Coding of Auditory Signals in Narrowband Neurons in the Inferior Colliculus of the Barn Owl

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Nomenclature

AAr     auditory arcopallium
AN      auditory nerve
ANN     auditory nerve neurophonic
AR      adaptation ratio
BF      best frequency
BF<sub>50</sub> center frequency at half maximum width of the FTC
CF      center frequency
FTC     frequency tuning curve
IC      inferior colliculus
ICC     central nucleus of the inferior colliculus
ICC<sub>core</sub> core of the central nucleus of the inferior colliculus
ICC<sub>ls</sub> lateral shell of the central nucleus of the inferior colliculus
ICX     external nucleus of the inferior colliculus
ILD     interaural level difference
ITD     interaural time difference
LLDa    anterior part of the dorsolateral lemniscal nucleus
LLDp    posterior part of the dorsolateral lemniscal nucleus
LSO     lateral superior olive
MR      masking ratio
MSO     medial superior olive
NA      nucleus angularis
NL      nucleus laminaris
NM      nucleus magnocellularis
NO      nucleus ovoidalis
OT      optic tectum
PSTH    peri-stimulus-time-histogram
RLF     rate-level function
RR      response ratio
SD      standard deviation
SEM     standard error of the mean
SFA     spike-frequency adaptation
SR      spontaneous ratio
τ       time constant
W<sub>50</sub> bandwidth of a FTC at half maximum rate
1 Summary

The barn owl (*Tyto alba*) is a well-known model system for auditory processing and sound localization. Several morphological and neuronal adaptations enable these birds to catch prey even in total darkness solely by using acoustic information such as the rustling of a mouse moving on the ground. Performing this task requires many complex computational processes as to discriminate time differences between both ears in the range of a few microseconds or exploiting level differences of only a few decibels. One of the nuclei involved in auditory processing is located in the midbrain and is called inferior colliculus. This nucleus can be roughly subdivided into a central nucleus (ICC) and an external nucleus (ICX) both with separate functions in neuronal processing of auditory information. Two major goals were pursued in this doctoral thesis. First, an alternative model of sound localization in reference to the Jeffress model was tested. Second, the response properties of ICC neurons to double-stimulation that mimics snapshots of complex signals varying either in temporal or level-dependent manner were recorded. Both approaches served to shed light on the complex processing in the auditory midbrain and to contribute to the long lasting debate on the different competing models of auditory processing.

The first part of this thesis tackles the question of whether an alternative model to the Jeffress model for sound localization could be involved in the encoding of extremely large interaural time differences (ITDs). While the animal’s head size limits the range of naturally experienced ITDs to the so-called physiological range, a neuronal response to ITDs far exceeding this range can be recorded in the IC of barn owls. Although the Jeffress model of sound localization is well established in the auditory system of the barn owl, the model is insufficient to explain the recorded responses to large ITDs. As an alternative to the Jeffress model, the stereausis model was tested for its ability to explain the described responses to large ITDs. This model assumes a systematic mismatch of frequencies between both ears that vary as a function of ITD. For the barn owl, neither the data recorded in ICC nor a comparison of the recorded data with the predictions of a cross-correlation model for sound localization could support the stereausis model.

The second part of the thesis investigated the effect of adaptation in the ICC. When tested with tonal double-stimulation, the response of recorded units to the second stimulus (probe) was generally reduced compared to the response to the first stimulus (masker). This effect is known as response adaptation. Subsequently, it was tested how response adaptation could be compensated for by changing the stimulus paradigm. Two different paradigms were applied: first, the stimulus level of the probe was varied to investigate how much increase in stimulus level was necessary to overcome response adaptation. Second, the interval between masker offset and probe onset was increased from 25 ms to 1600 ms to test for the recovery periods. It turned out that ICC neurons were very sensitive to faint changes in the stimulus level and an increase of about 5 dB was sufficient to release the neurons from adaptation. The temporal recovery could best be described by two time constants, one short time constant of 3.03 ms, and one longer time constant of 300 ms. Additionally, the spike-frequency adaptation in the neuron’s response to the masker and probe stimuli were investigated. Spike-frequency adaptation (SFA) describes the dynamics of the instantaneous spike rate during stimulus presentation. For the elicited spikes at steady state, the responses to the masker stimulus were reduced by about 70% compared to the maximum peak response at stimulus onset. Furthermore, the decay of SFA acted on time scales in the range of tens of milli-
onds. SFA to the probe stimuli was much more complex and generally influenced by the proceeding response to the masker, by the silent interval between the two stimuli and by the intensity of the stimulus.

Thus, the results of this thesis demonstrate that neurons in the barn owl’s ICC are capable to respond to changing stimulus parameters with high precision and the coding properties of these neurons contribute to the underlying processing of auditory signals, which finally are involved in the computation of auditory space needed for precise sound localization.
2 Zusammenfassung


3 General Introduction

It is of fundamental importance for animals to acquire information about their environment to find food, conspecifics or mates. The transduction of external stimuli in the acoustic, visual or chemical modality to neural signals is performed by specialized sensory organs. For instance, the auditory system has evolved high precision in the encoding of acoustic stimuli enabling the precise localization of sound sources (Payne, 1962; 1971; Konishi, 1973a; 1973b; Heffner and Heffner, 1988; May and Huang, 1996; Nelson and Stoddard, 1998; Populin and Yin, 1998; Nelson, 2000; Nodal et al., 2008; Hausmann et al., 2008; Dent et al., 2009; Devore et al., 2009; Hausmann et al., 2009; Singheiser et al., 2010b) or the filtering out of distracting background noises enabling the animal to focus on relevant acoustic information (Jiang et al., 1997; King et al., 2000; Kurt et al., 2008; Takahashi et al., 2008; Jones and Litovsky, 2008; Leech et al., 2009; Lambrecht et al., 2010). The neural coding of stimulus level requires mechanisms that are able to encode a range of up to 120 decibels, which corresponds to an increase of ten or twelve orders of intensity (Baccus, 2006). The hearing range of animals may cover a relatively narrow range of several kilohertz as for the barn owl (Konishi, 1973b; Dyson et al., 1998) or exceed more than twenty kilohertz and extend to the ultrasonic range enabling echo-location like in bats or marine mammals (Kastelein et al., 2003; Heffner, 2003; Au et al., 2004). To cope with many different aspects of acoustic information, specialized neural circuits have evolved and most of them were optimized to dedicated needs according to ecological niches or certain behavior like song learning. One example of the use of auditory information is the high-accuracy approaching and the striking of prey in total darkness by the barn owl. In the following introduction, I will briefly describe the sound localizing abilities of barn owls and give a short overview on the auditory pathway of these birds as well. Due to the outstanding coding properties of neurons in the inferior colliculus, being a prerequisite for barn owl’s extraordinary sound localization ability, this nucleus may be considered the main auditory midbrain nucleus. For a better understanding of why I focused my thesis on the coding properties in IC neurons, I will provide a brief description of its sub-nuclei and the response properties of its neurons. The general overview will be followed by an outline of my aims for this thesis.

3.1 Sound localization in the barn owl

The barn owl (*Tyto alba*) is a predatory bird that is considered an auditory specialist. Due to this ability, barn owls serve are well-known model systems for auditory processing and sound localization. Barn owls are able to approach distant targets even under dim illumination with high accuracy and precision (Payne, 1962; 1971; Konishi, 1973b; 1973a; Hausmann et al., 2008; Singheiser et al., 2010b). The hearing range of barn owls covers the frequencies from about 0.2 to 12 kHz and the lowest thresholds are found between 4-8 kHz (Konishi, 1973b; Dyson et al., 1998). The facial ruff and the asymmetrical arrangement of the ears mediate their outstanding sound localization abilities. The ruff functions as a sound collector guiding the incoming sound directly to the ear canals (Payne, 1971; Coles and Guppy, 1988), hereby enhancing the directionality for the two main cues involved in sound localization (von Campenhausen and Wagner, 2006; Hausmann et al., 2009; Hausmann et al., 2010): interaural time differences (ITDs) and interaural level differences (ILDs). Due to the asymmetrical ears of the barn owl, ITDs change with the horizontal displacement of a sound source whereas ILDs change with a dis-
placement in elevation for high frequencies (Moiseff, 1989a; 1989b; Keller et al., 1998) and with azimuth for low frequencies (Moiseff, 1989b; von Campenhausen and Wagner, 2006). The importance of ITDs on the localization in azimuth has clearly been demonstrated in behavioral studies involving barn owls when manipulated stimuli were replayed to the animals via headphones (Moiseff and Konishi, 1981; Saberi et al., 1998; Poganiatz et al., 2001) whereas the relevance of ILDs could be shown for sound localization in the elevational plane (Poganiatz and Wagner, 2001; Hausmann et al., 2009; Hausmann et al., 2010). To accomplish the computation of these spectral cues specialized and distinct auditory pathways in the owl’s auditory system have evolved. As a consequence, the relevant auditory nuclei are remarkably enlarged in comparison to other birds (Kubke et al., 2004).

