Neuronal Activity for Duration Discrimination in Guinea Pig Primary Auditory Cortex

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Chapter 1

GENERAL INTRODUCTION

1.1 Preface

Now we know that “the brain is a seat of the mind”. However, the relationship between material world (brain) and inner world (mind) is too complicated to clarify. Recently, it has been hotly debated among neuroscientists that some neurons in the brain evidently involved in higher cognitive brain functions, such as attention, consciousness, emotion, memory, learning, prediction, speech, and so on.

Far in advance, psychology has been contributed to clarify these functions on the basis of behavioral and theoretical study. While, from over a century

ago, physiologists have methodically examined the structure and function of the brain. For example, central nervous system including brain is a bilateral and essentially symmetrical structure with seven main parts: spinal cord, medulla, pons, cerebellum, midbrain, diencephalons, and cerebral hemispheres. The cerebral hemisphere consist a heavily wrinkled outer layer, the cerebral cortex, and three deep-lying structures: basal ganglia, hippocampus, and amygdaloid nuclei. In particular, the cerebral cortex involved with cognitive abilities.

These parts consists of an enormous number of neurons, that are electrically activated and continuously transmit information each other. The information is processed in parallel but sequentially in some perspective. The most general functional definition divides the brain into sensory systems like vision or hearing that acquire and process information from the environment, and motor systems that allow the organism to respond to such information by generating movements. Although individual limited neurons that play important roles in sensation and action get to understand, most of the neurons and its cognitive functions in the brain are remained unclear between the sensory systems and motor systems.

The cerebral cortex is categorized into four lobes by the sulcus of the brain

surface: frontal lobe, temporal lobe, parietal lobe, and occipital lobe. Early in the 1900's, K. Brodmann identified areas of cortex by the basis from cytoarchitectonic differences and numbered 52 separate areas that are known as the Brodmann areas (Fig. 1.1). However, the observation of localized functions in the brain cannot explain the higher cognitive brain functions, because the activation of the brain often appears multiple areas with various time scales.

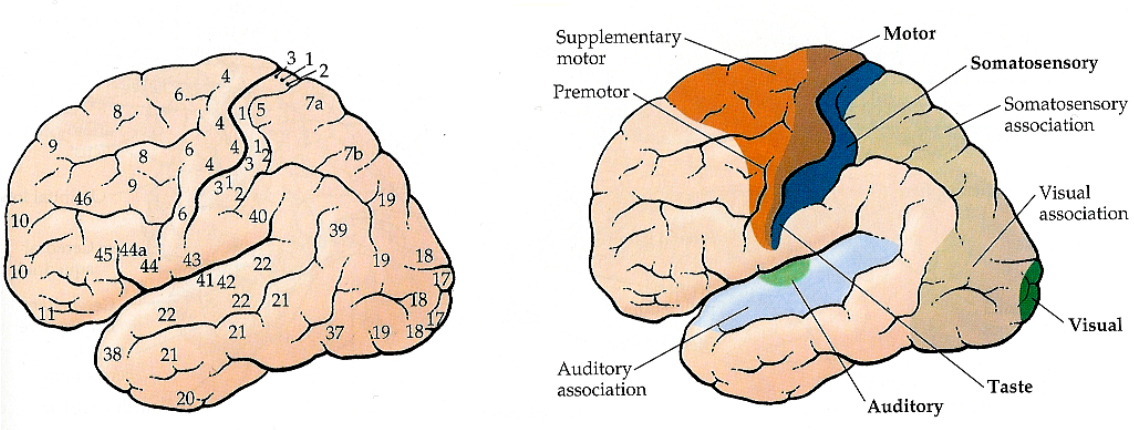

Figure 1.1 Anatomical and functional sub divisions of human cerebral cortex (left hemisphere). *Left*, Numbered anatomical areas of the cerebral cortex (Brodmann's area). *Right*, Localization of motor and sensory cortex. Auditory cortex corresponds 41, 42 areas in Brodmann's areas. This figure is cited from Ref. [1].

In late years, top down and bottom up approaches to investigate the brain function have been established. The former is strongly depend on an advance of new macroscopic and noninvasive measurements of human nervous system

such as electroencephalography (EEG; for abbreviations throughout all chapters, see Table 1.1), magnetoencephalography (MEG), and functional magnetic resonance imaging (fMRI). A lot of behavioral and psychological phenomena have been verified and confirmed by these measurements. The latter mostly involved in the accumulated findings of progressive and hierarchical sensory systems, mainly in non-human animals. Fundamental and universal neuronal mechanisms were fully investigated from several points of view and the associated function constructed from the mechanisms can be rapidly clarified. However, there is an unbridgeable abyss between these approaches due to discontinuity between human and animals. One of the key strategies to interpolate the discontinuity must be an establishment of animal models in higher order cognitive functions and their investigation based on the neuronal mechanisms. We should comprehend that a lot of species in non-human animals can achieve most of the higher order cognitive functions and we can study these function in the more suitable animal models.

Table 1.1 Abbreviations

AAF	anterior auditory field
AI	primary auditory cortex
AII	secondary auditory cortex
AIII	tertiary auditory cortex
ANOVA	analysis of variance
Assoc	associative areas
DC	dorsocaudal field
DP	dorsoposterior area
EEG	electroencephalography
Ep	posterior ectosylvian region
ERP	event-related potential
fMRI	functional magnetic resonance imaging
INS	insula
ISI	inter-stimulus interval
MEG	magnetoencephalography
MGB	medial geniculate body
MMN	mismatch negativity
OSD	onset of the stimulus difference
PAF	posterior auditory field
PSTH	peri-stimulus time histogram
SD	standard deviation
SEM	standard error of mean
SF	suprasylvian fringe
SI	stimulus-specific adaptation index
SOA	stimulus onset asynchrony
SSA	stimulus-specific adaptation
T	temporal area
V	ventral area
VPAF	ventroposterior auditory field

This strategy was applied to auditory perception in this study. Auditory system is sufficiently examined field of research and correlated with higher cognitive brain functions that are fundamental to the perception of speech and music. These functions are based on the auditory processing to an acoustic context, such as masking, stream segregation, and change detection that the animals can also achieve. Especially, auditory cortex is believed to play important roles for the perception of acoustic context. Here I report the following sequential experiments in this study. The first is to construct animal model of guinea pigs involved in the change detection. The change detection in human is well investigated by event-related potential (ERP) in EEG, which is called mismatch negativity (MMN). MMN reliably reflects behavior of change detection. To confirm the ERP, especially in MMN, in guinea pig provides the opportunity to apply the invasive electrophysiological methods for investigating the change detection. Finally, I search how the neurons in auditory cortex contribute to the MMN generation and will clarify neuronal mechanisms of the change detection.

1.2 Backgrounds

In this section, the common backgrounds of sequential experiments performed in this study are summarized. The contribution of guinea pigs for auditory researches, relationship between guinea pigs and human on the structure and function of auditory cortex, and the review of studies about the target brain function in this study are mentioned in detail.

1.2.1 Guinea pig: an experimental animal

Guinea pigs were used as an experimental animal for this study, because of its familiarity, handiness, and good audition, in short. Detail explanations are outlined below.

Taxonomically speaking, the guinea pig (*Cavia porcellus*) is a small mammal commonly accepted to be of the order Rodentia. Guinea pigs are further classified in the suborder hystricomorpha. This traditional phylogenetic classification, based on traditional morphological data, has been challenged in

recent years due to modern studies of DNA and RNA sequences. Evidence based on the mitochondrial genome strongly supports the possible future inclusion of the guinea pig in a new mammalian order [2].

A number of stocks and strains of guinea pigs have been described, but only 5 are often used. The short-haired American or English guinea pig (Fig. 1.2) is most popular and often used in researches. Research with guinea pigs is generally acknowledged to have started in the late-18th century, when Lavoisier used them in 1780 to measure heat production [3]. Since then, guinea pigs have been extensively used in studies of immunology, nutrition, otology, genetics, and infectious disease; and provided a lot of important information for these studies in human [4].

Figure 1.2 An adult, albino, short hair, Hartley strain guinea pig. The smooth, short-haired coat is found English and American Breeds. This figure is cited from Ref. [5].

Guinea pigs are docile and easily handled animals. Furthermore, its biological feature, husbandry, diseases, anesthetic care, and experimental methodology are enormously proposed and established [5]. One of the most interesting biological features of guinea pig is social behavior and communication. Close proximity to other guinea pigs is well tolerated both at rest and when moving, the group providing a measure of security. The primary physical contact between adult guinea pigs is huddling, and may be more related to conserving heat than a desire for contact. There is little or no grooming between individuals except at mating and by mothers rearing young. Social grooming, when it occurs, is performed by a female. She nibbles at the head and

ears of the recipient [6].

Guinea pig vocalizations have been identified as the primary means of communication within the species. Guinea pig has a good audition and its audible range in frequency is relatively low than that of other rodents. Berryman has analyzed the frequency and duration range of guinea pig calls, identifying and naming at least 11 distinct vocalizations, each heard in specific situations [7]. For example, exploratory behavior is often associated with the "chutt" and "putt", short duration sounds of varying frequency, while a "whistle" or "low whistle" is a longer call with a distinct rise in pitch. Guinea pigs use whistles when separated from each other, and when anticipating the arrival of food. For the above reasons, guinea pigs are used as an experimental animal in auditory research.

1.2.2 Structure and function of auditory cortex

Auditory cortex is the region of the brain that is responsible for processing of auditory information. As with other sensory cortical areas, auditory sensations at

the subcortical nuclei from cochlea to medial geniculate body (MGB) reach perception only if received and processed by a cortical area. Historically it has been delimited using electrical stimulation, microscopic observation (cytoarchitecture), and anatomo-pathological observations correlating the consequences of neuronal lesions on the auditory function (central deafness) with the extent of morphological changes such as neuronal and axonal degenerations. An important portion of the superior temporal gyrus has thus been identified as the auditory cortex in human (Fig. 1.3*A*). This identified portion corresponds to Brodman areas 41 (Henschel's gyrus) and 42 (Fig. 1.1).

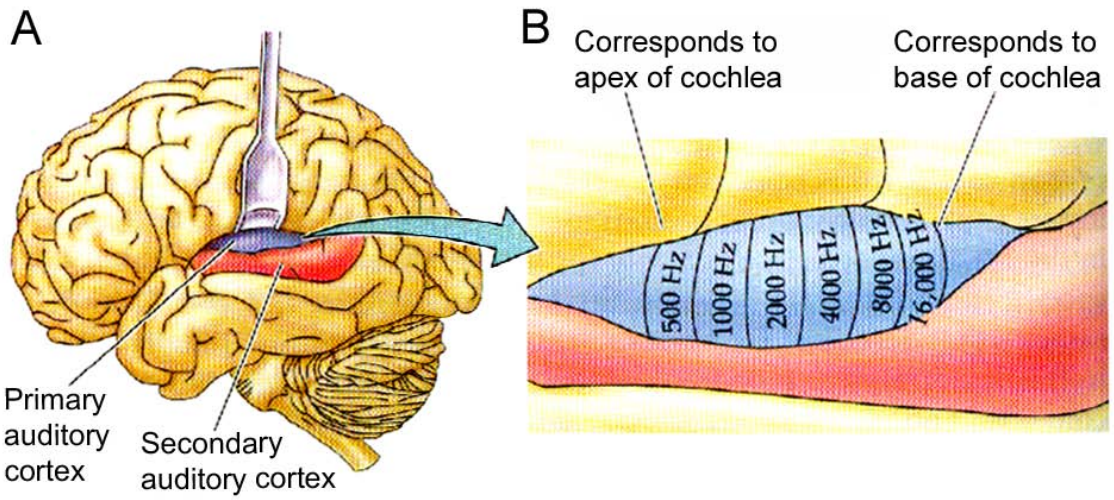

Figure 1.3 Human auditory cortex. *A*, Auditory cortices, primary auditory cortex (AI, blue area) and secondary auditory cortex (AII, red area), are located on superior temporal gyrus. *B*, AI, folded into lateral sulcus, represent tonotopic map, which is a position-dependent property of the response to sound frequency corresponding to the property in cochlea. Cochlear apex and

anterior side of AI selectively responds to low frequency while cochlear base and posterior side of AI responds to high frequency. This figure is cited from Ref. [8].

Neurons in the auditory cortex are aligned according to the frequency of sound to which they respond maximally. Neurons at one end of the auditory cortical areas shows maximal response to low frequencies and neurons at the other end respond best to high frequencies, which is known as a “tonotopic map”. There are multiple auditory areas like the multiple areas in the visual cortex, which can be distinguished anatomically and on the basis that they contain a complete tonotopic map. The meaning of this tonotopic map is unknown and is likely to reflect the fact that the sensory epithelium of the auditory system, the cochlea, is arranged according to sound frequency. Tonotopic map in human primary auditory cortex (AI) is arranged from low to high along anteroposterior axis (Fig. 1.3B).

In the cat, a detailed study of cortical potentials evoked by electrical stimulation of different cochlear regions [9] defined the auditory cortex as occupying a large cortical surface with many distinct tonotopic map (Fig. 1.4*A*): the AI, the secondary (AII) and tertiary (AIII) areas, the posterior ectosylvian region (Ep), the suprasylvian fringe (SF), and the insula (INS). Besides these

areas which are purely related to auditory processing, one can find associative areas influenced also by other sensory modalities (Assoc) that will not be considered here.

The use of acoustic stimulation with pure tones coupled with multiunit recordings along numerous electrode tracks more precisely defined the auditory fields surrounding the AI [10]. Four complete tonotopic maps are presented in the cat (Figure 1.4*B*): the anterior (AAF), AI, posterior (PAF), and ventroposterior (VPAF) auditory fields. These areas are surrounded by a belt of cortex that has neurons responsive to acoustic stimulation, but which have not been systematically studied: the ventral (V), temporal (T) and dorsoposterior (DP) areas. These fields are partly embodied in the anterior or posterior ectosylvian gyri and that becomes fully visible only on an “unfolded” view of the cortex (Fig. 1.4*C*). The tonotopic maps are organized in mirror images at each successive and adjacent field boundary. This mirroring structure of auditory cortical fields seems to be a constant characteristic of mammals, and has been found in rodents, carnivores, bats, and primates.

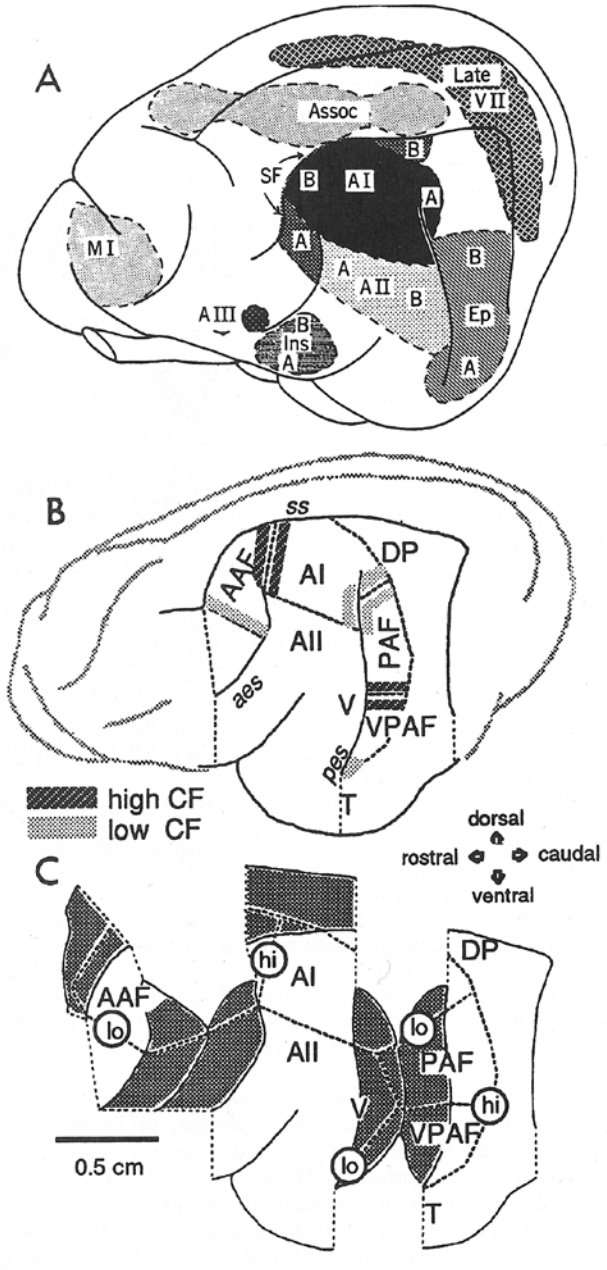

Figure 1.4 Representation of the auditory cortical areas in the cat (left hemisphere). Dashed and dotted lines indicate approximate borders of areas. *A*, Classical representation proposed by Woolsey (1960) based on cortical-evoked potentials to electrical or acoustical stimulation of the cochlea. The letters A and B on the cortex signify cochlear apex and base, or low and high frequencies, respectively. The auditory areas identified at that time are the primary (AI), secondary (AII), tertiary (AIII), posterior ectosylvian (Ep), insular (Ins) and the suprasylvian fringe (SF). The areas situated above the

suprasylvian sulcus are not purely auditory and present weak or delayed responses. MI, Assoc, and VII indicate primary motor cortex, polysensory associative area, and secondary visual area, respectively. *B*, Scheme of the auditory areas as actually defined, using a dense microelectrode sampling of the neuronal responses to pure tones recorded mainly in layer IV. The tonotopically organized areas have a tonotopic map that alternates as the mirror image of each other at boundaries between adjacent areas (anterior auditory cortical field (AAF), AI, posterior (PAF), and ventroposterior auditory cortical fields (VPAF)); surrounding areas do not have a clear tonotopic organization (AII, ventral (V), temporal (T), and dorsoposterior (DP) auditory cortical fields). *C*, Same representation as in B, but on an unfolded cortex so as to see the surfaces hidden in the depth of the sulci. The abbreviation of "lo" and "hi" mean low and high frequency, respectively. This figure is cited from Ref. [11].

