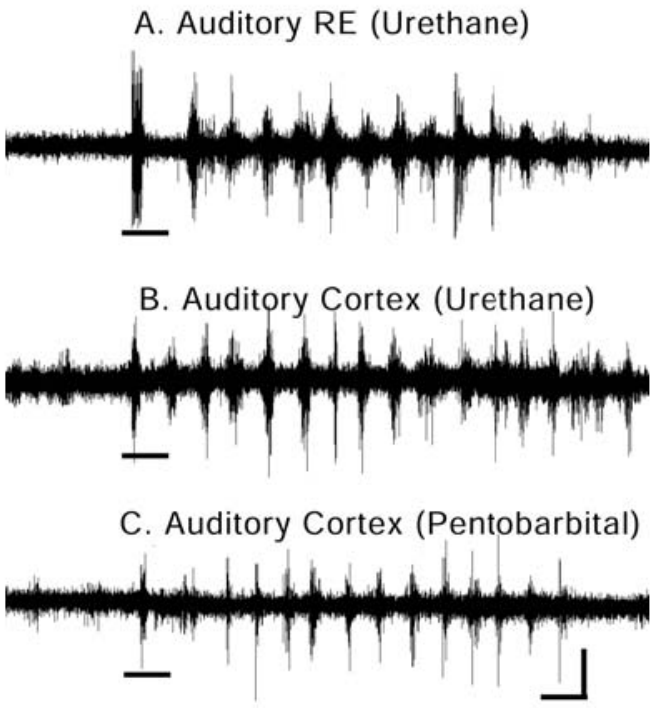

Figure 11. Examples of oscillations recorded from the auditory sector of the TRN (**A**) and the AC (**B** and **C**) in rat under urethane anesthesia (**A** and **B**) and pentobarbital anesthesia (**C**). The "on" tone-evoked response (bars of 100 ms below the traces) is followed by rhythmic oscillations for several hundreds of milliseconds. Scale bars: 100 ms, 150 μV (Cotillon and Edeline, 2000).

The origins of stimulus-evoked oscillations remain still largely unexplored. By inactivating each component of the TC loop, Cotillon and Edeline (2000) have looked for the rhythmic oscillations' origins. Muscimol (0.1 or 0.05 μl), a $GABA_A$ agonist having effects lasting up to several hours (Hikosaka and Wurtz, 1985a, , 1985b), was injected to perform the inactivation. After the inactivation of the auditory sector of the RTN, the stimulus-evoked oscillations in the auditory thalamus and AC were totally abolished. However, inactivation of AC did not affect the pattern of oscillations in the auditory sector of the RTN and inactivation of the auditory thalamus suppressed the oscillations in the auditory sector of the RTN evoked by electrical stimulation of AC (Figure 12) (Cotillon and Edeline, 2000). The stimulus-locked oscillations detected in three different nuclei of the TC loop results from the interactions between auditory thalamus and auditory sector of RTN which suggests that these low-frequency evoked oscillations share some functional properties with spontaneous spindle waves (Steriade et al., 1993b; Steriade et al., 1994; McCormick and Bal, 1997).

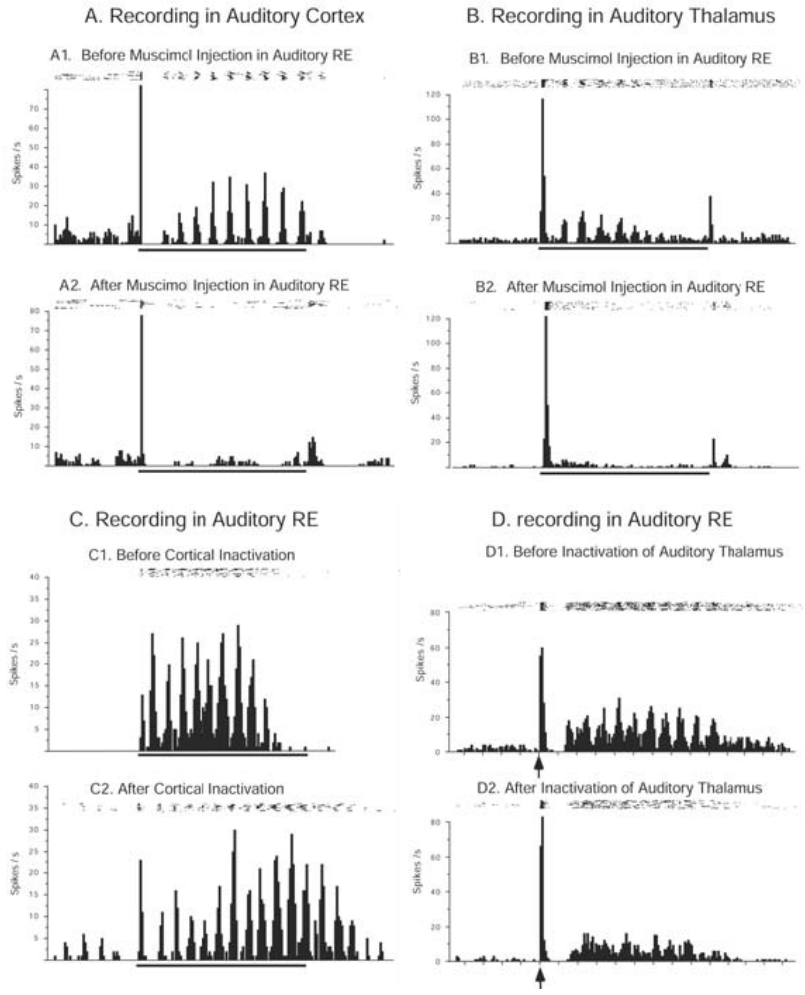

Figure 12. Origins of the stimulus-locked oscillations. **A**, **B**: Effect of auditory sector of the RTN inactivation. **Control**: oscillations were recorded simultaneously from AC and auditory thalamus (**A1**, **B1**). After inactivation of the auditory sector of the RTN by 0.05 µl of muscimol, the oscillations totally disappeared. **C**: Effect of AC inactivation: oscillations, detected from recordings in the auditory sector of the RTN, were still present after inactivation of AC. **D**: Effect of auditory thalamus inactivation: oscillations, triggered in the auditory sector of the RTN by cortical electrical stimulation disappeared after inactivation of auditory thalamus (Cotillon and Edeline, 2000).

2.5 Functional implications

The anatomical and physiological differences between MGm and MGv have been long recognized, however, the difference in their modulatory functions on different auditory cortical layers is yet to be investigated.

As the major part of the auditory lemniscal TC pathway, MGv is believed to carry fast, auditory specific input and peripheral auditory tonotopic maps to the AI (Hu, 2003). Recent anatomical studies (Huang and Winer, 2000; Lee and Winer, 2008a) showed that axons of MGv neurons ended in more than four auditory cortical layers which indicates that the MGv may have more complicated modulatory effects rather than a relay station in the central auditory acsending pathway.

MGm has extensive axonal projections to AC including its association cortex (Winer et al., 1977; Niimi and Matsuoka, 1979; Andersen et al., 1980; Imig and Morel, 1983; Huang and Winer, 2000; He et al., 2002; Lee and Winer, 2008a) and to the somotosensory cortex, prefrontal cortex, amygdala and basal ganglia (Wepsic and Sutin, 1964; Russchen, 1982; LeDoux et al., 1990; Shinonaga et al., 1994; Cruikshank et al., 2002; Kimura et al., 2003), MGm is involved in neuronal plasticity during auditory fear condition (Quirk et al., 1997; Duvel et al., 2001; Maren et al., 2001; Doyere et al., 2003). Learning (neuronal plasticity) under a fear condition is possibly associated with an increased alertness of AC and amygdala. As the axons of MGm neurons project widely to the cortex (Huang and

Winer, 2000; Lee and Winer, 2008a), the direct modulatory effect of MGm is likely to have a broad and general effect on the AC and/or other cortex. The direct projections from DCN to MGm bypassing IC mediated the short latency responses of MGm neurons to the acoustic stimuli (Anderson et al., 2006) which may induce short latency responses in the AC to prepare it for rapid analysis and recruit the amygdala for rapid emotional responses such as fear.

In our pilot extracellular recording results from pentobarbital sodium (Nembutal, Abott, 35 mg kg^{-1}, I.P.) anesthetized guinea pigs, mainly inhibitory effects in the middle cortical layer neurons to the noise stimuli were recorded when electrical stimulation was applied to the MGm (Figure 13). The MGm has majorly modulatory effects.

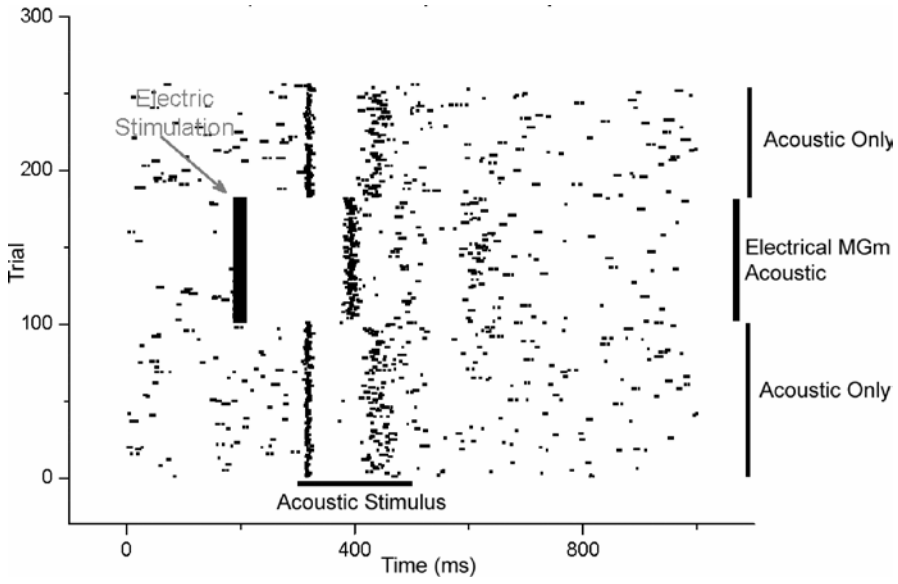

Figure 13. Responses of a deep layer (600 μm) neuron of AI recorded extracellularly to electrical stimulation of MGm and an acoustic stumulus. The first 100 and the last 80 trials of the raster display show the neuronal response to an acoustic stimulus before and after the middle 80 trials which is showing the

response to the combination of electrical stimulation of MGm and an acoustic stimulus with 100 ms interval between them. The neuron is totally inhibited for about 200 ms after the MGm was stimulated. The latency of auditory response is lengthened for about 100 ms. (Xu et al., unpublished result).

Many valuable studies have revealed that neurons from different MGB divisions with various firing patterns and tuning properties form the parallel lemniscal and non-lemniscal TC pathways involved in different cortical functions (Calford and Aitkin, 1983; Hu, 1995; Tennigkeit et al., 1996; He et al., 2002; Hu, 2003). Sukov and Barth (2001) found that MGB neurons may play a role in adjusting the general responsiveness of AC in addition to forwarding auditory information to the cortex (Sukov and Barth, 2001). Laminar difference of the acoustic responses in AI of urethane anaesthetized guinea pigs indicated that there are separate inputs to different cortical layers and the outputs from layers V/VI and layers II/III are different (Wallace and Palmer, 2008). Further studies on TC projection effects of different MGB pathways may offer more convincing evidence.

In this study, we pay special attention to the MGm which has some unique characteristics compared with the MGv and MGd (Smith et al., 2006) such as having many resident cell types (Winer and Morest, 1983), little topographic inputs from brainstem (Calford and Aitkin, 1983) even inputs directly from the DCN (Anderson et al., 2006), poorly tonotopic organized (Rouiller et al., 1989) and significant axonal projections to non-auditory cortical areas (Shinonaga et al., 1994) and so on. The largest TC axons arise from MGm neurons terminate in

cortical layer I (Huang and Winer, 2000). Layer I with GABAergic neurons has synaptic connections with the dendrites of neurons in the other 5 cortical layers (Mitani et al., 1985; Huang and Winer, 2000). Some progress in understanding the auditory TC transmission has been made using in vivo (Metherate and Ashe, 1993; Sukov and Barth, 2001) and in vitro (Cruikshank et al., 2002; Rose and Metherate, 2005) intracellular recording techniques, but little is known about the cellular and synaptic mechanisms by which the thalamic inputs are transmitted to and processed in the AC and the modulation of MGv and MGm on the AC.

In the present study, we adopt the *in vivo* intracellular recording combined with the multiple extracellular recording techniques to explore the modulation of MGm and MGv on different cortical layers in guinea pig AC, especially in AI.

Chapter 3

Methodology

The methods employed in this thesis consist of observational recordings and experimental designs. This chapter illustrates animal model, surgical procedures, recording techniques, electrical and acoustic stimulation, histological skills and analytical processes.

3.1 Animal preparation

Hartley albino guinea pigs of either sex, *Cavia porcellus*, from 400 to 700 g, provided by central animal facilities, the Hong Kong Polytechnic University, served as the subject (Figure 14). To reduce bronchial secretions, atropine sulfate (0.06 mg/kg, s.c, Sigma, U.S.A.) was administered at the start of the experiment. Anesthesia was initially induced with Urethane (1.3 g/kg, in 20% solution in 0.9% saline, ip, Sigma, U.S.A.) and maintained by supplemental doses of the same anesthetic (0.2 g/kg/hr) during the surgical preparation and physiological recording on indication by pedal withdrawal reflex. The subject was mounted in a stereotaxic device following the induction of anesthesia. A midline incision was made in the scalp and a craniotomy was performed to enable us to implant stimulation electrodes into the MGB and access vertically to the surface of the AC

on the right hemisphere of the brain (He, 2002; He et al., 2002; He, 2003c; Xiong et al., 2004). Cerebrospinal fluid was released through the foramen magnum to reduce the vibration of the brain during the process of physiological recording. Artificial respiration was applied to the animal, muscles were relaxed after administration of gallamine triethiodide (50 mg/kg initially 10 mg/kg/hr regularly, i.p.), the animal's chest was opened bilaterally, and its body was suspended to reduce the vibrations to the brain caused by intra-thoracic pressure. Core temperature was maintained at 37.6-38.5 °C via a heating blanket and rectal probe. Animal Subjects Ethics Sub-Committee of the Hong Kong Polytechnic University approved the experimental protocols.

Figure 14. Hartley albino guinea pig served as subjects in this study.

3.2 Acoustic stimuli

The subject was placed in a double-walled soundproof room (NAP, Clayton, Australia). Acoustic stimuli were generated digitally by a TDT system (System III; Tucker-Davies Technologies, U.S.A.) and delivered to the subject contralateral to the recorded AI via a dynamic earphone (Bayer DT-48) mounted in a probe.

Repeated white noise bursts with a spectral band of 0-30 kHz, intervals of 2 s, 200 ms in width, 70-80 dB SPL and a 5 ms rise-fall time were used to examine the neuronal responses of the AC. Tympanic sound pressure levels (expressed in dB SPL in reference to 20 μPa root mean square) was calibrated over the white noise and a frequency range of 100 Hz to 35 kHz under the control of a computer by using a condenser microphone (Brüel and Kjær, 1/4 inch, Norcross, GA). The calibration was saved in the computer and used to compensate for the output intensity for each frequency (Semple and Kitzes, 1993).