### 3.2 The auditory pathway

The sound arriving at the ears is decomposed into individual frequency components at the basilar papilla of the cochlea (Köppl et al., 1993) using hair cells tuned to only a very narrow range of frequencies. This frequency decomposition is in line with a reduction of spatial cues into many narrowband cues consisting only of single frequencies. Nonetheless, the information about spatial position of a sound source is represented in the cochlear output using rate and time codes. The temporal information is maintained by means of phase locking up to 9 kHz in the cochlea (Köppl, 1997a). Nerve fibers of the 8th nerve send this information via bifurcating axonal projections to the two cochlear nuclei (Carr and Bouderau, 1991): nucleus magnocellularis (NM) and nucleus angularis (NA, see Figure 1 for a schematic of the auditory pathway). From this stage of processing up to the convergence in the lateral shell of the central nucleus of the inferior colliculus (ICCl, Adolphs, 1993b), the information of time and level are computed in two distinct pathways (Takahashi et al., 1984). Whereas timing information is preserved by phase-locking to the stimulus phase in NM by responses preferentially occurring in a specific phase of the stimulus cycle (Sullivan and Konishi, 1984; Köppl, 1997b), information of the stimulus level is encoded by NA neurons having large dynamic ranges of spike rates (Sullivan and Konishi, 1984; Köppl and Yates, 1999). While level information is send from NA to the contralateral dorsal lateral lemniscal nucleus pars posterior (LLDp, previously referred to as VLVP, Figure 1), NM projects to nucleus laminaris (NL) (Takahashi and Konishi, 1988a; 1988b). NL neurons are the first site in the auditory pathway where input from both hemispheres converges and binaural information is computed. For this purpose, axons originating in NM project to both to the ipsi- and contralateral NL. These axons act as delay lines. The NL neurons form an array of coincidence detectors (Moiseff and Konishi, 1983; Carr and Konishi, 1988; 1990). Coincidence detector neurons fire maximally when information from both sides arrives simultaneously. Because the delay lines compensate individual interaural delays, spatial information is converted from a time code into a place code. Since this mechanism was first proposed by Jeffress in the middle of the last century (Jeffress, 1948), the model was termed Jeffress model. From NL, the auditory pathway transmits time information to the contralateral LLDa (previously referred to as VLVA) (Takahashi and Konishi, 1988b) and further on to the core of the central nucleus of the inferior colliculus (ICCore) (Moiseff and Konishi, 1983). The next synapse is located in the neighboring lateral shell of the central nucleus of the inferior colliculus (ICCls) where time and intensity pathways converge (Adolphs, 1993b). From ICCls, information about time and intensity is sent to the external nucleus of the inferior colliculus, where space-specific neurons encode the location of a sound source (Knudsen and Konishi, 1978). A more detailed description of the inferior colliculus (IC) will be given in the next chapter of the introduction (3.3). The IC is the major
relay station for acoustic information in the auditory pathway and two distinct branches of projections emerge in ICC (Arthur, 2005). As mentioned earlier, auditory information is send from ICCcore to ICCls and then to the external nucleus of the IC (ICX). From the ICX, the information is projected to the optic tectum (OT) where the auditory map computed in the ICX and the visual map merge (Knudsen and Knudsen, 1983). This so called midbrain pathway is currently thought to be the pathway for precise sound localization (Vonderschen and Wagner, 2009; Singheiser et al., 2010b). Originating in ICC, auditory information is also sent to different nuclei in the forebrain via the nucleus ovoidalis (NO) of the thalamus (Proctor and Konishi, 1997; Cohen and Knudsen, 1998). Upstream of NO information is passed to Field L (Cohen et al., 1998) and then to the auditory arcopallium (AAr) (Cohen and Knudsen, 1995; 1998). In these nuclei, neurons are also sensitive to best frequencies below 3 kHz (Pérez and Peña, 2006; Vonderschen and Wagner, 2009; Pérez et al., 2009). Since no map of auditory space could be shown in the forebrain (Cohen and Knudsen, 1995; 1996) that would be comparable to the map found in the midbrain, and since ITD tuning curves rather represent coarse auditory space (Vonderschen and Wagner, 2009), the forebrain pathway seems to be involved in coarse rather than precise sound localization.

**Figure 1: Auditory pathway.** The afferent auditory pathway is schematically illustrated starting from the auditory nerve upstream to the optic tectum (OT) where the auditory and visual maps merge. Nuclei shown in black transmit only ITD information (black solid lines) whereas nuclei indicated in white are responsible for ILDs only (dashed lines). Nuclei shown in grey are involved in the processing of both ITD and ILD information.
3.3 The inferior colliculus of the barn owl

The inferior colliculus of the barn owl is a large midbrain nucleus dedicated to encoding auditory signals. The ICC consists of four distinct sub-nuclei. Generally, the inferior colliculus may be subdivided into the central nucleus (ICC) and the external nucleus (Knudsen, 1983; Wagner et al., 2003). The ICC may be furthermore differentiated into three sub-nuclei. Going from medial to lateral, the first subnucleus is the medial shell of the central nucleus of the inferior colliculus (ICCms), followed by ICCcore and ICCls (Wagner et al., 2003). Lateral to ICCls is located the ICX, which itself is surrounded laterally by the optic tectum. All sub-nuclei of the IC can be distinguished by their morphology and the labeling of their neurons by different antibodies (Takahashi et al., 1987; Adolphs, 1993a; Wagner et al., 2003) as well as by their physiological responses to different stimuli (for a summary see supplementary material of Wagner et al., 2007). As mentioned before, the time pathway first enters ICCcore. The responses upon stimulation with either pure tones or broadband noise are cyclic and the heights of the peaks are similar (Wagner and Takahashi, 1990; Fujita and Konishi, 1991; Wagner et al., 2002; Bremen et al., 2006; Wagner et al., 2007; Singheiser et al., 2010a). The inter-peak distance in these tuning curves depends on the period of the best (BF) or characteristic (CF) frequency a neuron is tuned to. All neurons in ICCcore are narrowly tuned to frequency (tuning width of about 1 octave for low frequencies and about 1/3 octave for higher frequencies) and represent a clear tonotopy of BFs in the dorso-ventral axis covering the entire audible frequency spectrum of barn owls (Wagner et al., 2002; Wagner et al., 2007; Singheiser et al., 2010a). Neural responses to frequencies as low as 0.2 kHz can be found in the dorsal layers of ICCcore, whereas high frequencies can be recorded more ventrally (Wagner et al., 2002; Singheiser et al., 2010a). Since information about stimulus level converges in ICCls, no responses to varying ILDs can be recorded in ICCcore resulting in flat tuning curves (Wagner et al., 2002; Bremen et al., 2006). Rate-level functions clearly show an increase in spike rate with increasing stimulus level not only to binaural but also to ipsi- and contralateral monaural stimulation (Wagner et al., 2002).

Neurons from ICCcore project to the contralateral ICCls (Takahashi et al., 1989). The responses of ICCls neurons to ITD and frequency resemble those of ICCcore. Again, a cyclic tuning to varying ITDs can be observed (Wagner et al., 1987; Wagner, 1990; Fujita and Konishi, 1991; Bremen et al., 2006). However, unlike ICCcore, ICCls neurons display a strong tuning to varying ILDs. Generally, these tuning curves show a sigmoid or open-peak tuning favoring contralateral ILDs (Adolphs, 1993b). Frequency tuning curves are still narrow and a clear tonotopy is preserved (Takahashi and Konishi, 1986; Wagner et al., 1987). When stimulated monaurally, responses to contralateral stimulation act excitatory, whereas responses to ipsilateral stimulation are inhibitory (Adolphs, 1993b). Outputs from ICCls convey both ITD and ILD information to the external nucleus of the inferior colliculus where the map of auditory space is computed (Knudsen and Konishi, 1978; Knudsen, 1983). ITD and ILD information is combined in a non-linear way in ICCls. The majority of the responses are well described by a multiplicative model indicating that ICCls is the first station in the auditory pathway where multiplicative tuning to ITD and ILD can be observed (Fischer et al., 2007).

The responses of ICX neurons to varying ITDs with broadband stimulation show a reduced response rate of the side peaks relative to the main peak (Takahashi and Konishi, 1986; Wagner et al., 1987; Wagner, 1990; Fujita and Konishi, 1991). ILD tuning curves recorded in ICX are typically bell-shaped (Takahashi et al., 1984). Frequency tuning curves are broad and typically sensitive only to frequencies >2.5 kHz (Knudsen and Konishi, 1978; Mazer, 1998; Wagner et al., 2007). By integrating neural responses across several narrow frequency bands,
the true ITD is enhanced and ambiguous peaks - as observed in ICCcore and ICCIs giving rise to false position encoding of the sound source - are eliminated (Takahashi and Konishi, 1986; Wagner et al., 1987). This across-frequency integration takes place in ICCIs and resembles straightness weighting as proposed by Trahiotis and Stern (Trahiotis and Stern, 1989; Trahiotis and Stern, 1994). In contrast to ICCcore and ICCIs, ICX neurons are exclusively binaurally excitable while monaural stimulation evokes no neural responses (Takahashi et al., 1984; Takahashi and Konishi, 1986).

3.4 Aim of the thesis

The aim of this doctoral thesis was to investigate the role and function of neurons in the barn owl’s ICC that are involved in the processing of auditory information. The thesis is divided in two main chapters each focusing on different aspects in the computation of auditory space and the processing of auditory signals.

