Zur Homepage der Dissertation

Mouse auditory perception of temporal features of sound

Karin B. Klink

Ph. D. thesis

14 April 2008

Carl von Ossietzky Universität Oldenburg

Mouse auditory perception of temporal features of sound

Von der Fakultät für Mathematik und Naturwissenschaften
der Carl von Ossietzky Universität Oldenburg
zur Erlangung des Grades und Titels eines

Doktors der Naturwissenschaften (Dr. rer. nat.)

angenommene Dissertation

von

Karin B. Klink

geboren am 24. November 1974
in Königshütte (Chorzów)

Gutachter:	**Prof. Dr. Georg M. Klump**
Zweitgutachter:	**Prof. Dr. Jesko L. Verhey**
Datum der Disputation:	**14. April 2008**

Contents

Summary of the Ph.D. thesis

The house mouse (*Mus musculus*) has developed into a promising model organism for the study of auditory function, and a comparison of physiological and psychoacoustic data from the mouse can be used to investigate the neural mechanisms underlying the perception and processing of sound. The present psychoacoustic study was designed to provide the behavioral data for a comparison with physiological measurements obtained in other laboratories. The study investigated two different aspects of temporal processing in the mouse in a total of four experiments.

The aim of the first experiment (duration discrimination, chapter 2) was to investigate the ability of mice to detect an increment in signal duration compared to a reference duration. The performance of the subjects was found to fall within the range of performances of several different vertebrate species, and is best explained by a mechanism that sums up neural impulses over the duration of the stimulus. Comparisons with duration tuning of long-pass neurons in the mouse inferior colliculus (IC, Brand et al. 2000) revealed a discrepancy between the neural sensitivity to changes in duration and the sensitivity seen in the mouse behavior. This suggests that either the mice use a considerably less than optimal mechanism to integrate the information represented in the neural responses, or that the decision process underlying the perceptual discrimination of stimulus duration may be located in neuron populations at other levels of the auditory system with a reduced sensitivity to a change in duration compared with that reported for IC neurons by Brand et al. (2000).

The second experiment was conducted to examine the duration discrimination performance of mice with and without pharmacological manipulation of neural inhibition using strychnine (chapter 3). The aim of this experiment was to investigate whether the performance of mice in a duration discrimination task could be explained by a purely excitatory mechanism or whether a model based on excitation and (glycinergic) inhibition might be better suited to describe the behavioral data.

Pharmacological treatment with oral doses of the glycine antagonist strychnine did not affect the performance of the subjects, suggesting a mechanism based on excitation only. The failure to detect an effect of pharmacological manipulation with strychnine indicates that either the oral application of strychnine was not suited for eliciting changes in the duration discrimination performance, or that glycinergic inhibition is less likely to play a direct role in the mechanisms underlying duration discrimination in the mouse, at least for the range of stimuli tested. The effectiveness of oral application of strychnine in mice was tested using the acoustic startle response (ASR; experiment 3, chapter 3). The ASR has been often used in psychoacoustic experiments and the role of glycinergic inhibition on the performance of the subjects in this experiment is well known. The failure to find an increase of ASR amplitude (or startle threshold) in strychnine sessions in the present study points to the possibility that oral application of strychnine is unsuited for testing the role of glycine-mediated inhibition in neural processing of acoustic stimuli.

Experiment four (chapter 4) focussed on the ability of mice to exploit correlated amplitude fluctuations in a noise masker in order to enhance signal detection. This phenomenon, referred to as comodulation masking release (CMR), has been attributed to auditory processing within one auditory channel (relying on within-channel cues) and/or across several auditory channels (relying on across-channel cues). The present CMR experiment employed two narrow-band noise maskers. The experiment was designed to separate the effects of within- and across channel cues, and to investigate the role of within-channel cues on the amount of CMR in the mouse. The results demonstrated significant within-channel CMR in the mouse while no unambiguous evidence could be found for CMR occurring due to across-channel processing. The amount of within-channel CMR increased with decreasing frequency separation between both masker bands. The huge increase in CMR for the smallest frequency separation tested (i.e. 100 Hz) was likely to be due to the exploitation of changes in temporal cues of the stimulus upon the addition of the signal. The results were compared with results from similar experiments in humans and starlings, and with data obtained in a different CMR experiment in the house mouse. This experiment is intended to be the behavioral counterpart to physiological experiments on the neuronal processing of temporal information in mice in the same task, that are planned in cooperation with another laboratory.

Zusammenfassung der Dissertation

Die Hausmaus (*Mus musculus*) hat sich zu einem vielversprechenden Model für auditorische Studien entwickelt, da mit der Maus sowohl psychoakustische als auch physiologische Experimente durchgeführt werden können. Ein Vergleich der Ergebnisse von neuronalen Untersuchungen und Verhaltensexperimenten am gleichen Organismus ermöglicht ein besseres Verständnis der neuronalen Mechanismen, die der Wahrnehmung und Verarbeitung von Geräuschen zugrunde liegen. Die vorliegende psychoakustische Studie wurde entwickelt, um Verhaltensdaten für einen Vergleich mit physiologischen Messungen aus anderen Labors zu liefern. Die Studie untersuchte zwei verschiedene Aspekte der zeitlichen Verarbeitung in der Maus in insgesamt vier Experimenten.

Das Ziel des ersten Experiments (Dauerdiskrimination, Kapitel 2) war die Erforschung der Fähigkeit von Mäusen, eine Verlängerung der Signaldauer im Vergleich zu einer Referenzdauer zu erkennen. Die Dauerdiskriminationsleistung der Versuchstiere bewegte sich im Bereich der Diskriminationsleistungen anderer Wirbeltierspezies und kann am besten mit einem Mechanismus erklärt werden, der Nervenimpulse während der Dauer des Stimulus aufaddiert. Bei dem Vergleich mit dem Antwortverhalten von „long-pass“-Neuronen im Colliculus inferior (IC) der Maus (Brand et al. 2000) zeigte sich eine Diskrepanz zwischen der Sensitivität der Nervenzellen auf eine Änderung der Dauer und der Sensitivität der Tiere im Verhaltensexperiment. Dieses Ergebnis deutet darauf hin, dass a) die Mäuse einen suboptimalen Mechanismus benutzen, um die Informationen zu integrieren, die in den neuronalen Antworten repräsentiert werden, oder b) dass der Entscheidungsprozess, der der Wahrnehmung von Änderungen der Dauer zugrunde liegt, in Nervenzellpopulationen auf anderen Stufen des auditorischen Systems beheimatet ist, die weniger sensitiv auf eine Änderung in der Dauer reagieren als die von Brand et al. (2000) untersuchten IC-Neurone.

Im zweiten Experiment wurde die Dauerdiskriminationsleistung von Mäusen mit und ohne pharmakologische Manipulation mit Strychnin bestimmt (Kapitel 3). Das Ziel

dieses Experiments war es zu klären, ob die Dauerdiskriminationsleistung der Mäuse durch einen rein exzitatorischen Mechanismus erklärt werden kann oder ob ein Modell basierend auf Erregung und (glycinerger) Hemmung besser geeignet ist, die Verhaltensdaten zu beschreiben. Pharmakologische Behandlung mit dem oral verabreichten Glycin-Antagonisten Strychnin beeinflusste die Diskriminationsfähigkeit der Tiere nicht, was auf einen Mechanismus schließen lässt, der nur auf Erregung beruht. Das Fehlen eines Einflusses der pharmakologischen Behandlung mit Strychnin deutet darauf hin, dass a) die orale Verabreichung von Strychnin nicht geeignet ist, um Änderungen in der Dauerdiskrimiationsleistung zu verursachen, oder dass b) glycinerge Hemmung keine direkte Rolle in dem Mechanismus spielt, welcher der Dauerdiskrimination in der Maus zugrunde liegt, zumindest nicht bei der Verarbeitung der getesteten Stimuli. Die Effektivität einer oralen Gabe von Strychnin wurde in einem „acoustic startle response“-Experiment (ASR, Experiment 3, Kapitel 3) getestet. Das ASR-Paradigma wurde bereits oft in psychoakustischen Experimenten verwendet und der Einfluss der glycinergen Hemmung auf das Verhalten der Tiere ist gut erforscht. Im vorliegenden Experiment konnte keine Erhöhung der ASR-Amplitude (oder der Startle-Schwelle) in Strychnin-Sitzungen festgestellt werden, was darauf hinweist, dass die orale Verabreichung von Strychnin vermutlich ungeeignet ist, um zu testen, ob glycin-vermittelte Hemmung in der Verarbeitung der Stimuli involviert ist.

In Experiment 4 (Kapitel 4) wurde die Fähigkeit von Mäusen untersucht, korrelierte Fluktuationen in der Amplitude eines Rauschen zur Verbesserung der Tondetektion zu nutzen. Dieses Phänomen wird “comodulation masking release” (CMR) genannt, und wird einer Hörverarbeitung innerhalb eines auditorischen Kanals („within-channel“ CMR) und/oder zwischen mehreren auditorischen Kanälen („across-channel“ CMR) zugeschrieben. Im vorliegenden CMR-Experiment wurden zwei Schmalbandrauschbänder als Maskierer benutzt, um die Effekte der beiden Verarbeitungsarten voneinander zu trennen und die Rolle des spektralen Abstands zwischen beiden Rauschbändern auf die Größe des CMR-Effekts in der Maus zu untersuchen. Die Ergebnisse zeigten, dass die Hörverarbeitung innerhalb eines auditorischen Kanals zu einem deutlichen CMR-Effekt führte und dass es keinen eindeutigen Hinweis auf ein „across-channel“ CMR gab. Der “within-channel“ CMR-Effekt wurde größer, je näher beide Rauschbänder nebeneinander lagen. Der große

Anstieg des CMR-Effekts beim kleinsten getesteten Frequenzabstand zwischen den Rauschbändern (hier: 100 Hz) ist wahrscheinlich darauf zurückzuführen, dass das auditorische System der Maus in der Lage ist, die Änderungen in den zeitlichen Mustern des Rauschstimulus zu detektieren, die sich durch die Präsentation des Tonsignals ergeben. Die Ergebnisse aus dem vorliegenden Experiment wurden anschließend mit Ergebnissen aus ähnlichen Experimenten mit Menschen und Staren und mit den Ergebnissen eines anderen CMR-Experiments mit Mäusen verglichen. Die in diesem Experiment gewonnenen Daten sollen mit Daten aus geplanten physiologischen Experimenten aus einem kooperierenden Labor, welche die neuronale Verarbeitung zeitlicher Informationen bei Mäusen bei der gleichen Aufgabe untersuchen, verglichen werden.

Chapter 1

The mouse as a model in psychoacoustic research

Hearing research involves both behavioral procedures to access the ability of humans and animals to detect and discriminate sounds (psychoacoustics) and the recording of neuronal responses to different sound features at various levels of the auditory system (physiology). While each approach contributes to the understanding of hearing, it is the integration of both methods that allows to investigate the neuronal mechanisms underlying perception and processing of sound.

The best way to integrate both approaches is to compare physiological and psychoacoustic data from the same species. The prime candidate for such an approach is of course the human species, however, despite the abundance of psychoacoustic data (e.g. Yost et al. 1996), (electro)physiological recordings of neural activity in humans are rarely conducted. Therefore, for a study of both physiology and psychoacoustics in the same species a suitable animal model has to be found.

The house mouse (*Mus musculus*) is one of the animals that has developed into a promising model organism for the study of auditory function, and many data are available on mouse psychoacoustics and physiology (see chapter 1.1 and 1.2). Another advantage of the mouse is that its hearing sensitivity includes both frequencies within the range of the human auditory system and also within the ultrasonic range of up to 100 kHz (e.g. Birch et al. 1968, Ehret 1974, Mikaelian et al. 1974), allowing to examine auditory processing using wide auditory filters (e.g. Ehret 1975a, 1976a). Furthermore, the knowledge of the complete mouse genome (Waterston et al. 2002) and the availability of many naturally occuring and genetically engineered mutants (e.g. Willott et al. 2003, Zheng et al. 1999) offers the possibility

to study the genetic basis of the mechanisms underlying various aspects of auditory processing.

1.1 Mouse psychoacoustics

Psychoacoustic data on many basic auditory functions in the mouse have been obtained with various psychacoustic methods (for a review see also Heffner and Heffner 2001). In the mouse, both simple methods like the measurement of reflexive (i.e. unconditioned) responses and more elaborate conditioning experiments (classical and operant conditioning) have been employed to study different aspects of hearing.

There is a variety of data available in the mouse on auditory thresholds measured using **unconditioned responses** like the Preyer reflex (the movement of the pinna in response to loud sounds; e.g., Schleidt 1952), the galvanic skin response (a measure of skin conductance, e.g., Berlin 1963), or the acoustic startle response (ASR; a coordinated muscle contraction of the eyelid, the neck and the extremities upon presentation of sudden loud acoustic stimuli; e.g., Plappert et al. 2001). While most of these responses are elicited only by loud stimuli and therefore unsuitable to obtain low thresholds, the modification of the ASR by presenting a prepulse (prepulse inhibition, e.g., Ison and Agrawal 1998, Willott et al. 2003) before the startle eliciting stimulus may be used to measure sensitive thresholds. So far, only masked thresholds have been measured in the mouse using prepulse inhibition (Ison and Agrawal 1998). Prepulse inhibition has also been used to obtain data on spatial masking for different signal frequencies in young and aged mice (Ison and Agrawal 1998). A modification of the prepulse inhibition has also been used to measure the temporal resolution in the mouse. In the gap detection paradigm, the startle stimuli were presented in continuous background noise, and the “prepulse” consisted of partial (or total) offsets in the noise level (gaps) presented prior to the onset of the startle stimuli (e.g. Ison et al. 1998, Ison and Allen 2003a, Ison et al. 2005, Walton et al. 1997).

Since most unconditioned responses (except the prepulse inhibition of the ASR) can only be used to measure simple reactions (e.g. the detection of more or less loud stimuli), more elaborate psychoacoustic methods have to be employed for the

investigation of other aspects of hearing in mice. These methods usually involve the behavioral training of the subjects using either positive or negative feedback, or both. **Classical conditioning** is used to train the subjects to respond involuntarily to a previously neutral stimulus (e.g. a sound) with a conditioned reaction (e.g. the closing of their eyes) because the subjects have learned to associate the sound stimulus with reward or punishment in previous training. In the mouse, a conditioned eyelid reflex has been employed to obtain for example hearing thresholds (e.g. Markl and Ehret 1973, Ehret 1974) and masked thresholds (Ehret 1975b), and also to measure temporal integration of pure tones and noise the mouse (critical duration, Ehret 1976b). Furthermore, the conditioned eyelid reflex has also been used to calculate the width of auditory filters in the mouse using both indirect measurements like critical ratios (Ehret 1975b, 1976a) and direct measurements of the critical bands (Ehret 1976a). Another classical conditioning method, the conditioned suppression of behavior, was used to obtain hearing thresholds (Heffner and Masterton 1980, Koay et al. 2002), while frequency difference limens in the mouse were measured using avoidance conditioning (Kulig and Willott 1984).

Operant methods on the other hand are used to train the subjects to exhibit a specific behavioral response to the presentation of a target stimulus. The standard operant procedure in mouse psychoacoustics is the Go/NoGo procedure in which the subjects are required to wait until a target stimulus is presented and then to respond by crossing a border or jumping onto a platform within a certain time interval. Using water (or other liquids) or food reward, the Go/NoGo procedure has been used, for example, to obtain hearing thresholds (e.g. Ehret 1974, Klink et al. 2006, Markl and Ehret 1973, May et al. 2002, Mikaelian et al. 1974, Prosen et al. 2000, 2003), masked thresholds (e.g. Ehret 1975b, Weik 2004), frequency (Ehret 1975a) and intensity discrimination thresholds (Ehret 1975a, May et al. 2002), and critical durations (Ehret 1976b). As with classical conditioning, the width of auditory filters in the mouse was calculated using both critical ratios (Ehret 1975b, May et al. 2002) and critical bandwidths (e.g. Weik 2004, Weik et al. 2005, 2006), and the equivalent rectangular bandwidth (ERB) of auditory filters in the CBA/CaJ mouse strain has been obtained in young animals and aging subjects with hearing impairment (May et al. 2006). Operant conditioning was also used in a study on the localization of tones and noise in the horizontal plane in the mouse (Ehret and Dreyer 1984).

Furthermore, Ehret and coworkers conducted behavioral studies on auditory perception and grouping of multiharmonic communication sounds in the mouse (Ehret and Bernecker 1986, Ehret and Riecke 2002, Gaub and Ehret 2005, Geissler and Ehret 2002).

1.2 Physiology

Besides psychoacoustic experiments, also physiological experiments have been conducted in the mouse. Data on basic auditory functions in the mouse have already been obtained using distortion product otoacoustic emissions (DPOAEs), a measure of the health and functioning of outer hair cells (e.g. Carvalho et al. 2004, Liberman et al. 2004, Martin et al. 2007), and the auditory brainstem response (ABR), a physiological measure mostly used to estimate hearing thresholds (e.g. Barsz et al. 2002, Ison and Allen 2003b, May et al. 2002, Song et al. 2006, Zheng and Johnson 2001, Zheng et al. 1999).

A selection of studies on electrophysiological recording in the mouse is presented below. The frequency tuning, a basic characteristic of neurons, has been investigated, for example, in single auditory nerve fibers (Taberner and Liberman 2005), in cartwheel cells in the dorsal cochlear nucleus (Portfors and Roberts 2007) and in inferior colliculus (IC) neurons of the mouse (e.g. Egorova et al. 2001). The tonotopic organization of the auditory cortex in the mouse has been investigated as well (e.g. Stiebler et al. 1997).

Temporal characteristics of neurons like firing rate or first spike latency have been measured in IC neurons (Tan et al. 2008), and data on the spontaneous firing rate of single auditory nerve fibers are also available in the mouse (Taberner and Liberman 2005). The temporal resolution of IC neurons has been investigated in a gap detection task employing silent gaps imbedded in noise (e.g. Allen et al. 2003, Barsz et al. 2002, Walton et al. 1997, Walton et al. 1998, Walton et al. 2007). The timing patterns of spike discharges (e.g., the degree of phase-locking) and the dynamic range (i.e. the response rate in relation to the level of the presented tones) of single auditory nerve fibers have been measured (Taberner and Liberman 2005). Furthermore, data on responses of IC neurons to sinusoidally amplitude-modulated noise and tones (Tan and Borst 2007, Walton et al. 2002) and to tones of different

duration (Brand et al. 2000, Tan and Borst 2007) are available. Neuronal responses of single units in the auditory nerve and cochlear nucleus of the mouse to tone bursts presented in noise have been used to calculate masked thresholds and neural critical ratio bandwidths (Ehret and Moffat 1984).

1.3 Overview

A comparison of physiological and psychophysical data from the mouse has already been conducted to deepen the understanding of several aspects of auditory processing. For example, the effects of age-related hearing loss have been studied using both the auditory brainstem response and the acoustic startle response (e.g. Ison et al. 2007), and the temporal resolution in mice has been investigated using both neural minimal gap thresholds obtained from IC neurons and gap detection thresholds using auditory startle resonse (e.g. Walton et al. 1997). However, there are still many aspects of hearing in the mouse that have not been studied using both a behavioral and a physiological approach.

The present psychophysical study was designed to investigate two different aspects of temporal processing in the mouse and to supply the behavioral data for a comparison with physiological measurements obtained in other laboratories.

Chapter 2 consists of an already published paper by Klink and Klump (2004). Its aim was to investigate the ability of mice to detect an increment in signal duration compared to a reference duration. Three different reference durations and two different level conditions (fixed and roving) were used. The performance of the subjects was compared with the performance of several different vertebrate species (e.g., humans, rats, starlings). Furthermore, the performance was evaluated with regard to the expected performance described for different psychoacoustical concepts (e.g. clock and clock-counter models, including Weber's law) and also with results obtained by Brand et al. (2000) on the neuronal representation of acoustic signal duration in the IC of the mouse.

Chapter 3 is split into two parts, each describing a different experiment. The first experiment depicted the duration discrimination performance of mice with and without pharmacological manipulation with strychnine. The aim of this experiment was to investigate whether the performance of mice could be explained by an

interaction of excitation and (glycinergic) inhibition, or whether purely excitatory mechanisms might be involved. Similar to chapter 2, this experiment investigated the duration discrimination performance of mice for three different reference durations. The second experiment examined the influence of an oral dose of strychnine on the acoustic startle response (ASR) in mice. The ASR has been often used in psychoacoustic experiments and the role of glycinergic inhibition on the performance of the subjects in this experiment is well known. Using the same kind of pharmacological manipulation as in experiment 1, experiment 2 aimed to evaluate the effectiveness of an oral application of 1 mg/kg strychnine on the processing of auditory stimuli.