The auditory cortex was well studied electrophysiologically also in guinea pigs. Figure 1.5*A* shows the location of the auditory cortex of guinea pig. The auditory cortex is placed in the temporal cortex anterior to visual area and ventral to primary somatosensory area [12]. Its core areas are located at caudoventral side of sylvian fissure (or sulcus), which may correspond to AAF and AI in other mammals. Figure 1.5*B* shows the tonotopic map of the guinea pig auditory cortex. The core area of auditory cortex is separated into AI and Dorsocaudal field (DC). The tonotopic map in AI is arranged from low to high along anteroposterior axis and that in DC is inverted [13]. AI in guinea pigs is

similar to that in human on an arrangement in tonotopic map. However, it is similar to AAF in other mammals rather than their AI.

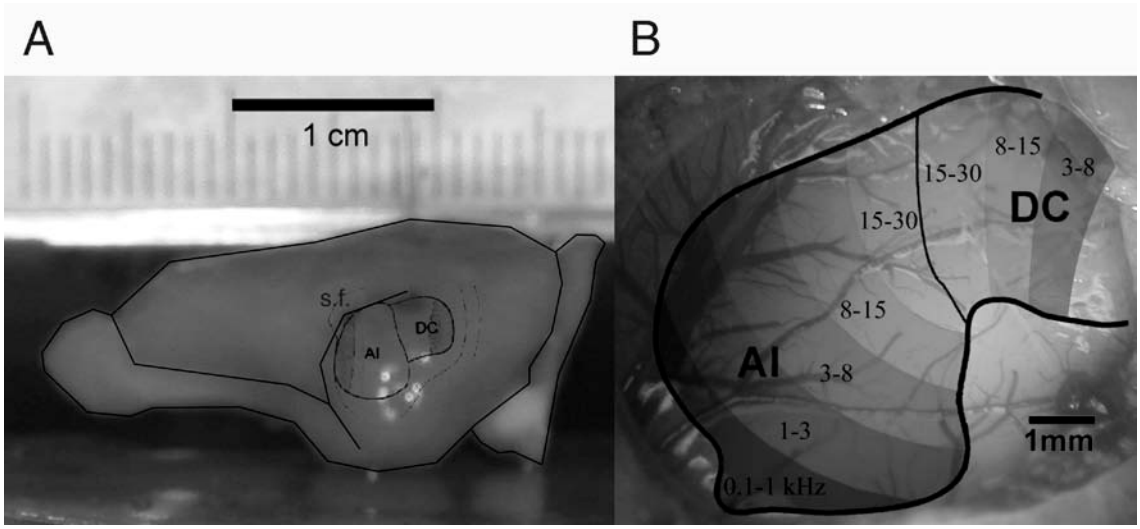

Figure 1.5 Core areas of auditory cortex in guinea pig. *A*, AI and DC are located ventroposterior side of sylvian fissure (s.f.). *B*, Tonotopic map in AI and DC. A scale bar is 1mm.

Processing of sensory stimulus features is essential for humans in determining their responses and actions. If behaviorally relevant aspects of the environment are not correctly represented in the brain, then the organism's behavior cannot be appropriate. Figure 1.6 illustrates the sequential and parallel processing on the auditory pathway. The processing in the auditory cortex will address the relatively higher stages such as “Sensory feature traces” and “Sensory stimulus representation” (Fig. 1.6).

There are some aspects of the function in auditory cortex in various time scales. First, auditory cortex is involved in tasks such as identifying and segregating auditory events and identifying the location of a sound in space. As well as frequency, intensity, binaural information, duration is represented in the auditory cortex. However, arrangement of auditory cortical neurons with sensitivity for specific duration is still unknown. These features are already extracted at the subcortical nuclei and integrated to relatively short and static auditory events by the processing in the auditory cortex. The perceptions of location, pitch, loudness, and harmonic pattern are represented in the auditory cortex by comparing and combining the information of the auditory features. Second, temporal information of the context of auditory events is also important

for perception of speech and music. Once a tone is presented, the excitatory and inhibitory effects that are elicited by the tone can last up to a few hundreds milliseconds [14]. Consequently, the response to succeeding tone is inhibited and facilitated depending on the inter-stimulus interval and their differences in frequency. Especially in auditory cortex, these effects are varied in time scales and longer than those in subcortical nuclei [15]. Sequence of sounds with different auditory features and probability of representation or combination of two sounds with various auditory features are used to identify the effects. Suppression of the following response by the inhibitory effects in the auditory cortex leads adaptation of auditory cortical neurons and involved in the buffering memory (sensory memory). Enhancement of the following response by the excitatory effects is supposed to contribute the coding of spectrotemporal pattern of two tones [14]. Third, long-term memory is also represented in auditory cortex [e.g. 16]. The organized representation for auditory features in auditory cortex including tonotopic map can reconstructed by learning in the long term. Punishment and reward combined with sound of a specific frequency may transform the response to sound with the frequency and tonotopic map in the auditory cortex.

The second aspect of the function in auditory cortex is the target of this study. Information of repetitive sounds is adapted and stored as sensory memory for comparing to the information of following sounds. It is available for animals to detect the change and novelty in the sound sequence. This function will be achieved in the early stage of auditory cortex, like AI.

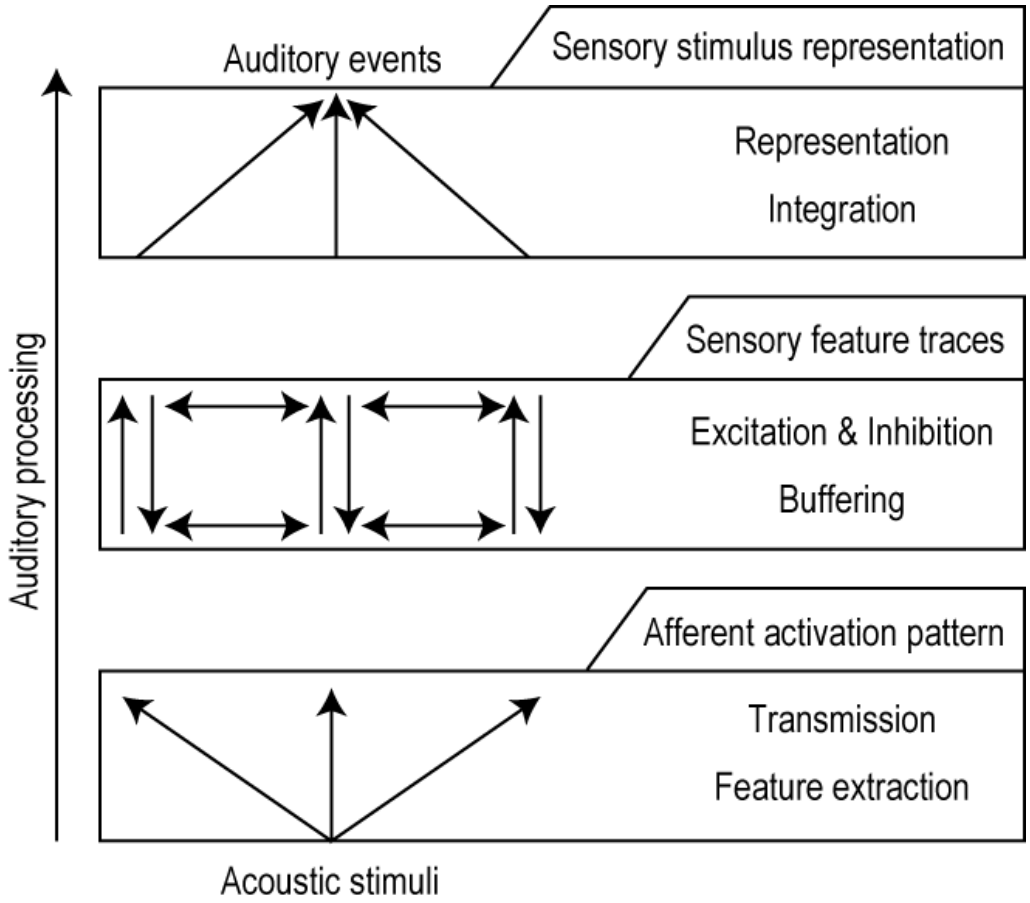

Figure 1.6 Sequential and parallel processing on the auditory pathway. The processing is functionally categorized into three hierarchical stages. One is “afferent activation pattern” which indicates a stage that the fundamental physical features were extracted from the environmental acoustic stimuli and characterized by the firing rate of the auditory neurons. This information is transmitted to the next auditory nuclei. Second is “sensory feature traces” which is a stage that the transmitted information is buffered successively and interacted by other information through the interconnected auditory neurons. Third is “sensory stimulus representation” which is a stage that the extracted and modulated information is integrated to each neuron. The important information for life is selected and represented as auditory events. The processing of the auditory cortex is ranged from the second to third stages. This figure is reconstructed from an article by Naatanen and Winkler [17].

1.2.3 Oddball paradigms and mismatch negativity

Temporal information of sequential acoustic stimuli is essential for auditory perception as described above. Attention and change detection are the very high ordered brain functions achieved through the use of the temporal information. Sound sequence that consists of frequently repeated acoustic stimuli and other acoustic stimuli different in auditory feature inserted infrequently have been used to investigate the functions. This sound sequence is called “oddball paradigm” (Fig. 1.7), that was classically applied in a lot of series of EEG studies in human. The frequent sound stimuli are termed “standards” or “standard stimuli”, while the infrequent sound stimuli are termed “deviants” or “deviant stimuli”. Each sound stimulus in oddball paradigm induced many types of responses in EEG. Especially, set of ERPs that are elicited by deviant stimuli specifically, “MMN” and “P300 or P3”, have been attracted many researchers. Mismatch negativity is an ERP that reflects pre-attentive automatic change detection of the brain and triggered P300 involved in attention in the brain. In fact, recent studies have shown that the automatic change detection governs attentive auditory discrimination ability in humans. This is evident in the finding that the latency of the MMN determines the

timing of behavioral responses to changes in the auditory environment [18]. The MMN is also a likely component of the chain of brain events causing attention switches to changes in the environment [19]. In the light of these observations, it seems that at present the MMN provides the best available physiological measure of automatic central auditory processing.

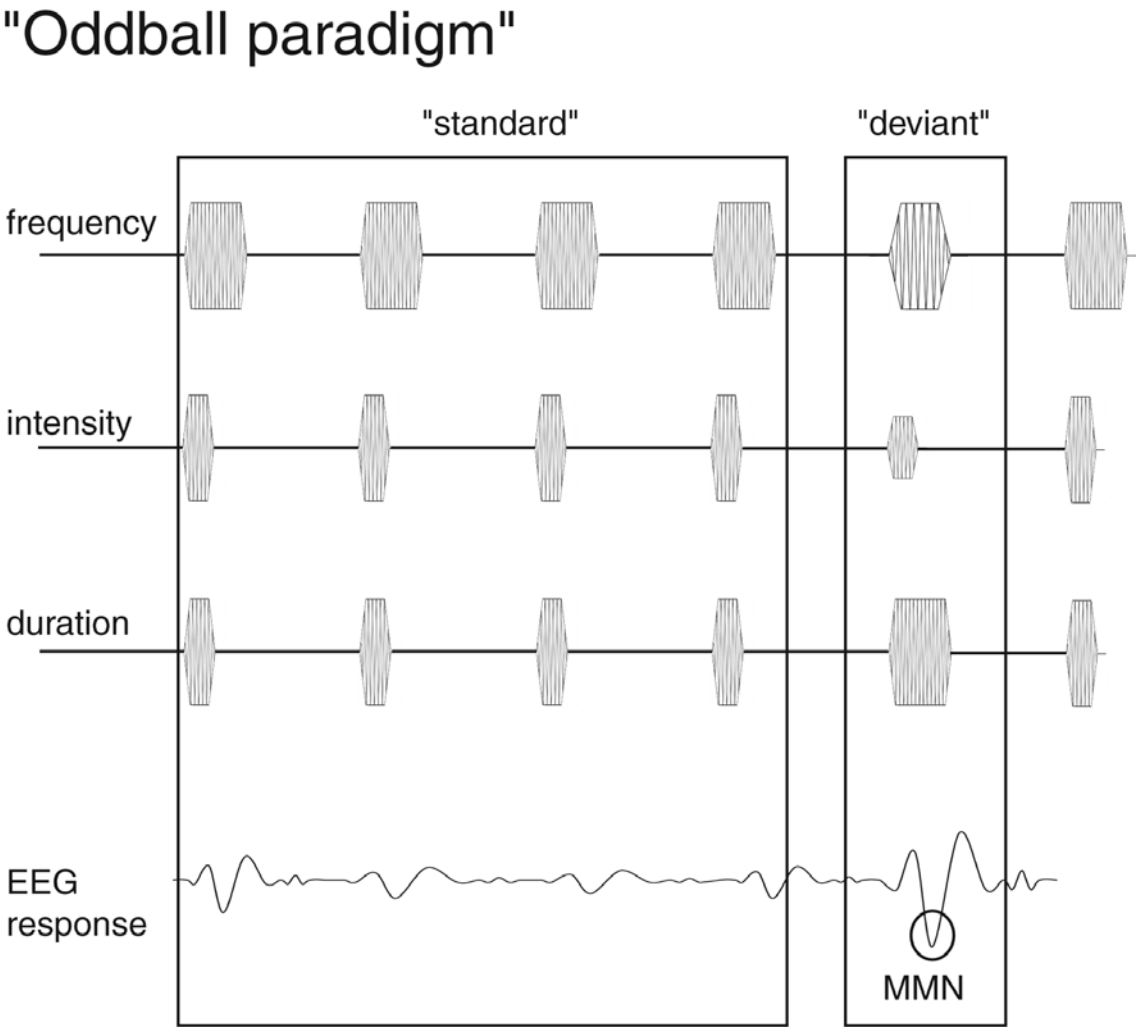

Figure 1.7 Scheme of oddball paradigm and EEG response. The oddball paradigm with a change of auditory features such as sound frequency, intensity, and duration are illustrated from the top. Bottom figure is an EEG response to the oddball paradigm. Open circle indicates MMN, which is a negative ERP component elicited by deviant stimuli specifically.

MMN was first reported by Naatanen, Gaillard, and Mantysalo in 1978 [20]. An in-depth review of MMN research can be found by Naatanen in 1992 [21] while other recent reviews also provide information on the generation mechanisms of MMN [22], its magnetic counterpart, MMNm [23], and its clinical applicability [24]. The MMN can be recorded in response to any discriminable changes in the stimulus stream. Change of sound duration [e.g. 25-29] as well as that of frequency [e.g. 30-32] can elicit the MMN (termed duration MMN and frequency MMN, respectively). Other discriminable changes of acoustic features, such as intensity [33, 34] and inter-stimulus interval [35-37], and the change of a complex sound [38-40] also induce the MMN. The MMN data provide evidence that stimulus features are separately analyzed and stored in the vicinity of auditory cortex. The close resemblance of the behavior of the MMN to that of the previously behaviorally observed "echoic" memory system strongly suggests that the MMN provides a non-invasive, objective, task-independently measurable physiological index of stimulus-feature representations in auditory sensory memory.

The MMN has been documented in a number of studies to disclose neuropathological changes. Presently, the accumulated body of evidence

suggests that while the MMN offers unique opportunities to basic research of the information processing of a healthy brain. MMN, which is elicited irrespective of attention, provides an objective means for evaluating possible auditory discrimination and sensory memory anomalies in such clinical groups as dyslexics and patients with aphasia, who have a multitude of symptoms including attentional problems. Recent results suggest that a major problem underlying the reading deficit in dyslexia might be an inability of the dyslexics' auditory cortex to adequately model complex sound patterns with fast temporal variation [41, 42]. Patients of Alzheimer's disease demonstrate decreased amplitude of MMN [43], especially with long inter-stimulus intervals; this is thought to reflect reduced time span of auditory sensory memory. Parkinsonian patients do demonstrate a similar deficit pattern [44], whereas alcoholism would appear to enhance the MMN response [45]. This latter, seemingly contradictory, finding could be explained by hyper-excitability of neurons in the brain resulting from neuronal adaptations taking place during a heavy drinking bout.

MMN is observed not only in humans, but also in many awake and anesthetized mammals, e.g. monkey [46], cat [47, 48], rabbit [49], rat [50-52], mouse [53], and guinea pig [54, 55]. To clarify the neuronal mechanisms of

MMN, it is important to establish animal models of the brain response involved in MMN. In fact, the research of animal models has contributed significantly to the understanding of the neuronal mechanisms of human MMN. However, it is still debatable about the generation mechanisms of MMN [56]. For example, MMN in guinea pig was reported that its generator is MGB [54, 55], while MMN in other mammals seem to be generated in AI. Furthermore, argument for the generation of MMN is confused due to its multiple generators, such as anterior and posterior part of auditory cortex [30, 56].

A previous study was probably decisive of the issue. It is demonstrated that cat AI neuron whose responses were specifically adapted by successive acoustic stimuli with an identical feature (stimulus-specific adaptation or SSA) could detect sound deviation in frequency and intensity [48]. Therefore, it has been suggested that SSA on individual AI neurons will contribute to generate the MMN (a neural substrate of MMN).

It is well known that the amplitude and latency of MMN depend on the magnitude of difference on both frequency [e.g. 32] and duration changes [e.g. 27]. However, time profile of duration MMN were quite different from frequency MMN. Duration MMN were elicited from the offset of the shorter

stimulus (the onset of the stimulus difference or OSD [53]). Thus, it is still unclear that the SSA also serves as a neuronal substrate of duration MMN. Therefore, it is a challenging problem whether or not SSA in AI neurons can be a common neuronal basis of duration MMN as well as frequency MMN.

1.3 Objectives

Sound duration is one of the fundamental auditory features and is essential for the perception of speech and music in human. Furthermore, also in non-human animals, perception of vocal communication with companions and awareness of chasing predator must require the information of sound duration. It is believed that animals can pre-attentively detect a change of sound duration over the regular inputs from the environment by means of automatic change detection in the brain. However, neuronal mechanisms of such a change detection of sound duration are still unknown.