The first chapter tackles the question of whether a coding model alternative to the Jeffress model may be implemented in the barn owls ICCcore. The background of this question is the observed sensitivity of neurons in ICCcore to array-specific ITDs that are much larger than the physiological range of barn owls. The Jeffress model is insufficient to answer this question since such ITDs lay outside the barn owl’s physiological range. For this reason, an alternative model - the stereausis model - was tested to explain the coding of these large best ITDs. The question was addressed by recording the responses to broadband ITDs as well as binaural and monaural pure tones in low best-frequency neurons in ICCcore. The results were analyzed with respect to fundamental principles of the stereausis model:

- Does the monaural input of best frequencies to an ICCcore neuron differ between the ipsi- and contralateral input channels and are they furthermore different from the binaural BF? If so, do these inputs correlate with the neurons’ array-specific ITD? This would be expected if the stereausis model were involved into the coding of large best ITDs in ICCcore.

- In a second analysis in this project, the recorded data obtained in ICCcore of the barn owl were fitted to a cross-correlation model. The results of this comparison allowed to draw conclusions about the theoretical implementation of the stereausis model and demonstrated how the correlation between frequency mismatches and array-specific ITD would look like if the stereausis model were implemented.

The second chapter takes a more detailed look on the coding properties of neurons in the barn owl’s ICC when stimulated with tonal double stimuli. In everyday life, auditory signals are composed of many frequencies, the levels of which may vary over time as for example in the rustling of a mouse moving on the ground. Although much is known about the coding properties of neurons in all stations of the auditory pathway when stimulated with a single signal - be it a pure tone or noise - less is known about the response properties of neurons when stimulated with two consecutive pure tone stimuli that vary either in the level or in the interval between both stimuli, thus resemble very narrowband snapshots of broadband signals. The coding properties of neurons in the barn owl’s ICC in response to double stimuli might be relevant for the computation of auditory space in ICX. To shed light on this question, neurons having different best frequencies were recorded and tonal double stimuli of the unit’s best frequency were presented. The level of the first stimulus was presented at five
different levels of the unit’s saturating rate-level-function where it was kept constant. The second stimulus was altered either in its level or the inter-stimulus interval between the first and the second stimulus. The responses of the neurons were analyzed with respect to several open questions:

- How do neurons respond when a second stimulus of the same frequency is presented shortly after the first stimulus? And how does the response to the second stimulus change with respect to the first one? Generally, the response to a consecutive stimulus is expected to be lower than the response to the first stimulus due to suppression or adaptation. This effect is called response adaptation. It was investigated to what extend this reduced firing capability depends on stimulus level or inter-stimulus interval between the two signals.

- The coding of broadband information is not a simple task for a neural network requiring high flexibility of neurons in responding to changes in the stimulus statistics. Therefore, the second point addresses the question of whether the adapted response to a second stimulus was static or whether it could be compensated by changing the properties of the second stimulus. Two different stimulus paradigms were applied to answer this question. In the first approach, it was asked if an increase in the stimulus level was sufficient to release the neurons from response adaptation. The second paradigm was based on the temporal recovery properties, realized by increasing the silent interval between offset of the first and onset of the second stimulus. This paradigm served to answer the question of what minimum time lag was required for the second stimulus to become unbiased by a preceding stimulus.

- The third approach of this chapter focused on the spike-frequency adaptation of the responding neurons. How do neurons in the barn owl’s IC generally respond to pure tones at different stimulus levels? Do they behave differently, for example in their response type (primary-like or tonic) when the stimulus is faint or loud? What are the basic properties for spike-frequency adaptation in a neural response with respect to the temporal decay? Is the degree of spike-frequency adaptation similar in all responses or does it depend on the stimulus level as well?
4 The Stereausis Model – an Alternative Coding Mechanism for ITDs?

4.1 Introduction

At present, the representation of interaural time difference (ITD) is controversially discussed (McAlpine et al., 2001; Brand et al., 2002; Wagner et al., 2002; McAlpine and Grothe, 2003; Harper and McAlpine, 2004; Tollin and Yin, 2005; Joris et al., 2006; Palmer et al., 2007; Wagner et al., 2007; Pecka et al., 2008; Carr et al., 2009; Vonderschen and Wagner, 2009; Leibold, 2010; Pecka et al., 2010; van der Heijden and Joris, 2010). Three possible mechanisms have been suggested for this representation (for a review see Joris and Yin, 2007; Grothe et al., 2010). Briefly, Jeffress (1948) proposed the existence of a network in which many ITDs are represented in an array of neurons. These neurons use the coincident arrival of spikes from the two brain sides to compute ITD in a central-auditory nucleus. External delays are compensated for internally by delay lines arising at the input to the coincidence-detector neurons. A systematic variation in the interaural delays leads to the formation of a map of ITD. I refer to this model as the "place-code model". A second model, the "stereausis model", was proposed by Shamma and colleagues (Shamma et al., 1989). These authors suggested that binaural differences in the excitation of cochlear locations could provide the interaural delays required for coincidence detection. This model does not require central delay lines, but uses a central, binaural nucleus for coincidence detection (for more details see below). Finally, it was pointed out that only two broad, hemispheric channels that act in a push-pull manner may underlie the representation of auditory space (McAlpine et al., 2001). These two channels would also work in a frequency-specific way and would mathematically reflect coincidence-detection at a particular interaural phase difference. I refer to this latter model as the "slope-code model". Harper and McAlpine (Harper and McAlpine, 2004) applied optimal-detection theory to the problem of ITD representation. They predicted an implementation of the slope-code model in the low-frequency range, and a map of ITD in the high-frequency range.

The available data favor the realization of the slope-code model in many mammals (McAlpine et al., 2001; Brand et al., 2002; Harper and McAlpine, 2004; Lane and Delgutte, 2005; Pecka et al., 2008a; Pecka et al., 2010). Cochlear mismatches as assumed for the stereausis model could be shown in the cat for low frequencies (Joris et al., 2006). No evidence for the stereausis model was found in high-frequency neurons (>3 kHz) of the barn owl (Penet al., 2001). Moreover, data from the barn owl were in line with the place-code model, but did not support the predictions of the slope-code model (Sullivan and Konishi, 1984; Carr and Konishi, 1988; 1990; Wagner et al., 2007). It remained unclear, however, why in low-frequency neurons (<3 kHz) of the barn owl array-specific ITDs may occur outside the physiological ITD range of this bird (> ±250-280 µs; (Keller et al., 1998; Poganiatz et al., 2001; von Campenhausen and Wagner, 2006; Wagner et al., 2007; Vonderschen and Wagner, 2009; Hausmann et al., 2009). This led to the question of whether these large delays in the low-frequency range may be caused by stereausis.

A prerequisite for coincidence detection in the stereausis model is a central array of coincidence-detector neurons that receives direct or indirect binaural input from the tonotopically organized ipsi- and contralateral basilar membranes (Figure 2). Sound entering the cochlea elicits a traveling wave along the basilar membrane
that first reaches and excites locations representing high frequencies at the base and subsequently locations representing low frequencies at the apex (von Békésy, 1960; Gummer et al., 1987). A matrix of binaural coincidence detectors receives phase-locked inputs from both ears via the auditory nerve and the cochlear nucleus. These inputs are snapshots of the excitation pattern of both basilar membranes and depend on the ITD of the stimulus (Figure 2). At the coincidence detector, the instantaneous spatial cross-correlation between the simultaneous input patterns from the two ears is computed. If there is no time disparity between the input to the two ears at the basilar membrane, the travelling waves of both basilar membranes are identical (Figure 2A bottom), and coincident input occurs at the diagonal of the stereausis network according to the characteristic frequencies (CF) of each basilar membrane. A time disparity between both ears (Figure 2B) leads to a frequency disparity at the two basilar membranes, since the ipsilateral ear becomes excited slightly earlier than the contralateral ear. Thus, the travelling wave in the contralateral ear is delayed in respect to the ipsilateral ear and the excitation pattern of the basilar membranes is phase shifted (Figure 2B bottom). This creates a spatial mismatch between the two input patterns in the stereausis network that can be used to estimate the spatial position of a sound source.

**Figure 2: Stereausis model.** The cross-correlation between the simultaneous input patterns from the two ears is computed by this network. A matrix of binaural coincidence detectors receives phase-locked input from the basilar membrane of both ears via the auditory nerve and cochlear nucleus. These inputs depend on the interaural time difference (ITD) of the stimulus (A: no ITD (0 µs), B: negative ITD). If there is no time delay between the ears, the travelling waves of the basilar membrane are identical (A bottom) and coincident input occurs at the diagonal of the stereausis network (open symbols and solid arrow) according to the characteristic frequencies (CF) of the basilar membrane. If there is a time delay between both ears (B), the excitation pattern of the basilar membrane is phase shifted (B bottom). This creates a spatial mismatch between the two input patterns in the stereausis network that can be used to estimate the spatial position of a sound source. Note that the maximum activation shifts to the ipsilateral side (open symbols and solid arrow). Figure adapted from Shamma et al. 1989.
In birds, hair cells are contacted by eighth-nerve fibers that send their afferent information to the cochlear nucleus. The cochlear nucleus magnocellularis (NM) projects bilaterally to nucleus laminaris (NL), the first binaural nucleus in the ascending auditory pathway. NL is the station in which coincidence detection takes place in distinct frequency bands (Sullivan and Konishi, 1984; Young and Rubel, 1986; Carr and Konishi, 1988; 1990; Seidl et al., 2010; Slee et al., 2010). NL projects to the core of the central nucleus of the inferior colliculus (ICCcore) (Takahashi and Konishi, 1988a). ICCcore neurons exhibit a reduction in response variability compared to NL neurons (Pena et al., 1996; Wagner et al., 2002), but otherwise resemble in their responses NL neurons, specifically with respect to both frequency and ITD tuning (Carr and Konishi, 1990; Pena et al., 1996; Viete et al., 1997; Wagner et al., 2002). Because of the similarity of response properties between NL and ICCcore, it was possible to analyze low-frequency ITD tuning in the owl using activity recorded in ICCcore.