Chapter 4 focussed on the ability of mice to exploit correlated amplitude fluctuations in a noise masker to enhance the detection of a tonal signal. This phenomenon is called comodulation masking release (CMR), and has been attributed to auditory processing within one auditory channel (relying on within-channel cues) and/or across several auditory channels (relying on across-channel cues). The present CMR experiment was designed to separate the effects of within- and across channel cues from each other, and to investigate the role of within-channel cues on the amount of CMR in the mouse. The results were compared with model predictions, results from similar experiments in humans and starlings, and with results from a different CMR experiment in the house mouse (Weik 2004; for a review of CMR paradigms see also Verhey et al. 2003). This experiment is intended to be the behavioral counterpart to physiological experiments conducted in a cooperating laboratory on the neuronal processing of temporal information in mice in the same task, which will be available in the near future.

1.4 Contributions to this thesis

The present thesis is based on psychoacoustical data obtained in NMRI mice trained for various behavioral tasks. The training of the animals and the conducting of the experiments has been done by myself. My supervisor Prof. Dr. Georg M. Klump and I selected the tasks to investigate different aspects of temporal processing in the mouse, and we discussed the results (especially chapter 2 and 4) in relation to the findings of other experiments.

Holger Dierker calculated the modulation spectra of the stimuli that had been presented to the mouse subjects in the CMR experiment (chapter 4; see also Fig. 4.3), and together with Prof. Klump and myself has contributed to the discussion and intepretation of the results. Holger also contributed greatly to the establishing of the setup for the auditory startle experiments (chapter 3).

1.5 References

Allen PD, Burkard RF, Ison JR, Walton JP (2003) Impaired gap encoding in aged mouse inferior colliculus at moderate but not high stimulus levels. Hear Res 186:17-29

Barsz K, Ison JR, Snell KB, Walton JP (2002) Behavioral and neural measures of auditory temporal acuity in humans and mice. Neurobiol Aging 23:565-578

Berlin CI (1963) Hearing in mice via GSR audiometry. J Speech Hear Res 13:359-368.

Birch LM, Warfield D, Ruben RJ, Mikaelian DO (1968) Behavioral measurements of pure tone thresholds in normal CBA-J mice. J Audit Res 8:459-468

Brand A, Urban A, Grothe B (2000) Duration tuning in the mouse auditory midbrain. J Neurophysiol 84:1790–1799

Carvalho S, Mom T, Gilain L, Avan P (2004) Frequency specificity of distortion-product otoacoustic emissions produced by high-level tones despite inefficient cochlear electromechanical feedback. J Acoust Soc Am 116:1639-1648

Egorova M, Ehret G, Vartanian I, Esser K-H (2001) Frequency response areas of neurons in the mouse inferior colliculus. Exp Brain Res 140:145-161

Ehret G (1974) Age-dependent hearing loss in normal hearing mice. Naturwissenschaften 61:506-507

Ehret G (1975a) Frequency and intensity difference limens and nonlinearities in the ear of the housemouse (*Mus musculus*). J Comp Physiol 102:321-336

Ehret G. (1975b) Masked auditory thresholds, critical ratios, and scales of the basilar membrane of the housemouse (*Mus musculus*). J Comp Physiol 103:329-341

Ehret G (1975c) Schallsignale der Hausmaus (*Mus musculus*). Behaviour 52:38-56

Ehret G (1976a) Critical bands and filter characteristics in the ear of the housemouse (*Mus musculus*). Biol Cybernetics 24:35-42

Ehret G (1976b) Temporal auditory summation for pure tones and white noise in the house mouse (Mus musculus). J Acoust Soc Am 59:1421-1427

Ehret G, Bernecker C (1986) Low-frequency sound communication by mouse pups (*Mus musculus*): wriggling calls release maternal behavior. Anim Behav 34:821-830

Ehret G, Dreyer A (1984) Localization of tones and noise in the horizontal plane by unrestrained house mice (*Mus musculus*). J exp Biol 109:163-174

Ehret G, Moffat AJM (1984) Noise masking of tone responses and critical ratios in single units of the mouse cochlear nerve and cochlear nucleus. Hear Res 14:45-57

Ehret G, Riecke (2002) Mice and humans perceive multiharmonic communication sounds in the same way. Pro Natl Acad Sci USA 99:479-482

Gaub S, Ehret G (2005) Grouping in auditory temporal perception and vocal production in mutually adapted: the case of wriggling calls in mice. J Comp Physiol A 191:1131-1135

Geissler DB, Ehret G (2002) Time-critical integration of formants for perception of communication calls in mice. Pro Natl Acad Sci USA 99:9021-9025

Heffner HE, Heffner, RS (2001) Behavioral assessment of hearing in mice. In J. F. Willott (Ed), Handbook of mouse auditory research (pp. 19-29). Boca Raton, FL: CRC Press.

Heffner H, Masterton B (1980) Hearing in glires: Domestic rabbit, cotton rat, feral house mouse, and kangaroo rat. J Acoust Soc Am 68:1584-1599

Ison JR, Agrawal P (1998) The effect of spatial separation of signal and noise on masking in the free field as a function of signal frequency and age in the mouse. J Acoust Soc Am 104:1689-1695

Ison JR, Agrawal P, Pak J, Vaughn WJ (1998) Changes in temporal acuity with age and with hearing impairment in the mouse: a study of the acoustic startle reflex and its inhibition by brief decrements in noise level. J Acoust Soc Am 104:1696-1704

Ison JR, Allen PD (2003a) A diminished rate of “physiological decay” at noise offset contributes to age-related changes in temporal acuity in the CBA mouse model of presbycusis. J Acoust Soc Am 114:522-528

Ison JR, Allen PD (2003b) Low-frequency tone pips elicit exaggerated startle reflexes in C57BL/6J mice with hearing loss. J Assoc Res Otolaryngol 4:495-504

Ison JR, Allen PD, O'Neill WE (2007) Age-related hearing loss in C57BL/6J mice has both frequency-specific and non-frequency-specific components that produce a hyperacusis-like exaggeration of the acoustic startle reflex. J Assoc Res Otolaryngol 8:539–550

Ison JR, Allen PD, Rivoli PJ, Moore JT (2005) The behavioral response of mice to gaps in noise depends on its spectral components and its bandwidth. J Acoust Soc Am 117:3944-3951

Klink KB, Bendig G, Klump GM (2006) Operant methods for mouse psychoacoustics. Behav Res Methods 38:1-7

Klink KB, Klump GM (2004) Duration discrimination in the mouse (Mus musculus). J Comp Physiology A 190:1039-1046

Koay G, Heffner RS, Heffner HE (2002) Behavioral audiograms of homozygous med mutant mice with sodium channel deficiency and unaffected controls Hear Res 171:111-118

Kulig and Willott (1984) Frequency difference limens of C57BL/6 and DBA/2 mice: relationship to auditory neuronal response properties and hearing impairment. Hear Res 16:169-174

Liberman MC, Zou J, Guinan JJ (2004) Otoacoustic emissions without somatic motility: Can stereocilia mechanics drive the mammalian cochlea? J Acoust Soc Am 116:1649–1655

Markl H, Ehret G (1973) Die Hörschwelle der Maus (*Mus musculus*): Eine kritische Wertung der Methoden zur Bestimmung der Hörschwelle eines Säugetiers. Z Tierpsychol 33:274-286

Martin GK, Vazquez AE, Jimenez AM, Stagner BB, Howard MA, Lonsbury-Martin BL (2007) Comparison of distortion product otoacoustic emissions in 28 inbred strains of mice. Hear Res 234:59–72

May BJ, Kimar S, Prosen CA (2006) Auditory filter shapes of CBA/CaJ mice: behavioral assessments. J Acoust Soc Am 120:321-330

May BJ, Prosen CA, Weiss D, Vetter D (2002) Behavioral investigation of some possible effects of the central olivocochlear pathways in transgenic mice. Hear Res 171:142-157

Mikaelian DO, Warfield D, Norris O (1974) Genetic progressive hearing loss in the C57/b16 mouse. Acta Otolaryng 77:327-334

Plappert CF, Pilz PKD, Becker K, Becker C-M, Schnitzler H-U (2001) Increased sensitization of acoustic startle response in spasmodic mice with a mutation of the glycine receptor alpha1-subunit gene. Behav Brain research 121:57-67

Portfors CV, Roberts D (2007) Temporal and frequency characteristics of cartwheel cells in the dorsal cochlear nucleus of the awake mouse. J Neurophysiol 98:744-756

Prosen CA, Bath KG, Vetter DE, May BJ (2000) Behavioral assessments of auditory sensitivity in transgenic mice. J Neurosci Methods 97:59-67

Prosen CA, Dore DJ, May BJ (2003) The functional age of hearing loss in a mouse model of presbycusis: I. Behavioral assessments. Hear Res 183:44-56

Schleidt WM (1952) Reaktionen auf Töne hoher Frequenz bei Nagern. Naturwissenschaften 39:69-70

Song L, McGee J, Walsh EJ (2006) Frequency- and level-dependent changes in auditory brainstem responses (ABRs) in developing mice. J Acoust Soc Am 119:2242–2257

Stiebler I, Neulist R, Fichtel I, Ehret G (1997) The auditory cortex of the house mouse: left-right differences, tonotopic organization and quantitative analysis of frequency representation. J Comp Physiol A 181:559-571

Taberner AM, Liberman MC (2005) Response properties of single auditory nerve fibers in the mouse. J Neurophysiol 93:557-569

Tan ML, Borst JGG (2007) Comparison of responses of neurons in the mouse inferior colliculus to current injections, tones of different durations, and sinusoidal amplitude-modulated tones. J Neurophysiol 98:454-466

Tan X, Wang X, Yang W, Xiao Z (2008) First spike latency and spike count as functions of tone amplitude and frequency in the inferior colliculus of mice. Hear Res 235:90–104

Verhey JL, Dau T, Kollmeier B (1999) Within-channel cues in comodulation masking release (CMR): Experiments and model predictions using a modulation-filterbank model. J Acoust Soc Am 106:2733-2745

Verhey JL, Pressnitzer D, Winter IM (2003) The psychophysics and physiology of comodulation masking release. Exp Brain Res 153:405-415

Walton JP, Barsz K, Wilson WW (2007) Sensorineural hearing loss and neural correlates of temporal acuity in the inferior colliculus of the C57bl/6 mouse. J Assoc Res Otolaryng, DOI: 10.1007/s10162-007-0101-z

Walton JP, Frisina RD, Ison JR, O'Neill WE (1997) Neural correlates of behavioral gap detection in the inferior colliculus of the young CBA mouse. J Comp Physiol A 181:161-176

Walton JP, Frisina RD, O'Neill WE (1998) Age-related alteration in processing of temporal sound features in the auditory midbrain of the CBA mouse. J Neurosci 18:2764-2776

Walton JP, Simon H, Frisina RD (2002) Age-related alterations in the neuronal coding of envelope periodicities. J Neurophysiol 88:565-578

Wang Y, Manis PB (2006) Temporal coding by cochlear nucleus bushy cells in DBA/2J mice with early onset hearing loss. J Assoc Res Otolaryngol 7:412-424

Waterston RH, Lindblad-Toh K, Birney E, Rogers J, Abril JF, Agarwal P, et al. (2002) Initial sequencing and comparative analysis of the mouse genome. Nature 420:520-562

Weik VN (2004) Untersuchungen zu Maskierungseffekten innerhalb und zwischen auditorischen Filtern bei der Hausmaus (*Mus musculus*). Unpublished diploma thesis, Carl von Ossietzky Universität Oldenburg, Oldenburg, Germany

Weik V, Klink KB, Klump GM (2005) Comodulation masking release in the house mouse (*Mus musculus*), in: Elsner N., Zimmermann, H. (Eds.), Proceedings of the 30th Göttingen Neurobiology Conference and the 6th Meeting of the German Neuroscience Society, p. 101B

Weik V, Klink K, Klump GM (2006) Comodulation masking release determined in the mouse (*Mus musculus*) using a band-narrowing paradigm, in: Abstracts of the twenty-ninth annual midwinter research meeting of the Association for Research in Otolaryngology 29:124

Willott JF, Tanner L, O'Steen J, Johnson KR, Bogue MA, Gagnon L (2003) Acoustic startle and prepulse inhibition in 40 inbred strains of mice. Behav Neurosci 117:716-727

Yost WA, Popper AN, Fay RR (Eds.) (1996) Human Psychophysics. Springer, New York

Zheng QY, Johnson KR (2001) Hearing loss associated with the modifier of deaf waddler (*mdfw*) locus corresponding with age-related hearing loss in 12 inbred strains of mice. Hear Res 154:45-53

Zheng QY, Johnson KR, Erway LC (1999) Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses. Hear Res 130:94-107

Chapter 2

Duration discrimination in the mouse (*Mus musculus*)*

2.1 Abstract

Detection thresholds for an increment in duration of a 10-kHz pure tone were determined in the NMRI mouse using a Go/NoGo-procedure and the method of constant stimuli. Thresholds for reference durations of 50, 100 and 200 ms were obtained presenting the signals at a fixed level or at a level varying by ±3 dB. Thresholds were determined using signal detection theory (d'=1.0 or d'=1.8) and the criterion of 50% correct responses. For a fixed level, the average Weber fraction $\Delta T/T$ (criterion of d'=1.8) significantly decreased from 1.18 or 1.23 at reference durations of 50 or 100 ms, respectively, to 0.97 at a reference duration of 200 ms. Thresholds were on average reduced by 46.8 or 55.4% for the threshold criteria d'=1 or 50% correct responses, respectively. There was no effect of randomizing the level on the discrimination threshold. Duration discrimination in the NMRI mouse does not follow Weber's law. The results are consistent with a mechanism summing up neural impulses over the duration of the stimulus. The psychoacoustic data are compared with results obtained by Brand et al. (J Acoust Soc Am 51:1291–1223, 2000) on the representation of acoustic signal duration in the mouse inferior colliculus.

2.1.1 Keywords

Animal psychoacoustics, Auditory processing mechanism, NMRI mouse

---

* This chapter has been already published as "Klink KB, Klump GM (2004) Duration discrimination in the mouse (*Mus musculus*). J Comp Physiol A 190: 1039–1046".

2.2 Introduction

Signal duration is an important parameter characterizing many sounds that are evaluated by the auditory system. In the inferior colliculus and in the cortex of mammals, neurons showing a duration-dependent response pattern have been found (e.g., Brand et al. 2000, Casseday et al. 1994, Chen 1998, Ehrlich et al. 1997, Galazyuk and Feng 1997, He et al. 1997). These neurons either show a monotonic change of the response with the duration of the sound, or they exhibit a non-monotonic response indicating a tuning to a limited range of durations. It has been suggested that the neural processing of duration provides the basis for the perception of signal duration that is evident in the behavioral context (e.g., Brand et al. 2000, Ehrlich et al. 1997, Galazyuk and Feng 1997). To relate neural response patterns to psychophysical measures of auditory processing, it is desirable to compare neurophysiological and psychophysical data from the same species. So far, studies on the processing of sound duration have either focused on the neural mechanisms (see references above) or on the behavior (e.g., Church et al. 1976, Dooling and Haskell 1978, Maier and Klump 1990, Sinnott et al. 1987, Yunker and Herman 1974) and to our knowledge there is no species in which both aspects of the processing have been studied.

The current psychophysical study on auditory duration discrimination in the mouse aims at filling this gap. Previously, Brand et al. (2000) have reported duration tuned responses in the inferior colliculus of the house mouse. Among other stimuli, they presented tones of a duration ranging from 1 to 100 ms to characterize the neurons' response patterns. Here we report the accuracy of duration discrimination in the mouse for reference tone durations of 50, 100 or 200 ms, i.e., a range of durations overlapping that of the neurophysiological study. Most mouse vocalizations also fall into this range. Ehret (1975a) observed that the average duration of seven out of eight natural vocalizations in NMRI mice was between 40 and 140 ms, and sound duration ranged up to 300 ms. Only the lip-smacking sound of infant mice was much shorter (on average 4 ms).

The NMRI mouse is one of the strains of laboratory mice that has been investigated extensively in behavioral studies (e.g., Ehret 1975b, c, 1976, Geissler and Ehret 2002). Therefore, a large data set on auditory perception is available in this strain

that can be built upon in the interpretation of new results. Furthermore, mouse psychophysics is becoming increasingly important because mutant strains and knock-out mice offer unique opportunities to study the neural mechanisms of auditory perception. This study will provide the first psychophysical data on duration perception in the mouse and thus help to build the mouse psychophysics databook (for a summary of mouse psychophysics data see Fay 1988).

2.3 Materials and methods

2.3.1 Subjects

The animals, four females and six males, from which data were collected in this experiment were adult house mice (Mus musculus) of the NMRI strain bred by Günter Ehret, Ulm, Germany. Four additional mice (two females and two males) could not be trained within a time of up to 3 months. The experimental subjects' age ranged between 5 and 20 months during the total period of testing of 7 months. They were housed in individual cages (42x26x15 cm) with a hiding possibility (''mouse house'', Tecniplast) and a layer of wood shavings as bedding material (Raiffeisen). All cages were stored in a ventilated cage rack (Tecniplast Slim Line Sealsafe). The feeding of the animals assured that their weight did not drop below 30.3±3.7 g (mean±SD of the minimum weight of the subjects). They had unrestricted access to water. The food rewards during the experiments consisted of 20 mg pellets (Bioserve: Dustless Precision Pellets, Formula#FO163), additional rodent pellets (Altromin 1314) were given after the experiments to keep the animals' weight about constant. Animals were moved from their cages to the experimental cage using a small transfer cage.

2.3.2 Apparatus

The animals were tested in sound-attenuating chambers (Industrial Acoustics type IAC 403 A: inside dimensions 245x227x240 cm or a custom-built chamber: inside dimensions 67x108x91 cm) lined with two to three layers of sound absorbing wedges (Illbruck Waffel type 70/125, mounted on Illbruck Plano type 50/0 SF or Illbruck

Illsonic Pyramide 100/100, mounted on one or two layers of Illbruck Plano). The wedges had an absorption coefficient of more than 0.99 for frequencies above 500 Hz.

The experimental doughnut-shaped cage (outer diameter 25 cm, inner diameter 13 cm; height 13 cm, made from Casanet wire mesh) was located in the middle of the chamber on a rack constructed of thin (5–7 mm) metal bars (IAC chamber) or a wire construction lifting the cage above the sound absorbing wedges (custombuilt chamber). The cage contained a small feeding dish with a feeder light as a secondary reinforcer and 10 cm from the feeding dish a pedestal (size: 4x2.5x2.5 cm, length·width·height) with a light-interrupting switch. A nearby pedestal light was used to provide feedback to the animals during testing. A custom-built feeder mounted at a distance of at least 31 cm was connected to the feeder dish by a flexible tube and dispensed the reward pellets. A loudspeaker (Canton Plus XS, 65 Hz–30 kHz) was positioned a minimum of 30 cm above the pedestal at which the mouse sat in the experimental cage.

2.3.3 Stimulus generation

The stimuli were generated with a Linux workstation (AMD-Processor, Sound Blaster PCI 128, 44.1-kHz sampling rate) and passed through a programmable attenuator (PA4; Tucker Davis Technologies). The overall signal levels were adjusted by an additional manual attenuator (Hewlett Packard 350D). The stimuli were amplified either by a Rotel RMB-1066 amplifier (in the IAC chamber) or a Harman/Kardon HK6350R amplifier (in the custom-built chamber) and presented via the Canton XS loudspeaker. Sound-pressure levels in both experimental set-ups were calibrated once a day with a sound level meter (Bruel and Kjaer 2238 Mediator) located at the position where the head of the animal would be during the experiment.