This brain function, change detection, is fortunately well investigated not only human but also various non-human animals under the MMN research. Additionally, comparative researches with respect to the change detection and MMN have been gradually developed. Thus, it is high time to investigate the neuronal mechanisms of the change detection under the animal model. Since the role of sound duration on auditory processing is less known than that of sound frequency, I had to start establishing an animal model of MMN involved in change of sound duration. When I once establish animal models of

duration MMN in guinea pig, further electrophysiological studies will propose strong evidences for neuronal mechanisms of duration MMN.

In this study, I examined duration MMN and corresponding response of AI neurons in anesthetized guinea pigs under an identical auditory paradigm. If SSA is available for neuronal substrates of frequency and duration MMN, the temporal relationship between SSA and AI neuron responses is most important problem to be investigated. Here I demonstrate that SSA represents temporal property changed according to the types of the responses in AI neurons and the response-dependent property of the SSA can clarify a neuronal generating mechanism of MMN and the change detection in the brain.

The results in this study will significantly broaden the theoretical scope of the MMN research and be consequently of great potential interest in attempts to understand central auditory function, its development, and various forms of its pathology.

Chapter 2 describes establishment of animal model of duration MMN in guinea pigs. Guinea pigs have been extensively used in neuroscience studies of auditory system as well as in other various types of researches. However, MMN in guinea pigs were still indefinite. First of all, I have investigated

about ERP in guinea pigs elicited by a traditional auditory paradigm in detail. Consequently, duration MMN can be induced in guinea pigs and may originate from the AI as well as other mammals. Thus, it is proposed that guinea pigs are available to investigate the neuronal mechanisms of duration MMN by direct electrophysiological methods.

Chapter 3 focuses on the electrophysiological study for neuronal activity involved in duration discrimination in guinea pig AI. More refined auditory paradigm also induced duration MMN in guinea pigs. By means of a unit recording technique, AI neurons were characterized under the identical auditory paradigm. It was shown that responses in AI neurons by successive auditory stimuli were adapted not only by identical feature of auditory stimuli specifically but also on individual types of the responses. MMN has two properties. One is that amplitude of MMN increases as difference of change in auditory stimuli. The other is that duration MMN is induced after the OSD. The characteristics on the adaptation in AI neurons had similar properties as duration MMN. Thus, they fulfill the condition to be a neural substrate of duration MMN.

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Chapter 2

ESTABLISHMENT OF ANIMAL MODEL OF DURATION MISMATCH NEGATIVITY IN GUINEA PIG

2.1 Introduction

MMN is known to exhibit physiological evidence of sensory memory and automatic change detection. As well as to clarify their mechanisms in the brain, for clinical application of pathology, MMN is widely examined in various fields of researches (see Chapter 1). It is proposed in a variety of non-human animals and in human that MMN is organized from multiple complicated neuronal mechanisms in the primary auditory cortex. However, it is believed that MMN

in guinea pig, which is elicited by frequency change, is not generated in AI [1]. Furthermore, duration MMN in guinea pig was not investigated in the past. Thus, aim of the study introduced in this chapter is to establish the animal model for investigating the neuronal mechanisms of MMN. When the classical auditory oddball paradigm with sound duration change was presented, ERP that elicited over the temporal lobe of guinea pig was observed.

It has been well known that the amplitude and latency of MMN change depending on the difference in change of the stimuli [2]. In addition, previous studies have reported that duration MMN is triggered by the onset of the stimulus difference (for example. [3]). It was confirmed in this study whether the characteristics (see also Chapter 1) of MMN were applicable to the candidate ERP components observed in guinea pig. Investigating the amplitude and the peak latency of ERP, I observed a negative ERP component that might address duration MMN, which demonstrated the asymmetry proportion in duration increment and decrement. There were two types of duration MMN in anesthetized guinea pigs. One was duration MMN whose increase in peak amplitude occurred immediately after OSD in a decrement oddball paradigm. The other exhibited a peak amplitude increase closer to the offset of the longer

stimulus in an increment oddball paradigm.

The result suggests that duration discrimination reflected in duration MMN probably consists of two types of processing in the brain: whether the stimuli changes or not (change detection) and how its difference magnitude is (difference detection). On the oddball paradigm in which the duration of the deviant stimuli is shorter than that of the standard stimuli (duration decrement), the difference of duration between the standard and deviant stimuli is recognized after OSD. While, in the duration increment oddball paradigm, difference of duration between standard and deviant stimuli are detected at the offset of deviant stimuli as opposed to the OSD.

These findings indicate a mechanism to percept the difference of duration change and reveal the importance of the end of a stimulus for this perception. Furthermore, this study will be helpful for further investigation of neuronal mechanisms in duration discrimination by an electrophysiological approach.

Materials and methods

This experiment was performed in accordance with Society for Neuroscience Policies for the Use of Animals and Humans in Neuroscience Research and endorsed by the animal experiment committee at Keio University and Tamagawa University. This declaration is applicable to the next study described in Chapter 3.

2.1.1 Subjects and Surgery

Seven guinea pigs weighing 250-350 g were used (See Chapter 1) and obtained 14 data sets (2 data in each guinea pig) in this study. Body temperature was maintained at 37±1 ℃ throughout the experimental procedure. Operation was performed under ketamine (40 mg/kg, i.m.) and xylazine (20 mg/kg, i.m.) anesthesia. Tracheotomy was performed for artificial respiration with dinitrogen monoxide (N_2O) and sevoflurane anesthesia. Then, the bone over the left AI (3.0 mm post. bregma 10.0 mm lat. midline [4]) and the occipital

bone (1 mm caudal from lambda) were drilled to attach a recording and ground electrode, respectively.

After the operation, N_2O (60 - 70 %), sevoflurane (0.5 - 2 %), and oxygen (30 - 40 %) were introduced into the artificial respiration (GENEQ SAR-830 ventilator, Canada) after spontaneous respiration was eliminated by pancuronium bromide (0.2 mg/kg), and absence of apnea was maintained by pancuronium bromide (0.2 mg/kg/3h) during observation.

2.1.2 Electroencephalography (EEG)

There are several types of recording techniques for electrophysiological study (Fig. 2.1). Truly invasive recording is only a scalp recording according to human EEG. In addition to scalp recording, epidural and surface recording were relatively less invasive than extra- and intra- cellular recordings and were used for investigating the brain function in human. Epidural and surface recording are sometimes called ECoG in human study. ECoG is a recording technique that directly obtains cortical electrical activity close to the surface of

the cerebral cortex. The EEG is capable of detecting changes in electrical activity in the brain on a millisecond-level. It is one of the few techniques available that has such high temporal resolution. In animal studies, epidural recording is frequently used to observe ERP corresponding to ERP on human EEG. Thus, epidural and surface recordings both are called EEG in animal studies. EEG data represent an electrical signal (postsynaptic potentials) from a large number of neurons. Electrical currents are not measured, but rather voltage differences between different parts of the brain. There are two types of recording procedures: monopolar recording and bipolar recording. Monopolar recording is achieved by an electrode placed to the center part of the brain activated to stimuli and that placed the part not activated, such as ear lobe. While, bipolar recording is achieved by two adjacent electrodes, so that observers can focus clearly on activation point by means of phase reversal.

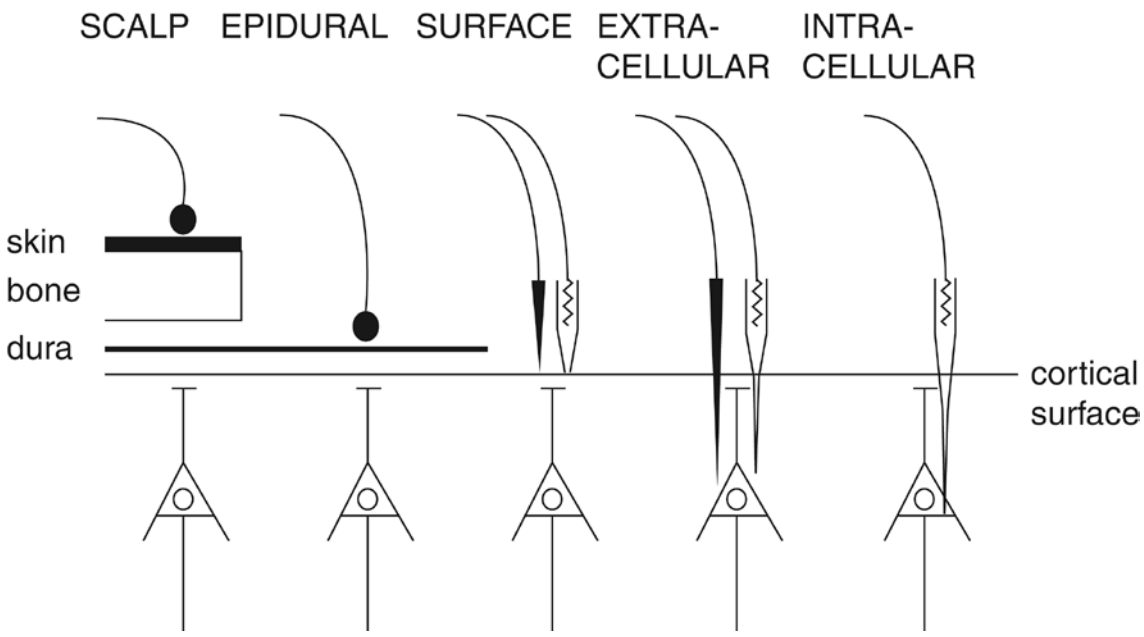

Figure 2.1 Electrode arrangements for different types of recordings. These are illustrated from the left in ascending order of Invasiveness, special resolution, and amplitude of the detected signals.

In this study, monopolar recording of EEG was employed. The reference electrode was clipped at the left ear lobe with conductive paste. The silver bead electrode for recording was inserted into epidural site on the temporal lobe (on top of the AI) and anchored with a small screw. EEG data were amplified by pre- (×20) and main-amplifiers (×250), and filtered on-line by 0.1 Hz high-pass and 300 Hz low-pass filters (Nihonkoden MEG-6116, Japan) and recorded with recording software (DataWave Technologies DISCOVERY, USA).

2.1.3 Acoustic stimulation

The duration increment and decrement oddball stimuli consisted of 4 kHz pure tones calibrated at 80 dB SPL were presented with 510 ms SOA. All oddball stimuli are composed of over 3000 standard stimuli and 10 % pseudo-randomly replacing deviants (3-15 standards between the deviants). Duration increment oddball paradigm was made up of increasing deviant stimuli (100, 150, and 200 ms) and standard stimuli (50 ms). Duration decrement oddball paradigm was made up of decreasing deviant stimuli (50 ms) and standard stimuli (100, 150, and 200 ms). These stimuli were delivered to the right ear through a tweeter (Tucker–Davis Technologies ES1, USA) with a conical tube.

2.1.4 Analysis

Band-pass filter (1-50Hz) was applied off-line to the measured EEG data. Averaged standard responses of individual animals were calculated from ERP traces of standard stimuli. The responses to the standard stimuli following

deviant stimuli were excluded from the averaging. The same applied to ERP traces with a maximum over 500 mV or a minimum below -500 mV. In the identification of authentic MMN, I employ the reverse condition that proposed by Jacobsen et al. 2003 [5]: the dominant negative component was calculated by subtracting the waveform of the responses of the standard on the increment oddball paradigm from the responses of the deviant on the decrement oddball paradigm and was defined as duration decrement MMN, and *vice versa*.

In each subject, responses to standards and deviants were compared with a two-way repeated measure ANOVA [6] and the significance of the MMN was computed from these comparisons within a time profile by using a two-tailed multiple t-test with Bonferroni correction (369 comparisons, Fig. 2.2). Peak amplitudes and peak latencies of the MMN in each subject were obtained at the time period of the subtracted waveform from 50 ms to 300 ms after the stimulus onset. These parameters across 6 conditions (increment or decrement, 3 duration differences) were evaluated statistically by Tukey-Kramer multiple comparison procedure following a one-way factorial ANOVA (15 comparison in 6 groups).

2.2 Results

Classical oddball paradigm of sound duration change and reverse condition were presented to 7 guinea pigs. Consequently, all 14 EEG data showed middle latency responses that were equivalent to those in previous study [4, 7] and MMN-like negative components of ERPs were observed.

2.2.1 Duration MMN in individual guinea pigs

An example of significant MMN components was illustrated in Fig. 2.2. Response to the standards and to the deviants was not significantly different by two way repeated measure ANOVA ($F(1, 3001) = 2.7687$, $\varepsilon_{GG} = 0.036$, $p = 0.0962$), however, interaction between the type of stimuli (standards and deviants) and time was significant ($F(368, 1104368) = 2.3422$, $\varepsilon_{GG} = 0.036$, $p < 0.001$). Within a time profile, response to the standards and to the deviants were compared by a two-tailed multiple t-test with Bonferroni correction following the ANOVA, revealing multiple components of significant negative

ERP during and after the stimulation (369 pairing comparison, $p < 0.05$). Effect sizes (ESs), which indicate a ratio of the difference of mean value against its standard deviation, were 0.19-0.25 on the significant MMN. All subjects showed significant duration MMN under the condition of a 150 ms duration change in the oddball paradigm.

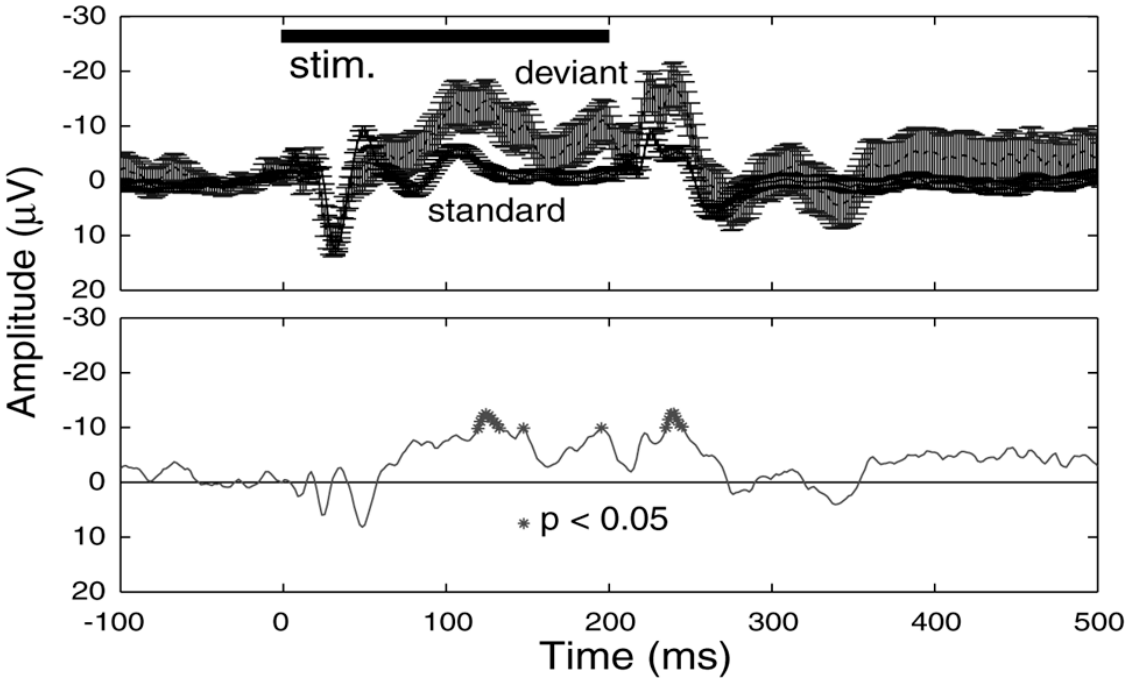

Figure 2.2 Top is the mean ± SE of the response to the standards (gray dotted line and error bars, 2717 trials) and deviants (black solid line and error bars, 286 trials) in one subject. Bottom is a subtracted waveform of these mean responses. * $p < 0.05$. Thick bar indicates the duration of the stimuli (200 ms). Effect size (ES) at the peak of MMN was 0.245. This figure is cited from Ref. [8].

2.2.2 Grand averaged duration MMN: mean of duration MMN obtained from all guinea pigs

ERP of EEG data are often evaluated by averaging the ERP over all subjects that is called “grand averaged” ERP. Figure 2.3 shows grand averaged responses and subtracted waveforms in each condition. Both increment and decrement oddball paradigms elicited negative ERP components having an enlarged peak amplitude as the duration difference increased (Fig. 2.3*A*-*F*). Temporal characteristics of the components were, however, quite different between duration increment and decrement conditions. Deviants in the decrement oddball paradigm elicited negative ERP components with a constant peak latency from OSD irrespective of duration difference (arrows in Fig. 2.3*H*). While, deviants in the increment oddball paradigm elicited negative ERP components that were sustained throughout the duration of the deviant stimuli and reached a peak close to the deviant stimuli offset (arrows in Fig. 2.3*G*). Especially with a duration difference of 150 ms, the ERP component showed two peaks.