I investigated whether the stereausis model could account for the observed frequency and ITD sensitivity of neurons with low best frequencies (BF) in the barn owl’s ICCcore. The data presented in the following argue against such a possibility.

### 4.2 Methods

#### 4.2.1 Owl handling and surgery

The data were obtained from four barn owls (*Tyto alba pratincola*) of both sexes taken from the breeding colony of the Institute for Biology II at RWTH Aachen University. All procedures were in accordance with the National Institutes of Health guidelines for animal experimentation and approved by the Landespräsidium für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen, Recklinghausen, Germany. On the day prior to an experiment, the weight of the owl was monitored, and the bird did not receive any food but had free access to water. On experimental days the owl’s weight was controlled again. Sedation was introduced by applying an intramuscular injection of diazepam (Valium, 1 mg/kg, Ratiopharm, Ulm, Germany). Anesthesia was initiated after a waiting period of about 30 minutes by applying an intramuscular injection of ketamine (30 mg/kg, Sanofi-Ceva, Düsseldorf, Germany). A single dose of atropine sulfate (0.065 mg/kg, B Braun AG, Tuttingen, Germany) was administered to prevent saliva secretion during anesthesia. Buprenorphine (Temgesic, 0.06 mg/kg, Essex Pharma GmbH, Munich, Germany) was injected intramuscularly as an analgesic.

As described earlier, a metal head plate was fixed onto the skull of the owl under anesthesia before it was used in electrophysiological experiments (e.g. (Vonderschen and Wagner, 2009). During experiments, the posterior edge of the head plate served as a stereotactic zero coordinate for both the rostro-caudal as well as the medio-lateral axis. To allow penetrations of the electrodes, a craniotomy was made over the recording site, which was sealed with petroleum jelly (Vaseline) during experiments and which was covered with antibacterial ointment (Nebacetin, Astellas Pharma, Munich, Germany) and dental cement (Paladur, Heraeus Kulzer, Wertheim, Germany) after the experiment had been terminated. During experiments, anesthesia was maintained with intramuscular injections of diazepam and ketamine as required. After terminating the experiment, a second injection of buprenorphine was administered before the skull was sutured and the wound was treated with antibacterial ointment (Nebacetin). The owl was removed from the setup and brought to a recovery box where it was provided
with food and was easy to monitor. Only when fully awake and showing no evidence of discomfort, the owls were returned to their aviary. Each owl was used more than once.

4.2.2 Signal generation and data acquisition

Experiments were conducted in a soundproof anechoic chamber (IAC 403A, Industrial Acoustic, Niederkrüchten, Germany). Acoustic signals consisted of 100 ms long dichotic noise or pure tone signals with 5 ms on- and offset cosine ramps. Signals were generated using Visual C++ software (Microsoft Corporation, Redmond, WA, USA) in combination with Brainware (Tucker-Davis-Technologies (TDT), Alachua, FL, USA) on a personal computer (PC). Digital-to-analog (DA) conversion was maintained using a DA converter (DA 34, System II, TDT) at a sampling rate of 50 kHz. DA-converted signals were individually attenuated for the left and right channel by programmable attenuators (PA4, TDT). Anti-alias filtering of the signals was done using a FT6 (TDT). Finally, the signals were power-amplified (Yamaha AX 590) and presented through calibrated earphones (Sony MDR-E831LP). Epoxylite-insulated tungsten microelectrodes (18-25 MΩ, FHC, Bowdoinham, ME, USA) were used for recording. The recorded electrophysiological signals were pre-amplified (M. Walsh Electronics, Pasadena, CA, USA), impedance matched, amplified and filtered (50 Hz notch filter; 300 Hz – 5000 Hz band pass filtered; M. Walsh Electronics, Pasadena, CA, USA), analog-digital converted (25 kHz, AD1, TDT) and read into a PC. Pre-analysis of the data and on-line spike sorting was done with Brainware (TDT). Final data analysis was performed off-line with self-written Matlab routines (MathWorks, Natick, MA, USA) and GraphPad Prism (La Jolla, CA, USA).

4.2.3 Stimulation protocol

Broadband noises (0.1-20 kHz) or tonal signals were used for stimulation. Cells were initially characterized by their responses to ITD ("ITD tuning curve", varying step size, positive ITD indicates right ear leading), ILD ("ILD tuning curve", -20 to 20 dB, step size 4 dB, positive ILDs refer to right ear louder), frequency ("iso-level frequency response function" or frequency-response curve, 200 to 9200 Hz, step size 500 Hz), and level ("RLF function", binaural and monaural paradigm, average binaural level at best ITD and ILD between 0 and 72 dB attenuation). During the recordings, the stimuli were presented once per second in random order. Each stimulus was repeated five to ten times. These recordings served to classify a response as originating from ICCcore (for details see next section)

After this initial physiological characterization of the unit, recordings of higher resolution were performed for further analyses: ITD step size was adjusted in accordance to the unit’s BF and varied from 30 to 250 µs so that four to six ITDs per period were presented. Furthermore, frequency-response curves were recorded with a step size of either 50 or 100 Hz to achieve a high resolution of the resulting frequency-tuning curve. The average level for binaural as well as monaural frequency-response curves was kept ~30 dB above threshold of the unit’s BF similar to Pena et al. (Pena et al., 2001).
4.2.4 Physiological characterization of the location of recording

All recordings were made from the ICCcore. Neurons were recorded from both sides of the brain. Coarse targeting of the nucleus was achieved by known stereotactic coordinates in reference to the head plate (~8-9 mm caudal, ~2-4 mm lateral, 12-15 mm below brain surface). The final classification was achieved by comparing the response characteristics with established physiological criteria for the ICCcore and for neighboring nuclei (lateral shell of the central nucleus of the inferior colliculus: ICCls, medial shell of the central nucleus of the inferior colliculus: ICCms, external nucleus of the inferior colliculus: ICX; see supplementary material in Wagner et al. 2007). In brief, both ICCcore and ICCls show a cyclic ITD-tuning curve to stimulation with broadband noise with no suppression of the side peaks in comparison to the main peak, while ICX neurons exhibit side-peak suppression. Neurons in ICCms are not tuned to ITD. ILD-tuning curves in ICCcore are flat, whereas ICCls neurons display a sigmoid ILD tuning favoring contralateral ILDs. Frequency-response curves in ICCls and ICCcore show a tonotopy from low to high frequencies with increasing recording depth and a tuning width, typically in the range of 1/3 of an octave at frequencies above 1.5 kHz (see below). Stimuli from both ears excite neurons in ICCcore (EE property), whereas neurons in ICCls show excitation to contralateral stimulation and inhibition to ipsilateral stimulation (EI property). Along the frequency axis neurons in ICCcore and ICCls exhibit functional ITD arrays. The response peak shared by all neurons in a dorso-ventral penetration, the array-specific ITD, served as a criterion for the representation of a given ITD in a neuron. The sign of the array-specific ITD corresponds to the sign of the stimulus ITD for neurons in ICCcore ("ipsi arrays"), while the signs are different for neurons in ICCls ("contra arrays"). The array-specific ITD was used in the final classification, because it had turned out earlier to be the most stable classification criterion (Wagner et al. 1987, 2002, 2007).

4.2.5 Data analysis

Several characteristics of a cell’s response were determined: 1) the average spontaneous discharge rate recorded during the 400 ms period preceding the stimulus, 2) the average response rate during stimulation minus the spontaneous rate. The response was calculated in an interval of 100 ms after stimulus onset and compensation for the response latency. 3) Frequency-response curves were characterized by three parameters. The first parameter was the BF of the unit, which is the frequency eliciting maximum response in the frequency-response curve. The width at half height of the tuning curve (W50) was also calculated. W50 corresponds to the range of frequencies over which the discharge rate of the unit corresponds to at least 50% of the maximum discharge rate (Pena et al. 2001; Wagner et al. 2002). Since BF may depend on sampling resolution, the center frequency at 50% of the maximum discharge rate (BF50) was calculated as well.

4.2.6 Computational model

A cross-correlation model was used to determine the predicted relationship between ITD and bilateral frequency mismatch under the stereausis model (Shamma et al., 1989). The cross-correlation model was applied in two ways. First, the model was used to compute the predicted ITD given the measured monaural best frequencies. Second, the measured ITD and ipsilateral best frequency were used to compute the predicted contralateral
best frequency, and therefore the predicted bilateral frequency mismatch. For each prediction, ITD-sensitive neurons were modeled using a modified version of the Bonham and Lewis (Bonham and Lewis, 1999) cross-correlation model, as in Pena et al. (2001).