The stimuli used in our experiments consisted of 10-kHz pure tones. The reference stimuli had equivalent durations of either 50, 100 or 200 ms, and all stimuli had a cosine rise/fall of 10 ms duration. They were repeated with a rate of one stimulus per 1.3 s. The median level of presentation was 40 dB above the individual’s auditory threshold for an 800-ms 10-kHz tone that was determined preceding the duration discrimination experiments in each subject using a Go/NoGo procedure (details are

Table 1 Auditory thresholds, sensation levels and stimulus types used in the tests with the ten different mice that served as subjects. Subjects 1–4 were tested with the complete stimulus set, subjects 5–10 were tested with partial stimulus sets

Subject ID	Female 81	Male 176A	Male 176B	Male 176D	Median	Median	Female 172	Female 94	Male 81	Male 90	Male 91	Female 97
Subject no.	1	2	3	4	Subjects 1–4	Subjects 1–10	5	6	7	8	9	10
Stimulus type[a]	50f/100f/200f 50r/100r/200r	50f/100f/200f 50r/100r/200r	50f/100f/200f 50r/100r/200r	50f/100f/200f 50r/100r/200r			50f/200f	50f/100f/200f	50f/100f/200f	50f	50f	50f/100f
Stimulus level (dB SPL)	80	60	59	69	65	60	59	80	53	43	64	43
Initial threshold (dB SPL)	21.0	19.6	18.6	28.8	20.3	18.8	19.0	−10.1	12.6	−10.9	23.6	2.8
Initial sensation level (dB)	59.0[b]	40.4	40.4	40.2	40.4	40.4	40.0	90.1[b]	40.4	53.9[b]	40.4	40.2
Final threshold (dB SPL)	67.4	23.2	46.8	33.3	40.0		13.2	71.3	ND	ND	ND	ND
Final sensation level (dB)	12.6	36.8	12.2	35.7	24.2		45.8	8.7	ND	ND	ND	ND

ND not determined[a]The number designates the reference duration in ms, the letter 'f' or 'r' designates fixed or randomly varying stimulus levels, respectively
[b]Higher sensation levels were used in subjects that did not respond reliably at a level of 40 dB above the previously determined threshold

provided in Table 2.1). Most subjects were presented with tones of 40 dB SL, two old subjects were presented with tones of 8.7 and 12.6 dB SL, respectively, when the sensation level was determined in relation to the threshold measured at the end of the experiment. The test stimuli had an increased duration which differed from the corresponding reference duration by a Weber fraction of either between 0.2 and 2 (for 50- and 100-ms reference tones) or between 0.25 and 1.75 (for reference signals of 200 ms). In the first of the experiments the level of the signals was constant; in the second experiment it was varied randomly over a range of ±3 dB from one presentation to the next.

2.3.4 Procedure

The experimental paradigm was a Go/NoGo-procedure reinforcing the subject with food rewards (Klink et al. 2006). The experimental protocol was controlled by the workstation using a custom-made program. An experimental session started with the repeated presentation of reference stimuli and the subject had to jump onto the pedestal. After a random waiting interval of between 1 and 5 s a single test stimulus with a longer duration than the reference signal was presented and then the reference stimuli commenced at the regular

interval of one stimulus per 1.3 s. The mouse was trained to jump off the pedestal when perceiving a test stimulus (Go condition), otherwise (i.e., if a reference stimulus was played, NoGo condition) it had to remain on the pedestal. If the subject responded correctly to a test stimulus (i.e. scored a ‘‘hit’’), a feeder light was switched on (secondary reinforcer) and a food reward was given and the next trial started. If the subject missed a test stimulus and remained seated, the pedestal light was switched off for 1 s before the next trial could be initiated. 30% of all trials consisted of sham trials in which a reference stimulus was given in place of a test stimulus. These trials were used to measure the false alarm rate. If the subject jumped off the pedestal in a sham trial (‘‘false alarm’’), the pedestal light was also switched off for 1 s and a new trial started.

Duration discrimination thresholds were obtained by the method of constant stimuli. A block of ten trials, consisting of a set of seven test stimulus trials differing in duration of the test stimulus and three sham trials, was repeated six times (i.e., the session had 60 trials in total and lasted mostly between 15 and 60 min). The first ten trials of each session were used as a ‘‘warm-up’’ period (only test stimuli with the longest durations were presented) and were discarded from the analysis.

2.3.5 Data analysis

Sessions were excluded from the analysis if the percentage of false alarms was greater than 20% or if the average hit rate in trials with the two most salient test stimuli (i.e., those with the largest deviation from the reference duration) was less than 80%. Sessions were accepted if they did not violate any of these conditions. The duration-discrimination threshold was generally calculated using signal-detection theory and a threshold criterion d’ of 1.8. For each animal, data from five consecutive valid sessions were combined in one psychometric function summarizing performance from 250 trials that was used to determine the threshold by linear interpolation. Sessions were only combined, if the threshold from single sessions with 50 data trials each did not vary more than by a factor of two. At the completion of the experiments, thresholds using additional criteria (d’=1 and 50% correct responses) were also calculated.

2.4 Results

Of the 14 subjects that were initially trained to report the occurrence of a tone in a signal-detection paradigm, ten could be trained to the threshold criterion to report the increase in duration of a test tone compared to a reference tone. This initial transfer turned out to be very difficult. The subjects that learned the task needed 13.4±8.5 (mean±SD) days to reach a performance that allowed us to determine duration discrimination thresholds. Only four of the subjects could be tested with all six stimulus conditions (reference durations 50, 100, 200 ms, and fixed and variable amplitudes). Not all sessions could be used for threshold estimation: in 29% of the sessions the false-alarm rate was above 20%, in 15% of the sessions the subjects responded less than 80% to the two most salient duration increments, in an additional 8% of the sessions both criteria for inclusion of a session into the analysis were violated.

Psychometric functions obtained in sessions that fulfilled the criteria for acceptance regarding the false-alarm rate and the most salient stimuli were generally steep in the region used for threshold estimation. Examples from the four subjects that were tested with all six conditions are shown in Fig. 2.1. On average, the false alarm rate was 13.4±3.0% (mean±SD for all animals tested). The discrimination measure d' increased linearly with the increase in tone duration of the test stimulus (r= 0.87±0.10, mean±SD). The slope of the psychometric function relating d' to the Weber fraction for the duration increment was on average 1.46, 1.46, and 2.24 for reference durations of 50, 100 and 200 ms, respectively. To make the visual comparison more easy, the x-axis in all subplots of Fig. 2.1 is scaled in such a way that a unit between two tic marks on the x-axis corresponds to an increment in the Weber fraction of 0.4, i.e., was 20, 40 and 80 ms at a reference duration of 50, 100, and 200 ms, respectively. A steeper slope indicates that a change in duration is more salient to the subject. The slopes varied significantly with the reference duration ($P=0.01$, two-way repeated-measures ANOVA). The slopes for the 200 ms reference duration were significantly steeper ($P<0.02$, Tukey test) than the slopes for the two shorter reference durations that did not differ significantly from each other. Although the two-way repeated-measures ANOVA revealed no significant difference between the presentation of the tones with a fixed or randomly varying amplitude, there was a

significant interaction (P=0.032) between the mode of amplitude variation and the effect of stimulus duration. In the fixed amplitude condition, the slope was larger for stimulus durations of 50 and 200 ms than of 100 ms (only the difference between slopes for stimulus durations of 200 and 100 ms were significantly different). In the randomly varying amplitude condition the slope monotonically increased from 50 to 200 ms stimulus duration. It was significantly steeper for a reference duration of 200 ms than for reference durations of 100 and 50 ms.

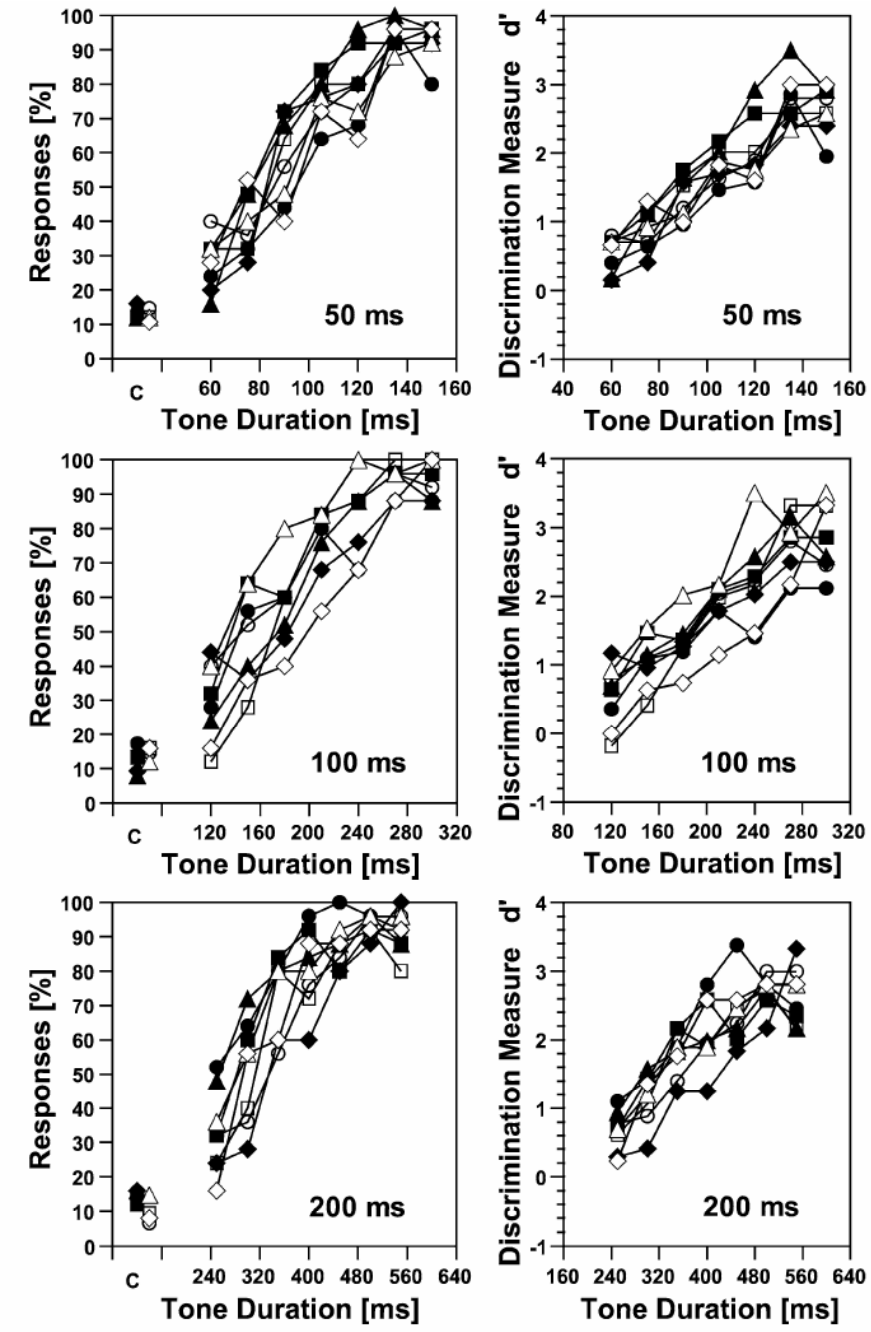

Fig. 2.1 Psychometric function showing the response probability (percentage reports of a change in duration) and the discrimination measure d' in relation to the duration of a test stimulus deviating in duration from that of a reference stimulus. The duration of the reference stimulus was 50, 100, or 200 ms as indicated in each graph. The symbols of different shapes represent the four individuals tested with all stimulus conditions. Filled symbols show data for a fixed amplitude of the tones. Open symbols show data for tones that were presented with an amplitude varying randomly by ±3 dB. C designates the rate of spontaneous responding without an increase in duration measured in ''catch'' trials (i.e., the false-alarm rate)

The thresholds for the detection of increments in tone duration expressed as the Weber fraction are shown in Fig. 2.2 and Table 2.2 (for different threshold criteria). For the threshold criterion d'=1.8 and reference durations of 50 or 100 ms an increment of more than 100% of the reference duration was necessary for detection. At a reference duration of 200 ms the increment at threshold was 97% and 77% for the fixed and variable conditions, respectively. The increment detection thresholds obtained with the criterion of 50% correct responses were found to be at a duration increment of between 48% and 77% of the reference duration. The increment-detection thresholds varied significantly with the reference duration ($P=0.009$, two-way repeated-measures ANOVA of the data of four individuals tested with all conditions), but did not differ between the fixed and variable amplitude condition. The increment-detection threshold was significantly lower for the 200-ms reference duration ($P<0.02$, Tukey test) than for the 100- and 50-ms reference duration that did not differ significantly from each other. The threshold criterion affected the value of the increment-detection threshold. Thresholds obtained with the criterion d'=1.8 were significantly elevated compared to thresholds obtained with the criterion d'=1 or the criterion of 50% responses when reporting an increment ($P=0.001$, Tukey test following significant results in a repeated measures ANOVA for the fixed and random-amplitude data). Thresholds obtained with the criterion d'=1 were not significantly different from those obtained using the criterion of 50% responses when reporting an increment.

2.5 Discussion

The thresholds for detecting an increment of the tone duration in the mouse were similar to the results reported by Sinnott et al. (1987) for Old World monkeys (Fig. 2.3). The monkeys' thresholds for a duration increment were found to range from 45 to 125 ms at a reference duration of 200 ms, whereas the mouse had an increment detection threshold of about 100 ms for this condition. Considerably larger increment-detection thresholds for duration were reported for the rat (Church et al. 1976) than for the mouse. In the other vertebrates tested so far (humans, Sinnott et al. (1987); bottlenose dolphin, Yunker and Herman (1974); parakeet, Dooling and Haskell (1978); European starlings, Maier and Klump (1990)), the threshold for detecting

increments in the duration of an auditory signal was much smaller. In general, an increment in signal duration by less than about 20% was sufficient for detecting the change in these four species (Fig. 2.3).

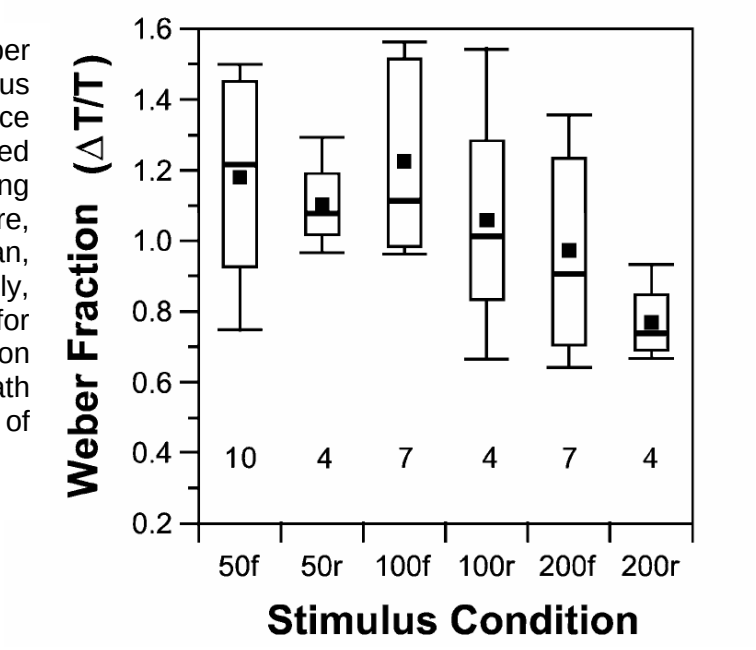

Fig. 2.2 Detection threshold (Weber fraction) for an increment in stimulus duration for different reference durations (50, 100, 200 ms) and fixed (f) and randomly (r, ±3 dB) varying amplitude of the tones (filled square, horizontal bar and box shows mean, median and quartiles, respectively, the whiskers show percentiles for 10% and 90%). Threshold criterion was a d' of 1.8. Numbers underneath each box plot indicate the number of individuals tested

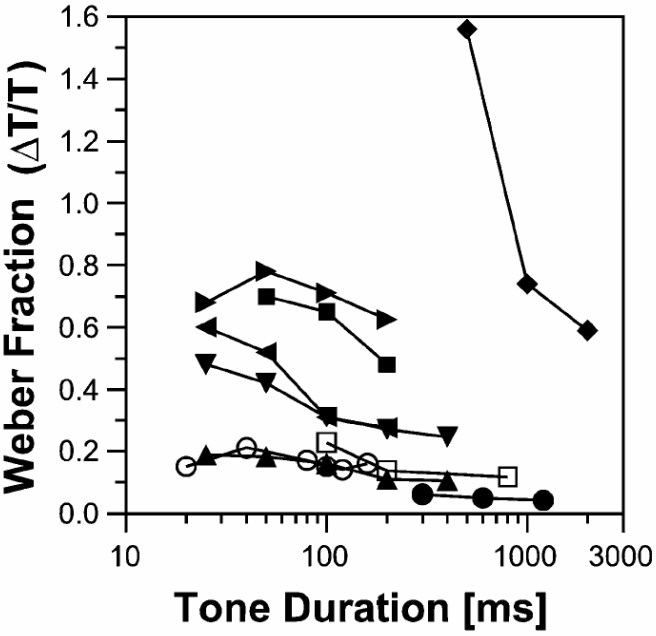

Fig. 2.3 Detection threshold (Weber fraction) for an increment in stimulus duration for different species in relation to the reference duration. Data are shown for Japanese macaque (▼, Macaca fuscata, criterion 50% correct, Sinnott et al. 1987), vervet monkey (►, Cercopithecus aethiops, criterion 50% correct in Go/NoGo, Sinnott et al. 1987), de Brazza monkey (◄, Cercopithecus neglectus, criterion 50% correct in Go/NoGo, Sinnott et al. 1987), human (▲, Homo sapiens, criterion 50% correct in Go/NoGo, Sinnott et al. 1987), bottlenose dolphin (●, Tursiops truncatus, criterion 75% correct in 2AFC, Yunker and Herman 1974), rat (♦, Rattus rattus, criterion 75% correct in 2AFC, Church et al. 1976), mouse (■, Mus musculus, criterion 50% correct in Go/NoGo, this study), parakeet (○, Melopsittacus undulates, criterion 50% correct in Go/NoGo, Dooling and Haskell 1978), European starling (□, Sturnus vulgaris, criterion ~50% correct in Go/NoGo, Maier and Klump 1990)

Table 2.2 Mean threshold (Weber fraction) and standard deviation for detecting an increment in stimulus duration for fixed and randomly varying amplitude of the tones.

Reference duration	$d' = 1.8$	$d' = 1.0$	50% correct
Fixed amplitude			
50 ms ($n = 10$)	1.18 ± 0.29	0.63 ± 0.25	0.70 ± 0.18
100 ms ($n = 7$)	1.23 ± 0.27	0.48 ± 0.21	0.65 ± 0.19
200 ms ($n = 7$)	0.97 ± 0.31	0.48 ± 0.20	0.48 ± 0.21
Variable amplitude			
50 ms ($n = 4$)	1.10 ± 0.14	0.57 ± 0.14	0.77 ± 0.10
100 ms ($n = 4$)	1.06 ± 0.36	0.59 ± 0.33	0.62 ± 0.29
200 ms ($n = 4$)	0.77 ± 0.12	0.46 ± 0.07	0.55 ± 0.10

n number of subjects tested

The comparison across the different species indicates a large variance in the performance. This raises the question whether the different species use different mechanisms for detecting the duration increment. One possibility for detecting an increment in duration is to use a loudness increase that may be associated with an increase in signal duration. In an experiment studying the perception of tones using multidimensional scaling in budgerigars, Dooling et al. (1987) found that these animals treated the stimulus parameters duration and intensity very similar. Also humans show a trading of duration and intensity in loudness perception (e.g., McFadden 1975). Loudness of short tone pulses can be attributed mainly to temporal summation (e.g., Poulsen 1981, Buus et al. 1997). Studying temporal summation for a wide range of stimuli in the mouse, Ehret (1976) reported a reduction of threshold by on average 6.3 dB for a tenfold increase in duration (range of durations 1– 1,000 ms) of a 10-kHz tone. If we assume that this threshold decrease is associated with a loudness increase of a similar magnitude, the increase of the duration of a 10-kHz tone at the duration discrimination threshold would result in a perceived intensity increment of about 2.2 dB for the largest Weber fraction determined in the present study. This increment is much smaller than the detection threshold for an intensity increment in the NMRI mouse that has been shown to lie above 4 dB for 10-kHz tones in a range of sensation levels of up to 80 dB (Ehret 1975b). These data suggest that it is unlikely that the NMRI mouse can use a loudness increment to detect an increase in duration in the psychophysical experiment. Furthermore, the failure to find an effect of a roving level (±3 dB) of the tones on the duration

discrimination performance of the mouse corroborates the notion that the mechanism involved in duration discrimination is not affected by moderate changes of the signal level.

Psychophysical studies of duration discrimination have led to the proposal of different kinds of models to explain the subject's performance. In their review, Killeen and Weiss (1987) contrasted two classes of models - clock models and clock-counter models - and proposed how to evaluate their fit to psychophysical data. In the clock model of duration perception, a single measure of the time interval from the beginning to the end of the stimulus is taken that shows some random variation (timing error) being proportional to the measurement value. Thus, duration discrimination performance according to the clock models generally follows Weber's law, i.e., the discrimination threshold is proportional to the stimulus duration. In the clock-counter model of duration perception, there is no direct timing of the interval. Instead, a counter sums up events generated during the occurrence of the stimulus (see for example Creelman 1962). We applied a sum-of-leastsquares fit of the general function proposed by Killeen and Weiss (1987) to the mouse data to estimate the model parameters. The values of the parameters allow to discriminate between the two different classes of models. Parameter values that are typical for a clock-counter model provide the best fit to the mouse data (model parameters according to Killeen and Weiss (1987), Eqn. 14: A=0.975, B=0.025, C=0). Clock-counter models also provide the best fit to the duration discrimination data in humans (e.g., Henry 1948, Abel 1972, Sinnott et al. 1987) and macaques (Sinnott et al. 1987; analysis of data from the literature by Killeen and Weiss 1987 and Maier and Klump 1990). In other species such as in the rat (Church et al. 1976) or in the European starling (Maier and Klump 1990), clock models provide the best fit to the data.