3.3 Electrical stimulation

In our lab, mapping studies of the MGB were performed before this study. In the present study, we omitted the mapping procedure to save time for the intracellular and extracellular recordings.

A tungsten electrode array consisting of three parallel low impedance electrodes with a constant inter-electrode distance of 0.8 mm was implanted into the MGB targeting the MGm and MGv. Electrical stimuli of 0.2 ms in width, 50 or 200 Hz in frequency, and 1, 5, or 50 pulses were used to activate the different divisions of MGB (He, 1997, , 2002; He et al., 2002). Once the electrode array was implanted, the skull opening was covered with low-melting temperature paraffin (42-44 °C, Wako, Japan). Electrical currents of 50-200 μA delivered by an isolator were applied to the MGB ipsilateral to the recording hemisphere. After a delay interval of 20, 50 or 100 ms, a sound stimulus was delivered to the contralateral ear after the end of the electrical stimulation on some subjests. An interval of 10 s in between different experimental trials was set in order to allow the neurons to recover from habituation and from their modulated state.

To examine if the electrical stimulating current spread to the sites where the nearby stimulating electrode was implanted, we recorded the neuronal response from the other stimulating electrode when the electrical current of 50-200 μA was given to the stimulating electrode nearby in some animals.

3.4 Intracellular recording

We used a glass-pipette filled with 1.0 M KCl (potassium chloride, Sigma, U.S.A.) or 3.0 M KAc (potassium acetate, Sigma, U.S.A.) to record neuronal activities of the AI neurons intracellularly according to the map obtained from previous work (see Figure 15) (Wallace et al., 2000).

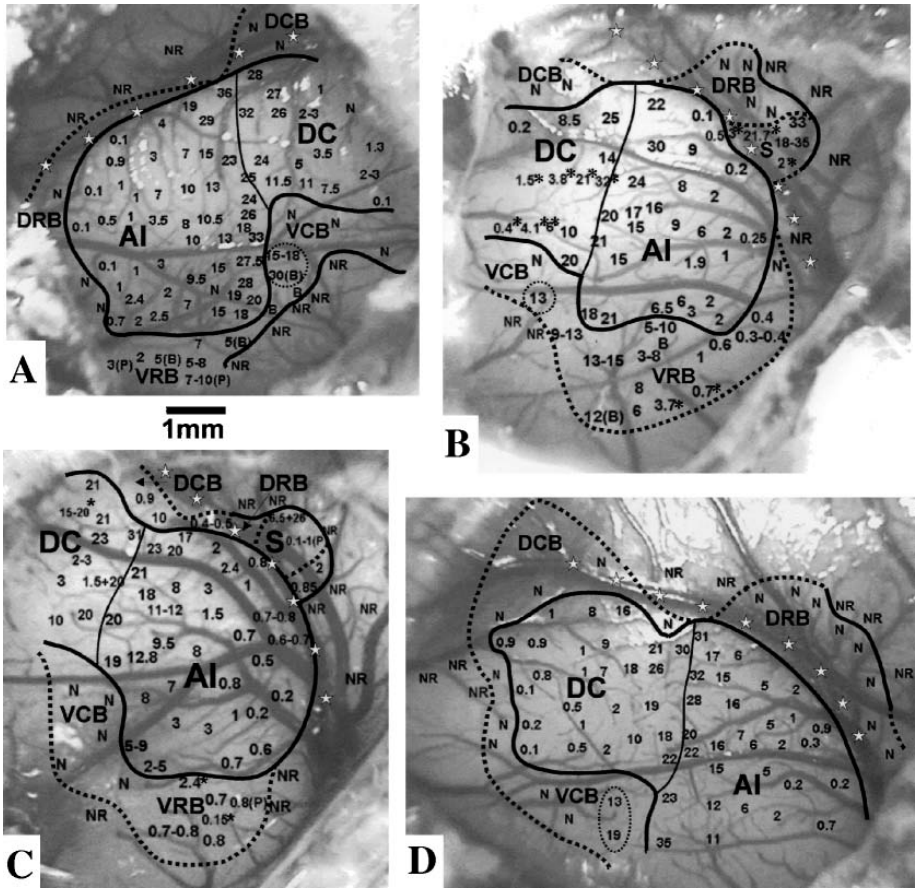

Figure 15. Superimposed maps of cortex obtained by evoked potential recordings. The *numbers* represent the characteristic frequency (CF) in kHz and those with *asterisks* indicate where a single unit or multi-unit was recorded. *Large white stars* indicate the position of pseudosylvian sulcus. **A:** Left cortex; **B-D:** right cortex (*N* stronger response to noise than pure tones, *NR* no response to noise or tones, *P* poor response) (Wallace et al., 2000).

The resistance of the intracellular recording electrode after filled with 1.0 M KCl or 3.0 M KAc was between 40 and 90 MΩ. The electrode was advanced vertically to the surface of the brain by the stepping motor (Narishige, PC-5N, Japan). The cortical exposure was sealed using low-melting temperature paraffin (42-44 °C, Wako, Japan) after the electrode was lowered into the surface of the brain. When the electrode was near or in the targeted area, it was slowly advanced at 1 or 2 μm steps. Only those neurons with a resting membrane potential lower than -50 mV and spikes that overshot the baseline were analyzed in the present study.

3.5 Extracellular recording

Together with the intracellular recording, multiple extracellular recordings were performed in the present study.

For extracellular recording, a tungsten electrode array consisting 4 electrodes with a fixed vertical tips offset of 250 μm and impedance of 2.0-4.0 MΩ (FHC, U.S.A.) was used to record the neuronal activities in different AC layers simultaneously.

After the physiological extracellular study, an electrical current of 0.1 mA was applied to one of the recording electrodes to lesion the tissue of the location recorded then the recording sites were confirmed morphologically using Nissl materials.

3.6 Anatomical confirmation

The intracellular recording pipette was filled with Neurobiotin™ (Vector Laboratories, Burlingame, CA, U.S.A. 1-2% in 1.0 M KCl or 3.0 M KAc) and the tracer was injected into 1 or 2 neurons in each subject after the physiological recordings. The tracer was delivered into the neuron recorded intracellularly by passing rectangular depolarizing current pulses (150 ms, 3.3 Hz, 2 nA) for 1-5 min using a function generator (Leader, Japan).

The position of the extracellular recording and electrical stimulating electrodes was marked via electrical lesions by applying 0.1 mA electrical current. The tracks and lesions were both used to confirm the location of electrodes.

The subject was deeply anesthetized with an overdose of urethane and perfused transcardially with 0.9% NaCl, followed by a mixture of cold 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The brain was quickly removed from the skull and post-fixed overnight in the same fixative. It was cryoprotected in 30% sucrose in PB (0.1 M, pH 7.4) for 2 days at 4 °C.

Coronal sections (with a thickness of 60 μm) of thalami and/or cortices were cut with a freezing microtome. 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 hrs at room temperature.

After the detection of peroxidase activity with 3', 3'-diaminobenzidine (DAB), sections were mounted on gelatin-coated slides and examined under the microscope. Those sections containing labeled neurons and stimulation tracks were counterstained with Nissl and photographed.

3.7 Criteria to define the cortical layers of AI

According to Wallace and Palmer's recent work (2008), the ventral borders of the layers in AI after correcting for shrinkage (10% during tissue processing) were: layer I, 240 μm (9.8%); layer II, 520 μm (21.2%); layer III, 800 μm (32.3%); layer IV, 1100 μm (44.9%); layer V, 1710 μm (69.8%) and layer VI, 2450 μm (100%). These measurements were taken at the high frequency end of AI in guinea pigs weighing 400-500 g. In the present study, we used their layer segregation as one of the criteria to judge which auditory cortical layer a neuron recorded intracellularly or extracellularly belong to.

Another criterion was referring the previous data using thionin staining technique (Figure 16). Briefly, there are very few cell bodies in the auditory cortical layer I. Layers II and III contain densely packed cells, many of which are pyramidal neurons. It is hard to distinguish between layers II and III. Layer IV is dominated by granule cells, which are smaller and rounder and packed more closely than the cells in adjacent layers III and V. Layer V is characterized by a low density of cells, most of which are pyramidal. Compared with layer V, Layer VI has higher density and smaller cells.

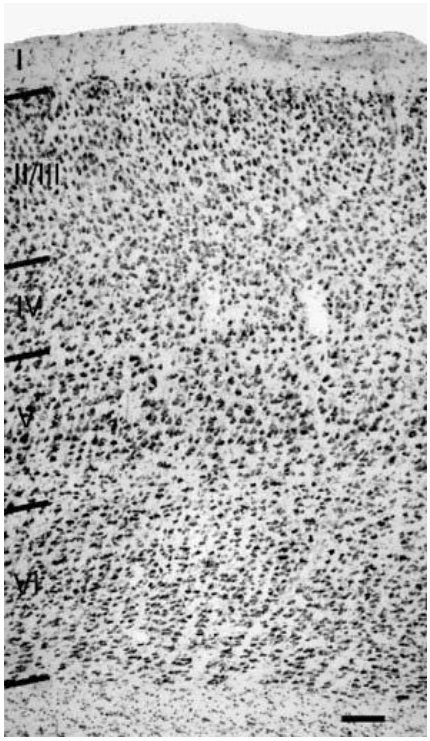

Figure 16. Digital photomicrograph of a thionin-stained section through AI showing laminar borders in guinea pig. Scale bar: 100 mm.

3.8 Data acquisition and analysis

After amplification, the intracellular and extracellular signals with artifacts of electrical stimulation and auditory stimulus signals were stored in the computer with the aid of commercial software (AxoScope, Axon Instruments, Foster City, CA, U.S.A.). No manipulations of membrane potentials of the neurons were made unless specifically stated. The neuronal activities were digitized by using Axon Digidata 1322A (Axon Instruments, Foster City, CA, U.S.A.) and the sampling rate was 20 kHz.

The latency of the EPSP/IPSP or spike was defined as the period between the onset of stimulation and the onset of the evoked amplitude. Meanwhile the amplitudes of EPSPs/IPSPs were calculated as the change to the membrane potential evoked by acoustic or electrical stimulation. The durations of EPSPs/IPSPs were calculated from their onset to offset.

Numerical results are expressed as mean ± standard deviation (S.D.). Comparison between the latency, duration and spike numbers was made using analysis of variance (ANOVA). Night-five percent confidence was set as statistically significant.

The frequency of the oscillatory neuronal activities was calculated by using the power spectrum function of the software Clampfit 9.0 (AxoScope, Axon Instruments, Foster City, CA, U.S.A.).

Chapter 4

Results

Modulation of the thalamocortical projections on different layers of auditory cortex in guinea pigs

We recorded one hundred and eighty nine AC neurons (intracellularly and/or extracellularly) to study their spontaneous neuronal firing patterns and responses to acoustic stimulation. Most AC neurons showed spontaneous neuronal activity independent of any acoustic stimulus. Thirty-one AC neurons responded to acoustic stimulation by showing EPSPs, APs, and/or rhythmic membrane oscillations (n=40 intracellularly recorded neurons). One hundred thirty out of 149 extracellularly recorded AC units showed spikes in response to the acoustic stimuli.

Recorded neurons were located in all six auditory cortical layers: 1) Five intracelluarly recorded neurons and 16 extracellularly recorded units resided in layer I; all but one extracellularly recorded unit showed a response to the acoustic stimuli; 2) Six of ten intracellularly recorded neurons and twenty of 22 extracellularly recorded units in layer II had acoustic responses; 3) In layer III, three of 14 intracelluarly recorded neurons and six of 42 extracellularly recorded units had no acoustic responses; 4) Three intracelluar recorded neurons and 17 extracellularly recorded units were located in layer IV, and only one extracellularly recorded unit had no acoustic response; 5) Two of three

intracellularly recorded neurons and 18 of 22 extracellularly recorded units in layer V had acoustic responses; and 6) In layer VI, four of five intracellularly recorded neurons and 25 of 30 extracellularly recorded units had acoustic responses.

To study the modulatory effect of TC projections arising from different divisions of the MGB on different layers of the AC in guinea pigs, we recorded the neuronal responses of 38 AC neurons to electrical stimulation of different divisions of MGB. Among them, thirteen intracellularly recorded neurons were located in layers I-IV (three neurons in layer I, five in layer II, three in layer III and two in layer IV) and twenty five extracellularly recorded units were located in all six cortical layers, five units in layer I, seven in layer II, ten in layer III, and one in each of layers IV, V and VI.

In this study, only AC neurons recorded intracellularly or extracellularly, with anatomical confirmation of the stimulation sites in the MGB, were sampled.

4.1 Acoustic responses of AC neurons during intracellular and extracellular recording

The resting MPs of the 40 intracellularly recorded AC neurons in this study were -65.45 ± 8.76 mV, ranging from -45.8 mV to -84.5 mV. The MGB neurons' resting MPs recorded in the previous study in our lab were -59.3 ± 5.5 mV and -57.8 ± 4.0 mV for those having EPSP and IPSP responses to the acoustic stimuli respectively (Yu et al., 2004). There were no significant differences among the resting MPs of neurons in the six auditory cortical layers (layer I: -70.14 ± 12.17 mV, layer II: -65.30 ± 6.89 mV, layer III: -64.71 ± 7.87 mV, layer IV: -67.83 ± 13.02 mV, layer V: -69.67 ± 2.27 mV and layer VI: -59.16 ± 10.74 mV. $P > 0.05$). The intracellularly recorded AC neurons were found in all six auditory cortical layers: five neurons were in cortical layer I, ten in layer II, fourteen in layer III, three in layer IV, three in layer V, and five in layer VI.

Among these 40 intracellularly recorded auditory cortical neurons, 31 neurons responded to an acoustic stimuli applied to the contralateral ear of the animals. The acoustic responses of intracellularly recorded neurons in AC were EPSPs, APs and/or rhythmic membrane oscillations.

In the present study, we also used a matrix metal electrode array to record the auditory cortical neuronal activity extracellularly or simultaneously with the intracellular recording. The extracellularly recorded AC neuronal units always

showed spikes (APs) synchronized with the intracellularly recorded neuronal activity (for example: Figure 17).

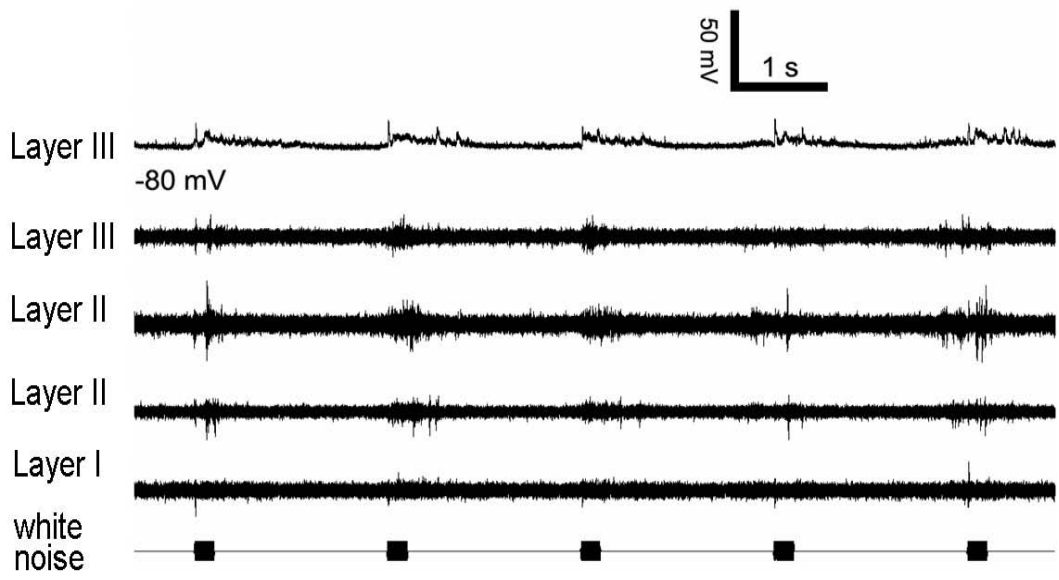

Figure 17. Acoustic responses of the AC neurons recorded intracellularly and extracellularly. *First panel:* the acoustic responses of an intracellularly recorded auditory cortical layer III neuron. EPSP followed by rhythmic oscillation was evoked by the acoustic stimuli. The *following four panels* are the acoustic responses of layers I-III units recorded extracellularly. Neuronal activities in different cortical layers were synchronized by the acoustic stimuli of white noise (*final panel*) applied to the contralaterial ear of the animal. These neurons also had spontaneous neuronal activities.