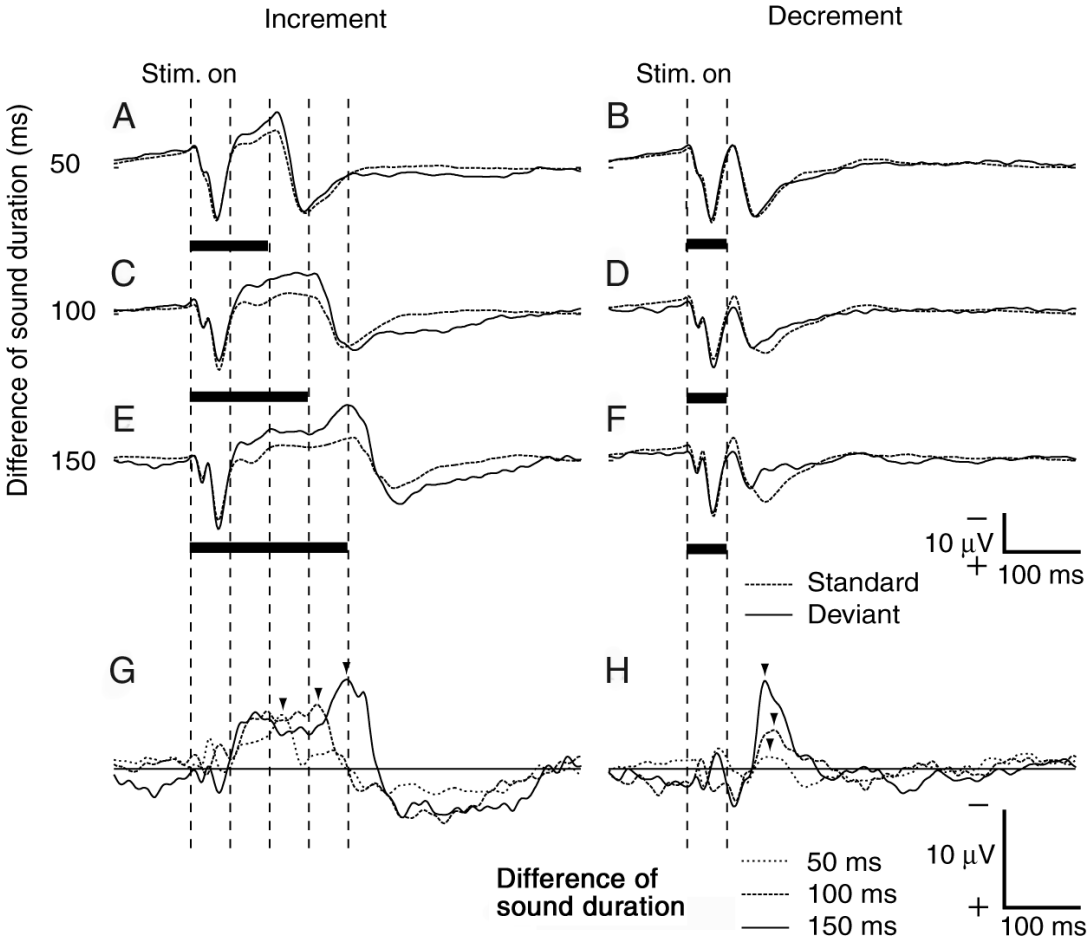

Figure 2.3 Grand averaged responses to standards (dotted line), deviants (solid line), and these subtracted waveforms (dotted and solid lines in lower figures). *A*-*F*, Grand averaged EEGs (N = 14) to the standard stimuli and the deviant stimuli of increment oddball paradigms (*A*, *C*, *E*) and decrement oddball paradigms (*B*, *D*, *F*). *G*, Subtracted waveforms of the EEGs in increment oddball paradigms. Each trace is indicative of the difference of tone duration (Thin dotted lines, subtracted waveforms in the oddball paradigm with 50 ms duration difference; thick dotted lines, those with 100 ms duration difference; solid lines, those with 150 ms duration difference). *H*, Subtracted waveforms of the EEGs in increment oddball paradigms as in *G*. Arrows indicate the peak of the dominant negative component of the subtracted waveforms. Peak amplitude was gradually enlarged as the difference of tone duration increased (-5.7, -6.4, and -8.0 mV in increment and -0.7, -4.4, and -8.9 mV in decrement). This figure is cited from Ref. [8].

2.3.3 Time profile of duration MMN in guinea pig

In Figure 2.4, the peak latencies of the negative ERP components in each animal were evaluated across the conditions (increment or decrement, duration difference). The peak latencies had a statistically significant difference across the conditions ($F(5, 78) = 4.31$, $p < 0.05$). A Tukey-Kramer multiple comparison revealed the significance of the following combinations: Peak latencies in the condition with increment 150 ms duration difference were longer than those with increment 50 ms duration difference, ($p < 0.05$), those with decrement 100 ms duration difference ($p < 0.05$), and those with increment 150 ms duration difference were longer than those with decrement 50 ms duration difference ($p < 0.01$). Mean of the peak amplitude enlarged slightly as the duration difference increased along with the grand averaged amplitude of subtracted waveforms (see Fig. 2.3) but showed no significant difference across the paradigms ($F(5, 78) = 1.25$, $p > 0.05$).

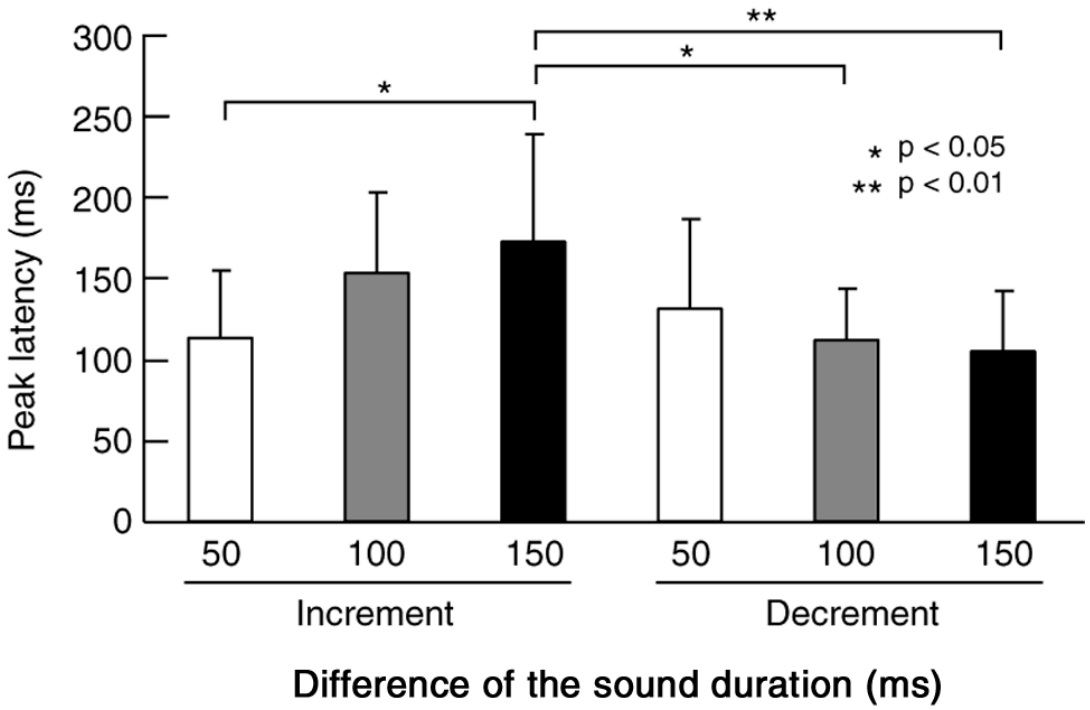

Figure 2.4 Mean and SD of peak latency across the condition of the paradigms (duration increment and decrement, duration difference). These are significantly different by ANOVA ($F(5, 78) = 4.31$, $p < 0.05$). Multiple comparison method was applied to all combinations of the two conditions. It revealed a significant difference of the peak latency between 50 ms increment and 150 ms increment, 150 ms increment and 100 ms decrement, and 150 ms increment and 150 ms decrement. The other combinations were not significant. This figure is cited from Ref. [8].

2.3 Discussions

In this study, the MMN-like negative components were observed using the duration oddball paradigm. I refer to the components that were derived by employing the reverse condition [5], as duration MMN. In this study, by comparing the duration MMN of duration increment and decrement, I can analyze two mechanisms of the MMN that are believed to correspond to change detection and difference detection.

The amplitude of the duration MMN in this study was increased as the duration difference increased. This result supports the previously reported duration MMN [9, 10] and indicates difference detection in the brain. Previous studies have focused on the MMN that are elicited from the offset of the shorter stimulus [11], the moment at which the stimulus deviation commences [12], and the deviant-standard-discrimination point [5], that are all equivalent to the OSD. Although, in this study, duration MMN in duration oddball paradigm initiated from the OSD, it presented a persistent response corresponding to the duration of the deviant stimuli in increment oddball paradigms. The peak latency of the duration MMN, involving in the difference detection, was prolonged until the

offset of the deviant stimuli. Furthermore, as in the case of a 150 ms duration difference, bimodal response was observed (Figs 2.2, 2.3). These results were quite different from previously reported duration MMN [3, 5, 9-11]. It implied the change and difference detection of the sound attributes are processed separately. Therefore, MMN may exhibit a bilateral character of change detection and difference detection.

These detections can coincide in the oddball paradigm of sound frequency, intensity, and duration decrement. However, in the increment oddball paradigm, only the change detection commences from the OSD, while the difference detection begins at the offset of the deviant stimuli. I was able to, thereby, discriminate the change detection and the difference detection with the duration increment oddball paradigm. Previous studies have been employed with a comparatively small duration difference (< 100 ms) (for example, [3]). In our results, there was no significance of the latencies between duration increment and decrement with smaller duration differences (50 ms, 100 ms). If the larger duration difference between standards and deviants emphasizes the temporal discrimination between change detection and difference detection, a duration increment oddball paradigm with a sufficiently larger duration

difference should allow these detections to be discriminated experimentally. This was, in fact, accomplished at 150 ms difference in our result.

Kraus et al. [1] reported that frequency MMN was not observed with epidural recording over the temporal cortex in the guinea pig as opposed to in other animals such as cats [13] and mice [3]. Moreover, a recent study has indicated that multiple generators of frequency MMN and duration MMN were revealed with intracranial recording [14]. Highly specific forms of echoic memory corresponding to different attributes of sound are assumed to differ in the contribution to the respective MMNs. This explains why I was successful in investigating the duration MMN by EEG recording over the temporal site.

2.4 Conclusion

Noting the different timing of the change detection and the difference detection in duration discrimination, I investigated the event-related potential from varying durations (duration MMN) on anesthetized guinea pigs. This study is the first report that clearly points out the difference between duration increment and decrement that is generated by separate mechanisms of change detection and difference detection. This suggests that duration MMN and also MMN with other physical attributes should be evaluated in consideration of these multiple detections. It will be a challenging process to clarify whether these detections are parallel neuronal processing mechanisms or not. Animal model of MMN in guinea pigs has good applicability for the future studies for neuronal mechanisms of duration discrimination in humans.

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Chapter 3

NEURAL SUBSTRATE OF DURATION MISMATCH NEGATIVITY IN GUINEA PIG

3.1 Introduction

Sound duration is behaviorally and semantically important for human and non-human animals. Shortening and lengthening vowel duration changes the vowel recognition in humans [1], and vowel duration often represents phonemic difference in some languages such as Japanese and Finnish [2-4]. As in the case of humans, situation-dependent animal vocalizations are varied in duration [5, 6]. The duration of animal vocalization can convey behaviorally important information [7, 8]. Therefore, it has been widely interested in how auditory neurons process the sound duration.

Many previous studies have investigated the response property to single stimulus of various durations and found that auditory neurons are tuned for specific sound duration in bat [9-13], cat [14], mouse [15], rat [8, 16, 17], and guinea pig [18-20]. Environmental sounds are, however, most often presented successively and are perceptible as an auditory sequence. Sound duration, as well as sound frequency and intensity, can be discriminated on the basis of their context of preceding stimuli [21]. It remains unclear how the duration-tuned auditory neurons are involved in the discrimination of sound duration during auditory sequence.

When the repetitive stimuli with rarely intervening stimuli that deviated in the sound features (termed as "oddball stimuli") were presented, we as humans and other animals can detect the deviation. Comparing event-related brain potentials to the rarely intervening stimuli (deviant stimuli) with those to the frequently repetitive stimuli (standard stimuli), a negative event-related brain potential termed as MMN [22] is elicited. Ulanovsky et al. used a similar experimental procedure and found that auditory cortical neurons contribute to the deviance detection of sound frequency and intensity during auditory sequence [23]. In the case that the deviant and standard stimuli are different in

sound duration, MMN is also elicited by the deviation in human [e.g. 24-28] and non-human animals [e.g. 29, 30]. Guinea pig is one of the rare animals that are well examined for both of duration-tuning in auditory neurons and the MMN. In this study, therefore, I demonstrated a neural substrate for the duration MMN in guinea pig.

It is noted that the change in sound duration during auditory sequence, different from sound frequency and intensity, cannot be recognized at the stimulus onset and until the succeeding stimulus shortens or lengthens comparing to the preceding stimuli. The timing is termed as OSD [28]. When the sound duration is suddenly lengthened, the duration increment can be detected even while the stimulus is still continued. Many previous studies proposed that auditory neurons with the response occurring immediately after the stimulus offset (offset response) are tuned for different sound duration and the offset response represents sound duration [9-13, 15, 16, 18, 20]. However, the duration discrimination initiated during the ongoing stimulus cannot be explained by the offset response [31].

I already proposed the possibility that there are 2 discrimination processes of sound duration during auditory sequence (see Chapter 2, [30]). One is a

detection initiated during the ongoing stimulus in duration increment. The other is a detection initiated after stimulus offset in duration decrement. A few studies reported that sustained response [17], a long-latency response [14], or pauser type of response [19] occurring before stimulus offset is also tuned for sound duration. I predict that these responses contribute to the duration discrimination initiated during the ongoing stimulus. In this regard, I focused on the relationship between the temporal characteristics of the responses in AI neurons and time course of the duration discrimination process. This study will shed light on how duration-tuned responses are involved in the processing of sound duration discrimination during auditory sequence.

3.2 Materials and Methods

3.2.1 Subjects and Surgery

The guinea pig has a large repertoire of vocal communication calls (11 distinct calls according to Harper, 1976 [5]). These calls fundamentally differ in their spectrotemporal features [32]. The components of each vocalization have a variety of duration (from a few tens of milliseconds to several hundred milliseconds) and frequency [33]. In addition, the guinea pig is one of the most established animal models for investigating neuronal representation of sound duration in subcortical areas [18-20]. More importantly, guinea pigs have been proposed as the animal model for investigating sound duration discrimination during an auditory sequence [30].

Eight female guinea pigs (3–5 weeks old) weighing 250–350 g were used in this study. Bone and dura overlying the left auditory cortex (3.0 mm posterior bregma, 10.0 mm lateral midline, 5 mm × 5 mm) [34] were removed for single-unit recording. Detailed procedures of subject maintenance and surgery

were identical with those in Chapter 2.

Figure 3.1 is schematic drawing of EEG (Chapter 2) and unit recording (Chapter 3) in this study.

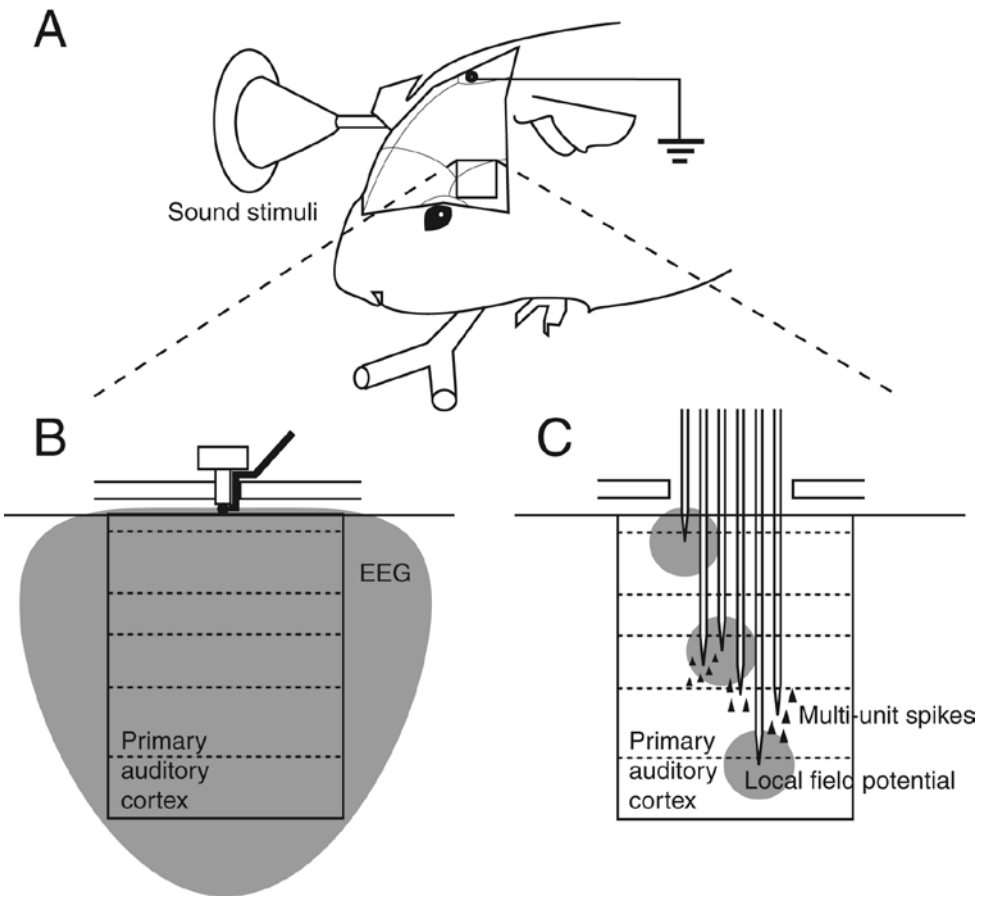

Figure 3.1 Schematic drawings of experimental preparation and arrangement of electrodes for EEG and unit recordings. *A*, Anterolateral view of experimental preparation. The guinea pig is anesthetized with artificial respiration and sound stimuli are presented to the right ear. Grand and reference electrodes are placed on occipital bone. The small square on the left temporal bone indicates the area including AI. *B*, Recording electrode and putative recording area of EEG. *C*, Recording electrode and putative recording area of unit recording and also local field potential recording.