Creelman (1962) suggested that a clock-counter mechanism functions by summing up Poisson distributed pulses generated during the duration of the stimulus and that the basis of the decision in the psychophysical discrimination of duration is the number of pulses. A number of neurophysiological studies in the auditory system have provided evidence for neuronal response patterns that could provide the basis for such clock-counter mechanisms. Here we concentrate on studies in mammals. For example, duration-tuned neurons have been reported for the inferior colliculus (e.g., Casseday et al. 1994, Ehrlich et al. 1997) and the cortex of bats (e.g., Galazyuk

and Feng 1997). They were found in the inferior colliculus of the chinchilla (Chen 1998) and the mouse (Brand et al. 2000) and in the auditory cortex of the cat (He et al. 1997). In general, three different types of neurons with duration-dependent response patterns were reported: (1) short-pass neurons which produced the largest number of spikes at short signal duration and a decreased number of spikes with increasing stimulus duration, (2) long-pass neurons which produced an increasing number of spikes with increasing signal duration, and (3) band-pass neurons which responded maximally to a specific duration and showed smaller responses to shorter and longer durations. One study (Chen 1998) reported no short-pass neurons but band-reject duration-tuned neurons which responded at short and long durations but not at intermediate durations. Short-pass or band-pass response patterns may be explained by a combination of excitatory and inhibitory mechanisms (e.g., see Casseday et al. 1994, Ehrlich et al. 1997, He et al. 1997, Casseday et al. 2000). Long-pass response characteristics represent some type of temporal summation that is indicative of the clock-counter mechanism proposed by Creelman (1962).

Since data on duration tuning of neurons in the auditory midbrain of the mouse are available, we can directly compare the psychophysical data in the mouse with the neuronal responses. Brand et al. (2000) found that 55% of 107 neurons in the mouse tested with signal durations of up to 100 ms showed some type of duration tuning when tested with a standard stimulus. Of these tuned neurons, 70% showed a long-pass characteristic, 22% a band-pass characteristic and 8% a short-pass characteristic. When tested with various types of stimuli, 11 of 19 long-pass neurons retained their tuning, however, in many neurons the cut-off duration was changed. Here we want to focus on the most common type, i.e., the long-pass neurons that can be used to implement a clock-counter mechanism, since they generate impulses that can be summed up during the ongoing stimulus. We were provided with the raw data obtained in the study of Brand et al. (2000) for further analysis. To evaluate the accuracy of the duration tuning of long-pass neurons, we computed their normalized response function from the number of spikes measured over a 120-ms time window starting with the stimulus onset (the maximum response rate was set to 100%). We then determined the slopes of the steep part of the response function by calculating a linear regression incorporating the points starting at or below the maximum response and down to two points below the 50% response level. The maximum value was only

included in the linear regression if the first two points below the maximum already had a value of less than 50%. The average slope of the response function of all long-pass neurons obtained was a relative change in spike number by 84±37% (mean±SD) per change in Weber fraction by a value of 1. If we limit our analysis to the neurons in which the sloping part of the response function included the stimulus duration of 50 ms and in which the linear regression provided a good representation of the raw data ($r>0.5$), we observe a relative change in spike number by 90% (±29% SD) per change in Weber fraction by a value of 1. At a stimulus duration of 50 ms, the average relative response strength of these neurons is 49±12% (mean±SD). In the psychophysical experiment, the mice detected an increase in duration from 50 to 81.5 ms with a d' of 1 if the amplitude was kept constant. If the same increase in duration is presented to the neurons, their relative response strength grows by 2.5 standard deviations (i.e., corresponding to a d' of 2.5). Thus, if the auditory system could selectively utilize the neurons' relative response, a better discrimination threshold would be expected than we found in the behavioral study.

It was suggested by Parker and Newsome (1998) that the psychophysical performance should optimally reflect the performance of the most sensitive neurons. However, if the neural system underlying the perception cannot selectively read out the information from the most sensitive neurons and therefore reflects the average response of the neuron population including the less sensitive cells, it can be expected that the psychophysical performance is ‘‘suboptimal’’ (i.e., worse than would be predicted from the sample of the most sensitive neurons). In our estimate of the neurons' discrimination performance, we only looked at a sub-sample of responses that would be optimally suited to detect a change in duration (i.e., had a steep response function with little random variation). Therefore, the discrepancy between the neurons' sensitivity in representing a change in duration and the animals' sensitivity in the behavior may suggest that the mice use a much less than optimal mechanism to integrate the information represented in the neuronal response. Alternatively, the decision process underlying the perceptual discrimination may be located in neuron populations at other levels of the auditory system that have a lower sensitivity to a change in duration than the sample of neurons that was analyzed in the present study.

2.6 Acknowledgements

We thank Antje Brand and Benedikt Grothe for providing us with the raw data of the response functions of neurons in the mouse inferior colliculus. We thank Günter Ehret for providing the mice. This study was funded by a grant from the DFG within the SFB 517. The care and treatment of the animals was approved by the Bezirksregierung Weser-Ems (permit No. 509.8-42502-35/7) and followed the NIH guide for the care and use of laboratory animals.

2.7 References

Abel SM (1972) Duration discrimination of noise and tone bursts. J Acoust Soc Am 51:1219–1223

Brand A, Urban A, Grothe B (2000) Duration tuning in the mouse auditory midbrain. J Neurophysiol 84:1790–1799

Buus S, Florentine M, Poulsen T (1997) Temporal integration of loudness, loudness discrimination and the form of the loudness function. J Acoust Soc Am 101:669–680

Casseday JH, Ehrlich D, Covey E (1994) Neural tuning for sound duration: role of inhibitory mechanisms in the inferior colliculus. Science 264:847–850

Casseday JH, Ehrlich D, Covey E (2000) Neural measurement of sound duration: control by excitatory-inhibitory interactions in the inferior colliculus. J Neurophysiol 84:1475–1487

Chen G (1998) Effects of stimulus duration on responses of neurons in the chinchilla inferior colliculus. Hear Res 122:142–150

Church RM, Getty DJ, Lerner ND (1976) Duration discrimination by rats. J Exp Psychol Anim Behav Process 2:303–312

Creelman CD (1962) Human discrimination of auditory duration. J Acoust Soc Am 34:582–593

Dooling RJ, Haskell RJ (1978) Auditory duration discrimination in the parakeet. J Acoust Soc Am 63:1640–1642

Dooling RJ, Brown SD, Park TJ, Okanoya K, Soli SD (1987) Perceptual organization of acoustic stimuli by budgerigars (*Melopsittacus undulatus*). I. Pure tones. J Comp Psychol 101:139–149

Ehret G (1975a) Schallsignale der Hausmaus (*Mus musculus*). Behavior 52:38–56

Ehret G (1975b) Frequency and intensity difference limens and nonlinearities in the ear of the housemouse (*Mus musculus*). J Comp Physiol 102:321–336

Ehret G (1975c) Masked auditory thresholds, critical ratios and scales of the basilar membrane of the housemouse (*Mus musculus*). J Comp Physiol 103:329–341

Ehret G (1976) Temporal auditory summation for pure tones and white noise in the house mouse (*Mus musculus*). J Acoust Soc Am 59:1421–1427

Ehrlich D, Casseday JH, Covey E (1997) Neural tuning to sound duration in the inferior colliculus of the big brown bat, *Eptesicus fuscus*. J Neurophysiol 77:2360–2372

Fay RR (1988) Hearing in vertebrates: a psychophysics data book. Hill-Fay Assoc, Winnetka

Galazyuk AV, Feng AS (1997) Encoding of sound duration by neurons in the auditory cortex of the little brown bat, *Myotis lucifugus*. J Comp Physiol A 180:301–311

Geissler DB, Ehret G (2002) Time-critical integration of formants for perception of communication calls in mice. Proc Natl Acad Sci U S A 99:9021–9025

He J, Hashikawa T, Ojima H, Kinouchi Y (1997) Temporal integration and duration tuning in the dorsal zone of cat auditory cortex. J Neurosci 17:2615–2625

Henry FM (1948) Discrimination of the duration of a sound. J Exp Psychol 38:734–743

Killeen PR, Weiss NA (1987) Optimal timing and the Weber function. Psychol Rev 94:455–468

Klink KB, Bendig G, Klump GM (2006) Operant methods for mouse psychoacoustics. Behav Res Methods 38:1-7

Maier EH, Klump GM (1990) Auditory duration discrimination in the European starling (*Sturnus vulgaris*). J Acoust Soc Am 88:616–621

McFadden D (1975) Duration-intensity reciprocity for equal loudness. J Acoust Soc Am 57:702–704

Parker AJ, Newsome WT(1998) Sense and the single neuron: probing the physiology of perception. Annu Rev Neurosci 21:227–277

Poulsen T (1981) Loudness of tone pulses in a free field. J Acoust Soc Am 69:1786–1790

Sinnott JM, Owren MJ, Petersen MR (1987) Auditory duration discrimination in Old World monkeys (Macaca, Cercopithecus) and humans. J Acoust Soc Am 82:465–470

Yunker MP, Herman LM (1974) Discrimination of auditory temporal differences by the bottlenose dolphin and by the human. J Acoust Soc Am 56:1870–1875

Chapter 3

Influence of strychnine on the duration discrimination performance and the acoustic startle response in the mouse (*Mus musculus*)

3.1 Abstract

The role of glycinergic inhibition on the behavioral performance of mice in a duration discrimination experiment was tested using pharmacological manipulation with the glycine antagonist strychnine (experiment 1). Detection thresholds for an increment in duration of a 10-kHz pure tone were obtained for reference durations of 50, 100 and 200 ms in control and strychnine (oral, 1mg/kg) sessions. The average Weber fraction $\Delta T/T$ (criterion of d'=1.8) in control sessions ranged from 0.81 to 0.92, while the mean Weber fraction in strychnine sessions ranged from 0.86 to 1.01. No significant influence of strychnine on the duration discrimination performance could be found. The effectiveness of oral application of strychnine in mice was tested using the acoustic startle response (experiment 2). Neither the amplitude of the startle response nor its corresponding d' value differed significantly between strychnine sessions (1 mg/kg, equal treatment as in experiment 1) and control sessions. No clear effect of strychnine treatment could be found. These findings are in contrast with the results of startle experiments with both rats treated with strychnine and mouse mutants with a defective glycinergic system. The effectiveness of an oral application of strychnine is discussed.

3.1.1 Keywords

Animal psychoacoustics, NMRI mouse, glycinergic inhibition, strychnine, startle

3.2 Introduction

Signal duration is an important property of sound, and the processing of signal duration has already been studied in both behavioral (e.g., Church et al. 1976; Dooling and Haskell 1978; Kelly et al. 2006, Klink and Klump 2004, Maier and Klump 1990; Sinnott et al. 1987; Yunker and Herman 1974) and physiological studies (e.g., Brand et al. 2000; Casseday et al. 2000; Chen 1998; Ehrlich et al. 1997; Galazyuk and Feng 1997; He et al. 1997, Kadner et al. 2006, Perez-Gonzáles et al. 2006).

So far, two general mechanisms underlying the processing of signal duration have been proposed. Casseday et al. (2000) have postulated that duration-specific responses might result from an adequately timed interaction of excitation and inhibition. They tested the duration tuning of short-pass and band-pass neurons in inferior colliculus (IC) of the big brown bat and showed that this tuning was often changed or abolished after the application of bicuculline or strychnine, antagonists of the inhibitory neurotransmitters GABA and glycine, respectively. On the basis of these data Casseday et al. suggested a model for duration tuning which consists of an onset inhibition, an offset excitation, and a delayed onset excitation. According to this model, band-pass tuning in neurons is created when the onset inhibition sets in before the excitatory input reaches the neuron. If the inhibitory input arrived later, it would be unable to counteract the influence of any excitation at short durations, and the neuron would show short-pass characteristics (Casseday et al. 2000). This model may also be able to explain the response patterns seen in short-pass and band-pass neurons in the mouse IC that show a reduced response if the duration is increased beyond a certain limit (Brand et al. 2000). Furthermore, Casseday et al. (2000) showed that while the application of either bicuculline or strychnine eliminated duration tuning in many of the tested neurons, the change in duration tuning was not always the same for the two antagonists, leading to the idea that these two inhibitory neurotransmitters may play different roles in creating duration tuning.

A second mechanism to explain duration tuning seems to be based on the integration of excitation. Creelman (1962) suggested in his psychophysical study on duration discrimination that the processing of signal duration can be seen as an mechanism that integrates action potentials over time similarly to a clock-counter mechanism. As seen in physiological studies, this type of processing might be represented by long-

pass neurons which respond only to stimuli exceeding a specific duration, and simply require a minimum integration time (Casseday et al. 2000). The performance of mice determined in a behavioral duration discrimination experiment (chapter 2, Klink and Klump 2004) is compatible with the clock-counter model and points to a solely excitation-based process. However, the experiment of Klink and Klump is not suited to rule out the possibility of inhibitory influences that may also play a role in the discrimination of duration.

A possible way to discriminate between the models based on excitation and inhibition on the one hand, and activity-integration on the other hand, might be the manipulation of the level of inhibition in behaving animals. As it has been shown in the physiological study by Casseday et al. (2000), both GABA and glycine seem to be involved in the duration tuning of IC neurons. According to Casseday et al., glycine could be the main source of onset inhibition for neurons with very short best durations. Therefore, if an interaction between excitation and glycinergic inhibition contributes to the duration tuning, a change of the level of the glycinergic inhibition should affect the processing of duration.

Glycine is the main inhibitory neurotransmitter in the spinal cord and the brain stem. It binds to postsynaptic receptors and causes the opening of anion channels leading to chloride uptake and hyperpolarization of the postsynaptic membrane (see Becker 1995 for a review on glycine receptors). The postsynaptic glycine receptors are typically composed of a pentamer of ligand binding α1-subunits and structural β-subunits (e.g., Langosch et al. 1988; for stoichiometry see also Grudzinska et al. 2005) and are reversibly associated with the peripheral membrane protein gephyrin which binds to polymerized tubulin and therefore the cytoskeleton of the cell (e.g., Kirsch and Betz 1995; see also Kirsch 2006 for a review on glycinergic transmission). Glycinergic inhibition can be impaired by the application of the glycine antagonist strychnine. Strychnine is an alkaloid and binds with high affinity to the α1-subunits of postsynaptic glycine receptors, thus reducing the glycine-mediated inhibition (e.g., Kirsch 2006). The binding of strychnine is competitive to that of glycine and involves overlapping but distinct binding sites on the glycine receptor (Vandenberg et al. 1992a, b). According to the data of Casseday et al. (2000), application of strychnine should affect the neurons' duration tuning in the mouse and should lead to an impaired performance in psychoacoustical duration discrimination experiments.

Applying the glycine antagonist strychnine should not affect the discrimination process if an activity-integrating mechanism is involved. To test this hypothesis, the duration discrimination performance of mice with and without manipulation with strychnine was measured in experiment 1.

Since it is obvious that direct application of strychnine to IC neurons can change the neurons' duration tuning, systemic application of strychnine should have a similar effect. The effectiveness of oral application of strychnine on the performance of animals in psychoacoustic experiments can be evaluated using the acoustic startle response (ASR), a coordinated muscle contraction of the eyelid, the neck and the extremities upon presentation of sudden loud acoustic stimuli (e.g., Plappert et al. 2001). The acoustic startle response is thought to be mediated by a neuronal pathway from different nuclei of the auditory pathway (e.g., the dorsal and ventral cochlear nucleus (CN), the lateral superior olive, and the cochlear root nucleus) via the caudal pontine reticular nucleus (PnC) to cranial and spinal motor neurons (e.g., Plappert and Pilz 2001, Walker and Davis 2002). Projections from the amygdala to the PnC can be involved in the sensitisation of the ASR (Plappert and Pilz 2001). Subletal strychnine poisoning leads to disinhibition of the auditory and motor centers involved in the ASR and to an increase of the ASR amplitude in rats (e.g., Bakshi et al. 1995, Kehne et al. 1981, Koch and Friauf 1995). In order to evaluate whether the oral application of 1 mg/kg strychnine in mice in experiment 1 had the desired effect, mice treated pharmacologically in the same way as the mice in experiment 1 were tested using the ASR (experiment 2), a paradigm in which the effects of strychnine have already been well established in rats.

3.3 Experiment 1: Influence of strychnine on the duration discrimination performance

3.3.1 Materials and methods

3.3.1.1 Subjects

Seven adult mice (*Mus musculus*) of the NMRI strain (3 females, 4 males) obtained from Günter Ehret, Ulm, Germany, participated in the duration discrimination

experiment. Six of the subjects had already participated in previous duration discrimination experiments (Klink and Klump 2004). Their age ranged between 8 and 16 months (5 subjects) and between 19 and 26 months (2 subjects) during the total period of testing. The animals were kept at an average weight of 32.6 g (SD $\pm$ 3.4 g) and had unlimited access to water. They were rewarded during the experiment with 20 mg pellets (Bioserve: Dustlesss Precision Pellets, Formula FO163), additional rodent pellets (Altromin 1314) and sunflower seeds were given to keep the subjects' weight about constant. The subjects were housed in individual cages (Eurostandard Type III H, 43 x 27 x 19 cm; Tecniplast) with a hiding possibility ("mouse house", Tecniplast) and a layer of wood shavings as bedding material (Raiffeisen). The cages were stored in a ventilated cage rack (Tecniplast Slim Line Sealsafe). Animals were moved to and from the experimenatal cage using a transport cage.

3.3.1.2 Pharmacological manipulation with strychnine

All subjects were water-deprived for at least 14 h before the experiments and did not show any striking changes in behavior due to the deprivation. Before the treatment all animals were weighted. Approximately 30 min before the start of each session the subjects were either given normal drinking water (control session) or a strychnine solution (the dose equalled 1 mg/kg; strychnine session) to drink. Pretests had shown that most subjects readily drank the water and the strychnine solution within 30 min. Subjects which did not seem to like the bitter taste of the strychnine solution were given several drops of a sugar solution mixed into the strychnine solution. After all of the strychnine solution (or an *ad libitum* amount of water) was drunk, subjects were placed into the experimental cage and the experiment was started. Each subject participated in both control and strychnine sessions.

3.3.1.3 Apparatus and stimulus generation

The setup for the experiment and the stimulus generation is the same as described in chapter 2 (Klink and Klump 2004). Briefly, the experiments were conducted in two sound attenuating chambers. The mice were tested in a doughnut shaped experimental cage which contained a feeder dish to distribute the reward pellets, a

nearby feeder light and a pedestal with a light interrupting switch. The rewards were dispensed by a custom-made feeder mounted on the chamber wall and connected with a flexible tube to the feeder dish. The stimuli were presented using a Canton Plus XS loudspeaker mounted at least 30 cm above the pedestal.

The pure tone stimuli were generated with a Linux workstation using a standard sound card, passed through a programmable attenuator and the base sound pressure level of the signal was adjusted by an additional manual attenuator. The signals were amplified by a Rotel RMB-1066 amplifier (in both chambers) and presented via the Canton XS loudspeaker. Sound pressure levels in the experimental setup were calibrated once a day with a sound level meter.

The stimuli consisted of 10- kHz pure tones of varying durations (see Klink and Klump 2004 for more details). The reference stimuli had equivalent durations of either 50, 100 or 200 ms, and a cosine rise/fall of 10 ms duration. They were repeated with a rate of one stimulus per 1.3 s. The test stimuli had an increased duration which differed from the corresponding reference duration by a Weber fraction of between 0.2 and 2 (for all reference durations). The median level of presentation was 40 dB above the individual's auditory threshold for an 800-ms 10-kHz tone that was determined preceding the duration discrimination experiments in each subject.

3.3.1.4 Procedure and data analysis

The experimental paradigm was a Go/NoGo-procedure reinforcing the subject with food rewards and has already been described in chapter 2 (for more details see also Klink et al. 2006). Briefly, a session started with the repeated presentation of the reference stimuli and the subject was required to jump onto the pedestal to start a trial. After a random waiting interval of between 1 and 5 s, a single test stimulus was played and then the presentation of the reference stimuli continued at the regular interval of one stimulus per 1.3 s. The mouse was trained to jump off the pedestal when perceiving a test stimulus. If the subject responded correctly to a test stimulus (i.e. scored a ‘‘hit’’), a food reward was given and the next trial started. If the subject missed a test stimulus and remained on the pedestal, the pedestal light was switched off for 1 s before the next trial could be initiated. 30% of all trials consisted of sham

trials in which a reference stimulus was played instead of a test stimulus. These trials were used to measure the false alarm rate.

Duration discrimination thresholds were obtained using the method of constant stimuli. A block of 10 trials, consisting of three sham trials and a set of seven test trials differing in the duration of the test stimulus, was repeated six times. The trials within each block were presented in random order; the first 10 trials of each session were used as a "warm-up" period (only test stimuli with the longest test duration were presented) and were discarded from the analysis.