To compute the predicted ITD for a given bilateral frequency mismatch, an input signal was filtered with two gammatone impulse responses and the resulting signals were cross-correlated. The input stimulus was a broadband noise signal with a flat spectrum between 0 and 8 kHz at a sampling rate of 100 kHz, which as generated on a computer and directly applied to the model. The ipsilateral and contralateral gammatone filters had impulse responses given by

\[ g_{ipsi}(t) = (t - t_d(f_i))^5 \exp(-(t - t_d(f_i))/\tau(f_i)) \sin(2\pi f_i(t - t_d(f_i)))H(t - t_d(f_i)) \]  \hspace{1cm} (Eq. 1)

and

\[ g_{contra}(t) = (t - t_d(f_c))^5 \exp(-(t - t_d(f_c))/\tau(f_c)) \sin(2\pi f_c(t - t_d(f_c)))H(t - t_d(f_c)) \]  \hspace{1cm} (Eq. 2)

respectively, where \( H(t) \) is the unit step function. The cochlear delay is a frequency-dependent parameter that was taken from latencies of auditory nerve fibers in the barn owl and is given by \( t_d(f) = 3.04 - 0.22 \ln(1000f) \) (Köppl, 1997a). The frequency-dependent time constant \( \tau \) was taken from Carney and Yin (1988) as \( \tau(f) = 1.3(f / 0.456 + 0.8)^{-2.985} + 0.4(f / 0.456 + 0.8)^{-0.347} \) (Figure 3A, B). The time \( \tau \) is given in ms, and the frequency \( f \) is given in kHz. The cross-correlation was performed in Matlab (MathWorks, Natick, MA) using the function xcorr. Because the input signals were not purely sinusoidal, the cross-correlation had a single dominant peak and the best ITD was given by the ITD at the peak of the cross-correlation.

To derive the predicted bilateral frequency mismatch for a given ITD, the mapping between ITD and bilateral frequency mismatch was computed for a fixed value of the ipsilateral best frequency. The value of the ipsilateral best frequency was fixed at the measured value and, for a range of values of the contralateral best frequency, computed the ITD at the peak of the cross-correlation as described above. This produced a mapping between ITD and contralateral best frequency, or equivalently between ITD and bilateral frequency mismatch. The predicted value of the contralateral best frequency under the stereausis model was found by interpolating this mapping at the measured ITD. The predicted bilateral frequency mismatch was then given by the difference between the measured ipsilateral best frequency and the predicted contralateral best frequency.
Figure 3: Cochlear and neural delays. A: Computation of cochlear delays using frequency-dependent latencies of primary auditory nerve fibers of barn owls (Köppl, 1997a) and the model introduced by Carney and Yin (Carney and Yin, 1988). The cochlear delays change as a function of best frequency. This frequency-dependent change is the basis of the stereausis model. Note that the change in delay is larger for low frequencies. B: Prediction of the required frequency mismatch to code a certain ITD. BF was changed in steps of 0.5 kHz from 3 kHz to 8 kHz.

4.3 Results

For this project, recordings from 44 sites in the low-frequency region (<3 kHz) of the ICCcore of four barn owls were collected. Of these 44 sites, 11 were well-isolated single units whereas the rest were classified as multi-units all showing homogeneous spike forms. The responses of single and multi-units were similar and showed no significant differences in the tuning parameters analyzed. Therefore, the data sets of the units were pooled for all analyses. In the following section, I will first describe the general tuning properties of the recorded units, and then compare the obtained results with predictions of the stereausis model.

4.3.1 General response properties

To determine whether binaural cochlear delays play a role in the representation of ITD, both ITD and frequency tuning are important. Therefore, the responses of the individual cells were characterized when ITD or frequency was varied. Generally, when the ITD of a broadband stimulus was altered, the neurons exhibited cyclic ITD tuning (Figure 4A, E). The period of the cyclic response was close to the period derived from the best frequency (BF) of the neuron (1/BF, see dashed lines in Figure 4A, E). The analyses were performed with the best ITD derived from the ITD shared by all neurons in a dorso-ventral array, termed the array-specific ITD (Wagner et al. 1987, 2007). Also the ITDs that were closest to zero in the ITD-tuning curves were applied for comparative analyses (data not shown). Generally, similar results with the latter ITD sample as with the array-specific ITDs were found.
Frequency-response curves typically exhibited a clearly identifiable peak with steep slopes on both sides of the BF (Figure 4B, D, F-H). The frequency at the peak response was termed the BF of a neuron. Stimulation with frequencies far away from BF sometimes evoked inhibition, as indicated by the negative spike rates in Fig. 2. Binaural frequency-response curves (Figure 4B, F) were generally more similar to contralateral (Figure 4D, H) than to ipsilateral response curves (Figure 4C, G).

Figure 4: Examples of tuning curves. A-D: A multi-unit (O21U163) with a very low best frequency (BF) of 0.4 kHz (B) and an extreme array-specific ITD of -1000 µs (A). E-H: A multi-unit (O72U006) with a BF of 2.1 kHz (F) and an array-specific ITD of -60 µs (see inset in E). The ITD tuning of both units is cyclic when stimulated with noise (A and E). Iso-level frequency response functions (ILFRF, frequency-response curve) obtained with binaural (B and F), ipsilateral (C and G) or contralateral (D and H) stimulation. In the upper case, BF_{ipsi} (0.6 kHz) and BF_{contra} (0.4 kHz) differed by 0.2 kHz, whereas in the lower case, BF_{ipsi} (2.3 kHz) and BF_{contra} (2.4 kHz) differed by 0.1 kHz and were slightly higher than the binaural BF (2.1 kHz). The definitions of BF, BF_{50} and W_{50} are given in H. Note that the spontaneous activity was subtracted from the driven responses, yielding negative spike rates in some cases that indicated inhibition (grey dotted lines). The dashed lines in A and E specify the duration of a period as predicted from the binaural BF. The solid lines in B-D and F-H denote W_{50}.

4.3.2 Distribution of best ITDs

In the stereausis model, the required frequency mismatch depends on the best ITD. Therefore, I attempted to record neurons with different best ITDs. In the sample, 23 units had their array-specific ITD peak within the barn owl’s physiological ITD range (±280 µs; Keller et al., 1998; von Campenhausen and Wagner, 2006; Hausmann et al., 2009). In the remaining 21 units the array-specific ITD was outside the physiological ITD range. Extreme best ITDs were as low as -1000 µs or as high as +800 µs (Figure 5). The units with array-specific ITDs outside the physiological ITD range were particularly interesting, because clear frequency mismatches were expected if stereausis would be responsible for the shift of the best ITD away from 0 µs.
Figure 5: Distributions of array-specific ITDs and best frequency. A: The number of neurons binned according to their array-specific ITD (bin size of 100 µs). Twenty-three neurons had their array-specific ITD within the physiological range of the barn owl (±280 µs) whereas twenty-one units had their best ITD outside this range. B: BF$_{50}$ [kHz] of each neuron is shown as a function of BF [kHz]. The slopes for the regression lines are plotted in the inset. C: Distribution of BF$_{50}$ values for the three stimulus paradigms (bin size 0.2 kHz; black: binaural; dark gray: ipsilateral; light gray: contralateral). The numbers on the x-axis in A and C denote the centers of the bins.

4.3.3 Measures and distribution of best frequency

The two measures of best frequency, BF and BF$_{50}$, were not significantly different for all stimulus paradigms (Figure 5B; binaural: P=0.626 (Wilcoxon matched-pairs test); ipsilateral: P=0.426; contralateral: P=0.226 (paired t-test for monaural paradigms)). Additionally, when BF$_{50}$ was plotted as a function of BF, the slopes of the linear regression for all comparisons were close to one (see inset in Figure 5B) and the correlations between BF$_{50}$ and BF were high (binaural: $R^2$: 0.977; ipsilateral: $R^2$: 0.988; contralateral: $R^2$: 0.973). Since BF and BF$_{50}$ were almost identical, the results indicated that frequency-tuning curves typically showed a highly symmetric shape (Figure 4B, D, F-H). For all further analyses the BF$_{50}$ values were exclusively used.

The BF$_{50}$ values followed a normal distribution for the monaural stimulus paradigms (Kolmogorov-Smirnov test, all P>0.05), but not for the binaural paradigm (Figure 5C; Kolmogorov-Smirnov test, P=0.033). A Kruskal-Wallis test yielded no significant differences in the median of BF$_{50}$ values for all paradigms (P=0.921). Best binaural frequencies in this sample ranged from 0.41 to 2.79 kHz.
4.3.4 Comparison of single and multi units

When the discharge rates of the whole sample were considered and single and multi-units were compared, no significant differences in discharge rate between these two populations (t-test for the binaural paradigm and Mann-Whitney tests for the monaural paradigms, all P>0.05) were found (Figure 6A). Nonetheless, standard errors of the mean (SEM) for single units were larger than for multi units (Figure 6A) indicating a higher variability in single units and more stable response rates in multi units. However, differences in discharge rates evoked by binaural or monaural stimulations were obvious in some cases (Figure 4B-D and F-H, Figure 6A, B) and discharge rates evoked by binaural and ipsilateral stimulation, as well as by contralateral and ipsilateral stimulation, differed significantly (Figure 6B, Wilcoxon matched-pairs test, P<0.004), with ipsilateral responses being lower. No significant difference was found between binaural and contralateral response rates (Figure 6B, Wilcoxon matched-pairs test, P=0.104).