3.2.2 Acoustic stimulation

There is a problem for estimating the neural substrate of the duration MMN by classical oddball paradigm. As shown in previous studies [26, 27], when the responses to the standard and deviant stimuli are compared directly, the difference reflects stimulus duration characteristics rather than the processing of sound duration discrimination [28]. To rule out this effect there are two possible methods. One is a paradigm using a reverse-standard-deviant condition [35, 36]. It is an oddball condition that standard and deviant stimuli are inverted in duration characteristics. Deviant stimuli in the reverse-standard-deviant condition and standard stimuli in a normal oddball condition have the same duration characteristics as compared, and vice versa [28, 30]. The other is a “roving-stimulus” paradigm [37] or the oddball paradigm of equal probability [38] that consists of stimulus trains of identical stimuli whose parameter (e.g. frequency) is varied from train to train. Accounting the first stimulus of the trains for the deviant stimuli and fully repeated stimulus at the end of the trains for the standard stimuli, the MMN can be elicited by comparing the response to the deviant stimuli with that to the standard stimuli [37, 38]. I

considered that the latter method is well suited in this study because neuronal responses sometimes drifted up and down during the long-term experiment and it is preferable that a time lag between the comparing sets of the deviant and standard stimuli should be as short as possible.

The paradigm in this study consisted of successive trains that pseudo-randomly changed in duration train-by-train (Fig. 3.2A) and the series of the trains were repeated over 50 times. Each stimulus in the paradigm were Gaussian noises of 4 durations (50, 75, 150, and 200 ms) formed by a trapezoidal window (rise/fall = 5 ms). These durations were selected in a reasonable range corresponding to guinea pig's vocalization [5, 33] and designed such that all combinations of durations have equally spaced duration difference of 25 ms (25–150 ms) at the time of changing duration. Note that these stimuli were made from an identical Gaussian noise and commenced with an identical signal (Fig. 3.1). The acoustic stimuli were delivered to the entrance of the right ear through an attenuator, a speaker driver, and a tweeter with a conical tube (PA5, ED1, and ES1, respectively; Tucker–Davis Technologies, Alachua, FL, USA). The sound pressure level of all stimuli was 76 dB SPL at the tube tip.

Each stimulus was presented for 7 to 13 times in a train, with an interval of 510 ms SOA. The number of stimuli in a train was pseudo-random and aligned such that the occurrence of each duration change was equal (10 %) over the stimulation. The first and seventh stimuli in the train were defined as “deviant” and “standard” stimuli, respectively.

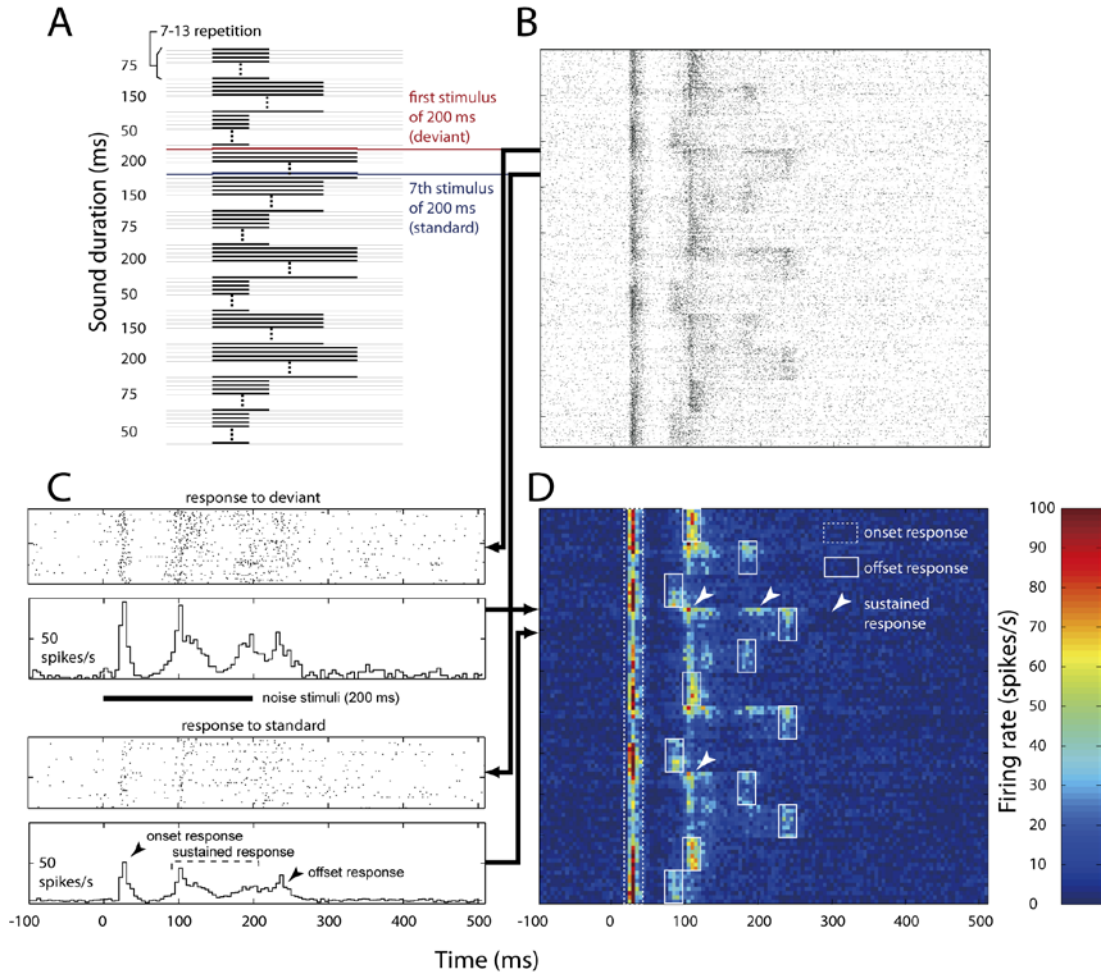

Figure 3.2 Auditory sequence used in this study and an example of the responses of a single AI neuron. A: Sequential noise stimuli with 4 durations (50, 75, 150, and 200 ms) repeated 7–13 times in each train, with the sequences being presented over 50 trials. Stimulus onset asynchrony was 510 ms. The first and seventh sound stimuli after the duration changes were defined as “deviant” and “standard” stimuli, respectively. B: Single unit discharges of responses to the sound sequences. C: Enlarged views of response discharges and peri-stimulus time histograms (PSTHs) of the responses to deviant and standard stimuli (200 ms, a horizontal solid bar). Arrowheads depict onset and offset responses. A dashed line depicts sustained responses. D: Color map distribution of PSTHs of all responses to the sound sequences. Onset (dotted-line square), offset (solid-line squares), and sustained responses (white arrowheads) are illustrated. This figure is cited from Ref. [39].

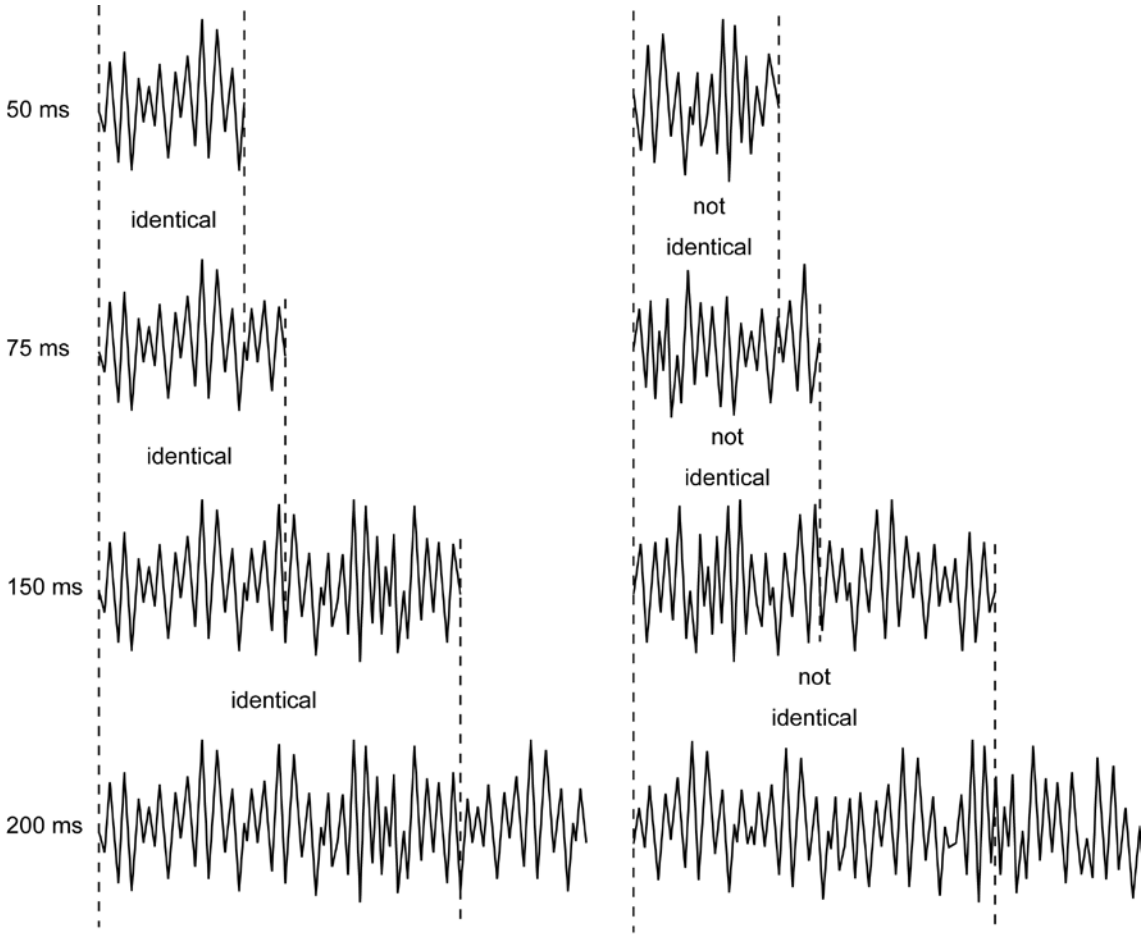

Figure 3.3 Schematic drawings of broad band noise stimuli which commence with an “identical” signal (left column) and do not (right column). Stimulus duration depicted at the left of the figure. Strictly speaking, the 5-ms ends of the stimuli do not identical due to application of trapezoidal window. I used the stimuli which commenced with an identical signal (left column) in the present study.

3.2.3 Unit recording

Platinum/tungsten electrodes coated with quartz glass and with tip diameters of 25 μm (impedance: 3–5 MΩ, ESI2ec; Thomas Recording, Giessen, Germany) were inserted perpendicular to the cortical surface of the left AI. Responses of a few neurons enduring approximately 1 h acquisition time were obtained for each penetration at a depth of 200 to 1400 μm from the surface. Response signals were amplified using pre-amplifiers (x20, EM112/R; Thomas Recording) and amplifier modules (x2000, MEG-6116; Nihon Koden, Tokyo, Japan) and then filtered by 500 Hz high-pass and 10 kHz low-pass filters (MEG-6116; Nihon Koden). The responses were digitized at 20 kHz. Neuron spikes were isolated from the responses by using appropriate threshold levels and were recorded with an acquisition window of 1.6 ms using an acquisition program (Discovery; DataWave Technologies, Longmont, CO, USA). Multiunit responses were decomposed to single-unit responses by a clustering method using add-on software (Autocut; DataWave Technologies). Waveforms of spikes were confirmed by comparison to a template waveform of initial several spikes on a MATLAB (The MathWorks Inc.; Natick, MA, USA) program.

Single-unit responses were displayed in raster diagrams (Fig. 3.2B) and peri-stimulus time histograms (PSTHs, 5 ms bins, Figs 3.2C, 3.5 and 3.7). A color map distribution of the responses during an auditory sequence was depicted by alignment of the PSTHs (Figs 3.2D and 3.8A)

3.2.4 Analysis

PSTHs of the response to standard and deviant stimuli were aligned across conditions of stimulus duration and duration of preceding (conditioner) stimuli. Responses within a time window of 50 ms following stimulus onset and offset were compared against the spontaneous activity during the 100 ms prior to stimulus onset for each PSTH (I used a standard 99.9999 % confidence limit such that no significance was observed during spontaneous activity, Fig. 3.4 E-H) in order to detect responses after stimulus onset and offset (respectively, onset and offset responses). A response component elicited during acoustic stimulation was also investigated (from 50 ms after stimulus onset to stimulus offset). This component was defined as a sustained response with onset inhibition. According to with or without the responses described above (onset,

offset, and sustained responses), I categorized all single AI neurons into 8 types (e.g. Fig. 3.4 A-D). In addition, effects of stimulus duration and duration of conditioner stimuli on the firing rate of overall responses (across bins during the analysis time window of 250 ms after stimulus onset) in AI neurons were evaluated by two-way repeated-measures ANOVA (4 stimulus durations ×4 durations of conditioner stimulus; e.g. Fig. 3.4 I-L).

For each neuron, I compared the response to deviant stimuli with that to standard stimuli at the 2 separate time windows configured before and after OSD (Fig. 3.5). One time window is of 50 ms from the stimulus onset (time window before OSD). The other time window is of 150 ms from the OSD (time window after OSD), which is defined to include the time range of MMN elicitation in previous studies [29, 30].

Many previous studies proposed that there is a suppressive effect by preceding stimulus which depends on stimulus duration [21, 40-43]. When I compared the response to deviant and standard stimuli (Fig. 3.5A), I uniformed the stimulus duration but did not the duration of preceding stimuli. Therefore the subtracted response between the responses to deviant and standard stimuli (Fig. 3.5B) might include the difference in the suppression effect by preceding stimuli (suppression

by 50-ms preceding stimuli < suppression by 200-ms preceding stimuli). To cancel the suppression effects, I averaged the responses to deviant stimuli and those to standard stimuli, respectively (e.g. response to deviant stimulus of 200 ms and that of 50 ms, Fig. 3.5C upper; response to standard stimulus of 200 ms and that of 50 ms, Fig. 3.5C lower). For each neuron, I compared these averaged responses across the manipulated duration difference.

Multi-comparison Wilcoxon tests were applied to the trial-varied responses in each time window (before and after OSD; multiple comparisons across 6 duration differences were corrected by Holm method). Significant differences are illustrated at the center of Fig. 3.5 C, E, and G). Finally, I counted the number of neurons with or without sustained responses that showed significant differences in the response to deviants and standards (Fig. 3.6).

During the time window after OSD I also compared the responses to deviant and standard stimuli over all neurons with or without sustained responses (Fig. 3.7). Three-way mixed-model ANOVA (2 neuron groups ×2 stimulus conditions ×6 duration differences) and multiple-comparisons Wilcoxon tests were applied to compare the responses to deviant and standard stimuli and these were corrected by Holm method (6 duration differences for each neuron group).

I then focused on neurons with sustained responses. To evaluate differences in response adaptation during the time windows before and after OSD I recorded response alterations during the auditory sequence (Fig. 3.8B). During each time window and each stimulus repetition I calculated the index of stimulus-specific adaptation (SI) as proposed previously [23]. SI was defined according to the equation given below, where the mean firing rates of the responses to standard and deviant stimuli with a stimulus duration *di* (50, 75, 150, or 200 ms) were *s(di)* and *d(di)*, respectively.

$$SI(di) = (d(di) - s(di)) / (d(di) + s(di))$$

This equation indicates that SI allows response adaptation to be normalized independently of differences in firing rates of the responses in each neuron and each time window. The SI of neurons that failed to show sufficient activity (4 out of 38 neurons) was not calculated to avoid zero division (these were 2 neurons without onset response and 2 neurons with only a weak sustained response). SI during the time window before OSD (SI before OSD) and that after OSD (SI after OSD) were compared across manipulated duration changes

and change directions (shortening or lengthening) and for 6 duration differences for each neuron (Fig. 3.8C). Because it was demonstrated that responses before OSD were affected only by the duration effect of the conditioner stimuli (see Results) I evaluated SI after OSD which was subtracted by that before OSD (SI after OSD – SI before OSD) for each neuron to exclude this duration effect (Fig. 3.8D). SI after OSD – SI before OSD over neurons was compared across duration change by 2-way repeated-measures ANOVA, post-hoc multiple t tests with Holm correction (Fig. 3.9). Correlation between duration difference and the difference in SIs were also analyzed.

3.3 Results

3.3.1 Response properties of AI single neurons in an auditory sequence

Figure 3.2 presents an example of a recording trace from an AI neuron. This neuron showed phasic onset and offset response and, in addition, there was a late component of the response during ongoing stimulation. I defined this component as a sustained response with an onset inhibitory period because it was sustained until stimulus offset.

I collected 51 single AI neurons. These were categorized into 8 types according to whether the onset, sustained, or offset responses were significant when compared against pre-stimulus spontaneous activity (See Materials and Methods and Table 3.1). The major 3 types were neurons with onset, offset, and sustained responses (N = 28, Fig. 3.4D); those with onset and sustained responses (N = 8, Fig. 3.4C); and those with onset and offset responses (N = 7, Fig. 3.4B). The remaining 8 neurons were categorized as 3 neurons with only onset response (Fig. 3.4A); 2 neurons with no response (but spontaneously

discharged); 1 neuron with sustained and offset response; 1 neuron with only sustained response; and 1 neuron with only offset response. No sustained responses without onset inhibition [17] were observed (Fig. 3.4C and D).

Repeated measures ANOVA revealed a significant effect of stimulus duration in 24 neurons (e.g. Fig. 3.4K and L), the duration of the conditioner stimuli in 38 neurons (e.g. Fig. 3.4I-L), and their interaction in 27 neurons (e.g. Fig. 3.4J-L) (blue numeric in Table 3.2). In particular, almost all AI neurons whose responses were significantly affected by the preceding conditioner stimuli showed a smaller response as the duration of the preceding stimuli increased (34 out of 38 neurons). This indicated that conditioner stimuli of longer duration caused stronger response reduction (duration-dependent response reduction). Furthermore, the significant interaction between stimulus duration and the duration of the conditioner stimuli indicated that the reduction effect was dependent on stimulus duration. This interaction was shown more frequently in AI neurons with sustained responses (23 out of 38, 61%) than in those without sustained responses (4 out of 13, 31%). No significant interaction was shown in all 3 AI neurons with only onset response (red numeric in Table 3.2).