At the end of each session, a psychometric function was compiled summarizing the results of the last 50 trials. Sessions were excluded from the analysis if the percentage of false alarms was greater than 20 % and/or if the average hit rate in the trials with the two most salient test stimuli (i.e. those with the largest deviation from the reference duration) was less than 80 %. Duration discrimination thresholds of single sessions were calculated using signal-detection theory and a threshold criterion of d' = 1.8. For each subject, data from five valid sessions were combined into a single psychometric function to summarize performance from 250 trials. Sessions were only combined, if the threshold from single sessions with 50 data trials each did not vary more than by a factor of two. Using a threshold criterion of d’ = 1.8, mean duration discrimination thresholds were calculated from the combined psychometric function.

3.3.2 Results

Thresholds for the detection of increments in tone duration for control and strychnine sessions were obtained in up to seven subjects. One of the subjects participated only in the experiments using the 50 ms standard duration because it stopped to respond reliably to the stimulus afterwards, and two others died before completing the experiments with the 200 ms standard duration. Mean duration discrimination thresholds expressed as the Weber fraction ($\Delta T/T$) are shown in Table 3.1. For a reference duration of 50 ms, the Weber fraction at threshold ranged from 0.59 to 1.28 for the control sessions, and from 0.69 to 1.36 for the strychnine sessions. The Weber fraction for a reference duration of 100 ms ranged from 0.54 to 1.18 (control

Table 3.1: Mean thresholds (Weber fraction) and standard deviation for detecting an increment in stimulus duration for 4 to 7 subjects in control and strychnine sessions. Threshold criterion was a d' of 1.8. n = number of subjects tested.

Reference duration	Control sessions	Strychnine sessions
50 ms (n=7)	0.92 ± 0.28	0.97 ± 0.27
100 ms (n=6)	0.89 ± 0.24	1.01 ± 0.19
200 ms (n=4)	0.62 ± 0.06	0.61 ± 0.16

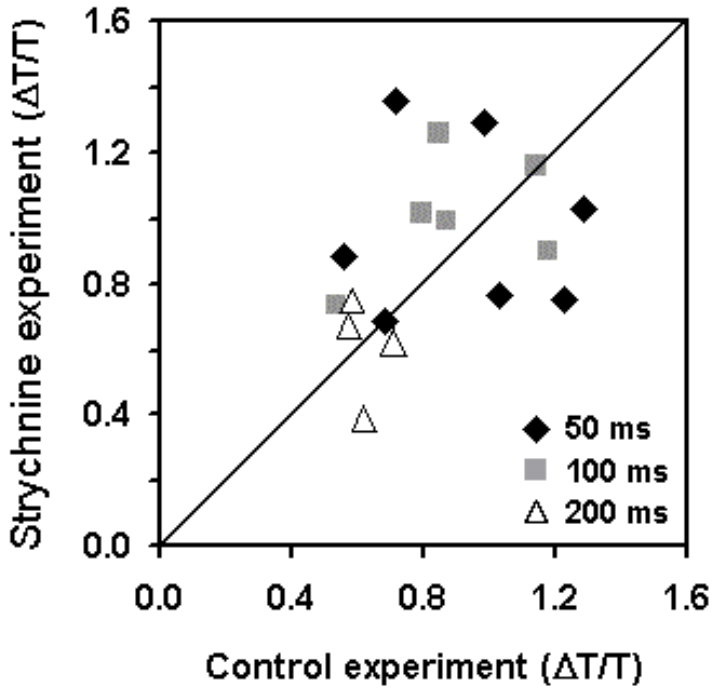

Figure 3.1: Duration discrimination thresholds (Weber fraction) of 4 to 7 subjects when given strychnine (ordinate) compared to thresholds obtained in the control condition (abscissa). Individuals were tested with multiple reference durations. The line bisecting the graph depicts equal performance in both experiments. Points below the line indicate better performance in the strychnine experiment, points above the line indicate better performance in the control experiment.

sessions) and from 0.74 to 1.26 (strychnine sessions). For a reference duration of 200 ms, a Weber fraction between 0.57 and 0.71 (control), and 0.39 and 0.75 (strychnine sessions) could be obtained. The mean performance of all subjects in both control and strychnine sessions are plotted in Fig. 3.1. Each symbol depicts the performance of a single subject in the control experiment (abscissa) versus its performance in the strychnine experiment (ordinate).

A Two-Way-Repeated-Measures ANOVA with the discrimination threshold (expressed as Weber fraction ΔT/T) as the dependent variable and reference duration and pharmacological manipulation as factors revealed that in the four

subjects tested with all conditions, reference duration (p=0.036) had a significant effect on the performance of the subjects while pharmacological manipulation did not (p=0.476). Pairwise comparisons between the discrimination threshold for different reference durations (Tukey test) showed that the increment-detection threshold for the 200 ms reference duration was significantly lower than for the 100 ms reference duration (p=0.040), while threshold for the 100- and 50-ms reference duration did not differ significantly from each other (p=0.855). There was a trend for the threshold in the 200 ms reference duration to be lower than for the 50 ms reference duration (p=0.077).

3.3.3 Discussion

The oral administration of the glycine antagonist strychnine did not significantly affect the duration discrimination performance as would be expected from a clock-counter model depending only on activity-integration (e.g., Creelman 1962). This result is compatible with the results from the duration discrimination experiment by Klink and Klump (2004). The failure to detect an effect of pharmacological manipulation with strychnine indicates that either glycinergic inhibition is unlikely to play a direkt role in the mechanism underlying duration discrimination in the mouse in the tested stimulus range or that the oral application of strychnine may not be suitable to elicit changes in the duration discrimination performance (for a test of this hypothesis see experiment 2). According to Casseday et al. (2000), glycinergic inhibition may be most effective in the early part of the response and could be the main source of onset inhibition for neurons with very short best durations. It cannot be ruled out that in the mouse neurons showing strychnine-induced changes in duration tuning are not able to give rise to any behavioral changes. Furthermore, the failure to find any influence of pharmacological manipulation with strychnine does not rule out the possibility of GABAergic influences on the duration discrimination mechanism (see Casseday et al. 2000), and the duration discrimination performance of mice treated with GABA antagonists (e.g bicuculline or gabazine) still needs to be investigated. According to Casseday et al., GABAergic inhibition may dominate over the sustained period of the response to different durations.

3.4 Experiment 2: Influence of strychnine on the amplitude of the acoustic startle reflex

In experiment 1, no changes in performance due to strychnine administration could be seen. While this indicates the absence of any treatment effect, it can not be ruled out that oral administration of strychnine may be unsuitable to influence the level of glycinergic inhibition in a behavioral experiment. Intraperitonally injected doses of strychnine ranging from 0.5 to 2 mg/kg are known to cause an increase in the amplitude of the acoustic startle reflex (ASR) in rats (e.g., Davis 1988, Harty and Davis 1985, Kehne et al. 1981). Thus, if the oral application of strychnine is effective in manipulating the level of glycinergic inhibition in mice, the ASR threshold should be reduced compared to control experiments without strychnine manipulation.

3.4.1 Materials and methods

3.4.1.1 Subjects

Ten adult mice of the NMRI strain (5 females, 5 males; obtained from Charles River Laboratories, Sulzfeld, Germany) participated in the startle experiments. Their age during the experiments ranged from 2 to 3 months (6 subjects) and from 12 to 14 months (4 subjects), respectively. All had unrestricted access to food (Altromin 1314 rodent pellets) and water and were given sunflower seeds during the experiment in order to keep them occupied. All subjects were housed in individual cages (Eurostandard Type III H, 43x27x19 cm; Tecniplast) with a hiding possibility (“mouse house”, Tecniplast) and a layer of wood shavings as bedding material (Raiffeisen). The cages were stored in a ventilated cage rack (Tecniplast Slim Line Sealsafe).

3.4.1.2 Pharmacological manipulation with strychnine

The pharmacological manipulation was the same as in experiment 1. However, since all subjects readily drank the strychnine solution, no addition of sugar solution was needed.

3.4.1.3 Apparatus

All tests were conducted in a sound attenuating chamber from Industrial Acoustics (IAC type 403) lined with two to three layers of sound absorbing wedges (for details on the chamber see Klink and Klump 2004, chapter 2). The mice were tested in a custom-made experimental cage (12x6.5x6.3 cm) made from stainless steel wire which was mounted on 4 springs on a metal plattform (15x14x2 cm) covered by a layer of sound absorbing material (Illbruck Plano). The metal platform was positioned on a panel lying on the sound-absorbing material covering the floor of the chamber. Positioned 10 cm from the cage was a Canton Plus XS loudspeaker. The startle amplitude was measured using an accelerometer (Bruel & Kjaer Model 4507) attached to the roof of the cage. During the whole experiment, the output of the acceleromenter was amplified by a measuring amplifier (Bruel & Kjaer Type 2525) and the resulting voltage was recorded by a custom-made program on a Linux workstation. In order to be able to discriminate between the voltage output before and after the onset of the startle stimulus, the startle stimulus was recorded in a second channel.

3.4.1.4 Stimulus generation

The generation of the pure tone stimuli was the same as in experiment 1. The stimulus design was adapted after Plappert et al. (2001). The stimuli consisted of 10-kHz pure tones with a 20 ms duration (incl. 4 ms rise-decay time). They were presented at an interstimulus interval of 15 s and had sound pressure levels ranging from 55 dB SPL to 100 dB SPL in steps of 5 dB SPL (i.e. the SPL of the startle stimuli was 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 dB, respectively). All stimuli were superimposed on 40 dB root mean square (RMS) broadband background noise generated using a real-time processor (RP2.1, Tucker-Davis Technologies). The noise level was adjusted using a programmable attenuator (PA4, Tucker-Davis Technologies).

3.4.1.5 Procedure

After consumption of water or strychnine, the subjects were transported into the startle cage and allowed to adapt for 5 min to the background noise. After the 5 minutes, 20 startle stimuli at highest SPL were played (warmup period) to allow the subjects to habituate to the sound. Following the warmup period, startle stimuli were presented in blocks of 10 trials using the method of constant stimuli. Each block consisted of all ten startle stimuli which ranged from 55 dB SPL to 100 dB SPL in steps of 5 dB and was repeated ten times. The stimuli within each block were presented in random order. Startle stimuli from the warmup period were not included in the analysis. Each subject participated in at least 6 sessions in each condition (strychnine / control).
For each stimulus condition, the startle amplitude was calculated as the difference between the peak-to-peak voltage during the 50 ms after stimulus onset and the peak-to-peak voltage during the 50 ms before stimulus onset.

3.4.1.6 Data analysis

At the end of each session, a psychometric function was compiled using the results from the last 100 trials. First, the mean startle amplitude and its standard deviation (in Volt) for each SPL of the startle stimulus was computed. Then, using signal-detection theory, the d’ value for each SPL was calculated using the following formula for unequal variance sensitivity (Harvey 2003):

$$d' = \frac{\mu_n - \mu_v}{\sqrt{\frac{(\sigma_n^2 + \sigma_v^2)}{2}}}$$

μ_n mean peak-to-peak amplitude after stimulus onset
μ_v mean peak-to-peak amplitude before stimulus onset
σ_n SD of the peak-to-peak amplitude after stimulus onset
σ_v SD of the peak-to-peak amplitude before stimulus onset

Startle thresholds for each session were calculated using linear interpolation for a threshold criterion of d' = 1.0. For each subject and condition, startle thresholds from up to 10 sessions were averaged.

3.4.2 Results

Startle amplitudes and startle thresholds were obtained for ten subjects. An example of an individual startle session is given in Fig. 3.2. On average, the startle

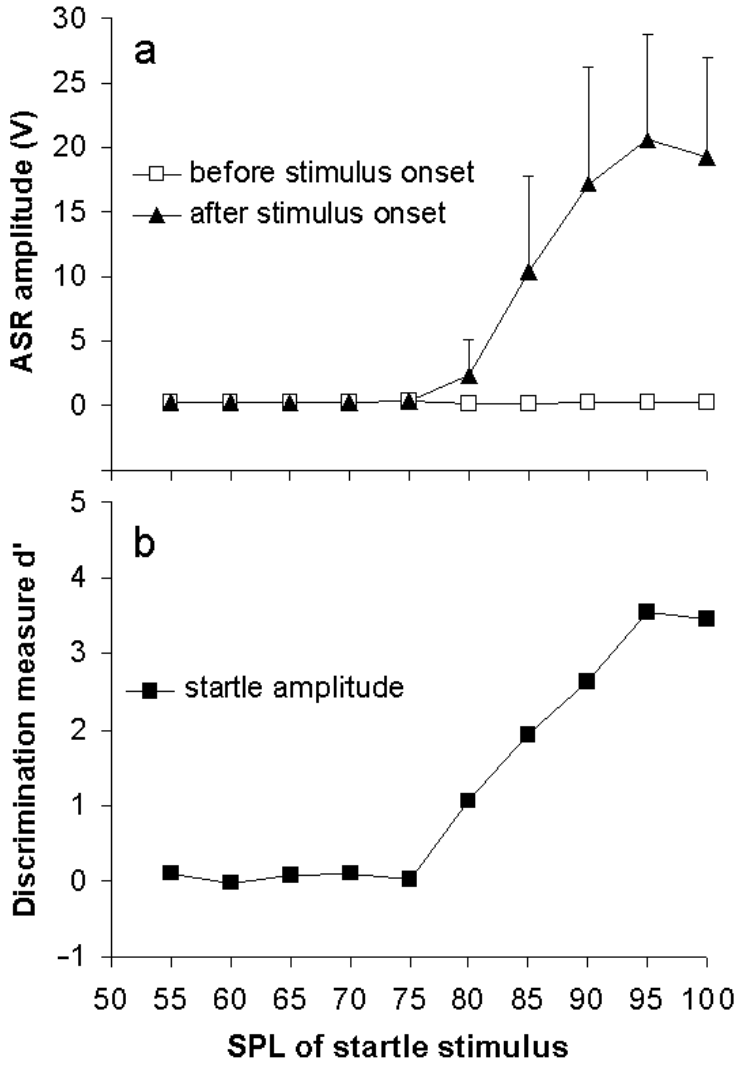

Figure 3.2: Psychometric function of a single session showing the ASR amplitude and the discrimination measure d' in relation to the SPL of the startle stimulus. (a) Mean peak-to-peak amplitudes and standard deviation (in Volt) during the 50 ms before stimulus onset (open squares) and during the 50 ms after stimulus onset (filled triangles). (b) Corresponding discrimination measure d' of the startle amplitude calculated from the difference between the mean peak-to-peak amplitudes during the 50 ms after stimulus onset and the mean peak-to-peak amplitudes during 50 ms before stimulus onset using the above formula (filled squares). The startle threshold in this session was 79.7 db SPL.

amplitude increased with increasing SPL of the startle stimulus in both control and strychnine sessions (see Fig. 3.3). A Two-Way-Repeated-Measures ANOVA with the startle amplitude (in Volt) as the dependent variable and SPL of the startle stimulus and pharmacological manipulation as factors revealed that in the four subjects tested with all conditions, the SPL of the startle stimulus ($p<0.001$) had a significant effect on the performance of the subjects while pharmacological manipulation did not ($p=0.072$). Furthermore, there was a significant interaction between the SPL of the

startle stimulus and the pharmacological manipulation (p=0.015). A Two-Way-Repeated-Measures ANOVA with the d' value as the dependent variable (and the same factors) showed similar results, namely a significant effect of the SPL of the startle stimulus (p<0.01) on the performance of the subjects but no effect of pharmacological manipulation (p=0.146) on the d' values.

Mean startle thresholds for a criterion of d'=1 ranged from 66.9 to 78.7 dB SPL in control sessions, while mean thresholds in strychnine sessions ranged from 67.9 to 81.0 dB SPL. On average, the startle threshold in the control sessions was 74.3 dB SPL while the threshold in the strychnine sessions was 75.9 dB SPL (see also Tab. 3.2). A One-Way-Repeated-Measures ANOVA with the startle threshold (in dB SPL) as the dependent variable and pharmacological treatment as factor revealed a significant effect of treatment (p=0.027) on the performance of the subjects. Thus, the ASR threshold of mice in strychnine sessions was significantly higher than the ASR threshold in control sessions when the same threshold criterion was applied. The threshold difference of 1.6 dB, however, was quite small and the effect of strychnine was not very pronounced and should be treated with caution.

Table 3.2: Mean startle thresholds (in dB SPL) and standard deviation for 10 subjects in control and strychnine sessions. Threshold criterion was a d' of 1.0.

Subject	Control session	Strychnine session
cr-m4d	70.6	72.7
cr-m4e	69.9	69.0
cr-w4a	66.9	67.9
cr-w4e	78.7	78.2
cr-m5a	77.3	76.5
cr-m5b	74.7	79.1
cr-m5c	71.0	74.8
cr-w5a	77.4	81.0
cr-w5b	78.2	79.4
cr-w5c	77.9	80.3
mean	74.3	75.9
S.D.	4.3	4.7

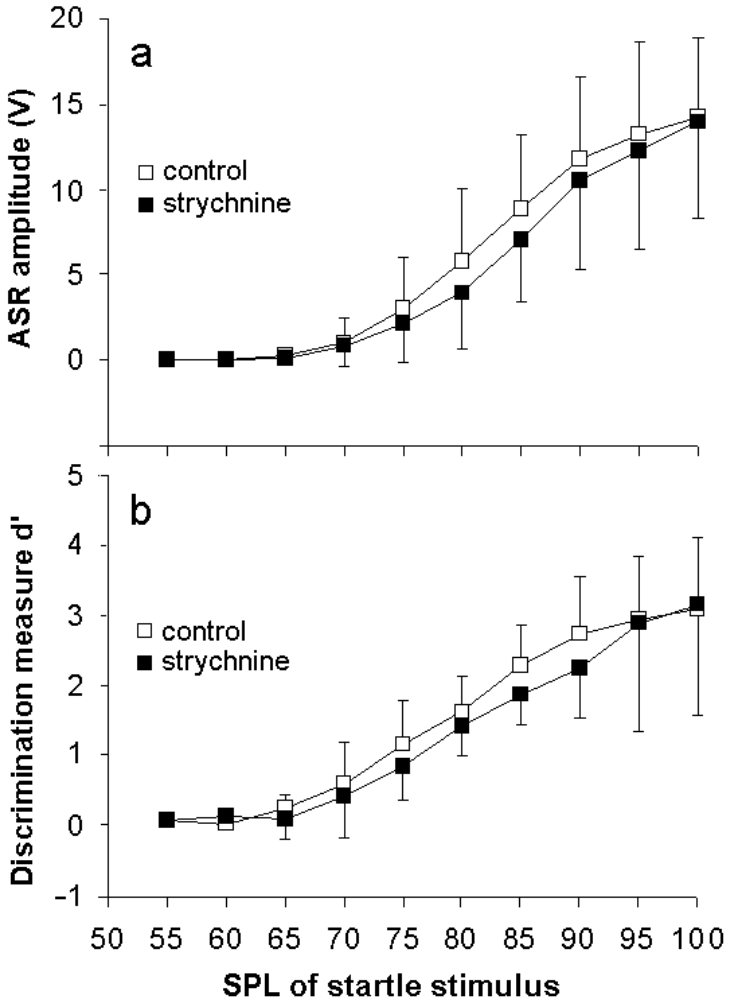

Figure 3.3: Average ASR amplitude and the discrimination measure d' of 10 subjects in control sessions (open squares) and strychnine sessions (filled squares) in relation to the SPL of the startle stimulus. (a) Mean peak-to-peak amplitudes and standard deviation (in Volt) for all startle stimuli tested. (b) Corresponding mean discrimination measure d' (and standard deviation) of the startle amplitudes depicted in (a).

3.4.3 Discussion

The failure to find a reduction of ASR amplitude (or startle threshold) in strychnine sessions in experiment 2 is quite puzzling and indicates that oral application of strychnine might be unsuitable to test whether glycine-mediated inhibition is indeed involved in the processing of the stimuli.

The failure to find an increase of startle amplitude due to application of strychnine in the present study is contradictory to the results of ASR studies in rats treated with strychnine or its derivates (strychnine sulfate, strychnine hypochloride). Both systemic (i.e. intraperitonal) injections (e.g., Kehne et al. 1981, Koch and Friauf 1995, Harty and Davis 1985) and microinjections into the spinal cord or the cisterna magna (Kehne et al. 1981) lead to an increase of the ASR amplitude in rats while microinjections into the lateral ventricle produced a reduction of the ASR amplitude

(Kehne et al. 1981), and injections into the PnC did not change the ASR amplitude (Koch and Friauf 1995). The strychnine-potentiated increase of startle amplitude could not only be seen in acoustically-elicited startle but also in startle elicited though electrical stimulation of the ventral cochlear nucleus (Harty and Davis 1985).