4.1.1 Latency of acoustic responses of AC neurons during intracellular and extracellular recording

Neurons in different auditory cortical layers had different response latencies to the acoustic stimuli. Twenty of 21 neurons recorded in AC layer I had acoustic responses. The latencies of these neurons could be divided into two types: long and short latencies. Fifteen layer I neurons, located in the entire layer I, had long latencies to the acoustic stimuli (12.32 ± 1.42 ms), and five other neurons, located in the upper half of layer I, had short latencies (7.32 ± 0.58 ms). In layer II, 26 of 32 neurons also had long (20 of 26 neurons, 12.56 ± 2.64 ms) or short (6 of 26 neurons, 8.42 ± 1.07 ms) latencies to the acoustic stimuli. Layer II neurons of long latencies were located in the entire layer II while those with short latencies in the lower sublayer of layer II. Forty seven of 56 layer III neurons recorded in this study had acoustic response latencies of 10.39 ± 2.77 ms while 19 of 20 layer IV neurons responded to the acoustic stimuli with latency of 9.40 ± 1.18ms. The layer V neurons (20 of 25) had relatively long acoustic response latencies of 11.91 ± 2.57 ms, and layer VI neurons (29 of 35) had relatively short latencies (9.89 ± 1.72 ms). To sum up, responding to the acoustic stimuli applied to the contralaterial ear, some neurons in the upper sublayer I and the lower sublayer II responded firstly, then layers IV, III, VI, followed by layers I, II, and V ($P < 0.05$).

In different animals in this study, the sequences of the auditory responses appearing among the auditory cortical layers were sometimes slightly different. Figure 18 shows that the six neurons located in six different AI layers were recorded extracellularly during one penetration of a single metal electrode in one animal. The acoustic responses of these six extracellular recorded units were of similar neuronal firing patterns and slight latency differences: layer VI started first, then layers III and V followed by layers I, II, and IV.

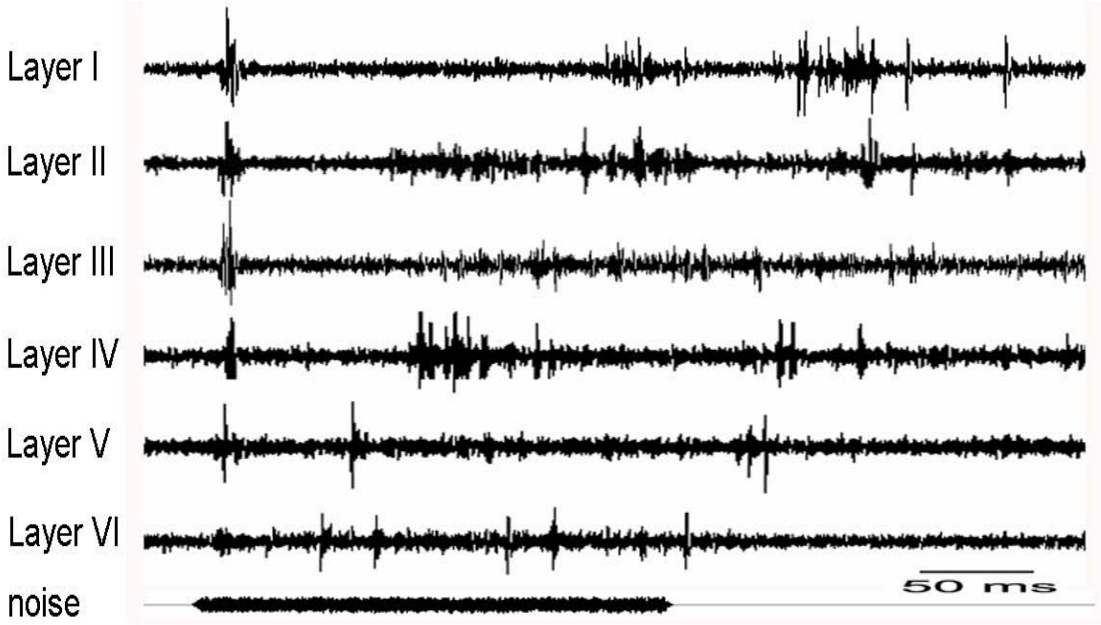

Figure 18. Acoustic responses in six cortical layers of AI recorded extracellularly within one penetration of the electrode on the same subject. The auditory responses appeared at layer VI before layers III and V, and layers I, II and IV responded finally. The firing patterns of six units in different layers were also slightly different.

4.1.2 Range of EPSP and number of spikes while responding to acoustic stimuli

The duration and amplitude of acoustic stimuli evoked onset EPSPs in intracellularly recorded AC neurons varied according to the resting MPs of the neurons. The durations of the acoustic stimuli evoked EPSPs were 28.58 ± 13.72 ms, while the amplitudes were 9.89 ± 4.06 mV. No significant difference was found in the duration and amplitude of EPSPs evoked by the acoustic stimuli on neurons in different auditory cortical layers.

The variation of the number of the onset APs evoked by the acoustic stimuli was relatively large, from one to four in the intracellularly recorded neurons. On some intracellularly recorded neurons there were one to nine APs on the membrane oscillations following the onset EPSPs/APs. For the AC neuronal units recorded extracellularly, one to eight acoustic stimuli spikes were evoked. However, there was no significant difference in the number of onset APs/spikes evoked by acoustic stimuli on neurons in different cortical layers.

4.1.3 Firing patterns of acoustic responses on neurons in different auditory cortical layers

The intracellularly recorded neurons in AC responded to the acoustic stimuli by showing onset EPSPs, APs and/or rhythmic membrane oscillations while the extracellularly recorded AC units showed spikes (APs) synchronized with the intracellularly recorded neuronal activities (for examples: Figures 17 and 18). Neurons in different auditory cortical layers had similar firing patterns to acoustic stimuli.

The frequencies of rhythmic membrane oscillations following the onset EPSPs/APs on the intracellular recorded neurons evoked by the acoustic stimuli were 14.77 ± 5.26 Hz. The latencies of the evoked rhythmic oscillations were 75.37 ± 26.15 ms with two neurons having shorter latencies (11.88 ms and 15.55 ms respectively). Neurons having acoustic evoked membrane oscillations were distributed in all cortical layers except layer V.

4.2 Responses to thalamic electrical stimulation

Neuronal responses of 13 intracellularly recorded neurons and 25 extracellularly recorded units in AC, primarily in AI, to the electrical stimulation applied in different divisions of MGB (MGv and MGm) were tested. Only auditory cortical neurons recorded from animals with anatomical confirmation of electrical stimulation sites in the MGB are reported. These AC neurons were located in all six cortical layers.

Nineteen of 38 neuronal responses to electrical stimulation of MGv and MGm were tested, while in 14 neurons, response to electrical stimulation of MGm only was tested. In five neurons, we tested the response to electrical stimulation of MGv only. Auditory cortical neuronal activity was mostly affected by electrical stimulation applied in both MGB divisions respectively. The modulatory effects of the TC projections were predominantly excitatory. On some AC neurons, spontaneous neuronal activities were inhibited or changed from tonic discharging to rhythmic oscillation by electrical stimulation of MGm. Meanwhile, AC neurons in different cortical layers received different modulatory effects from different divisions of MGB. In this section, we will discuss the modulatory effects of MGB divisions on AC neurons in different cortical layers.

On some animals, we used acoustic stimuli 20, 50 or 100 ms after thalamic electrical stimulation to test the effect of thalamic electrical stimulation on neuronal acoustic responses. When we applied acoustic stimuli to the animals 20

ms after the electrical stimulation of MGv or MGm, the acoustic responses were inhibited by the neuronal response induced by thalamic electrical stimulation (MGv: Figures 23 and 25; MGm: Figure 33). However, the acoustic responses still could be seen among the neuronal response induced by the thalamic electrical stimulation when the acoustic stimuli were applied to the animals 50 or 100 ms after the electrical stimulation of MGv or MGm (MGv: Figure 24; MGm: Figures 28 and 32).

4.2.1 Range of thalamic neurons excited by the stimulating current

To examine if electrical current spread to nearby regions, we recorded the neuronal activity from two stimulating electrodes when the electrical current was given to the middle stimulating electrode in some animals. The electrical stimulating current did not excite the neurons recorded by the nearby electrode (Figure 19), which indicates that the electrical stimulating current was limited to a single MGB division: MGv or MGm.

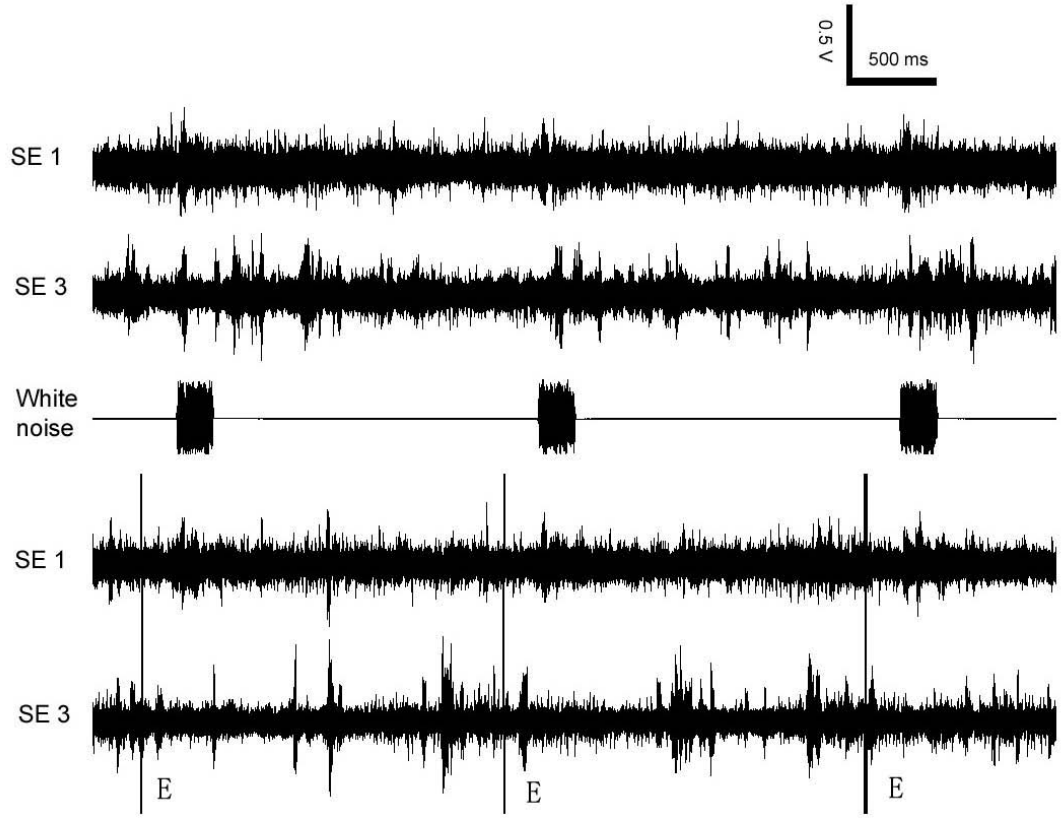

Figure 19. Effect of electrical stimulating current on the thalamic units recorded using nearby stimulating electrodes. **SE 1, 2 and 3** are an array of three stimulating electrodes inserted into MGB targeting different divisions with the distance between the electrodes of 0.8 mm. **E**: electrical stimulation. In this subject, SE 1 and 3 were used to record thalamic neuronal activity extracellularly while the stimulating current (50 - 200 μA) was given to the middle stimulating electrode SE 2. The stimulating current did not excite the units recorded by the electrode nearby.

4.2.2 Modulatory effect of electrical stimulation applied to MGv on AC

In the present study, nineteen AC neurons were recorded intracellularly or extracellularly when electrical stimulation was applied to MGv. Seven of them showed neuronal responses to the electrical stimulation of MGv (in detail: two of five in layer I; two of five in layer II; one of five in layer III; one of three in layer IV and one in layer VI). All responses to the stimulation of MGv were excitatory, including the onset EPSPs/APs and/or rhythmic oscillations.

In this section, we focus on the neuronal responses induced by the thalamic electrical stimulation in MGv. The effect of MGv stimulation on spontaneous neuronal activity of AC neurons will be described in the part 4.4.

4.2.2.1 Latency of the responses of AC neurons to electrical stimulation of MGv

Membrane oscillations in two layer I neurons (Nos. 1 and 2, Figure 20) and APs followed by rhythmic oscillations in each neuron in layers IV and VI (Nos. 18 and 20. Figures 24 and 25) induced by MGv stimulation, had shorter response latencies than the acoustic responses of these neurons. However, electrical stimulation of MGv could induce long delayed rhythmic oscillations in layers II and III neurons (Nos. 4 and 10, Figures 21 and 23). Meanwhile, one layer II neuron (No. 5. Figure 22) showed APs followed by rhythmic oscillations responding to MGv stimulation with similar latencies as its acoustic responses.

The latencies of neuronal responses to the electrical stimulation applied in MGv were different in different auditory cortical layers.

Layer II neurons (For example Neuron No. 4) had longer delayed rhythmic oscillations responding to MGv stimulation than layer III (for example Neuron No. 10. Latency: Neuron No. 4: 302.99 ± 19.95 ms and Neuron No. 10: 230.46 ± 15.74 ms. $P < 0.05$).

For the onset of short delayed responses, layer I neurons had longer latencies (Neuron No. 1: 7.77 ± 0.25 ms; Neuron No. 2: 7.03 ± 0.43 ms) than layers II, IV and VI neurons (No. 5: 5.74 ± 0.18 ms; No. 18: 5.48 ± 0.18 ms and No. 20: 6.22 ± 0.49 ms). The rhythmic oscillations following the onset of short delayed responses had similar latencies in layers II, IV and VI neurons (No. 5: 200.01 ± 22.38 ms; No. 18: 208.48 ± 10.18 ms and No. 20: 216.66 ± 18.74 ms. $P > 0.05$).