Table 3.1 Categorization of all AI neurons in this study and its statistical information (p-value) for the comparison between response to deviant and standerd stimuli across the manipulated duration differences (25 - 150 ms). Abbreviation of no, onset, sustained, and offset responses are “nr”, “on”, “sus”, and “off”, respectively. P-values of significant increase in response to deviant stimuli against that to standard stimuli are indicated by red-colored numeric. Bold numeric indicate the neurons whose response to deviant stimuli is significantly larger than that to standard stimuli at any duration differences (N = 12).

cell #	significance			response type								p value at the window after OSD (deviant > standard)					
	on	sus	off	nr	on	sus	off	on-sus	on-off	sus-off	on-sus-off	25	50	75	100	125	150
1	+	+	+								○	**0.122**	**0.1282**	**0.0358**	**0.0051**	**0.3959**	**0.6591**
2	+	+	+								○	0.8484	0.9889	0.4567	0.0227	0.1	0.0303
3	+	+	+								○	0.4338	0.6208	0.0643	0.9401	0.9693	0.6512
4	+	-	+						○			0.3493	0.4105	0.7523	0.394	0.1592	0.1299
5	+	-	+						○			0.0723	0.7486	0.6147	0.193	0.3003	0.8762
6	+	+	+								○	0.5192	0.4908	0.0243	0.6473	1	0.044
7	+	+	-					○				0.2616	0.3199	0.0403	0.3456	0.4116	0.0276
8	+	+	+								○	**0.1529**	**0.9101**	**0.9195**	**0.9913**	**0**	**0**
9	+	+	+								○	**0.2457**	**0.1403**	**0.1033**	**0.5856**	**0**	**0**
10	+	+	+								○	**0.4597**	**0.4259**	**0.9538**	**0.0081**	**0.2832**	**0.9753**
11	+	-	+						○			0.054	0.3848	0.5587	0.4029	0.2613	0.0151
12	-	-	+				○					0.1716	0.4547	0.6693	0.9524	0.6729	0.479
13	-	-	-	○								0.3113	0.0111	0.2696	0.0748	0.933	0.6627
14	+	+	+								○	**0.9722**	**0.3797**	**0.0006**	**0.0288**	**0.0283**	**0**
15	+	+	+								○	**0.0292**	**0.1317**	**0**	**0.0004**	**0.0082**	**0.0001**
16	+	-	+						○			0.1722	0.7297	0.3181	0.2962	0.03	0.1058
17	+	+	-					○				0.285	0.4502	0.8554	0.3308	0.0234	0.4177
18	+	+	+								○	**0.7327**	**0.1474**	**0.0013**	**0.0418**	**0.0483**	**0**
19	+	+	-					○				0.9878	0.2902	0.3694	0.381	0.8473	0.4349
20	+	+	+								○	**0.2782**	**0.8644**	**0.0033**	**0.0614**	**0.1165**	**0.0018**
21	+	+	+								○	0.0123	0.2239	0.0484	0.5167	0.6529	0.7437
22	+	+	+								○	0.5276	0.4124	0.8327	0.1579	0.947	0.6534
23	+	+	+								○	0.719	0.8426	0.7378	0.4608	0.2407	0.0846
24	+	+	-					○				**0.0071**	**0.3596**	**0.2699**	**0.1176**	**0.0335**	**0.0001**
25	-	+	-			○						**0.4087**	**0.2395**	**0.459**	**0.0045**	**0.2434**	**0.155**
26	+	+	+								○	0.1659	0.167	0.6092	0.8626	0.6868	0.1443
27	+	+	+								○	0.7323	0.0153	0.9145	0.06	0.5024	0.5246
28	+	+	+								○	0.2972	0.0571	0.8172	0.3214	0.315	0.4518
29	+	+	+								○	0.4508	0.5355	0.6572	0.2222	0.7992	0.3259
30	+	+	+								○	0.7275	0.1445	0.5876	0.6851	0.1201	0.0951
31	+	+	+								○	0.8973	0.9427	0.3147	0.2378	0.955	0.094
32	+	-	-		○							0.2737	0.9316	0.7441	0.3857	0.0656	0.9302
33	+	-	+						○			0.125	0.6357	0.5058	0.0622	0.0497	0.9285
34	+	-	+						○			0.438	0.2223	0.0768	0.4634	0.1088	0.3524
35	+	+	+								○	**0.7059**	**0.4054**	**0.2429**	**0.0208**	**0.0002**	**0.2052**
36	+	+	+								○	0.0693	0.8677	0.4355	0.2297	0.2665	0.1987
37	+	+	+								○	1	1	0.1875	1	0.2852	0.5
38	+	+	+								○	0.0935	0.3465	0.2028	0.3593	0.2363	0.5215
39	+	+	-					○				0.4774	0.9217	0.5078	0.1613	0.894	0.8333
40	+	+	+								○	0.4319	0.5399	0.3397	0.7603	0.8279	0.2171
41	+	+	+								○	0.9546	0.5938	0.1902	0.3044	0.3936	0.5488
42	+	+	+								○	**0.1769**	**0.0021**	**0.0027**	**0.0112**	**0.9508**	**0.4952**
43	+	+	-					○				0.2436	0.2827	0.9304	0.5065	0.0591	0.4404
44	+	-	-		○							0.8429	0.4275	0.3991	0.2725	0.9995	0.3073
45	+	-	-		○							0.7208	0.1826	0.4465	0.1146	0.6483	0.0499
46	+	+	-					○				0.7342	0.7333	0.0251	0.5513	0.8608	0.8803
47	-	+	+							○		0.5014	0.6441	0.7548	0.0172	0.9264	0.6852
48	-	-	-	○								0.3572	0.304	0.3976	0.5134	0.5902	0.1496
49	+	+	-					○				0.625	1	1	0.375	0.8418	0.8125
50	+	+	+								○	1	1	1	0.75	0.25	1
51	+	-	+						○			1	1	1	1	1	1
neurons with sustained response						1		8		1	28	1	1	5	5	4	7
neurons without sustained response				2	3		1		7			0	0	0	0	0	0

Table 3.2 Numbers of neurons whose response showed significant main effect of stimulus duration (D), that of duration of the conditioner stimuli (Dp), or significant their interaction (D×Dp). Abbreviation of no, onset, sustained, and offset responses are “nr”, “on”, “sus”, and “off”, respectively. Blue and red numeric indicates the data which are mentioned in the manuscript.

neuron types	# of neurons with siginificant effects and interaction			# of neurons
	D	Dp	D×Dp	
nr	0	1	0	2
on	2	3	0	3
sus	1	1	1	1
off	0	0	0	1
on-sus	2	5	6	8
on-off	3	6	4	7
sus-off	1	0	1	1
on-sus-off	15	22	15	28
response with sus	19 (50 %)	28 (74 %)	23 (61 %)	38
response without sus	5 (38 %)	10 (77 %)	4 (31 %)	13
total	24 (47 %)	38 (75 %)	27 (53 %)	51

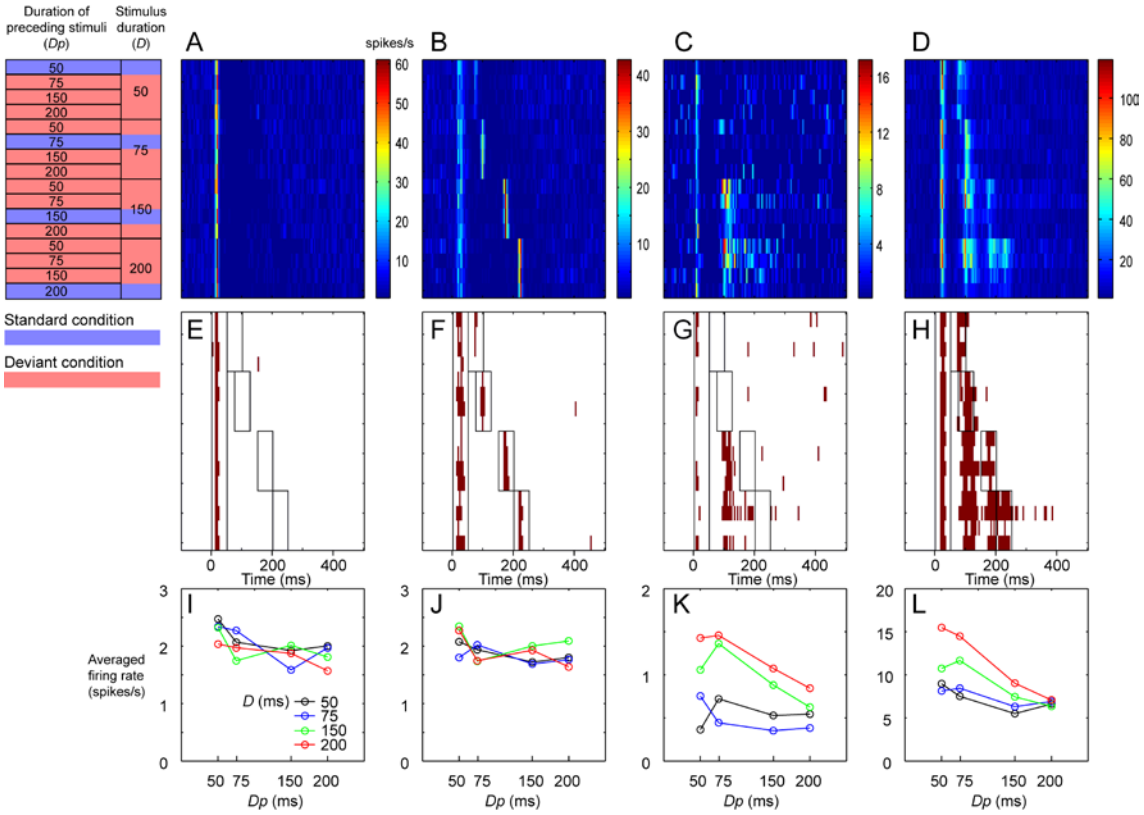

Figure 3.4 Examples of 4 typical AI neurons and the effects of stimulus duration and duration of the preceding stimuli on their responses. A - D: Color map distributions of PSTHs of an AI neurons with only onset response (A), with onset and offset responses (B), with onset and sustained responses (C), and with onset, sustained, and offset responses (D). The table inserted adjacent to the PSTHs indicates the conditions of stimulus duration and duration of the preceding stimuli for an alignment of the PSTHs. Red and blue shaded rows indicate deviant and standard conditions respectively. E – H: Significant response bins of the PSTHs in A - D are indicated by red squares. Solid squares indicate the time windows corresponding to the statistical definition for onset response and offset response in AI neurons. I: Averaged responses (during the time window of 250 ms after stimulus onset) of the AI neurons with only onset response shown in A are plotted across the conditions of stimulus duration and duration of the preceding stimuli. The main effect of the duration of the preceding stimuli was significant by 2-way repeated-measures ANOVA ($F(3,357) = 7.902$, $\varepsilon_{GG} = 0.913$, $p < 0.001$). J: The counterpart of I for an AI neuron with onset and offset responses in B. The main effect of the duration of the preceding stimuli and the interaction between stimulus duration and the duration of the preceding stimuli were significant ($F(3,357) = 5.382$, $\varepsilon_{GG} = 0.831$, $p = 0.002$; $F(9,1071) = 2.410$, $\varepsilon_{GG} = 0.774$, $p = 0.017$; respectively). K: The counterpart of I for an AI neuron with onset and

sustained responses in C. The main effect of the stimulus duration and the duration of the preceding stimuli were significant ($F(3,357) = 13.908$, $\varepsilon_{GG} = 0.608$, $p < 0.001$; $F(3,357) = 8.995$, $\varepsilon_{GG} = 0.793$, $p < 0.001$; respectively). The interaction between stimulus duration and the duration of the preceding stimuli was also significant ($F(9,1071) = 2.836$, $\varepsilon_{GG} = 0.741$, $p = 0.006$). L: The counterpart of I for an AI neuron with onset, sustained, and offset responses in D. The main effect of stimulus duration and the duration of the preceding stimuli were significant ($F(3,357) = 16.051$, $\varepsilon_{GG} = 0.714$, $p < 0.001$; $F(3,357) = 32.358$, $\varepsilon_{GG} = 0.465$, $p < 0.001$; respectively). The interaction between stimulus duration and the duration of the preceding stimuli was also significant ($F(9,1071) = 12.879$, $\varepsilon_{GG} = 0.650$, $p < 0.001$). This figure is cited from Ref. [39].

3.3.2 Responses of single AI neurons to standard and deviant stimuli

I compared the responses to standard and deviant stimuli in each AI neuron across the manipulated duration differences (25–150 ms at 25 ms increments). Figure 3.5 shows examples of 3 AI neurons that responded to standard and deviant stimuli when the duration difference was 150 ms (200 ms – 50 ms, Fig. 3.5A). In cases where the duration was short (50 ms) the firing rate of the responses to deviant stimuli was lower than to standard stimuli (Fig. 3.5B, left). In cases where the duration was long (200 ms) the firing rate of the responses to deviant stimuli was greater than to standard stimuli (Fig. 3.5B, right). These results indicated that the response reduction by conditioner stimuli is strengthened as the duration of the preceding stimulus is increased, an effect also described in the previous section. To cancel out the suppressive effect I compared the averaged responses to deviant stimuli and standard stimuli (Fig. 3.5C, E, and G). In the limited time range after OSD the averaged responses to deviant stimuli were greater than to standard stimuli (Fig. 3.5D, F, and H). Because onset response failed to show a difference between deviant and standard stimuli this would indicate that the suppressive effects of the preceding

conditioner stimuli were counterbalanced. This also suggested that sustained responses (after OSD) could not be explained purely by the reduction.

Twelve single AI neurons showed a significant difference in their responses during the time window after OSD and under some conditions of duration difference by multi-comparison Wilcoxon test (corrected $p < 0.05$ by Holm method; e.g. Fig. 3.5C, E, and G). The number of neurons that showed significant differences in their responses to deviants and standards after OSD was found to increase as the duration difference was increased (Table 3.1 and Fig. 3.6). Furthermore, this phenomenon was all observed in AI neurons with sustained responses.

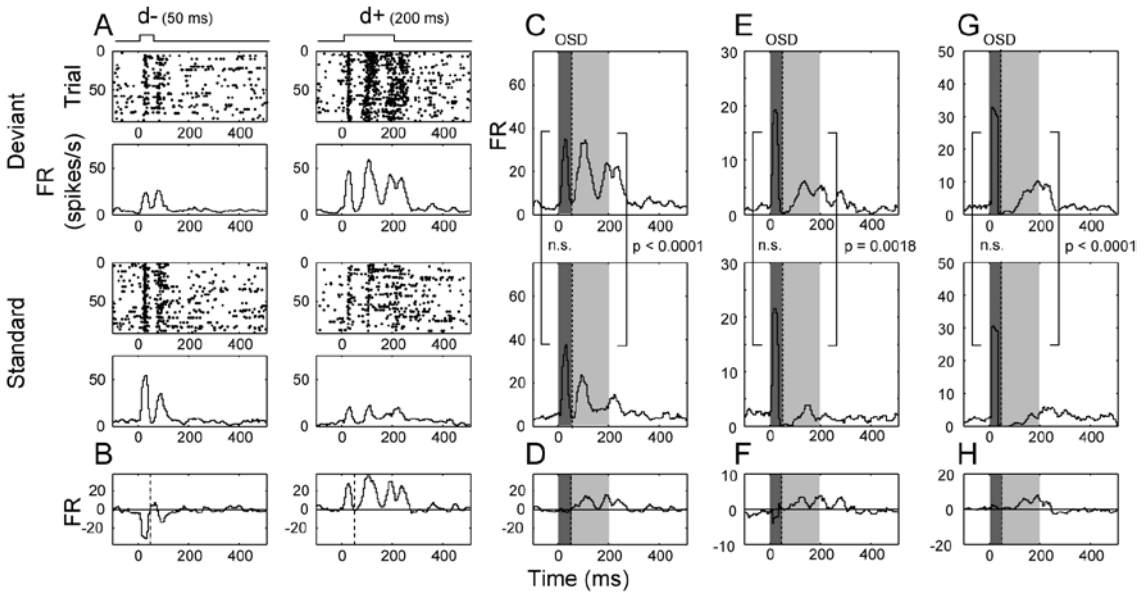

Figure 3.5 Examples of single AI neuron responses to deviant and standard stimuli with 150 ms deviance. A: Rasters and PSTHs of the responses to deviant stimuli of 50 ms (upper left), deviant stimuli of 200 ms (upper right), standard stimuli of 50 ms (lower left), and standard stimuli of 200 ms (lower right). B: Subtracted response of PSTHs in A with respect to each column. Vertical broken lines indicate OSD (50 ms from stimulus onset). C: PSTHs of the responses to deviants and standards calculated by averaging the responses in A at each row. Gray boxes indicate the time windows of the statistical analysis. The dark gray boxes indicate the time windows of 50 ms before OSD and light gray boxes indicate the time windows of 150 ms after OSD. Firing rates of the responses to deviants and to standards over the time window were compared. The value of the significance probability p is shown in the center of B. Vertical broken lines indicate OSD (50 ms from stimulus onset). D: Responses involved in change detection obtained by subtracting response to standards from that to deviants in C. In each bin during the analysis window (gray boxes), multiple comparisons were performed for trial-paired firing rates (N = 10 before OSD and N = 30 after OSD). The asterisk depicts the significance bin revealed by the comparisons. Vertical broken lines indicate OSD (50 ms from stimulus onset). E, G: Counterparts of PSTHs in B for other two neurons. F, H: Counterparts of PSTH in D for other two neurons. This figure is cited from Ref. [39].