Furthermore, an increase of startle amplitude was also measured in two mouse mutants showing deficits in their glycinergic system. The mouse mutant *spasmodic* carries a point mutation of the glycine receptor α1-subunit gene resulting in lowered receptor affinity to glycine and a reduced number of receptors. The mouse mutant *spastic* carries a mutation in the receptor β–subunit leading to a largely reduced number of receptors with unchanged substrate affinity (e.g. Becker et al. 2000; for a detailed study on glycinergic transmission in these mutants see Graham et al. 2006). Both mutants show excessive startle response resembling strychnine intoxication (e.g., Becker 1990, Plappert et al. 2001), and this indicates that intact glycine receptors are indeed involved in the transmission of the ASR.

The presence of a (small) increase of startle threshold in strychnine sessions measured in the present study is contradictory to the results of ASR studies in the mouse mutant *spasmodic* which showed a significantly lower startle threshold in wildtype mice with an intact glycinergic system (Plappert et al. 2001).

The failure to see a startle-potentiating effect of strychnine in the present ASR experiment is quite puzzling. It is unlikely that the strychnine dose used in the present study – which equaled half of the lethal dose (LD_{50}) – was not sufficient to elicit strychnine-influenced behavior. Rats treated with a strychnine dose less than half of the LD_{50} dose (LD_{50} = 2.5 mg/kg, intraperitoneal) were found to still show increased startle (e.g., Harty and Davis 1985: treatment with 1 mg/kg, Kehne et al. 1981: treatment with 0.5-2 mg/kg).

While it has been shown that intraperitoneal injections of strychnine are able to influence ASR, this has to the knowledge of the author not been tested so far with an oral application. Therefore, while strychnine seems to be quickly absorbed from the gastrointestinal tract and metabolized with a half-life of 10 hours (e.g., Borges et al. 1997), the absorption after oral ingestion can be delayed due to several factors like the contents of the stomach (i.e. quantity and quality of ingested food), or the time and rate in which strychnine has been drunk. Thus, the appearance of symptoms cannot be precisely timed. Furthermore, the effect of strychnine itself may vary with

several factors. It has been found that the resistance of mice and rats to the convulsive effects of strychnine (i.e. the latency of seizure and mortality) can vary with daytime and mouse strain: Torshin and Vlasova (2001) found that in mice receiving single intramuscular injections of 25 mg/kg strychnine (nitrate) the latency of seizure was higher when the injections were given at 9 a.m. (507 $\pm$ 128 s), than at 12 p.m. (210 $\pm$ 22 s) while the mortality rate of the animals treated at 9 a.m. and 12 p.m. was 50% and 100%, respectively. Engstrom and Woodbury (1988) showed that 8 week old mice of the DBA/2J strain had shorter convulsive latencies and lower convulsive CD_{50}s doses than mice of the C57BL/6J mice (CD_{50}s doses: 1.5 mg/kg (DBA/2J) and 2.3 mg/kg (C57BL/6J); latencies for CD_{50}s = 3 mg/kg: 2.6 min (DBA/J) and 5 min (C57BL/6J), respectively).

3.5 Conclusion

The aim of the present study was to investigate whether the duration discrimination performance of mice (e.g., Klink and Klump 2004) can be explained by a purely excitatory mechanism or whether a model based on excitation and inhibition might be better suited to describe the behavioral data. We found that pharmacological manipulation with the glycine antagonist strychnine did not affect the performance of the subjects, indicating an excitation-based mechanism. The performance of mice in the duration discrimination experiment can be explained solely on the basis of a mechanism similar to the clock-counter model proposed by Creelman (1962) and might reflect the activity pattern of long-pass neurons. The failure to find a reduction of ASR amplitude (or startle threshold) in strychnine sessions in experiment 2, however, indicates that oral application of strychnine may be unsuitable to test whether glycine-mediated inhibition is indeed involved in the processing of the stimuli in both experiments. Since intraperitoneal injections of strychnine are known to increase the ASR amplitude in rats, further experiments with intraperitioneally injected strychnine should be perfomed in mice to address this issue.

According to Casseday et al. (2000) both inhibitory neurotransmitters GABA and strychnine may be involved in the duration tuning. They suggest that – at least in the big brown bat - GABAergic inhibition may dominate over the sustained period of the response to sound of different duration, while glycinergic inhibition may be most

effective in the early part of the response. It cannot be ruled out that in the mouse the duration tuning is more dependent on GABAergic influences on the duration discrimination mechanism, and the duration discrimination performance of mice treated with GABA antagonists (e.g bicuculline or gabazine) still needs to be investigated.

3.6 Acknowledgements

This study was supported by a grant from the DFG within the SFB 517 and the Volkswagen-Stiftung (Joint Niedersachsen-Israel program ZN2040). The care and treatment of the animals was approved by the Bezirksregierung Weser-Ems (permit No. 509.8-42502-35/7) and followed the NIH guide for the care and use of laboratory animals.

3.7 References

Bakshi VP, Geyer MA, Taaid N, Swedlow NR (1995) A comparison of the effects of amphetamine, strychnine and caffeine on prepulse inhibition and latent inhibition. Behav. Pharmacology 6:801-809

Becker C (1990) Disorders of the inhibitory glycine receptor: the *spastic* mouse. FASEB J 4:2767-2774

Becker C (1995) Glycine receptors: molecular heterogeneity and implications for disease. Neuroscientist 1:130-141

Becker L, Hartenstein B, Schenkel J, Kuhse J, Betz H, Weiher H (2000) Transient neuromotor phenotype in transgenic spastic mice expressing low levels of glycine receptor b-subunit: an animal model of startle disease. Eur J Neurosci 12: 27-32

Borges A, Abrantes J, Teixeira MT, Parada P (1997) Strychnine. http://www.inchem.org/documents/pims/chemical/pim507.htm

Brand A, Urban A, Grothe B (2000) Duration tuning in the mouse auditory midbrain. J Neurophysiol 84:1790–1799

Casseday JH, Ehrlich D, Covey E (2000) Neural measurement of sound duration: control by excitatory-inhibitory interactions in the inferior colliculus. J Neurophysiol 84:1475–1487

Chen G (1998) Effects of stimulus duration on responses of neurons in the chinchilla inferior colliculus. Hear Res 122:142–150

Church RM, Getty DJ, Lerner ND (1976) Duration discrimination by rats. J Exp Psychol Anim Behav Process 2:303–312

Creelman CD (1962) Human discrimination of auditory duration. J Acoust Soc Am 34:582–593

Davis M (1988) Apomorphine, d-amphetamine, strychnine and yohimbine do not alter prepulse inhibition of the acoustic startle reflex. Psychopharmacology 95: 151-156

Dooling RJ, Haskell RJ (1978) Auditory duration discrimination in the parakeet. J Acoust Soc Am 63:1640–1642

Ehrlich D, Casseday JH, Covey E (1997) Neural tuning to sound duration in the inferior colliculus of the big brown bat, *Eptesicus fuscus*. J Neurophysiol 77:2360-2372

Engstrom FL, Woodbury DM (1988) Seizure susceptibility and DBA and C57 mice: the effects of various convulsants. Epilepsia 29:389-395

Galazyuk AV, Feng AS (1997) Encoding of sound duration by neurons in the auditory cortex of the little brown bat, *Myotis lucifugus*. J Comp Physiol A 180:301–311

Graham BA, Schofield RP, Sah P, Margrie TW, Callister RJ (2006) Distinct physiological mechanisms underlie altered gycinergic synaptic transmission in the murine mutants spastic, spasmodic, and oscillator. J Neurosci 26:4880-4890

Grudzinska J, Schemm R, Haeger S, Nicke A, Schmalzing G, Betz H, Laube B (2005) The beta subunit determines the ligand binding properties of synaptic glycine receptors. Neuron 45:727-739

Harty TP, Davis M (1985) Cocaine: effects on acoustic startle and startle elicited electrically from cochlear nucleus. Psychopharmacology 87:396-399

Harvey LO Jr (2003) Detection sensitivity and response bias. http://brahms.cpmc.columbia.edu/systems/SDT.pdf

He J, Hashikawa T, Ojima H, Kinouchi Y (1997) Temporal integration and duration tuning in the dorsal zone of cat auditory cortex. J Neurosci 17:2615–2625

Kadner A, Kulesza RJ, Berrebi AS (2006) Neurons in the medial nucleus of the trapezoid body and superior paraolivary nucleus of the rat may play a role in sound duration coding. J. Neurophysiol 95:1499-1508

Kehne JH, Gallager DW, Davis M (1981) Strychnine: brainstem and spinal mediation of excitatory effects on acoustic startle. Eur J Pharmacol 76:177-186

Kelly JB, Cooke JE, Gilbride PC, Mitchell C, Zhand H (2006) Behavioral limits of auditory temporal resolution in the rat: amplitude modulation and duration discrimination. J Comp Psychol 120:98-105

Kirsch J (2006) Glycinergic transmission. Cell Tissue Res 326:535-540

Kirsch J, Betz H (1995) The postsynaptic localization of the glycine receptor-associated protein gephyrin is regulated by the cytoskeleton. J Neurosci 15:4148-4156

Klink KB, Bendig G, Klump GM (2006) Operant methods for mouse psychoacoustics. Behav Res Methods 38:1-7

Klink KB, Klump GM (2004) Duration discrimination in the mouse (Mus musculus). J Comp Physiol A 190:1039-1046

Koch M, Friauf E (1995) Glycine receptors in the caudal pontine reticular formation: are they important for the inhibition of the acoustic startle response? Brain Res 671:63-72

Langosch D, Thomas L, Betz H (1988) Conserved quaternary structure of ligand-gated ion channels: the postsynaptic glycine receptor is a pentamer. Proc Natl Acad Sci USA 85:7394-7398

Maier EH, Klump GM (1990) Auditory duration discrimination in the European starling (Sturnus vulgaris). J Acoust Soc Am 88:616–621

Perez-Gonzales D, Malmierca MS, Moore JM, Hermandez O, Covey E (2006) Duration selective neurons in the inferior colliculus of the rat: Topographic distribution and relation of duration sensitivity to other response properties. J Neurophysiol 95:823-836

Plappert CF, Pilz PKD (2001) The acoustic startle response as an effective model for elucidating the effect of genes on the neural mechanism of behavior in mice. Behav Brain Res 125:183-188

Plappert CF, Pilz PKD, Becker K, Becker C-M, Schnitzler H-U (2001) Increased sensitization of acoustic startle response in spasmodic mice with a mutation of the glycine receptor alpha1-subunit gene. Behav Brain Res 121:57-67

Sinnott JM, Owren MJ, Petersen MR (1987) Auditory duration discrimination in Old World monkeys (Macaca, Cercopithecus) and humans. J Acoust Soc Am 82:465–470

Torshin VI, Vlasova IG (2001) Biorhytmologic aspects of seizure activity. Bulletin Exp Biol Med 132:1025-1028

Vandenberg RJ, French CR, Barry PH, Shine J, Schofield PR (1992a) Antagonism of ligand-gate ion channel receptors: two domains of the glycine receptor a subunit form the strychnine-binding site. Proc Natl Acad Sci USA 89:1765-1769

Vandenberg RJ, Handford CA, Schofield PR (1992b) Distinct agonist- and antagonist-binding sites on the glycine receptor. Neuron 9:491-496

Walker DL, Davis M (2002) Quantifying fear potentiated startle using absolute versus proportional increase scoring methods: implications for the neurocircuitry of fear and anxiety. Psychopharmacology 164:318-328

Yunker MP, Herman LM (1974) Discrimination of auditory temporal differences by the bottlenose dolphin and by the human. J Acoust Soc Am 56:1870–1875

Chapter 4

Comodulation masking release determined in the mouse (*Mus musculus*) using a flanking-band paradigm *

4.1 Abstract

Comodulation masking release (CMR) has been attributed to auditory processing within one auditory channel (within-channel cues) and/or across several auditory channels (across-channel cues). The present flanking-band experiment - using an on-frequency masker (OFM) centered at the signal frequency of 10 kHz and a single flanking band (FB) – was designed to separate the amount of CMR due to within- and across-channel cues and to investigate the role of temporal cues on the size of within-channel CMR in the NMRI mouse. The results demonstrated significant within-channel CMR in the mouse while no unambiguous evidence could be found for CMR occurring due to across-channel processing. The amount of within-channel CMR was dependent on the frequency separation between the FB and the OFM. It increased from a value of between about 4 and 6 dB for a frequency separation of 1 kHz to a value of 18 dB for a frequency separation of 100 Hz. The huge increase in CMR for a frequency separation of 100 Hz is likely to be due to the exploitation of changes in temporal cues of the stimulus upon the addition of the signal. Temporal interaction between both masker bands results in modulations with a large depth at a modulation frequency equal to the beating rate. Adding a signal to the maskers reduces the depth of the modulation. It seems possible that the auditory system of mice is able to use the change in modulation depth at a beating frequency of 100 Hz

* This chapter will be published as “Klink KB, Dierker H, Klump GM: Comodulation masking release determined in the mouse (*Mus musculus*) using a flanking-band paradigm”

as a cue for signal detection, while being unable to detect changes in modulation depth at other modulation frequencies. These results are consistent with experiments and model predictions for CMR in humans which showed that the main contribution to the CMR effect stems from processing of within-channel cues (Verhey et al. 1999, Piechowiak et al. 2007).

4.1.1 Keywords

NMRI mouse, psychoacoustics, operant conditioning, Go/NoGo, auditory scene analysis

4.2 Introduction

Most auditory signals in the natural environment are masked by background noise. This noise is often temporally structured and shows correlated amplitude fluctuations over certain frequency ranges (Nelken et al. 1999). It has been shown in psychoacoustical experiments that the auditory system of vertebrates is able to use these correlated, i.e. comodulated, amplitude fluctuations to improve signal detection (e.g. Hall et al. 1984, Klump and Langemann 1995, Klump et al. 2001, McFadden 1986, Weik et al. 2005). This effect has been termed comodulation masking release (CMR), and has been attributed to auditory processing within one auditory channel (relying on within-channel cues) and/or across several auditory channels (relying on across-channel cues).

CMR can be studied in a flanking-band (FB) paradigm employing two narrow bands of noise as maskers (e.g. Schooneveldt and Moore 1987). One masker (the on-frequency masker, OFM) is always centered at the signal frequency while the second masker (flanking-band, FB) can be positioned either within the same auditory channel as the signal to study the influence of within-channel cues, or in a separate auditory channel to measure the amount of CMR due to across-channel cues. Schooneveldt and Moore (1987), for example, showed in humans that CMR tends to be greatest at FB center frequencies close to the signal frequency (i.e. at a small frequency separation between OFM and FB and therefore still within the same auditory filter), and this increase might be caused by the exploitation of changes in

the temporal cues (i.e. the modulation pattern due to the beating between both maskers) when a signal is added.

Because human auditory filters are quite narrow and the equivalent rectangular bandwidth of the filter amounts to roughly 10 % of the signal frequency (e.g. Moore and Glasberg 1983, Oxenham and Shera 2003), it is not possible to study the effects of within-channel cues for a large range of frequency separations. Furthermore, the human filter bandwidth is similar to the bandwidth at which the envelope fluctuation becomes too fast to be of use for the auditory system (for a limit of modulation detection in humans see Viemeister 1979). This makes it difficult to clearly separate the effects of the masker spectrum from the effects of inherent temporal fluctuations of the maskers on the detection thresholds.

In the house mouse, the auditory filter at 10 kHz - a frequency within the best hearing range of the subject - is relatively wide and amounts to at least 3.4 kHz (Ehret 1976, Weik et al. 2005). The large width of the filter makes it possible to clearly separate the spectral and temporal effects on the threshold. Furthermore, the amount of within-channel CMR that is due to the varying temporal cues resulting from different frequency separations between FB and OFM can be measured. Therefore, we think that the mouse is a suitable model for studying spectral and temporal aspects of CMR independently from each other using narrow-band noise maskers.

4.3 Material and methods

4.3.1 Subjects

Masked thresholds were obtained for 14 adult house mice (*Mus musculus*) of the NMRI strain (bred by Charles River Laboratories, Sulzfeld, Germany). The subjects (7 females, 7 males) were between 2 and 18 months old during the total period of testing. Their hearing threshold for the 10-kHz signals at the end of the experiments was on average 21 dB SPL and did not exceed 34 dB SPL which is only moderately larger than the average threshold of the subjects at the beginning of testing (7 dB SPL). The subjects were housed in individual cages (Eurostandard Type III H, 43x27x19 cm; Tecniplast) with a hiding possibility ("mouse house", Tecniplast) and a layer of wood shavings as bedding material (Raiffeisen). All cages were stored in a

ventilated cage rack (Slim Line Sealsafe, Tecniplast). The animals had unrestricted access to water and were mildly food-deprived (their weight ranged from 28.2 to 46.9 g which is above the mean weight at reaching maturity). The food rewards during the experiments consisted of 20-mg pellets (Bioserve Dustless Precision Pellets, Formula FO163), and additional rodent pellets (Altromin Type 1314) were given after the experiments to keep the animals' weight about constant. Animals were moved from their cages to the experimental cage using a small transfer cage.

4.3.2 Apparatus

The wedges had an absorption coefficient of more than 0.99 for frequencies above 500 Hz.

The custom-made experimental cage was shaped like a doughnut (outer diameter 22 cm, inner diameter 9 cm, height 14 cm; made from stainless steel wire mesh) and was located in the middle of the chamber on a rack constructed of thin metal bars (IAC chamber) or a wire construction lifting the cage above the sound absorbing wedges (custom-built chamber). The cage contained a small feeding dish with a nearby feeder light as a secondary reinforcer and a pedestal with a light-interrupting switch. A nearby pedestal light was used to provide feedback to the animals during testing. A custom-built feeder mounted at a distance of at least 30 cm was connected to the feeder dish by a flexible tube and dispensed the reward pellets. A loudspeaker (Canton Plus XS) was positioned a minimum of 30 cm above the pedestal at which the mouse sat in the experimental cage.

4.3.3 Stimulus generation

Masked thresholds were obtained for a 10 kHz pure tone signal (duration 800 ms, cosine rise/fall times of 10 ms) presented in continuous narrow-band noise. The noise in each experiment consisted of two 25-Hz-wide noise bands centered at various frequencies. The OFM was always centered at the signal frequency (i.e. 10 kHz). In the standard conditions, in which the general amount of CMR due to within- and across-channel processing was investigated in all subjects, the FB was centered at frequencies of either 5, 9, 10, 11, or 15 kHz. To test whether the frequency

separation between both masker bands affects the size of within-channel CMR (due to changes in temporal cues), additional FB frequencies of 9.9 and 10.1 kHz were tested in a subgroup of the subjects (3 individuals). The envelope of the FB was either uncorrelated or correlated with that of the OFM. In the 10 kHz FB condition, both bands were presented simultaneously, and the SPL of the resulting noise – compared to a single OFM – was on average 3 dB higher in the uncorrelated condition (reference condition) and 6 dB higher in the correlated condition.