4.2.2.2 Firing patterns of the responses of AC neurons to electrical stimulation of MGv

Neuronal responses of AC neurons induced by MGv stimulation showed different firing patterns compared with their acoustic responses. Although AC neurons showed onset EPSPs/APs with or without rhythmic oscillations to the acoustic stimuli, they always showed onset EPSPs/APs followed by rhythmic oscillations to MGv stimulation. Thalamic stimulation induced responses had one more obvious silent component between the onset EPSPs/APs and the following rhythmic oscillations than the acoustic responses (for samples: see Figures 21-25). Unlike the neurons in other auditory cortical layers that had similar firing patterns, layer I neurons only had rhythmic oscillations without any EPSPs/APs responding to MGv stimulation (Figure 20).

The range of onset EPSPs and the number of the onset APs induced by MGv stimulation and acoustic stimuli were similar (see Figures 20-25).

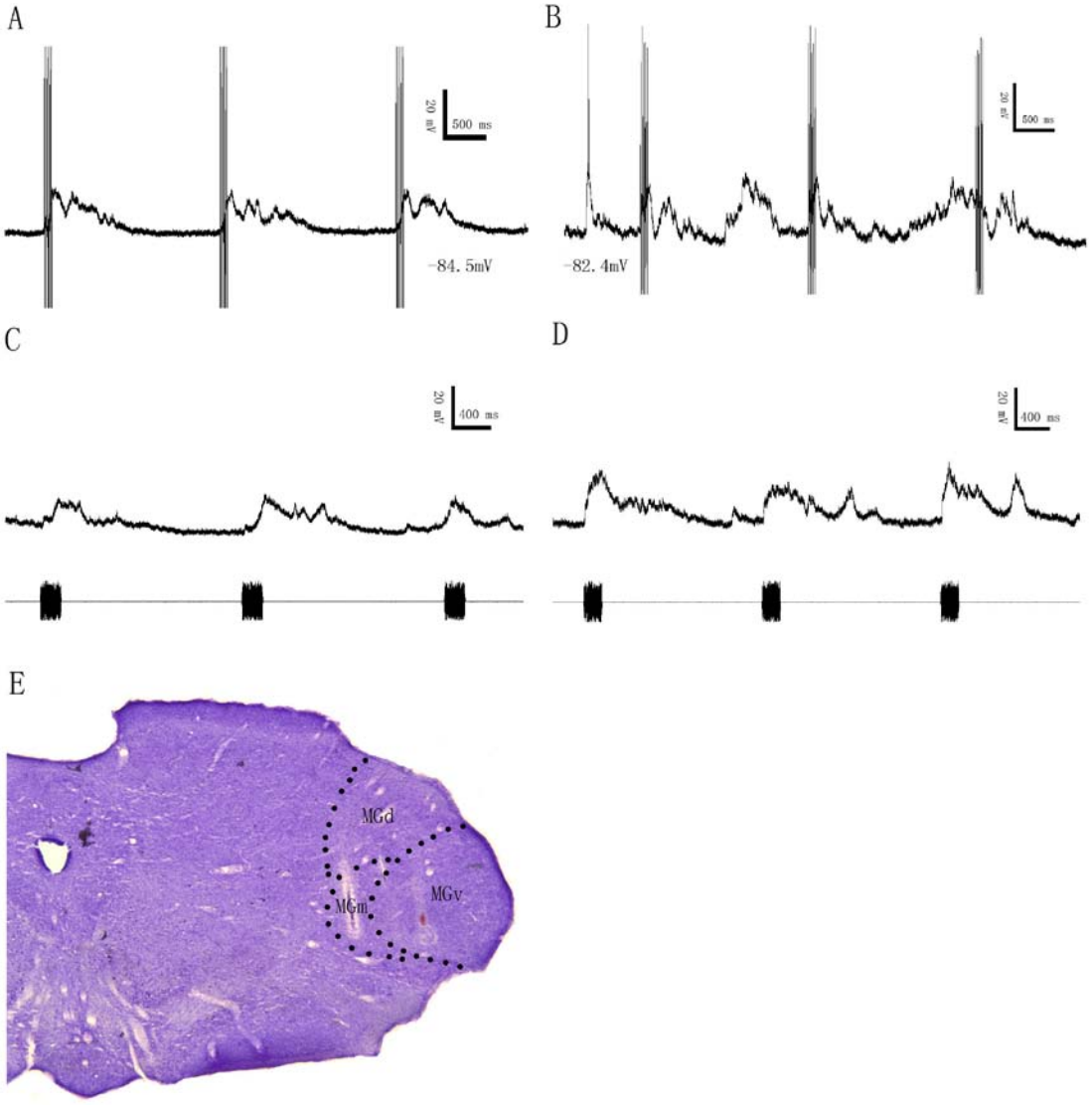

Figure 20. Comparison of membrane oscillations evoked by acoustic stimuli and electrical stimulating MGv. A, C: from neuron No. 1 and B, D: from neuron No. 2. Both neurons were located in layer I on the same subject. A, B: the membrane oscillations induced by electrical stimulating MGv and C, D: the membrane oscillations evoked by acoustic stimuli. The electrical stimulation induced oscillations had more and obvious silent components and shorter latencies than the acoustic stimuli evoked oscillations. E: Anatomical confirmation of electrical stimulating sites in MGB.

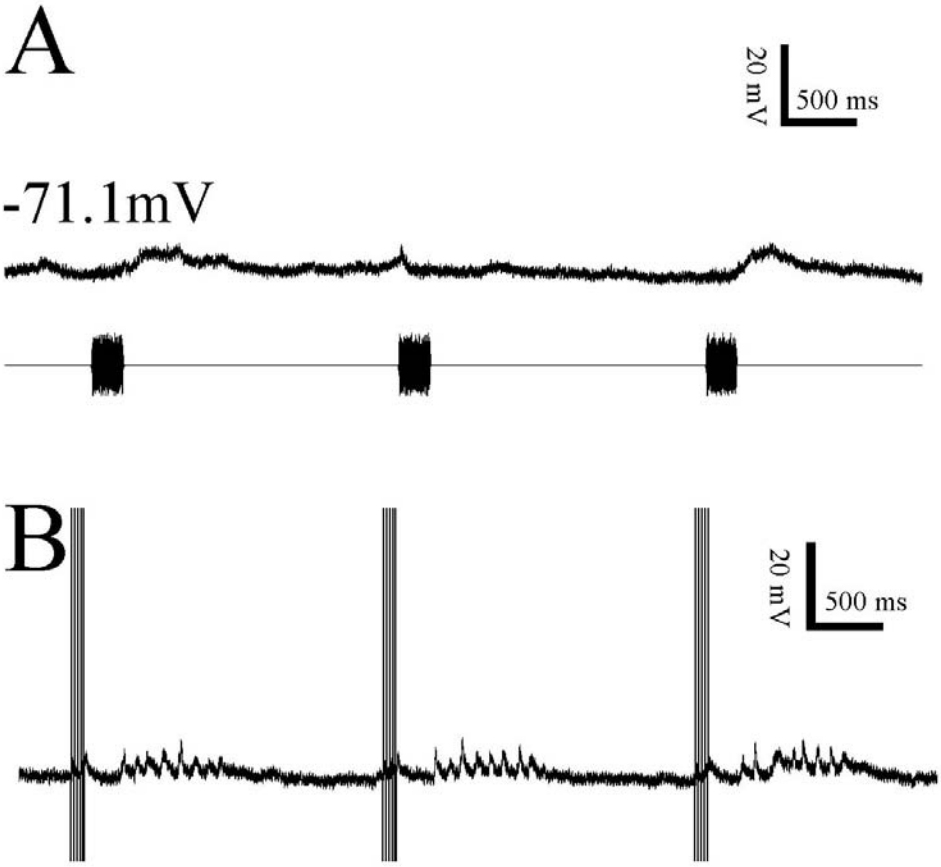

Figure 21. Membrane oscillations induced by electrical stimulating MGv on layer II neuron (No. 4). A: Neuronal activities when the acoustic stimuli were given to the subject. There was no acoustic response. B: Membrane oscillations induced by electrical stimulating MGv. Anatomical confirmation of electrical stimulating sites in MGB: see **Figure 20 E**.

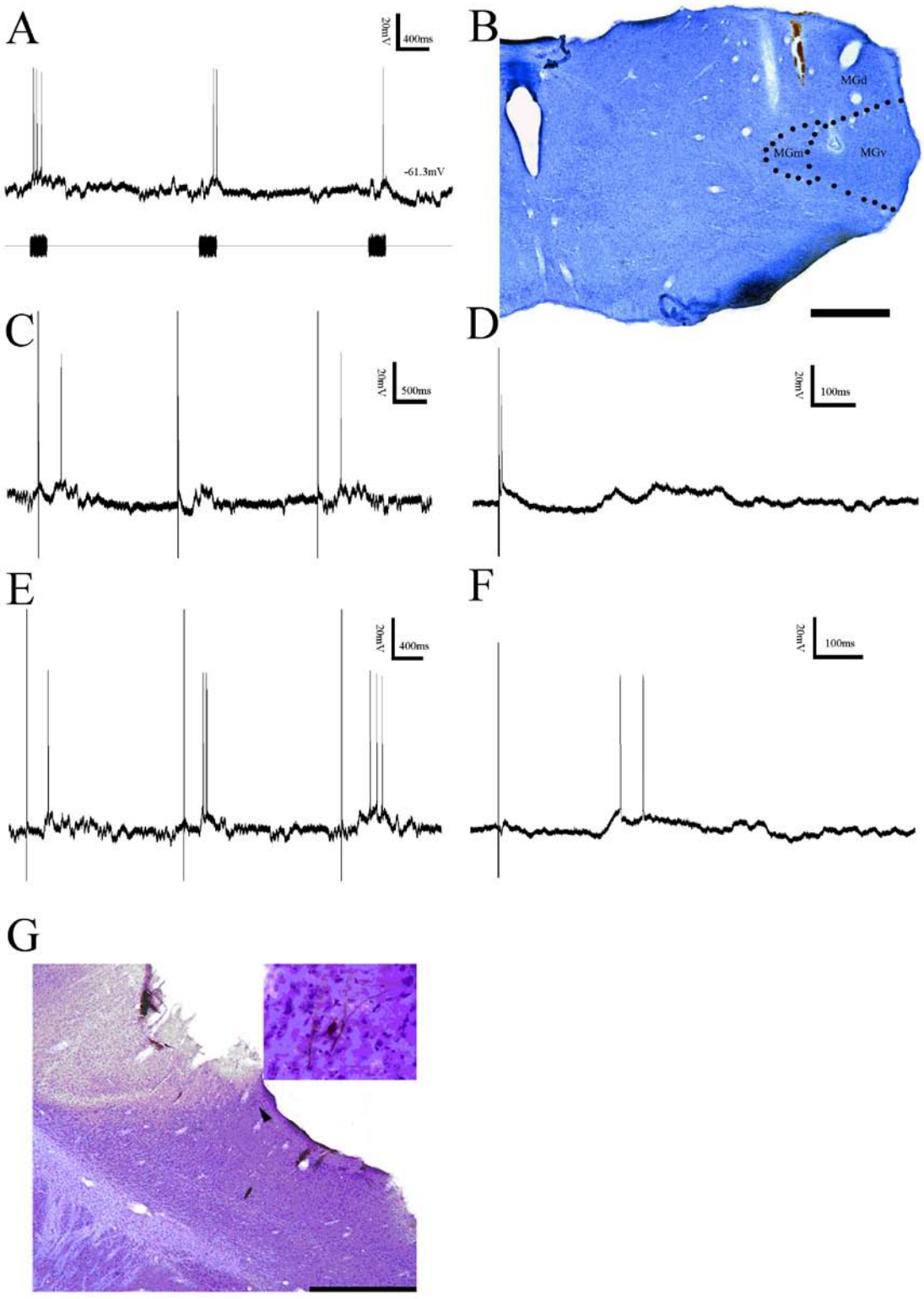

Figure 22. Neuronal responses (neuron No. 5) to the electrical stimulation of MGv and MGm respectively. A: acoustic responses. B: Anatomical confirmation

of electrical stimulating sites in MGB. *Scale bar*: 1000 μm. C, D: Neuronal responses to the electrical stimulation in MGv. AP followed by membrane oscillation were induced by the thalamic electrical stimulation. E, F: Neuronal responses when electrical stimulating MGm: EPSP followed by membrane oscillation. G: The labeled intracellular recorded neuron (pointed by *arrow head*) in layer II. *Scale bar*: 1000 μm. High magnification photo of the labeled neuron was inserted in figure G**.**

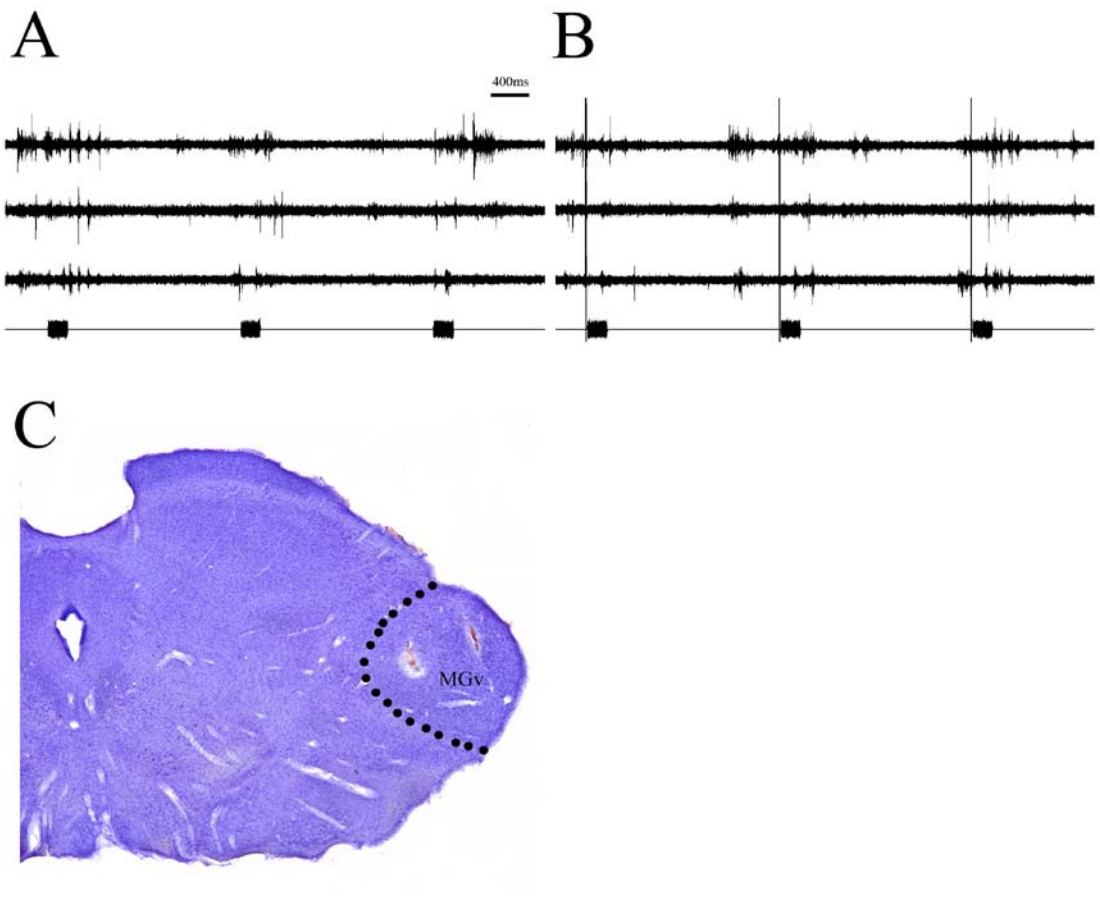

Figure 23. Responses in layers II, III (No. 10) and VI to the electrical stimulation applied in the marginal area of MGv. A and B: from upper to bottom trace: layers VI, III and II neurons. A: Acoustic responses of these neurons. B: Long delayed oscillations induced by electrical stimulating the marginal area of MGv. The acoustic responses were inhibited. C: Electrical stimulating sites in MGv.