Response latencies between single and multi-units (Figure 6C), as well as between binaural and monaural paradigms (Figure 6C, D) were not significantly different (Mann-Whitney and t-tests, Kruskal-Wallis and Dunn’s post-test, all P>0.05). Nonetheless, latencies at best frequencies for both monaural paradigms were slightly longer than for the binaural paradigm in multi and single units (Figure 6C, D). Pooled latencies were the following (mean ± SEM): binaural: 8.45 ±0.51 ms; ipsi: 9.81 ±0.71 ms; contra: 10.19 ±0.66 ms.

Figure 6: Response rates and latencies of frequency tunings. A: response rates (mean ±SEM) of multi and single units for the three stimulus paradigms. Response rates between single and multi units were not significantly different for each stimulus paradigm. B: Pooled response rates (mean ±SEM) of single and multi units. Response rates for the ipsilateral paradigm were significantly lower than the binaural (Kruskal-Wallis and Dunn’s post-test, P<0.01) and the contralateral paradigm (Kruskal-Wallis and Dunn’s post-test, P<0.05). C: Response latencies (mean ±SEM) for single and multi-units in the stimulus paradigms tested. Again, no significant differences occurred between single and multi units. D: Pooled latencies (mean ±SEM) for three stimulus paradigms. No significant differences were found.
4.3.5 Frequency tuning width and best frequency

In a next step it was analyzed whether there existed a relationship of frequency tuning width \( W_{50} \) and BF_{50} as has been shown in for different nuclei in the owl (Pena et al., 2001; Wagner et al., 2002) and other model systems (Semple and Kitzes, 1985). Figure 7A-C shows that \( W_{50} \) increased almost linearly with increasing BF_{50} (A binaural; B ipsilateral; C contralateral). Since no differences in tuning parameters between single and multi units have been found, the dataset was pooled for this analysis. For the binaural condition (Figure 7A), the linear regression yielded a positive correlation and \( W_{50} \) increased with increasing BF_{50}. The slope of the linear regression was \( y=0.2408x+0.5 \) and an ANCOVA analysis confirmed a slope significantly different from zero (P<0.0001). The coefficient of determination \( R^2 \) was 0.447. Comparable observations were made for the contralateral paradigm (Figure 7C). The slope of the linear regression was significantly different from zero (ANCOVA, P<0.0001) but the slope of the linear regression was slightly less steep than for the binaural paradigm (\( y=0.2056x+0.322 \)). Also, the coefficient of determination was lower compared to the binaural paradigm (\( R^2: 0.323 \)) indicating a higher variability of the data. When frequency-tuning widths for the ipsilateral paradigm (Figure 7B) were analyzed, the results confirmed the trend of broader frequency tunings with increasing BF_{50}. However, the slope of the linear regression was shallower compared to both the binaural (Figure 7A) and contralateral paradigm (Figure 2C): \( y=0.0598x+0.459 \). Furthermore, the slope was not significantly different from zero (ANCOVA, P=0.078) and the coefficient of determination was also small (\( R^2: 0.072 \)).

In a last step, it was tested whether \( W_{50} \) values were significantly different between the three stimulus paradigms. Mean and SEM frequency tuning width was broadest in the binaural paradigm (0.6 ± 0.039 kHz) followed by the contralateral (0.453 ±0.04 kHz) and ipsilateral paradigm (0.319 ±0.026 kHz). Frequency tuning widths differed significantly between the three stimulus paradigms (Kruskal-Wallis and Dunn’s post-test, P<0.0001): ipsilateral mean and median \( W_{50} \) was significantly narrower compared to both the binaural (Dunn’s post-test, P<0.001) and contralateral (Dunn’s post-test, P<0.05) paradigm, whereas no difference could be found between the binaural and contralateral paradigm (Dunn’s post-test, P>0.05). As may be seen in Figure 7, maximum \( W_{50} \) values for the binaural (Figure 7A) and the contralateral (Figure 7C) condition were in the range of 1.1 kHz whereas in the ipsilateral (Figure 7B) paradigm maximum frequency tuning width was in the range of 0.8 kHz, and the distribution was generally shifted to narrower tuning widths.

Generally spoken, frequency tuning widths increased linearly with increasing BF_{50} and compared to binaural stimulation, frequency tunings were narrower for monaural stimulation. However, the quality factor (ratio of \( W_{50} \) to BF_{50}) for the binaural as well as for both monaural paradigms decreased with increasing BF_{50} (Figure 7D). The decrease was strongest for the binaural condition (black squares) followed by contralateral (light gray circles) and ipsilateral stimulation (dark gray circles).
Figure 7: Relationship of $W_{50}$ and $BF_{50}$. Frequency tunings widths [kHz] increased with increasing $BF_{50}$ [kHz]: A binaural, B ipsilateral, C contralateral. The slopes of the linear regressions are indicated in each sub-figure. Binaural (A) and contralateral (C) frequency tuning widths were more similar than ipsilateral tunings widths (B). D: The quality factor ($W_{50}/BF_{50}$) decreased with increasing $BF_{50}$ for all stimulus paradigms tested and was therefore negatively correlated.

4.3.6 Relationship of ITD on frequency tuning width

Since the data showed a broadening of frequency tuning curves with increasing $BF_{50}$, it was also interesting to investigate a possible effect of ITD on $W_{50}$. The data in Figure 8A showed a significant decrease of frequency-tuning width with the absolute value of the array-specific ITD. At zero array-specific ITD the mean frequency-tuning width, as indicated by the $y$-increment in the linear regression, was 0.713 kHz. The regression had a negative slope (-0.35 kHz/1ms). The linear regression explained 12% of the variability. For the further analysis the sample was divided in two populations (Figure 8B). One population included all units with an array-specific ITD within the barn owl’s physiological ITD range of ±280 µs. The other population was composed of all neurons having their array-specific ITD outside this range. The population with array-specific ITDs within the physiological ITD range had a statistically broader $W_{50}$ (mean: 0.711 kHz) than the population with the best ITDs outside the physiological ITD range (mean: 0.478 kHz; t-test, P=0.0021).

It was also tested whether $W_{50}$ values were significantly different between the binaural and the two monaural stimulus paradigms (Figure 8B). The sample was divided into the two populations with their array-specific ITD either within or outside the physiological ITD range. When frequency-tuning widths were analyzed for the population with their array-specific ITDs within the physiological ITD range, $W_{50}$ differed significantly between the three paradigms (ANOVA and Bonferroni post-tests, P<0.0001). To find out whether $W_{50}$ between all three paradigms differed or whether only particular combinations yielded differences, post-tests were carried out.
These tests revealed that $W_{50}$ for the ipsilateral paradigm was narrower than $W_{50}$ for the binaural and contralateral paradigm (Figure 8B), and no significant differences were found between binaural $W_{50}$ and contralateral $W_{50}$. Similar results were found for the population having their best ITD outside the physiological ITD range (Figure 8B; Kruskal-Wallis test and Dunn’s post-tests, $P=0.0058$). Also for units having their best ITD outside the physiological ITD range a significant difference ($P<0.01$) was found for a comparison of the binaural and the ipsilateral $W_{50}$ with the post-tests.

Figure 8: Effect of array-specific ITD on $W_{50}$. A: Binaural frequency tuning width [kHz] decreased with absolute values of array-specific ITD [µs]. $W_{50}$ was significantly broader for ITDs within the physiological range compared to ITDs outside the physiological range. B: Comparison of binaural and monaural $W_{50}$ for ITDs inside and outside the physiological range. Box-and whisker plots denote the median values, the lower and upper 25 and 75 percentile as well as minimum and maximum values.

4.3.7 Frequency mismatch between contralateral and ipsilateral inputs

The stereausis hypothesis predicts a frequency mismatch between the contralateral and ipsilateral inputs that depends on both the BF and the best ITD of a particular neuron (Shamma et al., 1989). For an ITD of 0 µs, the BF$_{50}$ in the ipsi- and contralateral frequency-response curve should be almost identical. For best ITDs different from zero the expected frequency mismatch between the ipsi- and contralateral side should increase with the absolute values of the best ITD. Generally no significant differences in BF$_{50}$ between ipsi- and contralateral stimulation (paired t-test, $P=0.0558$) could be observed. When the frequency-tuning widths in the two populations introduced in the last section were compared (Figure 9A) it turned out that neurons that had their array-specific ITD outside the physiological range typically had BF$_{50}$ below 2 kHz, and, in fact, most best-frequencies (14 of 21) were below 1.0 kHz. In contrast, the BF$_{50}$ of neurons with an array-specific ITD within the physiological ITD range were scattered over a range of BFs higher than 1 kHz. The regression analysis between the ipsi- and contralateral BF$_{50}$ yielded the following results (in kHz): $BF_{50\text{contralateral}}=0.84*BF_{50\text{ipsilateral}}+0.4448$ for neurons with an array-specific ITD within the physiological ITD range ($R^2: 0.68$). Since the slope (0.84) was lower than 1, the BF$_{50}$ of the inputs from the two sides became more similar as BF$_{50}$ decreased. A similar relation held for the neurons having their array-specific ITD outside the physiological ITD range: $BF_{50\text{contralateral}}=0.9063*BF_{50\text{ipsilateral}}+0.1138$ (kHz, $R^2: 0.97$). Below 1 kHz, there were almost no differences between the ipsi- and contralateral inputs.
Next, the frequency mismatches of the same neurons were compared, but the neurons were separated into populations of neurons with either negative or positive array-specific ITDs (Figure 9B). The neurons with negative best ITDs had a mean frequency mismatch (BF\textsubscript{50\,ipsi} – BF\textsubscript{50\,contra}) of -0.0012 kHz and a median mismatch of -0.0362 kHz. Similar results were found for the neurons with positive best ITDs. Here, mean and median frequency mismatches were 0.1047 kHz and -0.0754 kHz, respectively. A Mann-Whitney test revealed no significant differences (P=0.770) between frequency mismatches of units with negative and positive array-specific ITDs. This finding was supported by the slopes as well as the y-intercepts of the linear regressions for both samples, which did not differ significantly (ANCOVA, P>0.05).