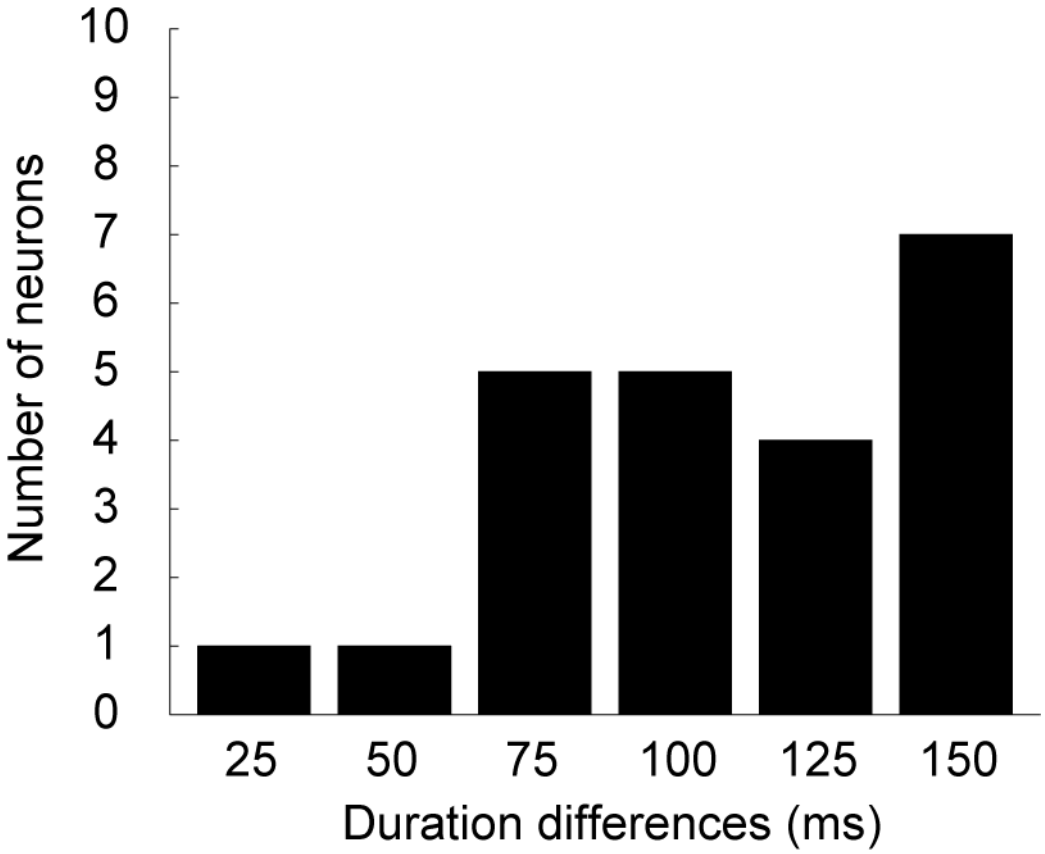

Figure 3.6 Numbers of neurons with significantly greater responses to deviants than to standards across the duration difference. Only AI neurons with sustained response showed a significantly greater response to deviants than to standards at some duration differences. Number of these neurons increased as the duration difference increased. The number of these neurons overlapped across the duration difference. This figure is cited from Ref. [39].

Comparison of the responses to deviant and standard stimuli across all 51 neurons with (N = 38) or without (N = 13) sustained responses was conducted. The upper and middle panels of Fig. 3.7 show the averaged responses over the neurons and their subtracted responses. First, mean firing rates of the responses during the time window of 150 ms after OSD were calculated for each neuron. Second, these were compared between stimulus conditions (deviant and standard) for each duration difference and each neuron type (with or without sustained response) by 3-way mixed-model ANOVA (2 neuron types × 2 stimulus conditions × 6 duration differences). The effect of neuron type was not significant ($F(1,49) = 0.005$, $p = 0.945$) but the effect of stimulus condition was found to be significant ($F(1,49) = 7.498$, $p = 0.009$). Although the main effect of duration difference was not significant ($F(5,245) = 0.813$, $\varepsilon_{GG} = 0.33$, $p = 0.432$) the interaction of neuron type and stimulus condition, and of stimulus condition and duration difference only narrowly fell short of achieving statistical significance ($F(5,245) = 3.058$, $p = 0.087$; and $F(5,245) = 2.616$, $\varepsilon_{GG} = 0.489$, $p = 0.066$; respectively).

Consequently, two-way repeated measures ANOVA (2 stimulus conditions × 6 duration differences) were applied for each neurons type. On the response in AI

neurons without sustained response, it was revealed that both effects of stimulus condition and duration difference were not significant ($F(1,12) = 1.511$, $p = 0.243$; $F(5,60) = 0.307$, $\varepsilon_{GG} = 0.448$, $p = 0.762$; respectively). Their interaction was also not significant but narrowly fell short of achieving statistical significance ($F(5,60) = 2.478$, $\varepsilon_{GG} = 0.63$, $p = 0.073$). On the response in AI neurons with sustained response, it was revealed that the effect of stimulus condition and the interaction between stimulus condition and duration difference were significant ($F(1,37) = 15.749$, $p < 0.001$; $F(5,185) = 4.067$, $\varepsilon_{GG} = 0.476$, $p = 0.015$; respectively). The main effect of duration difference was not significant ($F(5,185) = 2.232$, $\varepsilon_{GG} = 0.313$, $p = 0.127$).Based on the results so far, I therefore compared the responses to standard with those to deviant using the Wilcoxon test with a Holm correction in each neuron type ($N = 6$) for each neuron type and each duration difference. When duration differences were 75, 100, 125, and 150 ms, the responses to standard and deviant across the neurons with sustained responses were significantly different (corrected $p = 0.004$, $p < 0.001$, $p = 0.006$, and $p = 0.002$, respectively). The responses to standard and deviant stimuli across the neurons with sustained responses were not significantly different when the duration differences were 25 and 50 ms (uncorrected $p = 0.538$ and 0.551,

respectively). All comparisons of responses to deviant and standard stimuli across neurons without sustained responses showed no significance. These results indicate that AI neurons with sustained responses tended to be more activated by deviant stimuli, and particularly when the duration difference was increased. This prompted us to focus on neurons with sustained responses and to evaluate response adaptation occurring before and after OSD.

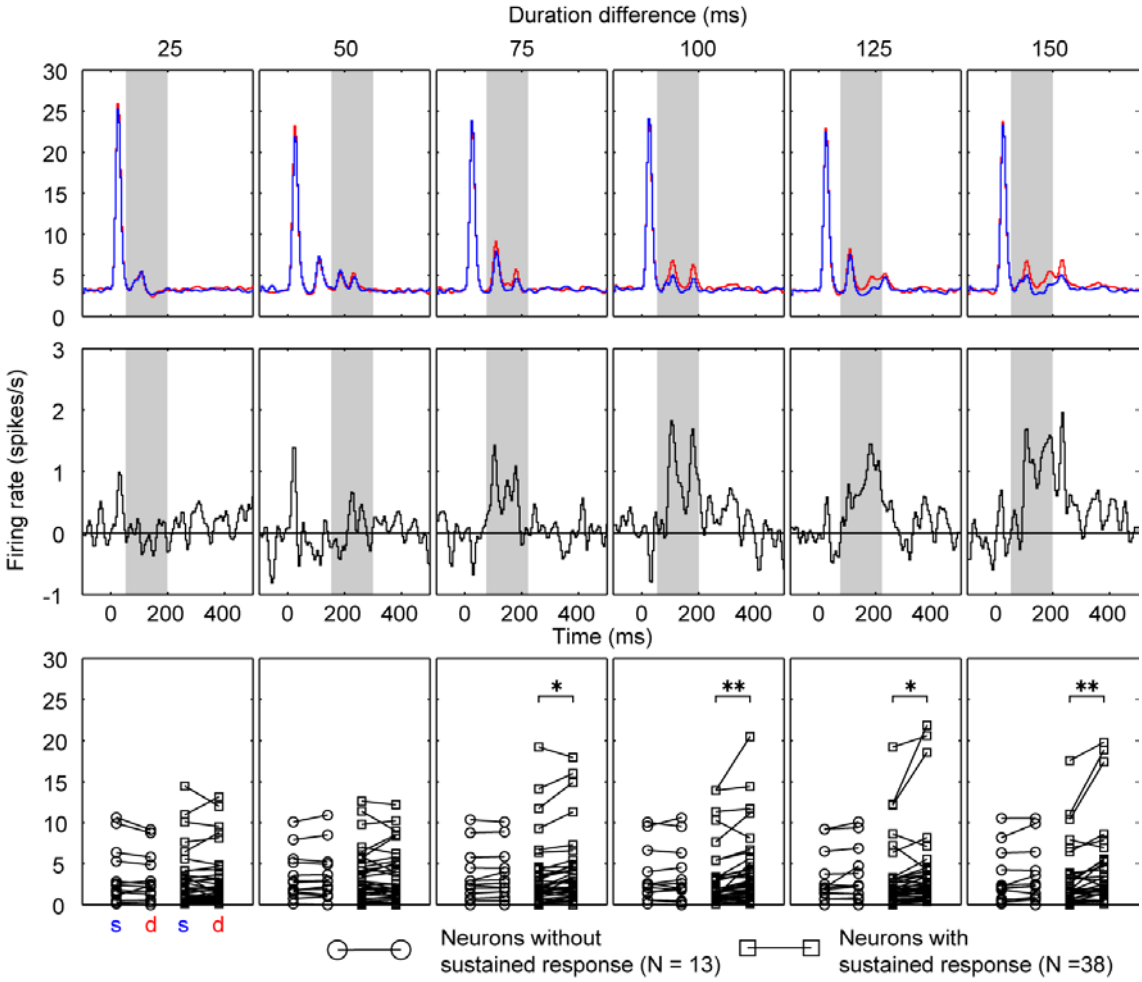

Figure 3.7 Comparison of the responses to deviant and standard stimuli in AI neurons with or without sustained responses. The upper panels illustrate the averaged responses over all AI neurons to deviant stimuli (red lines) and to standard stimuli (blue lines). The middle panels illustrate the subtracted responses of standard and deviant stimuli corresponding to the upper figure. Gray shaded areas indicate the analysis time window (150 ms). Lower panels illustrate the comparison between the responses to standard stimuli (s) and to deviant stimuli (d) over neurons. The comparisons were performed for neurons with or without sustained response (open squares or circles, respectively) and across duration difference using the Wilcoxon test with Holm correction (N = 6). Labels at the top of the figures indicate the duration differences for each column. Asterisks indicate significant differences (corrected $p < 0.05$). This figure is cited from Ref. [39].

3.3.3 Responses transition of AI neurons during an auditory sequence

To evaluate stimulus-specific adaptation, I calculated the SI before and after OSD and compared them. I analyzed 34 of the 36 single A1 neurons with onset and sustained responses for their activity during exposure to auditory sequences. The remaining 2 neurons were excluded from further analysis because this would involve zero division in SI calculations (Materials and Methods). Fig. 3.8 illustrates the firing rates of a single AI neuron during an auditory sequence and shows the calculated SI before and after OSD. The time windows before OSD included only onset response whereas those after OSD included both sustained and offset responses (Fig. 3.8A). During both time windows, the firing rate of the response to trains of shorter stimuli (e.g. 50 ms) tended to increase with stimulus repetition. In contrast, firing rates of the responses to trains of longer stimuli (e.g. 200 ms) tended to decrease on stimulus repetition (Fig. 3.8B). These results indicate that the longer stimuli exerted greater suppressive effects than shorter stimuli, and that the suppressive effects accumulated to bias the response to the following stimulus and resulted in the duration-dependent response reduction. In consequence the introduction of shorter stimuli after the

preceding longer stimuli caused the response to recover from this bias. For this reason the calculated SIs were directly proportional to duration change (Fig. 3.8C). Because the effects of the preceding conditioner stimuli on the response before and after OSD were different (Fig. 3.4) I calculated the difference between SIs before and after OSD (SI after OSD – SI before OSD) for each neuron (Fig. 3.8D). This revealed that there was slightly stronger adaptation after OSD than before OSD as the duration difference was larger (duration changes = ±125 and ±150 ms in Fig. 3.8D).

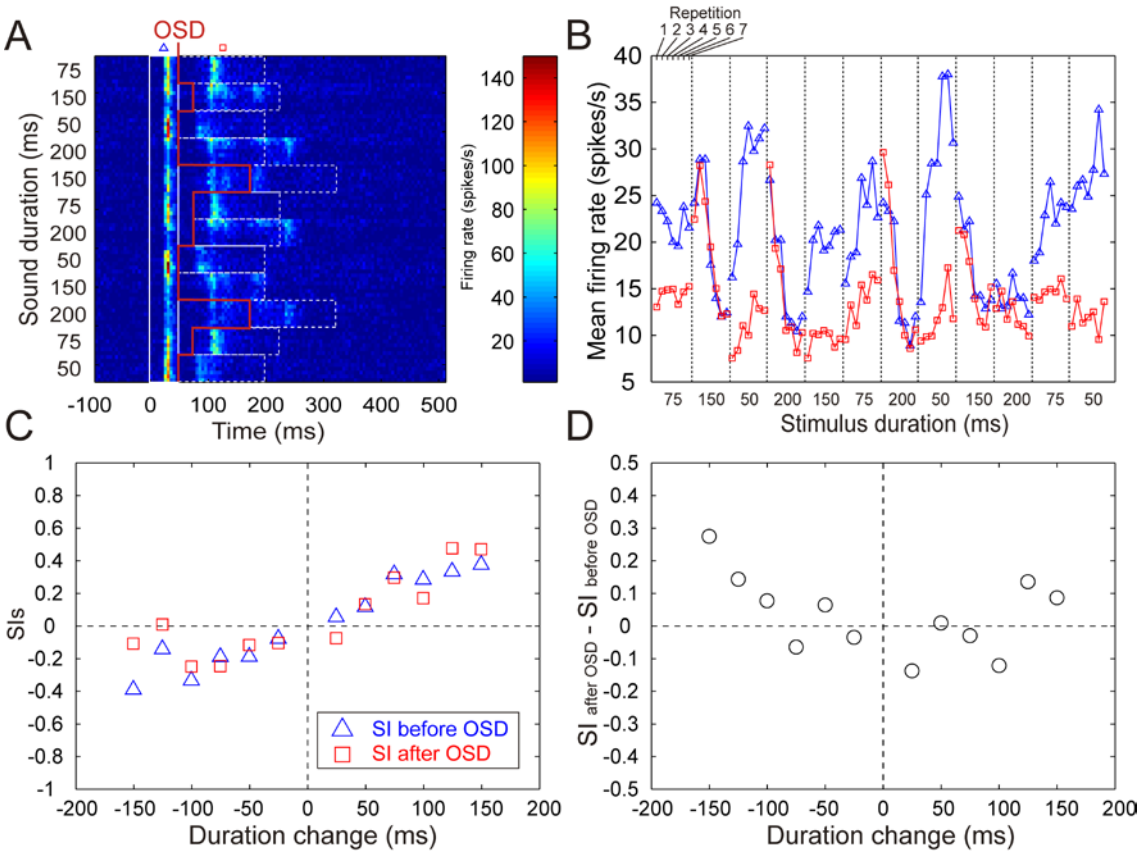

Figure 3.8 Response transition in a single AI neuron during auditory sequences. A: PSTH distribution of a single AI neuron as in the figure 1D. Red line indicates the OSD from when the change detection of sound duration initiated. White solid-line and dashed-line boxes depict the time window of 50 ms from the stimulus onset (before OSD) and those of 150 ms after OSD, respectively. B: Transitions of the mean firing rate in the time window before OSD (blue open triangles) and after OSD (red open squares) are illustrated. The first and seventh stimuli in each train are defined as “deviant” and “standard”, respectively. C: Relationship between duration differences and calculated Sis before and after OSD. Sis were calculated from the responses to deviant and standard stimuli described in B (see Materials and Methods). Blue open triangles and red open squares indicate SIs before and after OSD, respectively. D: Difference of Sis before and after OSD (SI after OSD – SI before OSD) was illustrated across the duration difference. This figure is cited from Ref. [39].

The difference in SIs before and after OSD in neurons with sustained responses was evaluated (Fig. 3.9). Two-way repeated-measures ANOVA across the duration change (2 change directions × 6 duration differences) revealed a significant main effect of duration difference ($F(5,165) = 2.653$, $p = 0.043$), no main effect of change direction ($F(1,33) = 2.042$, $\varepsilon_{GG} = 0.704$, $p = 0.162$), and no interaction ($F(5,165) = 0.69$, $p = 0.606$). This indicated that adaptation depended on the duration difference irrespective of duration shortening or lengthening (Fig. 3.9A). Subsequent one-way repeated-measures ANOVA ($F(5,335) = 3.191$, $\varepsilon_{GG} = 0.862$, $p = 0.012$) and post hoc multiple comparisons revealed that there were significant differences between the 25 ms duration difference and the125 ms duration difference ($p < 0.05$) and between the 25 ms duration difference and the 150 ms duration difference ($p < 0.05$) (Fig. 3.9B). The difference in SIs showed consistent increase with an increase in duration difference and their positive correlation was significant ($R = 0.178$, $p < 0.001$). These results indicated that difference in adaptation observed in the response before and after OSD depended on duration difference during the auditory sequences. It was also suggested that there is a different adaptation mechanism from simple duration-dependent response reduction produced by

preceding conditioner stimuli because the larger duration difference in duration shortening as well as that in duration lengthening causes greater SI difference (Fig. 3.9A).