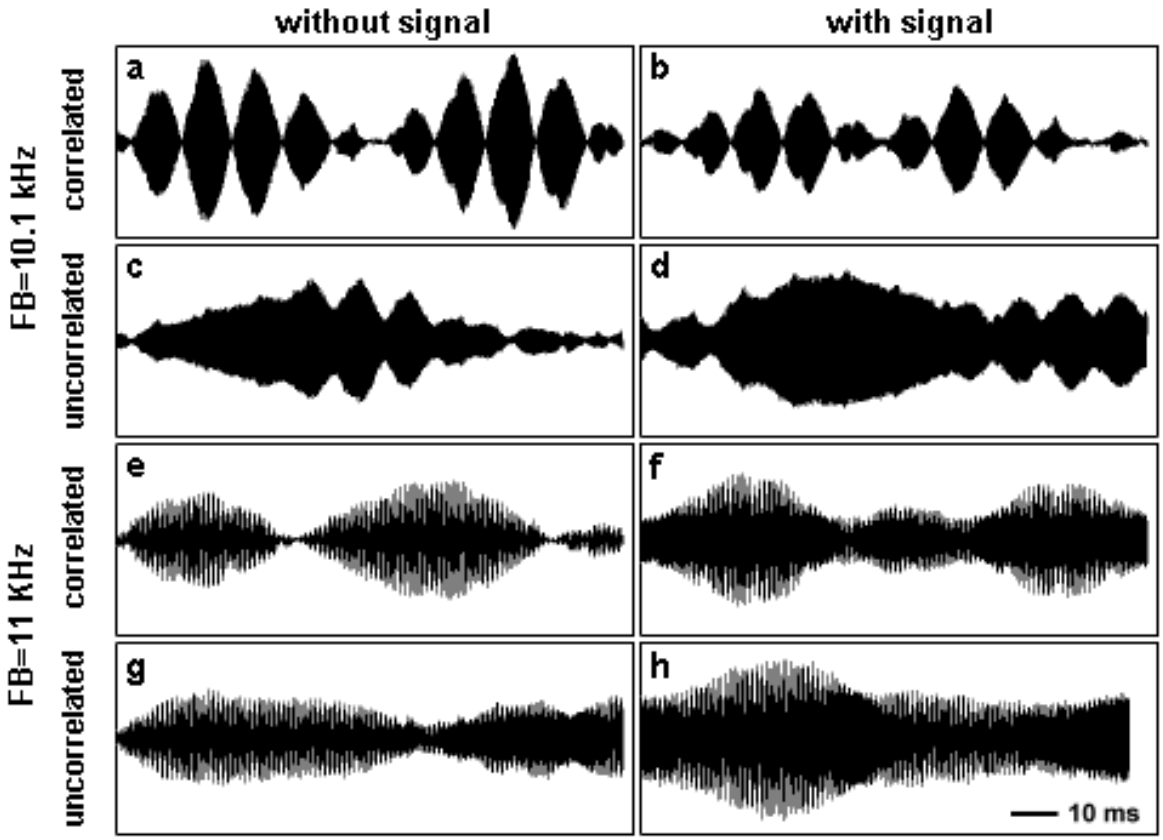

Figure 4.1: Examples of envelope fluctuations of correlated (a-b, e-f) and uncorrelated (c-d, g-h) 25-Hz narrow-band noise maskers without (left column) and with (right column) the pure tone signal present. The signal level equaled the mean SPL needed for the signal detection in the respective type of noise (i.e. at FB=10.1 kHz: 36.4 dB SPL (correlated) and 54.1 dB SPL (uncorrelated), at FB=11 kHz: 54.3 dB SPL (correlated) and 58.0 dB SPL (uncorrelated); noise spectrum level 40 dB SPL). FB=10.1 kHz: correlated: 54 dB SPL, uncorrelated. Each noise masker in this figure consisted of either the OFM and a FB at 10.1 kHz center frequency (a-d), or the OFM and a FB at 11 kHz center frequency (e-h). At a frequency separation of 100 Hz, the envelopes in both the correlated (a-b) and – to a smaller extent – the uncorrelated (c-d) noise show regular peaks and valleys at a modulation rate of 100 Hz in addition to the slow fluctuation of the 25 Hz noise bands. At a frequency separation of 1 kHz (e-g), however, the 1-kHz beating is too fast to be resolved by the auditory system and only the inherent fluctuation of the 25 Hz noise bands can be seen. Adding the 10 kHz signal to the noise results in a partial “filling” of the envelope minima (e.g. see fig e-f) and can be used as a cue for the detection of the signal.

The 10 kHz pure tone signal was generated with a Linux workstation with a standard sound card (Sound Blaster Model PCI 128, 44.1 kHz sampling rate; Creative Technology) and passed through a programmable attenuator (PA4, Tucker-Davis Technologies) to mute the signal completely between trials if necessary. The output sound pressure level of the signal was adjusted by an manual attenuator (Hewlett Packard 350D) in steps of 5 dB. The signal was amplified by a Rotel RMB-1066 amplifier and presented via the Canton XS loudspeaker. Sound pressure levels in the experimental setup were calibrated once per day with a sound level meter (Model 2238 mediator with Model 4188 microphone; Bruel & Kjaer) located at the position where the head of the animal would be during the experiment.

The continuous narrow-band noise was generated using a set of two real-time processors (RP2.1, Tucker-Davis Technologies). Each 25-Hz-wide noise band was produced by multiplying a 12.5 Hz low-pass noise (cut-off at 3 dB) with a sinusoid, thus generating a 25-Hz-wide noise band centered at the signal frequency of the pure tone. Using the same low-pass noise as the source two 25-Hz-wide noise bands with correlated amplitude fluctuations could be generated (see Fig. 4.1 a-b, e-f), while using two independent low-pass noise bands produced two uncorrelated 25-Hz wide noise bands (see Fig. 4.1 c-d, g-h). The noise spectrum level was adjusted to 40 dB SPL using a programmable attenuator (PA4, Tucker-Davis Technologies).

4.3.4 Procedure

The experimental paradigm was a Go/NoGo-procedure reinforcing the subject with food rewards (for initial training procedures and more details see Klink et al. 2006). The experimental protocol was controlled by the workstation using a custom-made program. A trial started when the subject jumped onto the pedestal in the experimental cage. After a random waiting interval of between 1 and 5 s, a single test stimulus was presented. The mouse was trained to jump off the pedestal when perceiving a test stimulus (Go condition), otherwise it had to remain on the pedestal. If the subject responded correctly to a test stimulus (i.e. scored a "hit") within 1 s, a feeder light was switched on, a food reward was given and the next trial started. If the subject missed a test stimulus and remained seated on the pedestal, the pedestal light was switched off for 1 s before the next trial could be initiated. 30% of all trials

were catch trials in which no stimulus was given (NoGo condition), and the subject had to remain on the pedestal ("correct rejection"). These trials were used to measure the false alarm rate.
Signal detection thresholds were obtained with the method of constant stimuli. A block of 10 trials, consisting of three catch trials and a set of seven test trials in which the sound pressure level of the tone differed in steps of 5 dB, was repeated six times. The trials within each block were presented in random order, thus making it impossible for the mouse to anticipate the next stimulus. The first 10 trials of each session were used as a "warm-up" period (only test stimuli with the highest sound pressure level were presented) and were discarded from the analysis.

4.3.5 Data analysis

At the end of each session, a psychometric function was compiled summarizing the results of the last 50 trials. Sessions were excluded from the analysis if the percentage of false alarms was greater than 20 % and/or if the average hit rate in the trials with the two most salient test stimuli (i.e. those with the largest sound pressure level) was less than 80 %. Threshold estimates of single sessions were calculated using signal-detection theory and a threshold criterion of d' = 1.8. For each noise condition, two to three consecutive valid 50-trial sessions which did not differ from each other in threshold by more than 3 dB were combined into a single psychometric function (i.e. this corresponded to 10-15 repetitions of the stimulus per sound pressure level). Using a threshold criterion of d’ = 1.8, mean signal detection thresholds were calculated from the combined psychometric function. The difference between the signal detection threshold in the uncorrelated and the correlated masker condition was defined as CMR.

4.4 Results

The present flanking-band experiment was designed to demonstrate the effects of spectral and temporal cues on the amount of CMR in the mouse. Three different frequency separations between the OFM and the FB were examined: 1) very large frequency separations of 5 kHz (i.e. for FBs centered at 5 and 15 kHz, respectively)

which addressed the processing of across-channel cues, 2) large frequency separations of 1 kHz within a single auditory filter (i.e. for FBs centered at 9 and 11 kHz, respectively), and 3) small frequency separations of 100 Hz within a single auditory filter (i.e. for FBs centered at 9.9 and 10.1 kHz, respectively). The latter two conditions only provide within-channel cues, but with different temporal characteristics.

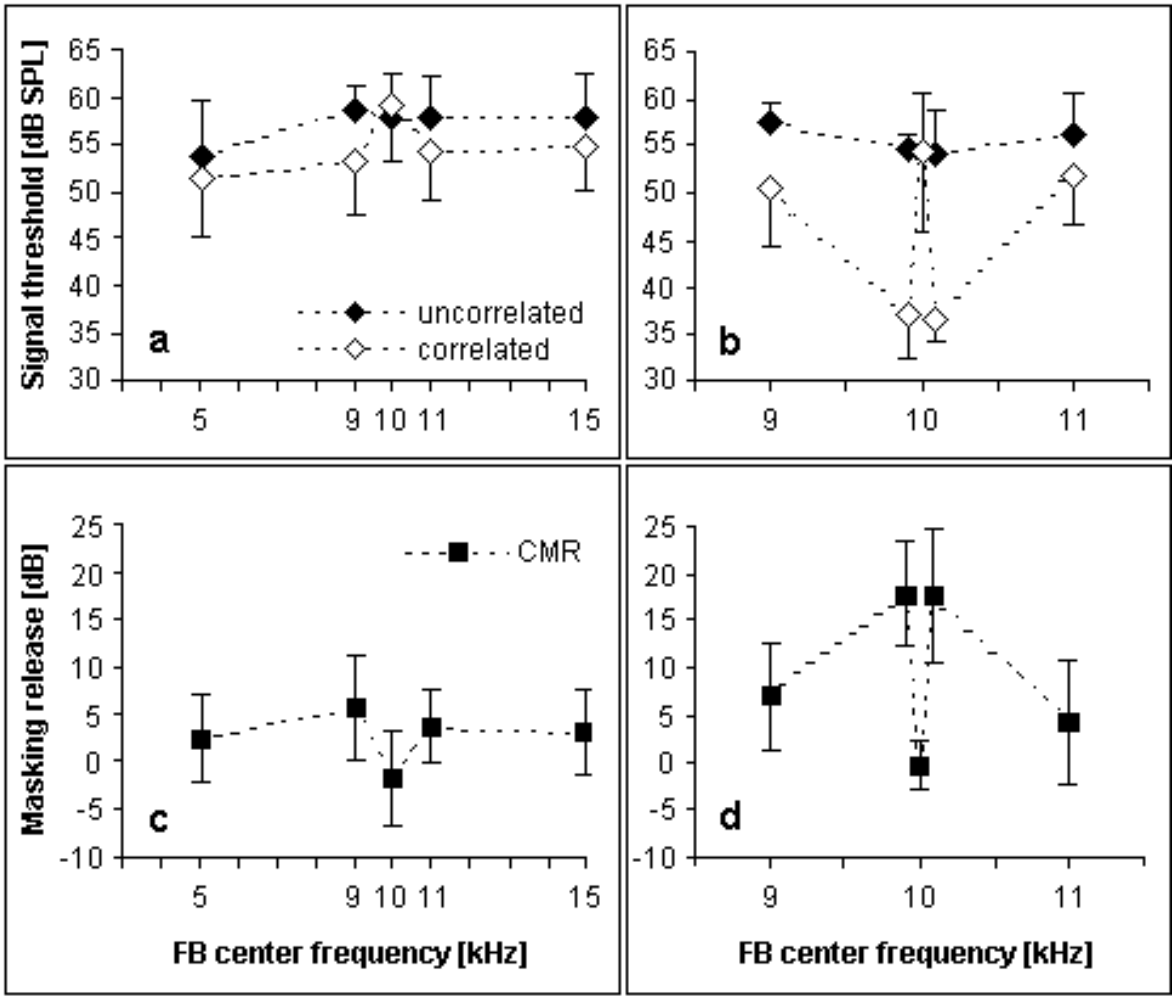

Figure 4.2: Mean signal thresholds for up to 14 NMRI mice for the detection of a 10-kHz tone masked by 25-Hz narrowband noise in relation to the center frequency of the FB (filled diamonds: uncorrelated noise bands, open diamonds: correlated noise bands, filled squares: CMR). Error bars show the standard deviation across subjects. The left column (a+c) depicts the results for the standard conditions for 14 subjects. The right column (b+d) shows a close-up of the results of FB conditions between 9 and 11 kHz including the additional FB conditions 9.9 and 10.1 kHz for the 3 subjects tested with all conditions. Figures a and b show the signal threshold for the uncorrelated and correlated conditions; figures c and d show the amount of masking release between the uncorrelated condition and the correlated condition.

To investigate the role of spectral cues on CMR (i.e. the ocurrence of within- and across-channel CMR), masked thresholds were measured in 14 NMRI mice. Figure 4.2a shows the mean signal detection thresholds (± standard deviation) for a 10-kHz tone presented either in a correlated or an uncorrelated masker condition in relation to the center frequency of the FB. In the reference condition a mean signal threshold of 57.5 dB SPL could be obtained. Positioning an uncorrelated 25-Hz-wide FB at a center frequency below or above 10 kHz did not affect the signal threshold much. The mean signal threshold for FB center frequencies between 9 kHz and 15 kHz ranged from 57.8 to 58.8 dB SPL, for a FB center frequency of 5 kHz it was 53.8 dB SPL. Positioning a correlated 25-Hz-wide FB at a center frequency 1 or 5 kHz below or above the center frequency of the OFM (i.e. 10 kHz) lowered the detection threshold to a value of 53.1 to 54.7 dB SPL (except for a FB of 5 kHz, where the threshold was 51.4 dB SPL). A Two-Way-Repeated-Measures ANOVA with the signal threshold (in dB SPL) as the dependent variable and type of correlation and FB center frequency (in kHz) as factors revealed a significant effect of type of correlation ($p=0.001$) and FB center frequency ($p=0.002$), and a significant interaction between the two factors ($p=0.003$).

To test the influence of correlation on the masked thresholds, pairwise comparisons between the masked thresholds for different FB frequencies (Tukey test) were obtained which revealed a significant difference between correlated and uncorrelated thresholds for FBs centered at 9 kHz ($p<0.001$), 11 kHz ($p=0.005$) and 15 kHz ($p=0.019$), indicating a possible CMR at these conditions. Furthermore, there was a significant difference in threshold in the correlated condition between the FB centered at 10 kHz and FBs centered at 5 kHz ($p<0.001$), 9 kHz ($p=0.002$), 11 kHz ($p=0.021$) and 15 kHz ($p=0.042$), respectively, indicating a beneficial effect of the frequency separation between FB and OFM at these conditions. In the uncorrelated condition, only thresholds for the FBs centered at 5 and 9 kHz differed significantly from each other ($p<0.001$).

CMR was calculated as the difference between the signal threshold in an uncorrelated and a correlated masker condition (see Fig. 4.2c). A One-Way-Repeated-Measures ANOVA with the CMR as dependent variable revealed a significant effect of FB position ($p=0.003$). Pairwise comparisons between the amount of CMR for different FB conditions (Tukey test) showed that the difference between

the correlated and the uncorrelated threshold for both the OFM and the FB centered at 10 kHz (i.e., -1.6 dB) was significantly different from the amount of CMR for FBs centered at 9 kHz ($p=0.001$) and 11 kHz ($p=0.026$), respectively. On average, the CMR was 5.6 dB and 3.7 dB for FBs centered at 9 and 11 kHz, respectively. No significant CMR effect in comparison to the threshold difference at 10 kHz could be found for the largest frequency separations tested (i.e., with a FB of 15 kHz, $p=0.068$, and with a FB of 5 kHz, $p=0.148$). Thus, in the current study, no unambiguous evidence for CMR occurring due to across-channel cues could be found.

To investigate the role of temporal cues on CMR, additional masked thresholds at FB conditions of 9.9 and 10.1 kHz were measured in 3 NMRI mice (see also Fig. 4.2b). Please note that for all following analyses, only the masked thresholds of the three subjects tested with the additional FB conditions were taken into consideration. For the three subjects, a mean signal threshold of 54.3 dB SPL could be obtained in the reference condition. Positioning an uncorrelated 25-Hz-wide FB at a center frequency of between 5 and 15 kHz did not change the detection threshold significantly (the detection thresholds ranged between 49.8 and 56.7 dB SPL). Positioning a correlated 25-Hz-wide FB at a frequency separation of 100 Hz lowered the detection threshold of the three subjects tested with all FB conditions to a mean value of 36.6 dB SPL, while the thresholds for the remaining FB conditions with a greater frequency separation (1 and 5 kHz) ranged between 46.0 and 52.4 dB SPL. A Two-Way-Repeated-Measures ANOVA with the masked threshold as the dependent variable and type of correlation and FB center frequency as factors revealed a significant effect of type of correlation ($p=0.043$) and FB center frequency ($p=0.005$). Furthermore, a significant interaction between type of correlation and FB center frequency could be found ($p=0.007$). To test the influence of correlation on the masked thresholds, pairwise comparisons between the masked thresholds for the correlated and uncorrelated conditions (Tukey test) were obtained. Concentrating on the data within each FB condition revealed a significant difference between uncorrelated and correlated thresholds for FBs centered at 9.9 kHz ($p<0.001$) and 10.1 kHz ($p<0.001$) only, indicating a CMR at these conditions. To test whether the FB position had any influence on the size of the masked threshold, pairwise comparisons between the masked thresholds for different FB center frequencies within the same type of correlation (Tukey test) were conducted. An analysis within

the uncorrelated condition showed no influence of FB condition on threshold (all $p>0.3$). In the correlated condition, however, there were significant differences in thresholds between the additional conditions (9.9 and 10.1 kHz, respectively) and the 9, 10, 11, and 15 kHz conditions, respectively (all $p\leq0.008$), indicating a beneficial effect of the frequency separation between FB and OFM only for the smallest separations measured (100 Hz). A One-Way-Repeated-Measures ANOVA with the CMR as dependent variable revealed a significant effect of flanking-band position ($p=0.007$). Pairwise comparisons (Tukey test) showed that the "amount of CMR" for the FB centered at 10 kHz - here only defined as the difference between the correlated and uncorrelated condition - was significantly different from the amount of CMR for FBs centered at 9.9 kHz ($p=0.016$) and 10.1 kHz ($p=0.018$), respectively. The mean CMR was 17.8 dB for FBs centered at 9.9 and 10.1 kHz (see Fig. 4.2d). Please note that the failure to obtain significant CMR at FBs of 9 and 11 kHz was mostly due to high standard deviation across the three tested subjects; if all subjects were taken into consideration (see above), the amount of CMR in both conditions became significant.

4.5 Discussion

The results of the flanking-band experiment demonstrated significant within-channel CMR in the mouse while no unambiguous evidence could be found for CMR occurring due to across-channel processing. The amount of within-channel CMR was dependent on the frequency separation between the FB and the OFM. It increased from a value of between about 4 and 6 dB for a frequency separation of 1000 Hz to a value of 18 dB for a frequency separation of 100 Hz. For both frequency differences between FB and OFM the CMR was slightly smaller for flanking bands positioned above rather than below the signal frequency, a tendency which is also found in humans (Hall et al. 1988, Piechowiak et al. 2007).

The largest CMR in the mouse in this FB experiment was higher than the CMR in other FB studies using 25 Hz narrow-band noise maskers. In European starlings, a within-channel CMR of 11.7 dB could be measured at a signal frequency of 2 kHz and a frequency separation between OFM and FB of 113 Hz (Klump et al. 2001). However, the starling also exhibited a large across-channel CMR of between 9.3 and

14.4 dB in the same experiment. CMR in humans measured with 25 Hz narrow-band maskers falling within the limits of an auditory filter ranged between 6 and 9 dB for 8 kHz tone signals; the largest CMR was observed for a frequency separation between OFM and FB of 100 Hz (Schooneveldt and Moore 1987). Schooneveldt and Moore also observed an across-channel CMR in this flanking-band experiment. Similarly to the results for 8-kHz signals, also at lower frequencies the largest CMR was observed at a frequency separation between OFM and FB of 100 Hz (e.g., for 2-kHz signals Schooneveldt and Moore 1987 and Piechowiak et al. 2007 observed a CMR of up to 14 dB in humans). If the amount of CMR is dependent on temporal processing mechanisms, a larger value would be expected in the mouse compared to both starlings and humans, since the mouse has wider auditory filters which may allow for a better temporal resolution.

The absence of across-channel CMR in the current study is consistent with the results of a previous study in the mouse in which CMR was measured using a band-widening paradigm (Weik et al. 2005, 2006). In this experiment a CMR of up to 13 dB could be found for masker bandwidths well below the auditory filter bandwidth of the mouse (i.e. below 3.4 kHz) and no additional CMR due to across-channel processing could be obtained. Both, the current study and the study of Weik et al. support the hypothesis that the occurrence of CMR in the mouse can be explained on the basis of within-channel cues only. Also in humans it has been suggested that the main contribution to the CMR effect stems from processing of within-channel cues (Verhey et al. 1999, Piechowiak et al. 2007).

What is the mechanism underlying CMR in the mouse that can explain both the lack of CMR for across-channel conditions and the huge increase in CMR for small frequency separations of 100 Hz? Moore (1992) suggested that the release from masking in the presence of correlated maskers within a single auditory channel might be attributed to the use of cues such as a change in the pattern of neuronal phase locking during the presentation of a signal. Phase locking can occur to the fine structure of a stimulus (i.e. to the signal frequency, or the carrier frequency of the masker) and/or to its envelope fluctuation. In the mouse, phase-locking to the fine structure ceases at a frequency of 4 kHz (Taberner and Liberman 2005). Therefore, in the current experiment phase locking to the fine structure of signal and carrier cannot be used to explain the results in the mouse. Locking of action potentials to the

masker envelope, however, still might provide usable cues. The envelope of the composite masker may be dominated by a temporal interaction between both maskers, leading to a beating with a rate depending on the frequency difference between both center frequencies (Schooneveldt and Moore 1987, Piechowiak et al. 2007). At a frequency separation of 1 kHz the beating occurs at a mean rate of 1 kHz while a frequency separation of 100 Hz leads to a mean beating rate of 100 Hz. The addition of a tone signal to the masker reduces the depth of modulation which may leadd to a reduced amount of locking of neural activity to the masker envelope (“locking suppression”); this change could be used as a cue for the detection of the signal (Nelken et al. 1999). This cue can be exploited efficiently when the frequency separation between OFM and the FB is small. The ability of the neurons to lock to the stimulus envelope depends on the modulation frequency. Tan and Borst (2007) found that in the mouse inferior colliculus locking to sinusoidal amplitude fluctuations ceased for modulation frequencies of 160 Hz or more. This indicates that while locking to the masker envelope beating at 100 Hz might be likely and a change in this locking might be useable as a detection cue, locking to the 1 kHz beating rate is not probable. This is further supported by data of Kelly et al. (2006) who found that the modulation detection threshold in the rat, a species closely related to the mouse with a similar auditory filter bandwidth (according to critical masking ratio data from Gourevitch 1965 and Ehret 1976), increased considerably at modulation frequencies above 100 Hz.