Figure 9: Relationship between ipsilateral and contralateral inputs. A: Open gray diamonds represent units with their array-specific ITD within the physiological range of the barn owl. Open black circles show neurons with their best ITD outside this range. Solid lines show the linear regression for both populations, respectively. The slopes were not significantly different and the correlation in both populations was high (R\textsuperscript{2} >0.68). The regression equations are given in the inset. B: Gray downward oriented triangles represent units with negative array-specific ITDs, while black open squares show neurons with positive array-specific ITD. Linear regressions are depicted color-coded as well. The slopes were not significantly different, and the correlation in both populations was high (R\textsuperscript{2} >0.80). The regression equations are given in the inset.

In a further analysis, the measured frequency mismatch (BF\textsubscript{50\,ipsi} – BF\textsubscript{50\,contra}) was plotted as a function of measured array-specific ITD (Figure 10). The correlation between frequency mismatch and array-specific ITD was low (R\textsuperscript{2}: 0.02) and no systematic change of frequency mismatch with ITD could be observed.

Figure 10: Frequency mismatch in ICCcore neurons. The frequency mismatch [kHz] between the left and right ear is plotted as a function of array-specific ITD [ms] and is not correlated with the measured ITD (R\textsuperscript{2}: 0.02).
4.3.8 Implications of the stereausis model

A cross-correlation model was used to quantitatively investigate whether the coding of best ITDs as demonstrated so far could be achieved by a computation as described in the stereausis model (Shamma et al., 1989; Pena et al., 2001; see Materials and Methods for further details). In a first approach, the BF_{50} and the measured mismatches in BF_{50} between the ipsi- and contralateral frequency-response curves of a particular neuron were used as input to the model. From these parameters, the best ITD under the stereausis model was predicted. The predicted and measured best ITD showed a very weak correlation (R^2: 0.06; Figure 11A). Moreover, the predicted and the measured ITDs often showed different signs (34% of all recording sites). In general, the stereausis model predicted best ITDs that were much closer to 0 µs than the actually measured best ITDs.

In a second step, the same model was applied using the measured array-specific ITD and the best frequency of a neuron as input. The frequency mismatch in BF_{50} required for stereausis to code the neuron’s particular best ITD was calculated. Since the cochlear representation of best delays depends on the logarithm of the best frequency, neurons having higher BF_{50} require a larger mismatch in frequency compared to neurons with lower BF_{50} if the ITD is held constant. For small ITDs, the stereausis model predicts a small mismatch in frequency. In contrast, the measured mismatches varied over a large range (Figure 11B). For example, in unit O72U019 (Figure 11B, black filled circle), having a small array-specific ITD (30 µs) and medium BF (binaural BF_{50}: 1.91 kHz) the measured mismatch was 1.02 kHz whereas the predicted mismatch was 0.06 kHz. On the other hand, in neuron O72U054 (Figure 11B, gray filled circle), which had a similar BF_{50} (binaural BF_{50}: 2.05 kHz) and an array-specific ITD of ~300 µs, the measured frequency mismatch was 0.06 kHz, whereas the model predicted a mismatch of ~0.31 kHz. Again, measured and predicted frequency mismatches often were of different signs (31.82% of all recording sites). For example, unit O72U80 had a positive array-specific ITD of 800 µs and a negative measured frequency mismatch, whereas unit O21U163 had a negative ITD of ~1000 µs but a positive measured frequency mismatch. In total, only 1.4% of the variability between measured and predicted frequency mismatches could be explained by a linear regression.

Figure 11: Comparison of the data with the stereausis model. A: Predicted ITDs [µs] as a function of the measured array-specific ITDs [µs] in the sample of 44 ICCcore neurons. B: Comparison of recorded [kHz] and predicted frequency mismatches [kHz]. Unit O72U19 is indicated by filled black circle, while unit O72U54 is indicated by the filled gray circle. The gray lines in both sub-figures resemble lines of identity.
Stereausis predicts large frequency mismatches for units with best ITDs outside the physiological ITD range. However, the measured frequency mismatches were typically in the same range as for the units having best ITDs within the physiological ITD range (Figure 11B), and the mismatches often had a sign opposite to the prediction of stereausis.

4.3.9 Comparison of frequency mismatches

Finally, the measured frequency mismatches between the ipsi- and contralateral input were compared to the frequency mismatches predicted by the stereausis model as a function of recorded array-specific ITD. As may be seen in Figure 12A, substantial differences between measured (black open circles) and predicted (gray filled circles) mismatches were found for the sample of 44 recording sites. Measured frequency mismatches clustered around ±0.3 kHz with the exception of two recordings sites at array-specific ITDs of +30 and +250 µs. In contrast, predicted frequency mismatches varied systematically with ITD. The slope of the linear regression for the measured frequency mismatch (solid black line) revealed no significant difference from zero (ANCOVA, P=0.36), whereas for the predicted frequency mismatches the slope (solid gray line) differed significantly from zero (ANCOVA, P<0.0001). A comparison of the slopes for both measured and predicted frequency mismatches resulted in a significant difference as well (ANCOVA, P=0.0014). A similar analysis with the recorded and predicted frequency mismatches of high-frequency neurons recorded in NL (Pena et al., 2001) yielded similar results.

In a last analysis, the results obtained in low best-frequency (0.4–2.7 kHz) neurons in ICCcore were compared with the results of high best-frequency (3.2–7.3 kHz) neurons recorded by Pena et al. (2001) in the barn owl’s NL. The distribution should be broader for the high-frequency data than for the low-frequency data, if stereausis would underlie the representation of ITD. However, most frequency mismatches in either sample ranged from -0.3 to +0.3 kHz (Figure 12B). In both samples the distribution of frequency mismatches was not normally distributed (Kolmogorov-Smirnov test, both P<0.05). The median frequency mismatches were 0.035 kHz for the ICCcore sample and -0.044 kHz for the NL sample. While these two samples differed significantly (Mann-Whitney test, P=0.0136) from each other, their medians were not different from zero in a statistical sense, as demonstrated by Wilcoxon signed-rank tests (NL neurons: P=0.058; ICCcore neurons: P=0.077). When the distribution of ICCcore mismatches was tested without the two extreme positive mismatches at 0.82 and 1.02 kHz, the distribution was Gaussian (Kolmogorov-Smirnov test, P>0.10). The mean and median of the absolute frequency mismatch were calculated as well. The values were 0.138 kHz and 0.083 kHz, respectively.
Figure 12: Frequency mismatches. A: Comparison of measured and predicted frequency mismatches for array-specific ITDs in ICCcore of the barn owl. The slopes of the linear regressions between measured and predicted frequency mismatches differed significantly (ANCOVA, P=0.0014). Note that measured frequency mismatches have been presented in Figure 10 before. B: Comparison of frequency mismatches in low and high frequency neurons. Histograms of measured mismatches in the core of the central nucleus of the inferior colliculus (ICCcore (light gray) and nucleus laminaris (NL, dark gray) demonstrate that most neurons in both nuclei had frequency mismatches below ±0.3 kHz. Bin size is 0.1 kHz.

4.4 Discussion

ITD- and frequency-response functions in low best-frequency neurons (<3 kHz) of the barn owl’s ICCcore were investigated in relation to possible mechanisms of sound localization as described by the stereopsis model. No systematic frequency mismatch between the ipsi- and contralateral inputs to ITD sensitive neurons with different array-specific ITDs could be observed. In the following I shall first discuss the basic physiological data before I relate the findings to the stereopsis model.

4.4.1 ITD and frequency tuning

ITD-tuning curves in single and multi-units were not different (Figure 4). Such observations had been made before in ICCcore (Wagner et al., 1987; Wagner et al., 2002; Wagner et al., 2007). Both samples showed cyclic ITD tuning, where the period depended on the BF (Wagner et al., 2002). Array-specific ITDs covering a wide range were observed (Figure 5A). Several low best-frequency units had array-specific ITDs at 0 μs. This is consistent with earlier reports (Wagner et al., 2002; Wagner et al., 2007) and confirms the conclusion drawn in these earlier studies that the data do not follow the predictions of the slope-code model (Harper and McAlpine, 2004). Similarly, a considerable percentage of neurons had array-specific ITDs outside the physiological ITD range of the barn owl (Figure 5A), as also reported earlier (Wagner et al., 2002; Wagner et al., 2007). While we do not yet know why such neurons occur, we can now exclude differences in cochlear delays as the source of these large best ITDs.
The frequency tuning observed (Figure 4) also resembled earlier observations (Wagner et al., 2002; Wagner et al., 2007). Specifically, very low-best frequency neurons (down to a BF of 200 Hz) can be observed in ICCcore (Figure 5B, C). This is lower than BFs measured in other collicular sub-nuclei like the neighboring ICCls (Wagner et al., 2007) and similar to the lower limit seen in the eighth nerve, NM or NL (Köppl, 1997a; Carr and Köppl, 2004). Therefore, the ICCcore is ideal for studying low-frequency effects. BF and BF50 measures did not exhibit significant differences (Figure 5B). Therefore, the BF50 was used for the analysis, as was also done by Pena et al. (2001). The typical tuning was primary-like as in mammals: cat (Aitkin et al., 1975; Kuwada et al., 1984); chinchilla (Nuding et al., 1999; Langner et al., 2002); gerbil (Semple and Kitzes, 1985); guinea pig (Popelár and Syka, 1982); mouse (Yan et al., 2005); rat (Kelly et al., 1991).