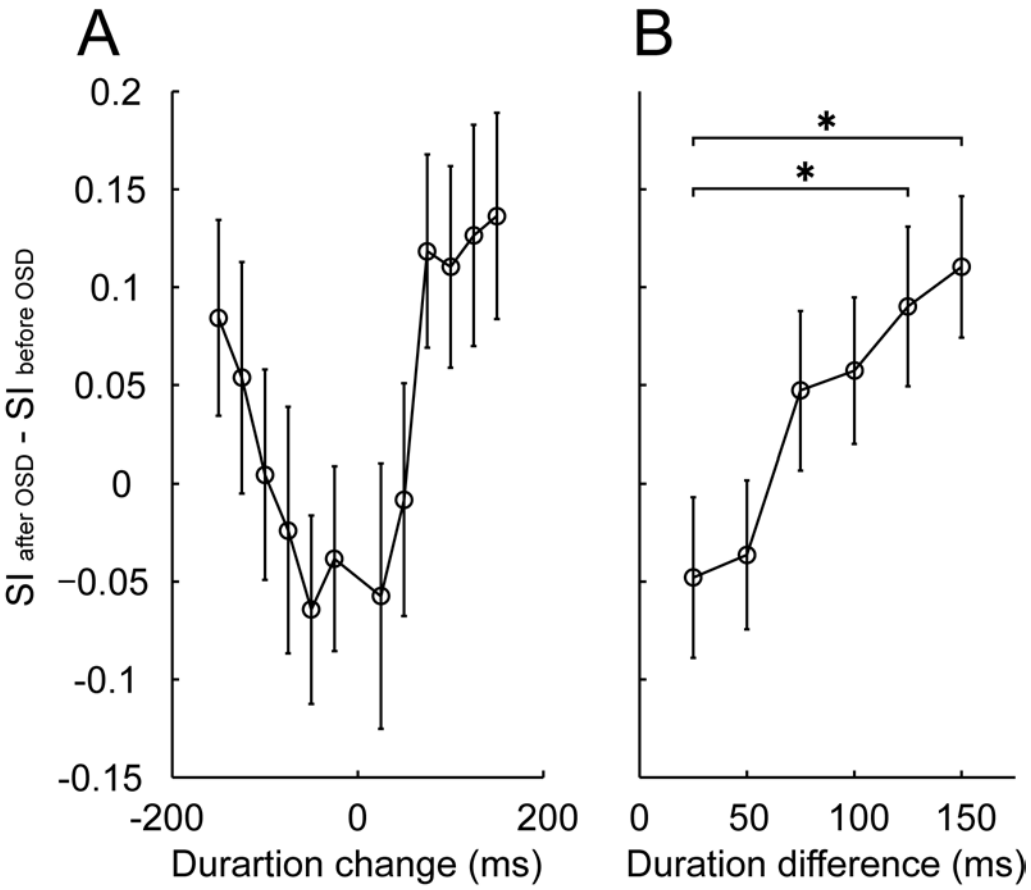

Figure 3.9 Differences between SIs before and after OSD over AI neurons involved in duration discrimination. A: Difference between SIs before and after OSD over AI neurons with sustained responses were compared across the manipulated duration change (2 change directions × 6 duration differences by 25-ms steps) by 2-way repeated-measures ANOVA. A significant main effect of duration difference ($F(5,165) = 2.653$, $p = 0.043$), no main effect of change direction ($F(1,33) = 2.042$, $\varepsilon_{GG} = 0.704$, $p = 0.162$), and no interaction ($F(5,165) = 0.69$, $p < 0.606$) were observed. Error bars represent the standard error of the mean. B: Combining the duration increment and decrement. The difference of SIs was compared across the duration difference by 1-way repeated-measures ANOVA and Holm method for post hoc multiple comparisons. Error bars represent the standard error of the mean. Asterisks indicate a significant difference (*, $p < 0.05$). This figure is cited from Ref. [39].

3.4 Discussions

Sound features can be discriminated on the basis of their context of preceding stimuli. Previous neurophysiological studies have investigated the influence of 2-tone stimuli [44-50]. It was found that the preceding conditioner stimulus could induce prolonged suppression or facilitation of the responses to the succeeding stimulus. The preceding stimulus whose sound features (such as frequency and intensity) are similar to those of the succeeding stimulus produces the strongest suppression (stimulus-specific effect [21]). However, there has been no evidence of the stimulus-specific effect in sound duration so far.

In this study, I showed that the responses of single AI neurons altered depending on sound duration of the preceding and succeeding sound stimuli during an auditory sequence. Various types of the response in AI neurons, such as onset, offset, and sustained responses, were each affected by the context of sound duration. By comparing the effect on the response before and after OSD, I found an adaptation on the response after OSD which is not observed before OSD. On the basis of the results in this study, I discuss herein the properties and functions of AI neurons involved in the discrimination of sound duration

during an auditory sequence.

3.4.1 Validity of the acoustic stimulation in this study

It was recently reported that there is a problem in estimating duration MMN by classical oddball stimuli. For example, when the responses to the standard and deviant stimuli are directly compared (e.g. [26, 27]) the difference reflects stimulus duration characteristics rather than the processing of sound duration change [28]. The apparently different time profile of offset responses in AI neurons (e.g. Fig 3.4B and F) would support this proposal. To rule out this effect there are 2 possible protocols. One is a protocol using a reverse-standard-deviant condition [28, 30, 35, 36], an oddball condition where standard and deviant stimuli are inverted in duration characteristics. Deviant stimuli in the reverse-standard-deviant condition and standard stimuli in a normal oddball condition have the same duration characteristics as compared, and vice versa. The other is an oddball paradigm of equal probability [38] or a "roving-stimulus" paradigm [37] that consists of stimulus trains of identical stimuli whose sound features are varied from train to train. Taking the first

stimulus of the trains as the deviant stimulus, and the repeated stimulus at the end of the trains as the standard stimulus, MMN can be revealed by comparing the responses to the deviant stimuli with the responses to the standard stimuli [37, 38]. The time lag between the deviant and standard stimuli to be compared should be as short as possible because neuronal responses can often increase or decrease during long-term experiments. The latter protocol was therefore employed in this study.

3.4.2 Duration-dependent response reduction by preceding stimuli

Many neurons showed significant main effect of preceding conditioner stimulus on the responses (38 out of 51, 75%) and almost all these neurons (34 out of 38, 89%) showed greater response reduction as the duration of the preceding conditioner stimuli increased (Fig. 3.4I-L). In cases where stimulus duration was long, firing rates of responses to deviant stimuli were greater than the firing rates of responses to standard stimuli (right column in Fig. 3.5A and B). Conversely, in cases where the stimulus duration was short, firing rates in response to deviant stimuli were lower than to standard stimuli (left column in

Fig. 3.5A and B). This phenomenon may be explained by two-tone suppression that is strengthened as the duration of preceding conditioner stimulus is increased [21, 51]. Because this suppressive effect has been proposed to be long-lasting over 1 sec [21], our results (Fig. 3.8B) can be also explained by the two-tone suppression that accumulates by stimulus repetition with short SOA (500 ms). In this study, the 50 ms trains in this study were all preceded by longer duration trains (75, 150, 200 ms). The responses at the end of the 200 ms trains were highly reduced, while the introduction of the 50 ms stimuli caused the neuronal response to recover from this reduction (Fig. 3.8B). Calculated SI for the response showing the duration-dependent reduction, therefore, increased with an increase of the duration change rather than the duration difference (Fig. 3.8C).

3.4.3 Stimulus-specific adaptation for sound duration

Many AI neurons with late-component of the responses (Fig. 3.4J-L) showed a significant interaction between the effects of stimulus duration and of the duration of the preceding conditioner stimuli on the responses. This indicates

that the late component of the responses is modulated by the relationship between the stimulus duration and the duration of the conditioner stimuli. AI neurons with only onset response, meanwhile, showed no interaction (Fig. 3.4I). This suggests that the onset response is modulated by the stimulus duration and the duration of the conditioner stimuli independently. To compensate for the effect of the conditioner stimulus and resulting duration-dependent response reduction I employed two analytical methods. One was to counterbalance the effect of the preceding conditioner stimuli and that of the stimulus duration by averaging the responses. Averaging was carried out across the sets of stimulus trains with the same duration transition (e.g. 150 ms, from a 50 ms train to a 200 ms train and from a 200 ms train to a 50 ms train). This method, in fact, allowed exclusion of duration-dependent response reduction at the onset response (before OSD, Fig. 3.5D, F, and H) and revealed the component of the response that is specific to the duration transition (Figs 3.5C, E, G, and 3.7) after OSD. The other was to normalize the adaptation after OSD to that before OSD. In this analysis I evaluated the difference in the SIs before and after OSD (SI after OSD – SI before OSD); this indicated that SI differences were dependent on the difference in sound duration (Figs 3.8D and 3.9) and showed a correlation

with it ($R = 0.178$, $p < 0.001$). These results suggest that the differentiated component of the response after OSD (middle panels in Fig. 3.7), and the subtracted SIs (Figs 3.8D and 3.9), may reflect stimulus-specific adaptation [23]. Since they corresponded temporally to the late component of the response such as offset and sustained responses, it is proposed that the adaptation of these responses contains the stimulus-specific adaptation.

A recent study proposed a possibility that the adaptation of feature-selective neurons in thalamus, whose inputs are convergent to the cortical neurons, causes the stimulus-specific adaptation in AI neurons [52]. In this regard, responses showing the stimulus-specific adaptation may correspond to the response that displays duration-selectivity such as offset [9, 15, 18], sustained [17], and pauser responses [19]. These findings support our proposal that stimulus-specific adaptation can be observed in AI neurons with the late component of the responses such as offset and sustained responses.

3.4.4 Sustained and offset responses in single AI neurons and their implications for the neural substrates of duration MMN

Several studies have proposed that the offset response is involved in the processing of sound duration [10-16]. However, it was pointed out that lengthening of sound duration must be detected before the offset of the longer following probe stimulus (after OSD), and this phenomenon cannot be explained purely by the offset response [30, 31]. I found that many AI neurons showed a sustained response (38 out of 51, 75 %) and these neurons showed response enhancement at the duration transition depending on the duration difference (Figs 3.5-3.9). Taken together with previous studies, these results suggest that not only offset response but also sustained response may be involved in the generation of the duration MMN and the duration discrimination.

Stimulus-specific adaptation and the resulting enhancement of responses to deviant versus standard stimuli were observed in the 150 ms time window following OSD. Although this time window contains the sustained and offset responses at duration lengthening, offset responses to 200 ms were not contained within the time window when the duration difference was 150 ms (Fig. 3.5). Conversely, at duration shortening, only the offset response was in this time window. I previously proposed that stimulus lengthening can be detected from the time of OSD but differing extents of lengthening cannot be detected at this

time. In consequence, “change detection” initiates from OSD but “difference detection” is performed at offset. Conversely, in cases of duration shortening both detections can operate simultaneously at offset [30]. In this regard, it is inferred that stimulus-specific adaptation on sustained response and offset response play important roles in the change detection of duration lengthening and shortening, respectively.

Stimulus-specific adaptation in this study was significantly larger when the duration difference was greater than 75 ms (Fig. 3.7) and the normalized SIs were suddenly increased over the 75 ms duration difference (particularly at duration lengthening, Fig. 3.9). These findings imply that the discrimination threshold for sound duration in guinea pigs is approximately 75 ms. Animals can learn to discriminate different sound durations (e.g. rat [53], macaque [54], and mouse [55]). Klink & Klump (2004) proposed that average Weber fraction ($\Delta T/T$) for the detection of duration lengthening in mouse is approximately 1, which is correspond to 75-ms lengthening against 75-ms reference sound. This is comparable our conclusion regarding discrimination threshold.

3.5 Conclusion

I already established animal model for duration MMN in guinea pig in Chapter 2. To extend the study, it was demonstrated here that sustained response in AI neurons and its adaptation can serve as a neural substrate of duration MMN. It has been previously inferred that offset response to the acoustic stimuli play an important role for sound duration processing. This study indicates the importance of the sustained response for the duration processing and its functional difference from the offset response.

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Chapter 4

GENERAL DISCUSSION, CONCLUSION, AND PERSPECTIVE

Temporal features of sound sequence are most essential information to be processed in the brain. It is well known that animals can automatically discriminate the change in the temporal features from the auditory stimuli in environment. Especially, auditory duration discrimination is most important for the perception of speech and music in human. An ERP in human indexing the discrimination is well investigated. This ERP generally termed MMN widely developed for clinical application as well as for clarifying the mechanisms of the discrimination. MMN involved in the duration discrimination is called duration MMN. The neuronal basis of the duration

discrimination is, however, remained unclear since direct electrophysiological methods are hard to apply in human. Because the duration MMN can be observed in non-human animals, the neuronal basis of auditory duration discrimination was investigated in the animal model (guinea pigs) of duration MMN in this study. Two consecutive studies constructing this thesis are summarized as follows.

Chapter 2 describes about an establishment of animal model for MMN. The MMN is known to characterize the change in auditory features during auditory sequence and exhibit physiological evidence of sensory memory. I employ the auditory oddball paradigm varying sound durations and observed two types of duration MMN in anesthetized guinea pigs. One was duration MMN whose increase in peak amplitude occurred immediately after onset of the stimulus difference in duration decrement. The other exhibited a peak amplitude increase close to the offset of the longer stimulus in duration increment. These results suggested that there is a new duration discrimination mechanisms, the detection of duration difference (difference detection), in addition to the detection of duration change (change detection) that previously proposed. This study allows me to investigate the neuronal mechanisms for these detections by an electrophysiological approach.

Chapter 3 describes the consecutive study following the study in Chapter 2. Here I

indicated an issue that the response occurred at the stimulus offset (offset response), which has been proposed to play important role for duration representation, cannot explain the neuronal mechanism for the change detection. In this study, I examined the effects of changing stimulus duration on the responses of neurons in the primary auditory cortex by using the sequence of noises varied in duration. I found that the effect of the preceding stimuli was different for the types of the response such as onset or sustained response. I demonstrated that the effect on the onset response could explain by the duration of preceding stimuli but that on sustained response could not. Furthermore, the effect on the sustained response was also sensitive to the decree of the changing stimulus duration during auditory sequence. It was suggested that the effect might be regulated by the neurons tuned for sound duration and contribute to the sound duration discrimination that is initiated even while the stimulus is still ongoing.

These consecutive studies contribute to link the separated research fields, psychophysiology and neurophysiology, for the limited target of brain function (Fig. 4.1). It was demonstrated that the duration discrimination process during auditory sequence consists of the change detection and difference detection and the change detection were explained by the adaptation of the sustained response in the neurons in the primary auditory cortex. The neuronal substrate for the difference detection,

however, could not be clearly demonstrated. Although it is speculated that the offset response were related to the difference detection, further investigation is necessary to clarify whether the offset response can be a neural substrate for the difference detection.

Recently, the duration discrimination process during auditory sequence was spotlighted for clinical application. MMN can be measured during passive listening in the absence of attention, which makes it particularly suitable for testing various clinical populations and infants. In fact, missing or attenuating the duration MMN has shown in persons with schizophrenia [1], dyslexia [2, 3], Parkinson and Alzheimer diseases [4, 5], which suggest the aberrations of the discrimination process in these disorders. In this regard, the results in my studies will provide important information for the clinical researches.

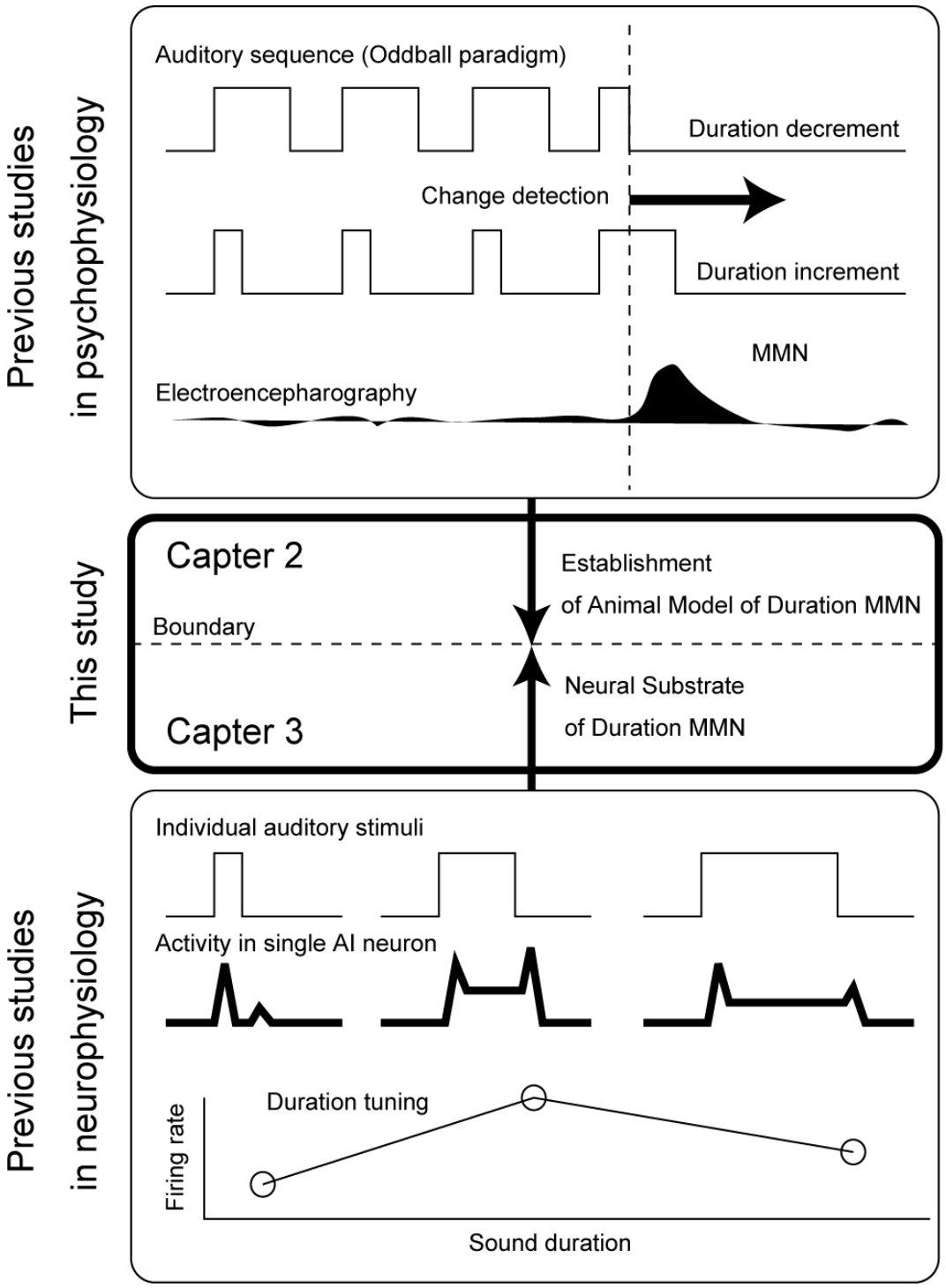

Figure 4.1 Position of the consecutive studies in this thesis against the peripheral research fields. These studies are centered on a boundary of psychology and neurophysiology and liked their obstacle.

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