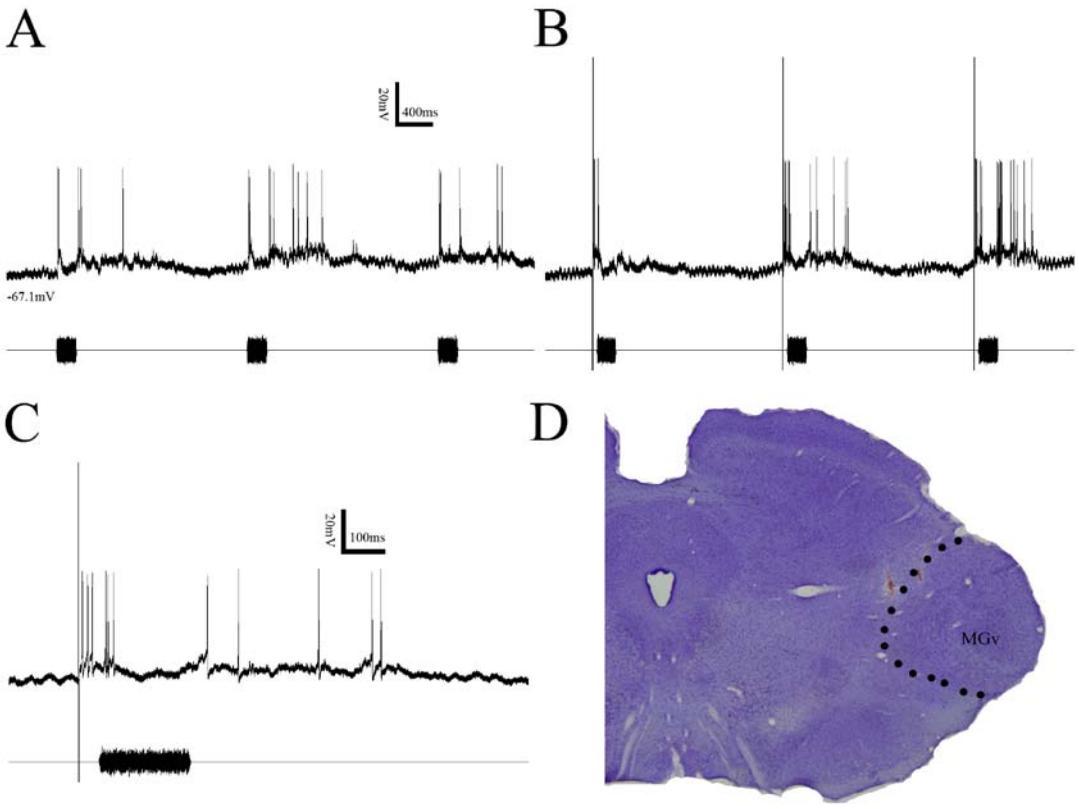

Figure 24. Neuroanl response of layer IV neuron (No. 18) to the electrical stimulation applied in MGv. A: Acoustic response. B and C: EPSP followed by membrane oscillation induced by electrical stimulating MGv. D: Electrical stimulating sites in MGv.

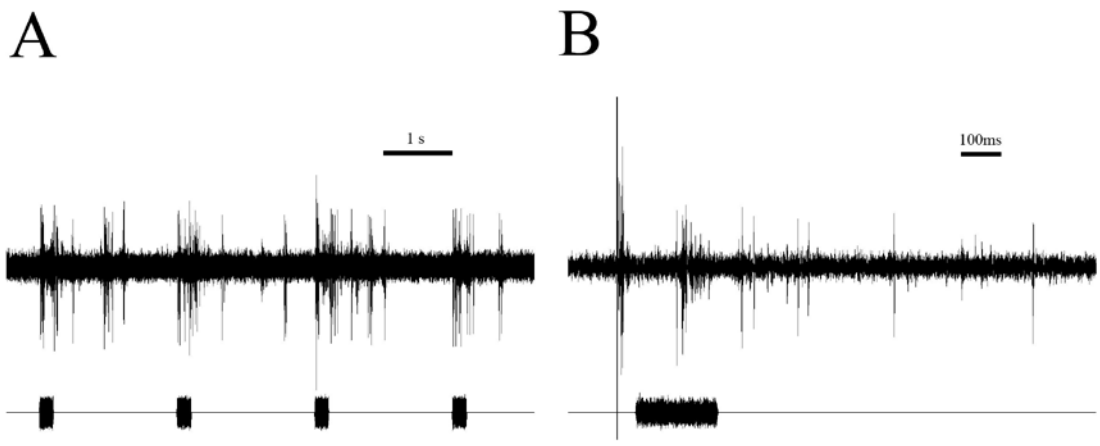

Figure 25. Neuroanl response in layer VI (No. 20) to the electrical stimulation applied in MGv. A: Acoustic response. B: AP followed by oscillation induced by electrical stimulating MGv. The acoustic responses were inhibited. Electrical stimulating sites in MGv: see **Figure 24 D**.

4.2.3 Modulatory effect of electrical stimulation of MGm on AC

In the present study, thirty-one AC neurons were recorded intracellularly or extracellularly when electrical stimulation was applied to MGm. Twenty-two neurons of them showed neuronal responses to MGm stimulation (in detail: five of eight in layer I; nine of eleven in layer II; five of eight in layer III; two of three in layer IV and one in layer V). The effect of MGm stimulation on spontaneous neuronal activity of AC neurons will be described in part 4.4.

4.2.3.1 Latency of the responses of AC neurons to electrical stimulation of MGm

In general, the onset EPSPs/APs of AC neurons induced by MGm stimulation had shorter response latencies than their acoustic responses. In auditory cortical layers II and III, rhythmic oscillations induced by MGm stimulation without the onset EPSPs/APs that had longer latencies than the acoustic responses of these AC neurons. Only two layer III neurons (Nos. 12 and 14) were the exceptions: in neuron No. 12, the responses to MGm stimulation and the acoustic stimuli had similar latencies (7.08 ± 0.35 ms and 7.42 ± 0.25 ms. Figure 30) and neuron No. 14 had membrane oscillations induced immediately following the offset of the electrical stimulation (Figure 31).

Similar to the layer II neuron No. 6 (Figure 28), the layer III neuron No. 15 had long delayed rhythmic membrane oscillation induced by the electrical stimulation of MGm (latency: 906.32 ± 116.55 ms, duration: 710.60 ± 92.91 ms) while the neuron No. 6 had shorter latencies (299.48 ± 10.54 ms. $P < 0.05$).

The latency of onset responses induced by MGm stimulation on neurons in different auditory cortical layers was different. The layer V neuron (No. 19) had electrical stimulation induced APs with short latencies (3.59 ± 0.15 ms), while the latencies of its acoustic response were 8.43 ± 1.41 ms (Figure 33). Neurons in layers II (for examples: No. 7 see Figure 29 and No. 8) and IV (for examples: No. 16 see Figure 32. latency: 1st electrical pulse: 4.18 ± 0.38 ms. 3rd: 7.67 ± 0.58 ms;

4^{th}: 7.52 ± 0.16 ms; 5^{th}: 7.59 ± 0.40 ms. No. 17: 4.14 ± 0.23 ms) had similar response latencies, which were longer than the layer V neuron No. 19 (Figure 33) and shorter than layer I neurons ($P < 0.05$. for example: No.3 Figure 26).

The acoustic response latency of the neuron (No. 11) in layer III was prolonged by MGm stimulation from 13.20 ± 1.45 ms to 17.29 ± 4.27 ms ($P < 0.05$), while the amplitude of the acoustic response did not change.

4.2.3.2 Firing patterns of the responses of AC neurons to electrical stimulation of MGm

The onset EPSPs/APs and/or rhythmic oscillations were induced by the electrical stimulation of MGm. Similar to responses when MGv was excited electrically, the neuronal responses had obvious silent components between the onset EPSPs/APs and the following rhythmic oscillations, while the acoustic responses of these AC neurons had not.

The resting MPs and neuronal firing patterns were affected differently by applying electrical current into the different areas of MGm. When the central area of MGm was stimulated electrically, the resting MPs of some auditory cortical layer I neurons decreased. The resting MPs increased when the marginal area of MGm was excited electrically. Together with the change of the resting MP, the neuronal activity changed between tonic dischargings and evoked APs (for example: neuron No. 3 Figure 26).

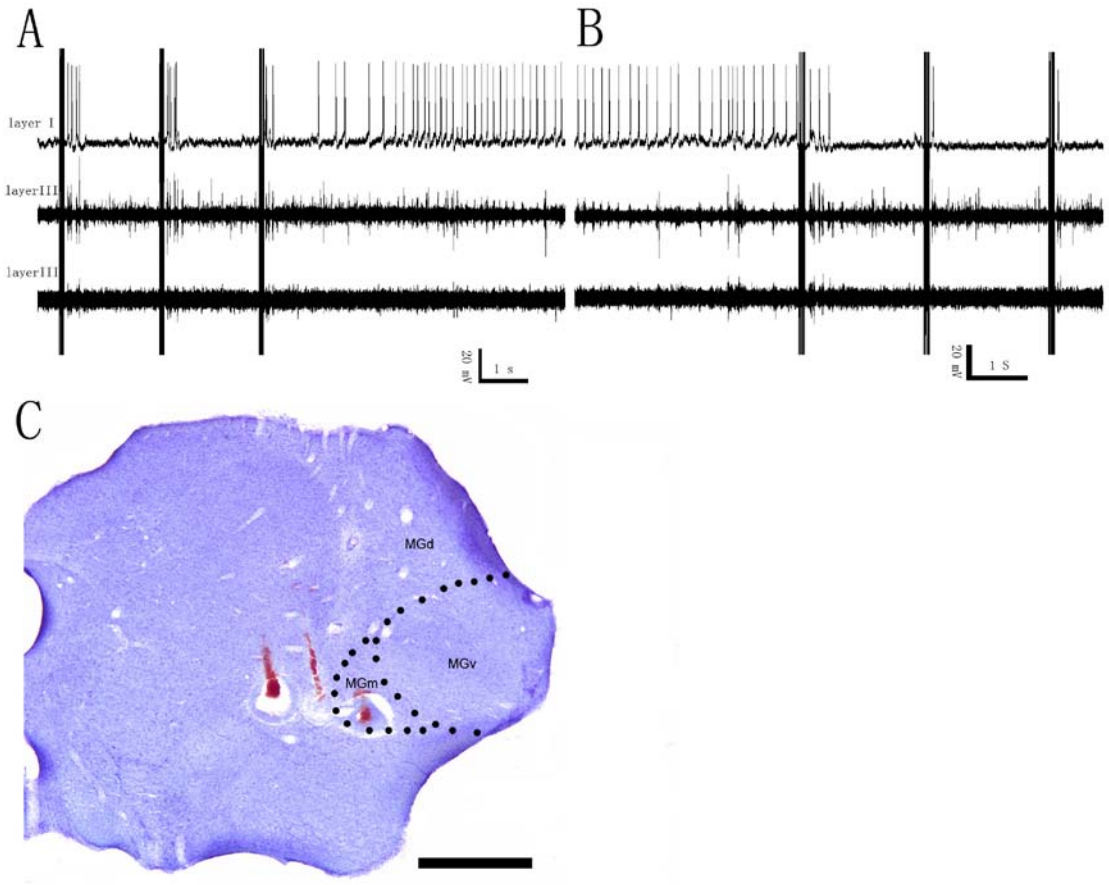

Figure 26. Auditory cortical layer I neuron's (No. 3) membrane potential could be changed by stimulating different area of MGm sequentially. A: Neuronal activities of layer I neuron when the marginal area of MGm was excited electrically. The MPs enhanced and the tonic neuronal discharging replaced the evoked APs. B: Neuronal activities of the same neuron when the central area of MGm was excited electrically. The MPs decreased and the tonic discharging disappeared. The electrical stimulation applied in both areas of MGm evoked APs. The two panels of extracellular recording were from layer III, both layer III neurons showed synchronized spikes. C: Anatomical confirmation of electrical stimulating sites in MGB. *Scale Bar*: 1000 μm.

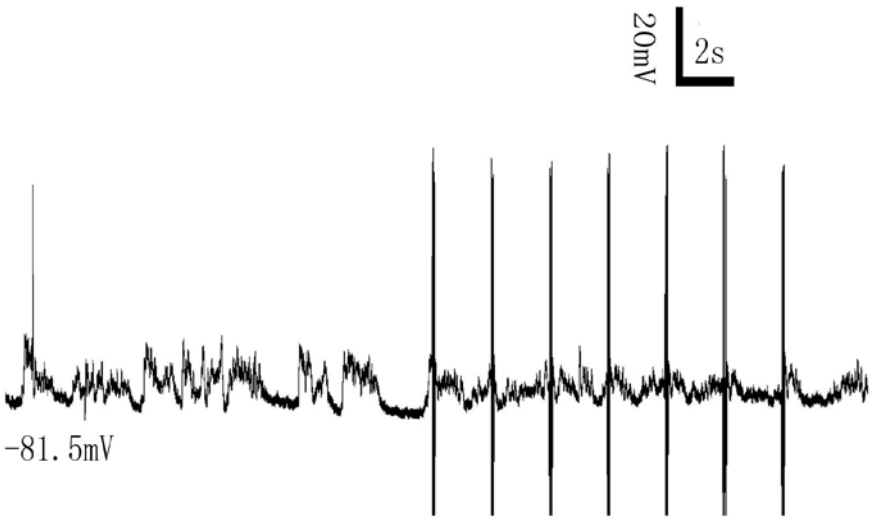

Figure 27. Modulatory effects of electrical stimulation in the marginal area of MGm on layer I neuron (No. 2). The resting MP was increased by the electrical stimulation applied in the marginal area of MGm and the spontaneous membrane oscillation was inhibited. Anatomical confirmation of electrical stimulating sites in MGB: see **Figure 20 E**.