The ability of vertebrates (for a overview see also Dent et al. 2002) to detect amplitude modulation depends not only on the modulation frequency but also on the modulation depth of the stimulus. Adding a signal to the masker results in both a local increase in level and a reduction of the modulation depth and a change in the modulation pattern of the resulting stimulus (e.g. Eddins 2001, Moore 1992) which could be used for signal detection. However, the auditory system is not equally sensitive to changes in modulation depth across all modulation frequencies. Similar to humans (Viemeister 1979, Dau et al. 1997), the modulation detection threshold in rats (Kelly et al. 2006) increases with increasing modulation rate, and the required changes in modulation depth become increasingly higher until they stop to be usable as a cue (see Fig. 4.3).

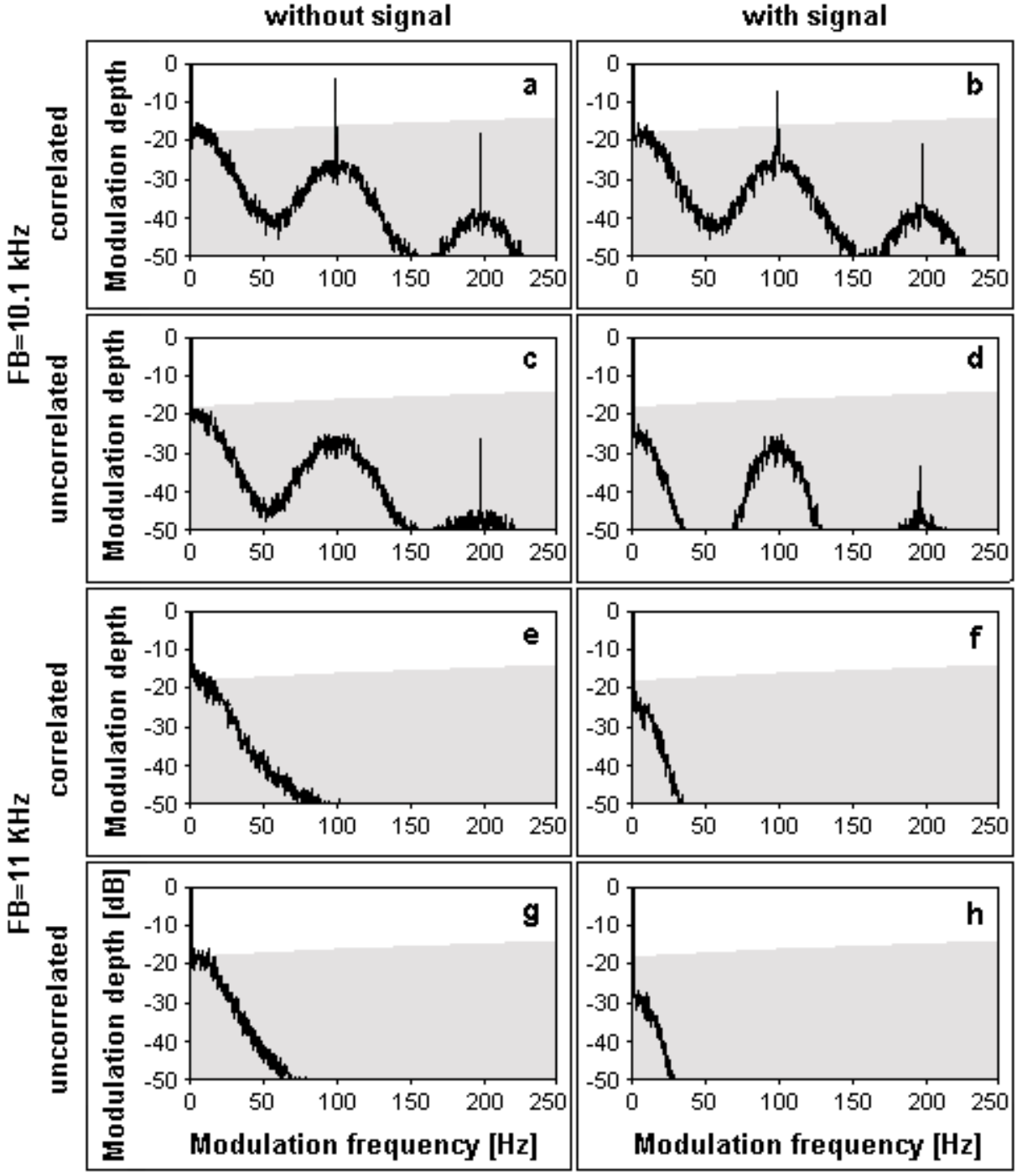

Figure 4.3: Modulation spectra of the resulting noise of the OFM and the FB at 10.1 kHz center frequency (a-d), or the OFM and a FB at 11 kHz center frequency (e-h), either correlated (a+b, e+f) or uncorrelated (c+d, g+h) with each other. The left column shows spectra with only the maskers present, the right column shows spectra with the signal added at a mean SPL level needed for the signal detection in the respective type of noise (for level see fig 1). The “peaks” in the 10.1 kHz condition (a-b) indicate the beating frequency (and multiples thereof). The grey area in each graph depicts the limit of modulation detection calculated from the modulation transfer fuction of rats (Kelly et al. 2006).

We have plotted the modulation spectra for different masker conditions and FB center frequencies in Fig. 4.3. The black curve depicts the modulation depth of the respective stimulus at different modulation frequencies, the grey colour marks the area in which the modulation cannot be detected by the rat (Kelly et al. 2006) and is likely not to be detectable by the mouse too. The modulation spectra for correlated maskers and a frequency separation of 100 Hz are depicted in Fig. 4.3a-b. Before the signal is added (Fig. 4.3a), the modulation spectrum shows high modulation depth (-3.6 dB corresponding to 66.1 % modulation depth) at modulation frequencies corresponding to the beating rate of the composite masker (sharp peak at 100 Hz modulation frequency) and to the inherent fluctuation rate of the narrow-band masker (broad peak at modulation frequencies below 50 Hz). Adding the signal (using a level equal to the mean SPL at threshold, Fig 4.3b) to the masker reduces the modulation depth at the 100 Hz modulation frequency (peak) by 3.4 dB (which is equivalent to a reduction of 21%). This reduction of modulation depth could be a sufficient cue for the signal detection, as indicated by data from Wakefield and Viemeister (1990) who showed that humans were able to detect changes in depth of 1-2 dB at high modulation depths (for low modulation rates of 100 Hz). If the auditory system of mice exhibits the same range of sensitivity, the mouse may be able to use this change in modulation depth provided by stimuli of this CMR experiment as a cue for signal detection. In the correlated condition, this might lead to especially low signal detection thresholds and to CMR of nearly 18 dB. Adding a signal to the masker hardly changes the modulation depth at low frequencies, making the use of this modulation cue questionable. In the uncorrelated condition (Fig. 4.3c-d), there is no peak in the modulation spectrum at 100 Hz modulation rate. Only the lowest modulation frequencies due to the inherent fluctuations of the masker show a modulation depth which may allow the mouse to detect the modulation. Adding a signal to the masker, however, reduces the modulation depth below the threshold of modulation detection in rats (Fig. 4.3d), and it is unclear whether this change can be detected by the mice and can be used as a cue for detection of the signal. In conditions with a frequency separation of 1 kHz (Fig. 4.3e-h), the change in modulation depth at low modulation frequencies after addition of the signal is similar to that of the uncorrelated condition with a frequency separation of 100 Hz. If the mouse is at all able to use changes in modulation depth at the slow modulation rate

caused by the inherent fluctuation of the narrow-band noise as a cue, this cue would be present in both conditions (i.e. 100 Hz and 1 kHz frequency separation), and might be able to explain a small CMR effect. However, it is likely that the CMR seen at frequency separations of 1 kHz might be a result of the auditory system's ability to detect a local increase in level of the masker after the addition of the tone (see Moore 1992).

4.6 Acknowledgements

This study was supported by a grant from the Deutsche Forschungsgemeinschaft (SFB 517) and the Volkswagen-Stiftung (Joint Niedersachsen-Israel program ZN2040). We thank Mathias Dietz and Jesko Verhey for their input.

4.7 References

Dau T, Kollmeier B, Kohlrausch A (1997) Modeling auditory processing of amplitude modulation. I. Detection and masking with narrow-band carriers. J. Acoust. Soc. Am. 102:2892-2905

Dent ML, Klump GM, Schwenzfeier C (2002) Temporal modulation transfer functions in the barn owl (*Tyto alba*). J Comp Physiol A 187:937-943

Eddins DA (2001) Monaural masking release in random-phase and low-noise noise. J Acoust Soc Am 109:1538-1549

Ehret G (1976) Critical bands and filter characteristics in the ear of the house mouse (Mus musculus). Biol Cybernetics 24:35-42

Gourevitch G (1965) Auditory masking in the rat. J. Acoust. Soc. Am. 37:439-443

Hall JW, Haggard MP, Fernandes MA (1984) Detection in noise by spectro-temporal pattern analysis. J Acoust Soc Am 76:50-56

Hall JW, Grose JH, Haggard MP (1988) Comodulation masking release for multicomponent signals. J Acoust Soc Am 83:677-686

Kelly JB, Cooke JE, Gilbride PC, Mitchell C, Zhang H (2006) Behavioral limits of auditory temporal resolution in the rat: amplitude modulation and duration discrimination. J Comp Psychol 120:98-105

Klink KB, Bendig G, Klump GM (2006) Operant methods for mouse psychoacoustics. Behav Res Methods 38:1-7

Klump GM, Langemann U (1995) Comodulation masking release in a songbird. Hear Res 87:157-164

Klump GM, Langemann U, Friebe A, Hamann I (2001) An animal model for studying across-channel processes: CMR and MDI in the European starling. In: Breebaart DJ, Houtsma AJM, Kohlrausch A, Prijs VF, Schoonhoven R (eds). Physiological and psychophysical bases of auditory function. Shaker Publishing, Maastricht, pp. 266-272

McFadden D (1986) Comodulation masking release: Effects of varying the level, duration, and time delay of the cue band. J Acoust Soc Am 80:1658-1667

Moore BCJ (1992) Across-channel processes in auditory masking. J Acoust Soc Jpn 13:25-37

Moore BCJ, Glasberg BR (1983) Suggested formulae for calculating auditory-filter bandwidths and excitation pattern. J Acoust Soc Am 74:750-753

Nelken E, Rotman Y, Yosef OB (1999) Responses to auditory-cortex neurons to structural features of natural sounds. Nature 397:154-157

Oxenham AJ, Shera CA (2003) Estimates of human cochlear tuning at low levels using forward and simultaneous masking. J Assoc Res Otolaryngol 4:541-554

Piechowiak T, Ewert SD, Dau T (2007) Modeling comodulation masking release using an equalization-cancellation mechanism. J Acoust Soc Am 121:2111-2126

Schooneveldt GP, Moore BCJ (1987) Comodulation masking release (CMR): Effects of signal frequency, flanking-band frequency, masker bandwidth, flanking-band level, and monotic versus dichotic presentation of the flanking band. J Acoust Soc Am 82:1944-1956

Taberner AM, Liberman MC (2005) Response properties of single auditory nerve fibers in the mouse. J Neurophysiol 93:557-569.

Tan ML, Borst JGG (2007) Comparison of responses of neurons in the mouse inferior colliculus to current injections, tones of different durations, and sinusoidal amplitude-modulated tones. J Neurophysiol 98:454-466

Verhey JL, Dau T, Kollmeier B (1999) Within-channel cues in comodulation masking release (CMR): Experiments and model predictions using a modulation-filterbank model. J Acoust Soc Am 106:2733-2745

Viemeister NF (1979) Temporal modulation transfer functions based upon modulation thresholds. J Acoust Soc Am 66:1364-1380

Wakefield GH, Viemeister NF (1990) Discrimination of modulation depth of sinusoidal amplitude modulation (SAM) noise. J Acoust Soc Am 88:1367-1373

Weik V, Klink K, Klump GM (2006) Comodulation masking release determined in the mouse (*Mus musculus*) using a band-narrowing paradigm. Abstracts of the twenty-ninth annual midwinter research meeting of the Assiociation for Research in Otolaryngology 29:124

Weik V, Klink KB, Klump GM (2005) Comodulation masking release in the house mouse (*Mus musculus*), in: Elsner N., Zimmermann, H. (Eds.), Proceed 30th Göttingen Neurobiol Conference and the 6th Meeting of the German Neuroscience Society:101B

Danksagung

Allem voran gilt mein Dank Prof. Dr. Georg Klump, der mich als Doktorandin in seiner Arbeitsgruppe „Zoophysiologie und Verhalten“ aufgenommen und es mir ermöglicht hat, verschiedene Aspekte des Hörvermögens bei Mäusen zu untersuchen.

Ein herzliches Dankeschön geht auch an Dr. Ulrike Langemann, die immer ein offenes Ohr für mich hatte und die eine unerschöpflicher Quelle an Anregungen war.

Ein weiteres Dankeschön geht an Prof. Dr. Jesko Verhey für die Diskussionen, die viel zu meinem Verständnis so mancher tückischer Sachverhalte beigetragen haben.

Bei Garnet Bendig bedanke ich mich für die gute Zusammenarbeit in meinem ersten Jahr an der Uni Oldenburg, als wir beide noch keine Ahnung hatten, wie man Verhaltensversuche mit Mäusen macht.

Ganz besonders herzlich bedanke ich mich bei Julia Maier, Verena Weik und Melanie Zokoll für die ihre tolle Unterstützung, sowohl wissenschaftlich als auch privat. Ich konnte mich immer auf sie verlassen, und ich werde stets mit Freude an die gemeinsam verbrachte Zeit denken (und hoffen, dass sie nicht so schnell endet).

Ich bedanke mich bei allen Mitarbeitern der AG „Zoophysiologie und Verhalten“ für die Schaffung eines angenehmen Arbeitsklimas und dafür, dass ich mich in der AG immer gut aufgehoben gefühlt habe. Ich danke Astrid Klinge, Nina Pohl, Ralf Edler, Alice Aidnik und Markus Metz für die vielen Diskussionen und für alle Verbesserungsvorschläge, vor allem bei den Vorträgen. Ein großer Dank geht an Colette Pokar (nicht nur fürs Mäusesaubermachen), Elisabeth Groscurth, Susanne Groß und Killian Köbrich. Prof. Dr. Peter Jaros danke ich für alle „Guten Morgen, Karin“, mit denen ein Arbeitstag gleich viel besser anfing. Peter Kanje danke ich für alle angeregten Diskussionen über „Gott und die Welt“. Dr. Mark Bee, der sicher einer der nettesten Amerikaner ist, die ich je getroffen habe, danke ich nicht nur für

die Hilfe bei so manchen Problemen mit der englischen Sprache. Bei Priv.-Doz. Dr. Thomas Friedl bedanke ich mich für die Hilfe bei der Statistik und für alle Anregungen bei den Vorträgen. Ein ganz großer Dank geht an Holger Dierker, nicht nur für die Erstellung der Modulationsspekten, sondern auch für seine schnelle Hilfe bei allen Computerproblemen und auch für seine Geduld, wenn ich ihm mal wieder mit Kleinigkeiten genervt habe.

Ich bedanke mich bei allen Mitgliedern des Internationalen Graduiertenkollegs “Neurosensorik” für den regen Gedankenaustausch, durch den ich Einblicke in viele wissenschaftliche Disziplinen gewonnen habe. Ein besonderer Dank geht an Michael Buschermöhle für die Diskussionen über CMR, and Stefan Strahl, Rainer Beutelmann, Marc Nitschmann, Stephan Heise, Suzan Emiroglu, Bastian Epp (vor allem für die Organisation des letzten Workshops) und alle anderen Mitglieder.

Ein herzlicher Dank geht auch an Prof. Dr. David McAlpine für seine aufmunternden Worte und seine Hilfe bei der Verbessung meiner Englischkenntnisse.

Der DFG und der Volkswagen-Stiftung danke ich für die Finanzierung der Projekte, über die auch meine Forschung bezahlt wurde. Dem Internationalen Graduiertenkolleg Neurosensorik danke ich für die Ermöglichung meiner Teilnahme an wissenschaftlichen Tagungen, Summerschools und anderen Projekten.

Als letztes bedanke ich mich bei meinen Eltern und meinem Bruder, die immer zu mir gehalten und mich nach Kräften unterstützt haben, auch wenn die ständigen „Wann bist du endlich fertig?“ mir manchmal doch auf die Nerven gingen.

Lebenslauf

Name:	Karin B. Klink
Geburtsdatum:	24.11.1974
Geburtsort:	Königshütte (Chorzów), Polen
Staatsangehörigkeit:	deutsch

Sep 2004 – Apr 2008	Promotionsstudium der Biologie, Carl von Ossietzky Universität Oldenburg
seit Jan 2004	Assoziiertes Mitglied im Internationalen Graduiertenkolleg “Neurosensorik”, Carl von Ossietzky Universität Oldenburg
Jun 2002 - Dez 2007	wissenschaftliche Mitarbeiterin in der AG Zoophysiologie und Verhalten, Carl von Ossietzky Universität Oldenburg
Okt 1995 - Feb 2002	Studium der Biologie, Universität Bielefeld Titel der Diplomarbeit: *Kann das Kopieren der weiblichen Partnerwahl beim Breitflossenkärpfling Poecilia latipinna die Durchsetzbarkeit eines neuen Männchenmerkmals fördern?*
Jun 1995	Abitur, Marienschule der Ursulinen, Bielefeld

Publication list

Publications in peer reviewed journals

Klink KB, Bendig G, Klump GM (2006) Operant methods for mouse psychoacoustics. Behav Res Methods 38:1-7

Witte K, Klink KB (2005) No pre-existing bias in sailfin molly females, *Poecilia latipinna*, for a sword in males. Behaviour 142:283-303

Klink KB, Klump GM (2004) Duration discrimination in the mouse (*Mus musculus*), J Comp Physiol A 190:1039-1046

Conference abstracts

Klink KB, Weik VN, Klump GM (2007): Comodulation masking release determined in the mouse *(Mus musculus*) using a flanking-band paradigm, in: Proceedings of the 31th Göttingen Neurobiology Conference and the 7th Meeting of the German Neuroscience Society, p. T19-8C

Klink K, Weik V, Klump GM (2007): The use of two different paradigms in studying comodulation masking release in the mouse (*Mus musculus*), in: Abstracts of the thirtieth annual midwinter research meeting of the Association for Research in Otolaryngology, vol 30, p. 315

Weik V, Klink K, Klump GM (2006) Comodulation masking release determined in the mouse (*Mus musculus*) using a band-narrowing paradigm, in: Abstracts of the twenty-ninth annual midwinter research meeting of the Association for Research in Otolaryngology 29:124

Klink K, Klump G (2005) Duration discrimination in the mouse (*Mus musculus*), in: Abstracts of the twenty-eighth annual midwinter research meeting of the Assiociation for Research in Otolaryngology 28:255

Weik V, Klink KB, Klump GM (2005) Comodulation masking release in the house mouse (*Mus musculus*), in: Proceedings of the 30th Göttingen Neurobiology Conference and the 6th Meeting of the German Neuroscience Society, p. 101B

Klink KB, Bendig G, Klump GM (2004) Operant methods for mouse psychoacoustics, in: Abstracts of the the twenty-seventh annual midwinter research meeting of the Association for Research in Otolaryngology 27:104

Klink KB, Bendig G, Klump GM (2003) Methods for mouse psychoacoustics, in: Elsner N., Zimmermann, H. (Eds.), The Neurosciences from basic research to therapy - Proceedings of the 29th Göttingen Neurobiology Conference and the 5th Meeting of the German Neuroscience Society 2003, Thieme, p. 460

Erklärung

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbstständig verfasst habe und nur die angegebenen Hilfsmittel verwendet habe. Die Dissertation wurde weder in Teilen noch in ihrer Gesamtheit einer anderen Hochschule zur Begutachtung in einem Promotionsverfahren vorgelegt.

Oldenburg, 24.09.2008

.....

Karin B. Klink