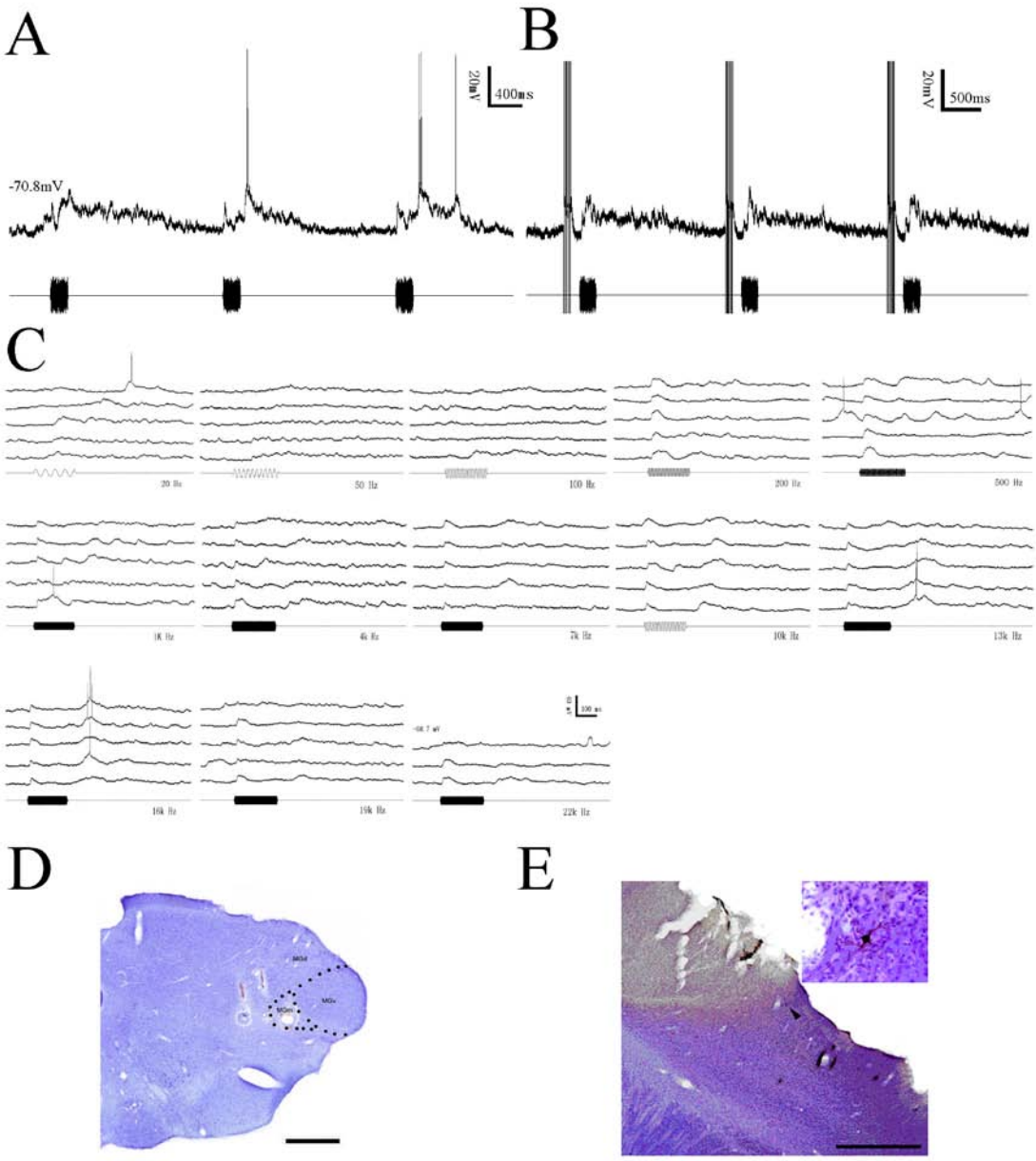

Figure 28. Neuronal responses of neuron No. 6 when the electrical stimuli were applied in MGm. A: Acoustic responses. Latency: 10.37 ± 2.20 ms, amplitude: 14.18 ± 1.88 mV. B: Long delayed membrane oscillations induced by the electrical stimulation applied in MGm. Latency: 299.48 ± 10.54 ms ($P < 0.05$). C: Tuning of the neuron to 13 pure tones. The largest acoustic responses appear when 200 and 500 Hz pure tones were given to the subject. D: Anatomical confirmation of electrical stimulating sites in MGB. *Scale bar*: 1000 μm. E: The labeled intracellular recorded neuron (*arrow head*) in layer II. *Scale bar*: 1000 μm. High magnification photo was inserted**.**

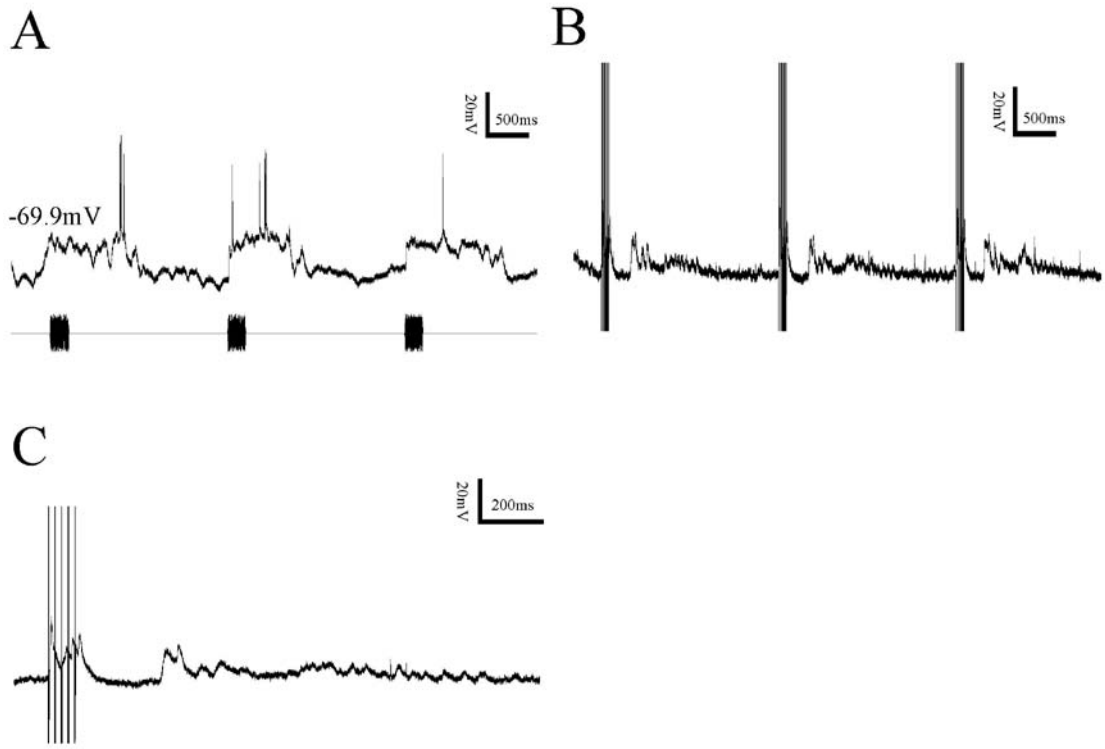

Figure 29. Neuronal responses of neuron No. 7 when MGm was stimulated electrically. A: Acoustic responses. Latency: 12.45 ± 1.32 ms. B: Short delayed EPSP and long delayed membrane oscillations induced by the electrical stimulation of MGm. Latency: 6.91 ± 0.91 ms and 330.24 ± 10.45 ms. ($P < 0.05$). Anatomical confirmation of electrical stimulating sites in MGB: see **Figure 28 D**.

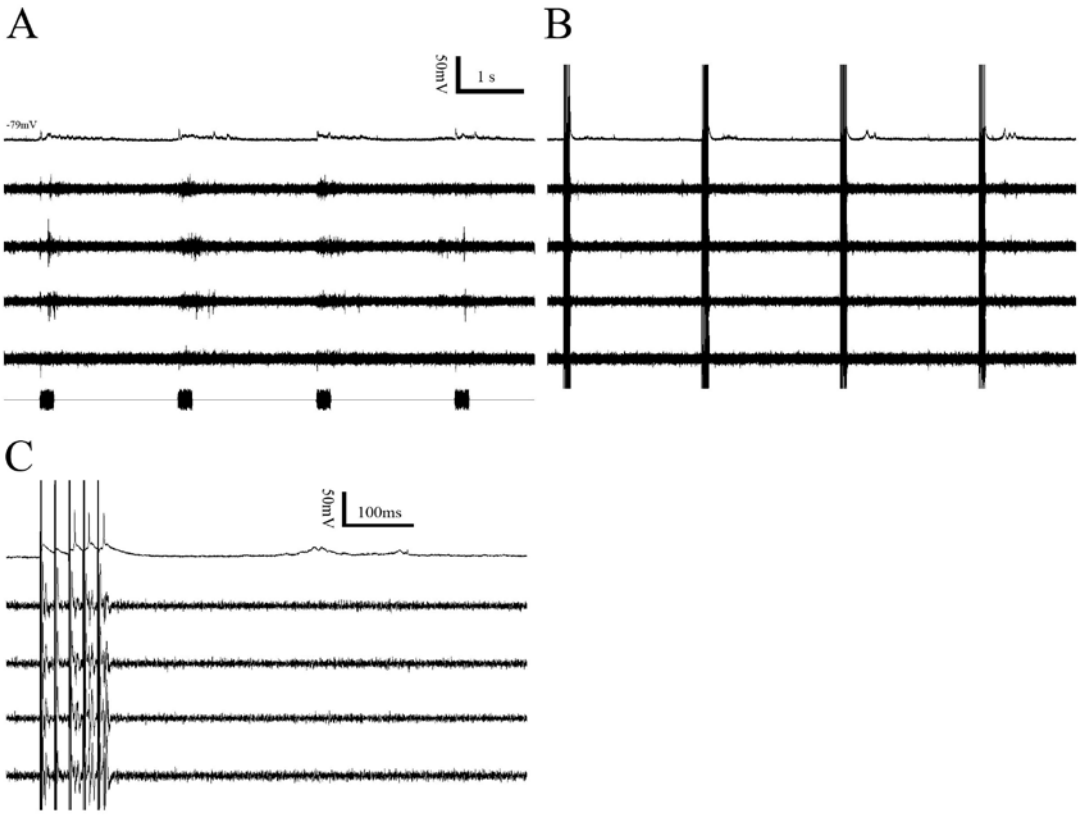

Figure 30. Responses of layers III, II and I neurons to the electrical stimulation of MGm. From the upper to the bottom trace: layers III (No. 12), III (No. 13), II, II and I neurons. A: Acoustic response. B and C: EPSP/AP followed by membrane oscillation was induced by the electrical stimulation of MGm on layer III neuron (No. 12). Electrical stimulating sites in MGm: see **Figure 28 D**.

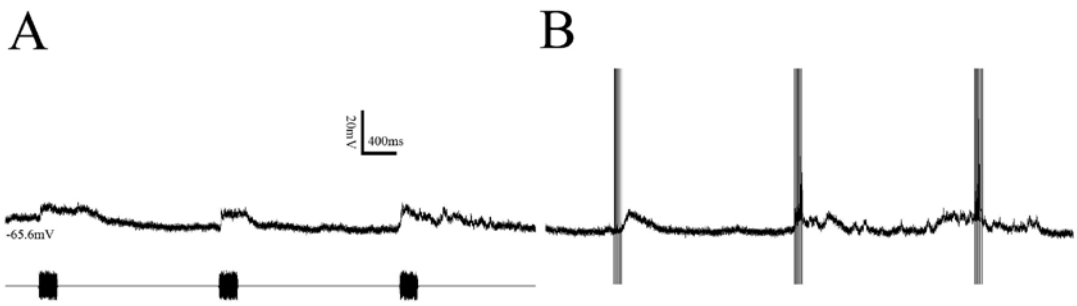

Figure 31. Response of layer III neuron (No. 14) to the electrical stimulation applied in MGm. A: Acoustic response (latency: 12.79 ± 0.76 ms). B: Membrane oscillation induced by electrical stimulating MGm. Electrical stimulating sites in MGm: see **Figure 26 C**.

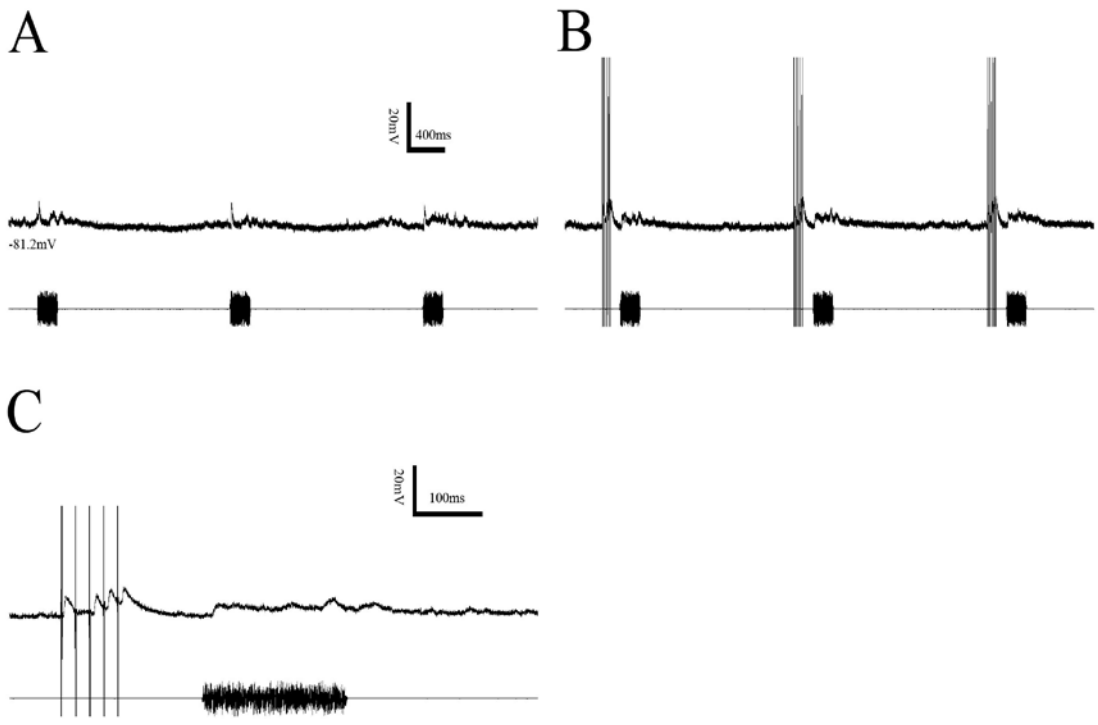

Figure 32. Neuronal response of layer IV neuron (No. 16) to the electrical stimulation applied in MGm. A: Acoustic response. B and C: EPSP induced by electrical stimulating MGm. The latency of the 1st induced EPSP was shorter than those of 3rd, 4th and 5th EPSP ($P < 0.05$). The acoustic responses were not affected by the electrical stimulation. Electrical stimulating sites in MGm: see **Figure 28 D**.

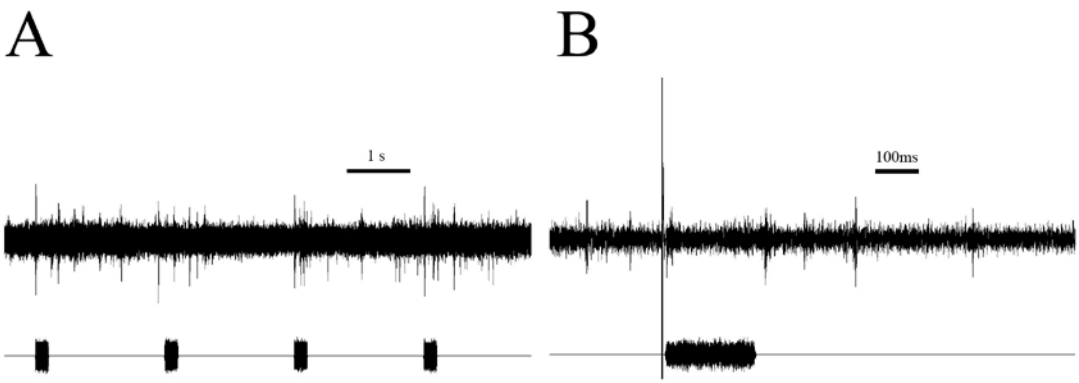

Figure 33. Neuroanl response of layer V neuron (No. 19) to the electrical stimulation applied in MGm. A: Acoustic response. B: AP induced by electrical stimulating MGm.

4.3 Spontaneous neuronal activities of AC neurons

Spontaneous rhythmic oscillations were the most common spontaneous neuronal activities of AC neurons found in the present study. Similar spontaneous rhythmic oscillations of 9-15 Hz were discovered in all six auditory cortical layers.

The spontaneous oscillations in AC were mostly at the spindle frequency (7-14 Hz) band. Oscillations appeared in different cortical layers in a certain sequence: from the bottom cortical layers to the upper layers (Figure 34). In some cases, the oscillations showed opposite sequence of appearance immediately (Figure 35).

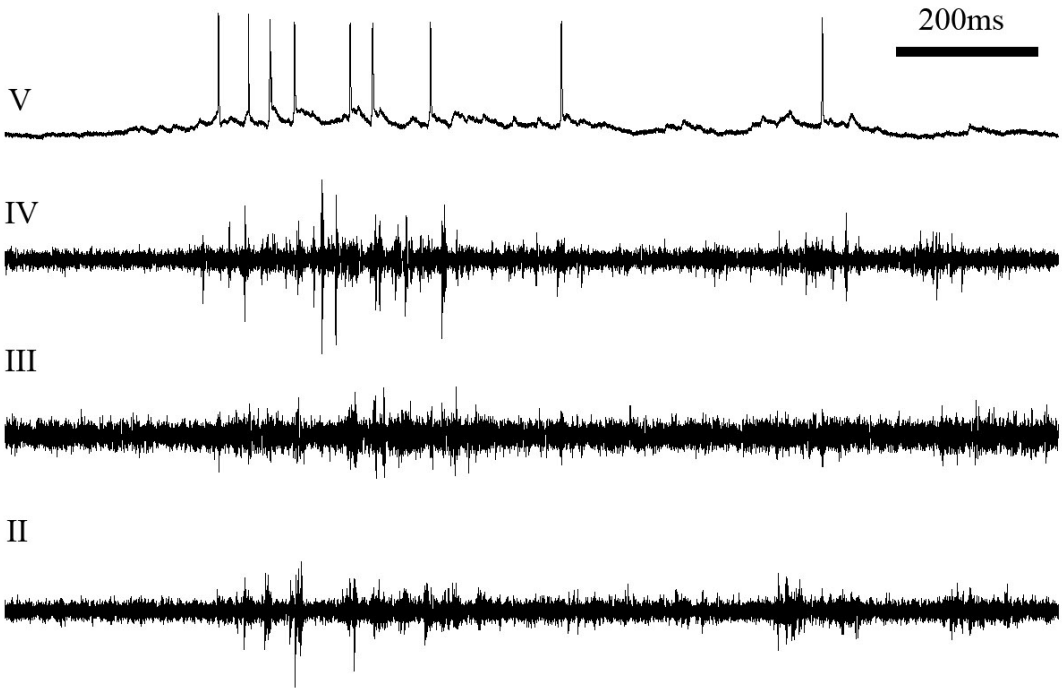

Figure 34. Spontaneous oscillations appeared in different cortical layers in a certain sequence: from layer V to layer II.

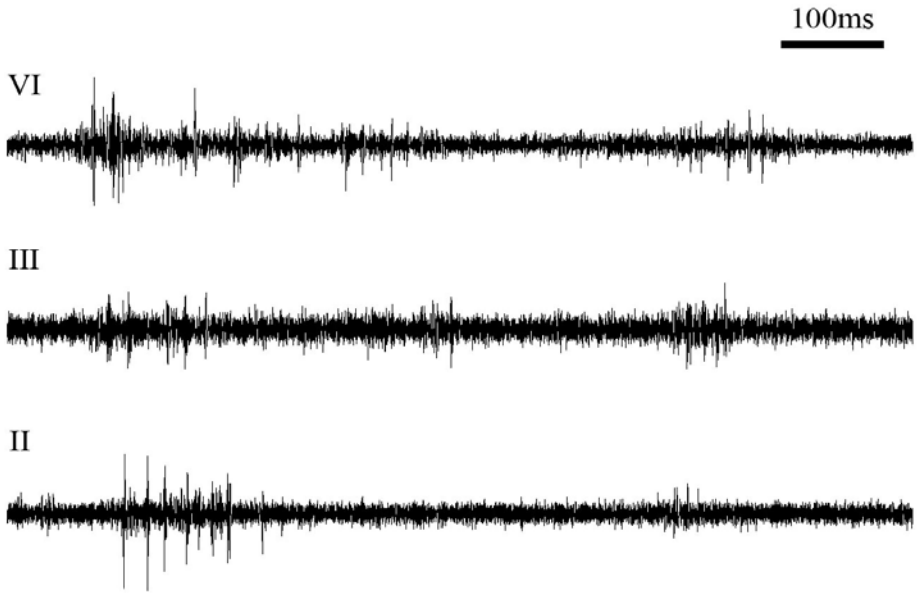

Figure 35. Oscillations appeared in different cortical layers in a certain sequence from layer VI to layer II and then the oscillations showed opposite sequence of appearance immediately.

4.4 Effects of thalamic electrical stimulation on spontaneous activity of AC neurons

4.4.1 Effects of thalamic electrical stimulation on spontaneous activity of AC neurons

Electrical stimulation of MGv had no effect on the spontaneous activity of AC neurons, while MGm stimulation inhibited the spontaneous activity, including tonic and/or oscillatory activity, of layers I, II and III neurons (for examples: layer I: Figures 26 and 27; layer II: Figures 22 and 29; layer III: Figures 28 and 31).

The inhibition of the spontaneous activity of AC neurons was mediated by the hyperpolarization in resting MPs induced by the electrical stimulation of MGm.

4.4.2 Thalamic electrical stimulation induced long lasting oscillatory activities in AC neurons

MGm stimulation could change AC neuronal firing patterns from tonic discharging to rhythmic oscillation at the spindle frequency band on some auditory cortical neurons in layers IV and V (for example: Figure 36). However, MGv stimulation had no such effect on AC neurons.

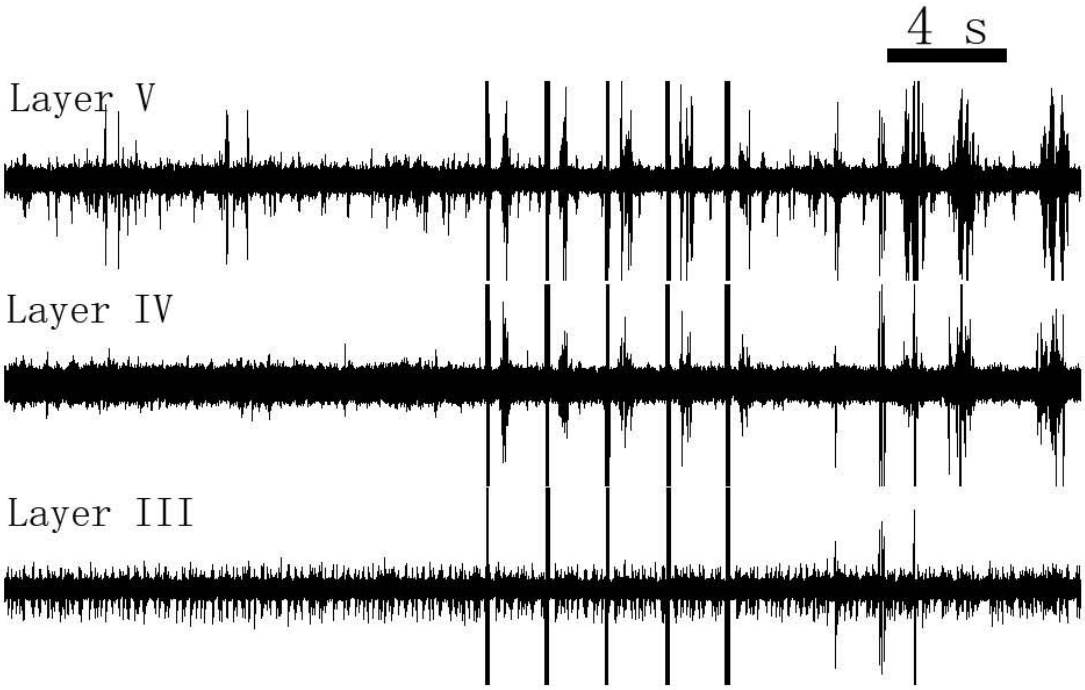

Figure 36. Neuronal spontaneous tonic discharging changed by the electrical stimulation applied in MGm to the rhythmic oscillation. Neuronal units in layers V, IV and III were recorded extracellularly. The spontaneous tonic dischargings in layers V and IV were changed by the electrical stimulation applied in MGm to the rhythmic oscillation. The thalamic electrical stimulation had no effect on the layer III neuron.

4.4.3 Modulatory effects of current injection on AC neurons

There was one special layer II neuron (No. 9) that showed no responses to the electrical stimulation of MGv and MGm. However, its spontaneous neuronal activity was inhibited by the injection of neurobiotin into the neuron.

We used neurobiotin to label the intracellular recorded neurons. Neurobiotin was delivered into the neuron by passing rectangular depolarizing current pulses (150 ms, 3.3 Hz, 2 nA) for 1-5 min. After the injection of neurobiotin, the resting MP of this neuron changed from -69.0 to -60.1 mV. Spontaneous neuronal activities disappeared, while acoustic responses remained (Figure 37).

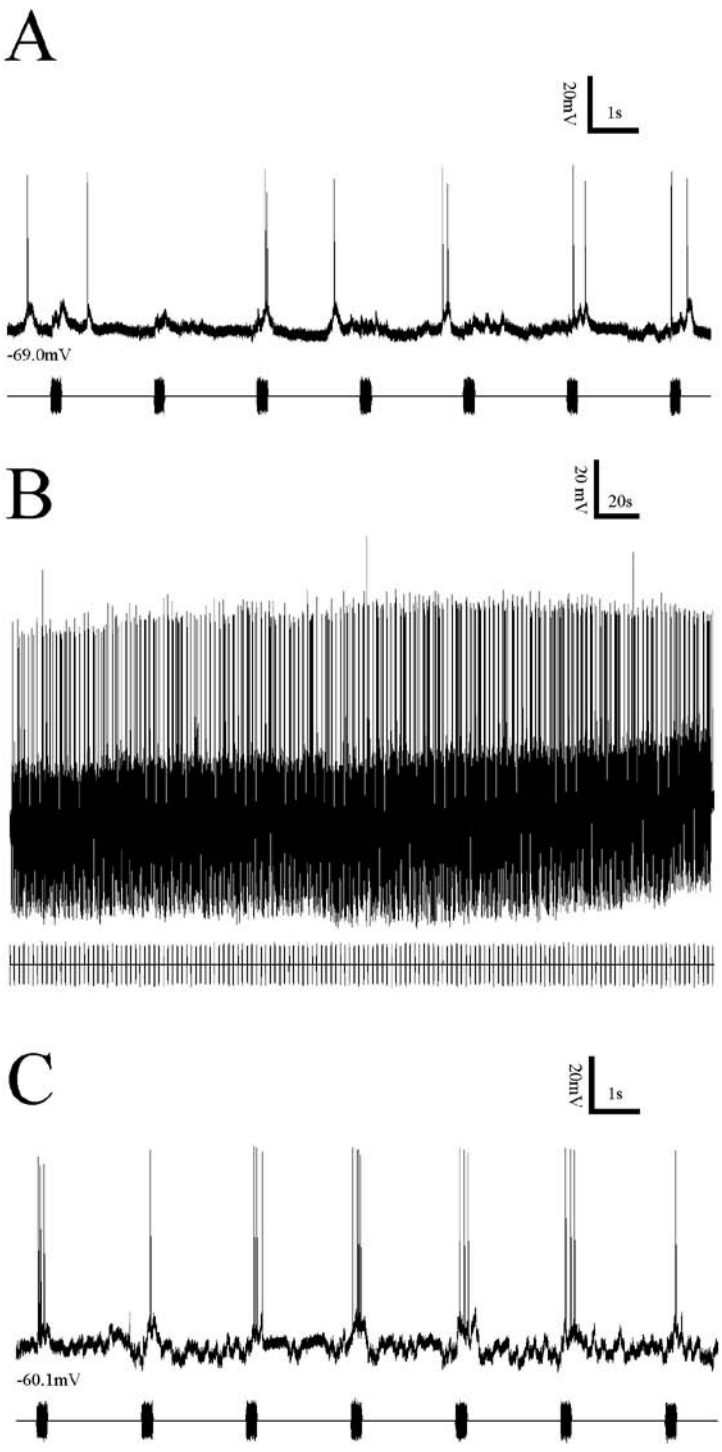

Figure 37. Neuronal activities (No. 9) affected by the injection of neurobiotin using rectangular depolarizing current pulses. A: Spontaneous activities (APs) and acoustic responses (EPSPs) of neuron No. 9. MP: -69.0 mV. B: Injection of neurobiotin by passing rectangular depolarizing current pulses for 5 min. C: After the injection of neurobiotin, the resting MP changed from -69.0 to -60.1 mV and

the spontaneous neuronal activities disappeared while the acoustic responses were changed from EPSPs to APs. Anatomical confirmation of electrical stimulating sites in MGB: see **Figure 24 B**.

Chapter 5

Discussion

5.1 Auditory responses of AC neurons to the noise-burst stimuli

In the present study, we used noise burst stimuli to test neuronal auditory responses of AC neurons. Most neurons showed excitatatory responses to the acoustic stimuli. The resting MPs of intracellularly recorded AC neurons was slightly lower than MGB neurons, as shown in previous work (Yu et al., 2004). The lower resting MPs of the auditory cortical neurons than MGB neurons may be the primary reason for the unitary excitatory responses of these auditory cortical neurons to the acoustic stimuli. The reason for the formation of the low MPs on AC neurons may be due to the input of auditory cortical layer I GABAergic neurons on the neurons in other five layers (Mitani and Shimokouchi, 1985; Mitani et al., 1985; Matsubara and Phillips, 1988; Ojima et al., 1991; Wallace et al., 1991; Lee and Winer, 2008b, , 2008c).

We also used a metal electrode array to record neuronal activities extracellularly or simultaneously with the intracellular recording. The extracellularly recorded AC units always showed spikes synchronized with APs and/or membrane oscillations in the intracellular recorded neurons.

Although the sequences that the auditory responses appeared among the auditory cortical layers were slightly different, the basic sequence of cortical

layers responding to the acoustic stimuli was similar (Figure 18). That is, some neurons in the upper sublayer I and the lower sublayer II responded first, followed by layers IV, III, VI, then layers I, II, and V ($P < 0.05$). This result indicates that the auditory signals are transmitted to the AC mediated by both MGv and MGm. The auditory signals mediated by MGv first go to auditory cortical layer IV, then up to layer III and back to layer V, via the lemniscal TC pathway (Andersen et al., 1980; Lee and Winer, 2008a). Because a direct, short latency projection from the DCN to the MGm exists (Anderson et al., 2004; Anderson et al., 2006), the auditory signals mediated by MGm could go to the upper sublayer I, lower sublayer II and layer VI first which confirms the previous anatomical studies (Andersen et al., 1980; Huang and Winer, 2000; Lee and Winer, 2008a).

Previous studies using multiunit or single unit recordings showed that long latency oscillations of about 10 Hz were observed in response to tones (Sally and Kelly, 1988; Maldonado and Gerstein, 1996; Cotillon and Edeline, 2000) or clicks (Eggermont, 1992) under various anesthetic conditions (urethane, ketamine, or pentobarbital) (Figure 11). Similar rhythmic oscillations (frequency: 14.77 ± 5.26 Hz; latency: 75.37 ± 26.15 ms) were evoked by noise burst stimuli under urethane anesthesia in this study (see Figures 20, 22, 24-26 and 29-33). The long delayed acoustically evoked rhythmic oscillations indicate that it is a polysynaptic response and may result from the interactions between auditory thalamus and auditory sector of TRN, similar to those evoked by tones (Cotillon and Edeline, 2000).

Meanwhile, there were two cortical neurons (in layers II and IV) with surprisingly short latency acoustic evoked oscillations (latencies: 11.88 ms and 15.55 ms). This indicates that other mechanisms of evoked oscillations may exist in auditory pathways. The interactions among different cortical layers may be involved.

Spontaneous oscillations of similar frequencies as the acoustic evoked oscillations were discovered in all six auditory cortical layers. They appeared in different cortical layers in a certain sequence: from the bottom cortical layers to the upper layers. In some cases, the oscillations showed opposite sequence of appearance immediately (Figures 34 and 35).

5.2 Range of electrical stimulation in MGB

We applied electrical stimulation (50-200 μA) to MGv and MGm separately to test the neuronal response of AI neurons in guinea pigs. It is vital to confine the electrical stimulation in a certain division of MGB.

To define the range of electrical stimulation we recorded neurons in one division of MGB, while electrical stimulation was given to another division. The results (Figure 19) confirmed that no electrical current could reach the nearby division of MGB. Thus the electrical stimulation performed in this study excited only one single division of MGB.

5.3 AC neurons labeled with Neurobiotin

Among the thirteen intracellularly recorded AC neurons when electrical stimulation was given to MGv or MGm, only two neurons were successfully labeled by neurobiotin.

The following reasons may explain the low success rate of neurobiotin labeling:

(1) Auditory cortex is on the surface of the brain and easily affected by breathing and heartbeats of the animal during intracellular recording. It is hard to record cortical neurons intracellularly for a period of time long enough to label the neuron with the tracer.

(2) During the electrophysiological recording, penetrations of the electrodes for intracellular and extracellular recording caused damage to AC, which may result in poor uptake of the tracer by the neurons.

5.4 Effects induced by electrical stimulation of MGB divisions on AC neurons

Responses of intracellularly and extracellularly recorded AC neurons to electrical stimulation of MGv and MGm were tested to investigate the modulatory effects of different MGB divisions on AC.

As the major part of the ascending primary TC pathway, MGv plays an important role in acoustic signal transformation (Creutzfeldt et al., 1980; Miller et al., 2001). Neurons in MGv project to several tonotopic auditory cortical areas, including AI, ending in an average of 4.6 layers of AI and mostly in cortical layers III and IV (Huang and Winer, 2000; Lee and Winer, 2008a). Although MGm has TC axonal projections to an average of 5.4 layers in all auditory cortical areas, projections from it target the cortical layers I and VI principally (Calford and Aitkin, 1983; Huang and Winer, 2000; Lee and Winer, 2008a). Both MGB divisions have overlapping and extensive projections to AC (Huang and Winer, 2000; Winer et al., 2005; Lee and Winer, 2008a). Considering the interactions among different auditory cortical areas and layers, as well as the interactions between MGB and the auditory sector of TRN, AI neurons show both monosynaptic and polysynaptic responses to electrical stimulation applied to different divisions of MGB.

Auditory cortical layer I has fewer neurons than other auditory cortical layers and most of them are GABAergic neurons. Besides their intrinsic local connections within layer I, layer I horizontal cells receive monosynaptic inputs

from the fibers of MGm neurons. Layer I neurons connect to neurons in layer II as well as other cortical layers (Matsubara and Phillips, 1988; Ojima et al., 1991; Wallace et al., 1991; Lee and Winer, 2008b, , 2008c). AC layer I may play an important role in modulating other auditory cortical layers. This is the first study to investigate the modulatory effect of thalamic electrical stimulation on auditory cortical layer I.

The rhythmic membrane oscillations in two AC layer I neurons (latency: 7.38 ± 0.35 ms) induced by MGv stimulation (Figure 20) may confirm physiologically that MGv has ascending axonal projections to AC layer I (Huang and Winer, 2000). However, the projections from MGv to AC layer I are in the minority because the other three layer I neurons isolated in this study were not affected by MGv stimulation. Although acoustic stimuli evoked similar membrane oscillations on these layer I neurons, thalamic stimulation induced membrane oscillations of shorter latencies and with more obvious silent components. Thus, the formation of membrane oscillations evoked by acoustic and thalamic electrical stimulation may arise by different mechanisms.

There were no APs from the stimulation of MGv in some AC neurons, which indicates that MGv may be more than an important relay station of acoustic information. EPSPs and/or membrane oscillations induced by MGv stimulation, which could not relay the information to AC, indicate that MGv may have modulatory effects on AC layer I by changing its resting MP.

Most layer I neurons (5 of 8) were modulated by electrical stimulation of MGm showing short delayed EPSPs, APs or rhythmic oscillations. It is interesting that electrical stimulation of different areas of MGm could induce different modulatory effects on layer I neurons. Electrically stimulating the marginal area of MGm increased the MP of layer I neurons. As a result of the depolarization of the resting MPs, spontaneous oscillations were inhibited (Figure 27), while neuronal activity changed to tonic discharge (Figure 26). When the central area of MGm was stimulated, the resting MPs of layer I neurons decreased. Neuronal activity changed from the tonic discharge back to the APs induced by the thalamic electrical stimulation (Figure 26). This two-direction modulatory effect of different MGm areas on layer I may be involved in the maintenance of the state of layer I neuronal activity.

In AC layers II and III, more neurons responded to the electrical stimulation applied to MGm than MGv. Stimulating MGv induced long delayed oscillations or short delayed APs followed by long delayed oscillations on layer II neuons (Figures 21, 22). Similar long delayed oscillations were induced by electrical stimulating the marginal area of MGv on layer III neuron (Figure 23). Together with this layer III neuron, two neurons in layers II and VI were recorded simultaneously. The sequence of thalamic stimulation induced oscillation appeared among these three cortical layers were the same as that of the acoustic response: layers III, VI to layer II.

Electrically stimulating MGv or MGm induced EPSPs/APs of shorter latency than that of acoustic responses in AC layer IV. One layer IV neuron was recorded when MGm was stimulated by using 5 electrical pulses, and the latencies of EPSP induced by the first electrical pulse are shorter than those induced by the third, fourth, and fifth pulses. Therefore, the mechanisms for the formation of the thalamic electrical stimulation induced EPSPs are different.

In auditory cortical layers V and VI, neurons could be excited antidromically by the thalamic electrical stimulation, showing very short delayed APs. This result confirms that AC layers V and VI have axonal projections back to MGB (Andersen et al., 1980).

Acoustic responses were inhibited by neuronal responses induced by thalamic electrical stimulation, in relation to the time window between the acoustic stimuli and the thalamic stimulation. The acoustic responses were inhibited during the APs and/or the following silent components induced by the thalamic stimulation may be due to the lower excitability of the AC neurons.

5.5 Sources of acoustic and thalamic electrical stimulation induced rhythmic oscillations in AC

Rhythmic oscillations induced by both acoustic and electrical stimulation of MGv or MGm were at the spindle (7-14 Hz) and beta (15-30 Hz) bands. The spindle oscillation, generated in the thalamus (Morison and Bassett, 1945), typically occurs at the early stages of sleep in animals and humans. The AC receives the spindle oscillation arising from the interactions between the auditory thalamus and the auditory sector of TRN via the TC projections (Cotillon and Edeline, 2000). Meanwhile, the corticothalamic feedback (Figure 2) plays a potentiating role in the genesis of spindles (Contreras and Steriade, 1996). The prolonged hyperpolarization of MP preceding the long latency evoked spindle oscillations may come from the generation of LTS (low threshold calcium spike) in the thalamic reticular nucleus (Steriade et al., 1985).

Another type of rhythmic activity evoked by both acoustic and electrical stimuli is of the higher frequency in the beta (15-30 Hz) frequency band. Although the source is unclear, the beta oscillation reflects different aspects of sensory signal processing (Traub et al., 1999; Kopell et al., 2000). Generation of these beta oscillations requires intact TC circuitry (Lopes da Silva, 1991). Recent work (Hong et al., 2008) in the human auditory system showed that beta oscillations mediate sensory gating, a certain brain state in which the response to repetitive stimuli becomes weaker.

5.6 Modulatory effects of electrical stimulation of MGB divisions on spontaneous activity of AC neurons

Electrical stimulation of MGv had no effect on spontaneous activity of AC neurons while spontaneous activities of neurons in the upper layers (I, II and III. see Figures 22, 27-29 and 31) were inhibited by electrical stimulation of MGm.

Although MGm stimulation could induce EPSPs, APs or membrane oscillations, as well as inhibit spontaneous activity of layers II and III, the responses were slightly different. Membrane oscillations induced in layer II neurons had longer latencies than layer III neurons (Figure 28, 31). Induced EPSPs or APs followed by membrane oscillations, in layer III neurons had similar latencies to their acoustic responses. Alternatively, layer II neurons (Figure 22, 29) had shorter delayed stimulation-induced respones than acoustic responses. This difference may result from the different projections from MGm to auditory cortical layers II and III (Andersen et al., 1980; Huang and Winer, 2000; Lee and Winer, 2008a). The modulation of MGm via the direct effect of layer I on layer II exists under physiological conditions (Mitani and Shimokouchi, 1985; Mitani et al., 1985) and the MGm stimulation may activate the cross modality pathways in AC layer III. Electrical stimulation of MGm not only inhibited their spontaneous activity but also prolonged the latencies of acoustic responses from 13.20 ± 1.45 ms to 17.29 ± 4.27 ms ($P < 0.05$). However, the amplitude did not change in some neurons (for example: layer III neuron No. 11). Auditory cortical layer I

GABAergic neurons or GABAergic neurons in layer III may be involved in this process.

Unlike neurons in the upper layers, neuronal firing patterns of some auditory cortical neurons in the bottom layers (IV-VI) could be changed by electrical stimulation of MGm from tonic discharging to rhythmic oscillation. MGv stimulation, alternatively, does not have this effect (Figure 36). The differential effects of MGm stimulation on upper and lower layers indicate that neuronal activity in these layers is modulated in different ways. More inter-layer interactions or interactions between AC and the auditory thalamus may be involved in the modulation of electrical stimulation of MGm on the deeper AC layers.

The resting MP of a layer II neuron (No. 9) changed from -69.0 to -60.1 mV and its spontaneous neuronal activity was inhibited by the injection of electrical current into the neuron. The acoustic responses of the neuron were not affected (Figure 37). This indicates that the repetitive stimulation may modulate the state of auditory cortical layer II neurons, while the sustained firing patterns of MGm neurons may be the source of this repetitive stimulation.

Chapter 6

Summary of findings and conclusions

6.1 Summary of findings

The present study investigated the modulation of different divisions of MGB on AI in urethane-anesthetized guinea pigs. Neuronal responses of AI were tested intracellularly and extracellularly to acoustic stimuli and/or electrical stimulation of different divisions of MGB (MGv and MGm).

EPSPs, APs and/or rhythmic oscillations were evoked by noise burst stimuli applied to the contralateral ear of the animals.

Both direct and long latency modulatory effects of different divisions of MGB on neurons in the AI were found. The modulatory effects of the thalamic electrical stimulation were mainly in AC layers I, II and III. MGm had more modulatory effects on them than MGv. Layer I neurons had opposite responses to electrical stimulation of different areas of MGm. Layers II and III had less modulatory effects by thalamic stimulation than the layer I neurons. Neuronal responses to thalamic stimulation and acoustic stimuli were different in layer II neurons, while in layer III, they were similar.

Acoustic responses were inhibited during the onset EPSPs/APs and the following silent component induced by thalamic stimulation because of the lower excitabilities of the neurons caused by thalamic stimulation.

Spontaneous neuronal activity in neurons of AC layers I-III were always inhibited by electrical stimulation of MGm, while spontaneous activity could be changed from tonic to oscillatory discharge in layers IV and V.

Layer IV received information mediated directly by MGv and MGm while layers V and VI had antidromic responses to electrical stimulation of MGB divisions.

6.2 Conclusions

Acoustic stimuli evoked synchronized excitatory responses, including EPSPs, APs and/or rhythmic oscillations on AC neurons recorded intracellularly and/or extracellularly.

The direct, short latency projection from DCN to MGm and the projection from MGm to cortical layers I, II and VI are involved in acoustic information processing in the auditory ascending system.

Electrical stimulation of MGv and MGm evoke mainly synchronized excitatory output, including direct excitation and long latency rhythmic oscillaitons in layers I to V. Electrical stimulation of MGm had more modulatory effects than MGv on auditory cortical layers I, II and III. The spontaneous neuronal activities are always inhibited by electrical stimulation applied to MGm. AC layer I is the major target of the modulation of the electrical stimulation applied in MGm. Opposite modulations are performed by different areas of MGm on layer I neurons, which may be involved in the maintanence of the general state of AC layer I. GABAergic neurons in layer I may mediate the modulatory effects of the MGm on other auditory cortical layers.

Thalamic electrical stimulation modulates AC layers II and III in different ways. Neuronal responses to thalamic electrical stimulation and acoustic stimuli are of different latencies on layer II neurons while on layer III neurons they have similar latencies. This indicates that layer II receives information mediated by

MGB more directly than layer III. Such modulation may be involved in the cross modality activation of the AC.

The acoustic responses could be inhibited due to the lower excitabilities of neurons, induced by the thalamic electrical stimulation.

The upper (I-III) and bottom (IV-VI) auditory cortical layers are modulated by electrical stimulation applied to MGm in different ways since their spontaneous neuronal activities are modulated differently. Electrical stimulation applied to MGm directly inhibits spontaneous neuronal activity in the upper layers, perhaps via the GABAergic neurons in these layers. Electrical stimulation changes spontaneous tonic discharging to oscillatory activity in the bottom layers. This may be mediated by inter-layer interactions or interactions between AC and the auditory thalamus.

Auditory cortical layer IV, the major layer receiving the acoustic information from auditory thalamus, and layers V and VI, the major source of the corticothalamic projection, are less modulated by thalamic electrical stimulation.

The present results demonstrate a functional segregation of the parallel pathways from the MGv and MGm to the AC. The thalamocortical projection from the MGv is the major pathway of auditory information, while the projection from the MGm is likely modulatory. The pathway from the MGm may regulate the state or the general arousal of the auditory cortex. A fast feedforward modulation of the upper layers of the auditory cortex through the MGm pathway

might enable a preparation of the auditory cortex to receive auditory information forwarded from the MGv.

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