4.4.2 Frequency tuning width

Half maximum frequency tuning width (W50) increased in all stimulus paradigms with increasing BF50 (Figure 7A-C). For the binaural paradigm frequency tuning widths show a striking similarity to the dataset of Wagner et al. (2002, their figure 3B). For neurons with BF <1 kHz, W50 was about 0.5 kHz, for BFs <2 kHz W50 was slightly broader whereas maximum tuning width was below 1.5 kHz for neurons having BFs <3 kHz. An increase in frequency tuning width was also observed by Pena et al. (2001) for high frequency neurons in the barn owl’s NL, where maximum W50 was limited below 2 kHz for BFs of 7 kHz. However, neither study investigated a relationship of frequency tuning width and BF for monaural paradigms. For the ipsilateral paradigm (Figure 7B), the units showed a significantly narrower frequency tuning width compared to the binaural (Figure 7A) as well as to the contralateral paradigm (Figure 7C). This was not the case for the contralateral paradigm, which was not significantly different from the binaural one. Recordings made in anesthetized gerbils by Semple and Kitzes (1985) revealed also significantly broader frequency response functions of binaurally excitable IC neurons for the contralateral side compared to the ipsilateral one. Furthermore, significantly higher discharge rates as described in this study (Figure 6A, B) for the contralateral paradigm compared to the ipsilateral paradigm was also previously reported by Semple and Kitzes (1985) for monaural excitation of IC neurons in the gerbil. In a study of Kuwada et al. (1984), discharge pattern of binaural IC neurons were also investigated. Unfortunately, they did not analyze discharge rates but the discharge pattern for ipsi- and contralateral stimulation was similar. A comparison of latency data derived from frequency tuning curves with other published work yielded diverse consistency with the results presented here. Whereas Kuwada et al. (1984) also found similar latencies for ipsi- and contralateral paradigms in the cat as we did in the owl (Figure 6C, D), mean latency in the gerbil was generally longer for the ipsi- than the contralateral paradigm (Semple and Kitzes, 1985). A decrease in the quality factor (W50/BF50) was observed for increasing BF50 for both the binaural paradigm (Figure 7D) and the monaural paradigms. A similar observation was made by Wagner et al. (2002) for binaural neurons in the ICCcore and for earlier stations in the auditory pathway of owls (Köppl 1997).
4.4.3 Comparison with the stereausis model

The stereausis model predicts a frequency mismatch between the ipsi- and contralateral input to a binaural neuron if the best ITD differs from 0 µs (see Figure 2). This mismatch needs to increase systematically with increasing best ITD of a neuron (Shamma et al., 1989). The analysis yielded no differences between ipsi- and contralateral BF50 (Figure 9A, B and Figure 10). Similar observations were made in several other preparations. For example, Aitkin and Reynolds (1975) and Kuwada et al. (1984) also found no differences in frequency tuning between ipsi- and contralateral inputs to neurons in the inferior colliculus of cats. The same holds for the study by Köppl and Carr (Köppl and Carr, 2008) who investigated low best frequency neurons (1–2.5 kHz) in the chicken NL. On the other hand, Semple and Kitzes (1985) found significantly lower BFs in response to ipsilateral than contralateral stimulation in gerbil IC. The difference was small, and it remains unclear whether the difference may be explained by cochlear delays because the corresponding ITD tuning curves are not available. Pena et al. (2001) observed significantly lower ipsilateral than contralateral BFs in high frequency neurons in the barn owl’s NL. However, the frequency mismatch was not large enough to produce the observed ITD tuning and did not always exhibit the sign expected from the stereausis model. Therefore, Pena et al. (2001) concluded that stereausis couldn’t explain the representation of ITDs in high-frequency neurons of barn owls. Similar observations were also made for low best-frequency neurons in the alligator NL (Carr et al., 2009), where the calculated frequency mismatch was not correlated with best ITD. This is also true for the analyses presented here, since frequency mismatch was not correlated with ITD for low-frequency neurons in the barn owl’s ICCcore (Figure 10 and Figure 12A).

The available data, therefore, suggest that stereausis cannot explain the array-specific ITDs observed in ICCcore of the barn owl. To substantiate this conclusion, a model of stereausis was applied to the data. The quantitative predictions of the model were far off the measured data. Neither did the predicted ITDs match the measured best ITDs (Figure 11A), nor did the predicted frequency mismatches comply with the measured differences (Figure 11B). A comparison of measured and predicted frequency mismatches (Figure 12A) supported this conclusion. Furthermore, the distribution of frequency mismatches had a median value of 0.083 kHz at 1 kHz that converts to about 2% of cochlear length or to less than 1/10 of an octave (Köppl et al., 1993). Such a low frequency mismatch seems to require mechanisms other than genetics to be established.

In summary, both in the high-frequency range (Pena et al., 2001; Fischer and Pena, 2009a) and in the low-frequency range (this study), stereausis is not responsible for the coding of ITD in the barn owl.
5 Adaptation in Narrowband Neurons in the Inferior Colliculus

5.1 Introduction

Adaptation is a widespread effect observed in neural systems. Hereby, response adaptation can be distinguished from spike-frequency adaptation (SFA). Recently the term adaptation has also been used in a broader sense to encompass the shaping of the activity of neurons in response to environmental needs like input statistics (Dean et al., 2005). SFA is thought to serve three main functions in neural systems: First, to select rapid transients over slow ones as a high pass filter (Benda and Herz, 2003; Benda et al., 2005), second, as a selectivity filter for stimulus-specific temporal inputs (Peron and Gabbiani, 2009), and third, to shift the cell’s dynamic range according to the high probability region of the stimulus intensity (Brenner et al., 2000; Dean et al., 2005; 2008). Adaptation may be characterized by the level of the tonic response relative to the phasic onset response, the time it takes until the tonic level of response is reached (Ingham and McAlpine, 2004; Crumling and Saunders, 2007; Dean et al., 2008), and the reaction of a neuron to a second stimulus (Ingham and McAlpine, 2004; Gutfreund and Knudsen, 2006).

Adaptation has been observed at almost all levels of the auditory system, including the inferior colliculus (Ulanovsky et al., 2004; Ingham and McAlpine, 2004; Dean et al., 2005; Gutfreund and Knudsen, 2006; Crumling and Saunders, 2007; Dean et al., 2008). In the barn owl, the inferior colliculus (IC) may be subdivided into the central nucleus (ICC) and the external nucleus (ICX). The central nucleus itself consists of a core (ICCore) and a lateral shell (ICCls) (Knudsen, 1983; Takahashi and Konishi, 1988a; Wagner et al., 2003). The ICC is tonotopically organized (Knudsen and Konishi, 1978; Wagner et al., 1987; Takahashi and Konishi, 1988a; Wagner et al., 2002; Wagner et al., 2007). The neurons exhibit narrow frequency tuning (Wagner et al. 2002; 2003; 2007) and are sensitive to a variation of interaural time difference (ITD) (both neurons in ICCcore and ICCls) and interaural level difference (ILD) (only neurons in ICCls) (Wagner et al., 1987; Wagner, 1990; Fujita and Konishi, 1991; Adolphs, 1993b; Mazer, 1998; Wagner et al., 2002; Bremen et al., 2006; Wagner et al., 2007; Singheiser et al., 2010a). Gutfreund and Knudsen (2006) showed that the responses to an auditory stimulus in the ICX are strongly suppressed by a preceding auditory stimulus with matching values of binaural cues.

What has not been quantified in detail in auditory adaptation is the effect of an initial stimulus on a second stimulus that may vary in its delay, quantified by the inter-stimulus interval (ISI), and its level relative to the stimulus level of the first stimulus, termed here 2nd level. To close this gap, tonal double-stimuli at the neuron’s best frequency (BF) and at different stimulus levels of the neuron’s dynamic range were presented and either varied in the level of the second stimulus or the ISI. A strong deterministic influence of the stimulus level on SFA in ICC neurons could be shown, whereas the influence of the ISIs tested was less predictable.
5.2 Methods

5.2.1 Owl handling

Seven adult barn owls (*Tyto alba pratincola*) of both sexes from the institutes breeding colony at RWTH Aachen University were used. All procedures were in accordance with the National Institutes of Health guidelines for animal experimentation and approved by the Landespräsidium für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (Recklinghausen, Germany). The day before an experiment, the weight of the owls was monitored and the birds received no food but had free access to water. On the experimental day, the weight of the owls was controlled again and when no signs of discomfort were obvious, sedation was introduced by applying an intramuscular (i.m) injection of diazepam (Valium, 1 mg/kg; Ratiopharm, Ulm, Germany). After a waiting period of about half an hour, anesthesia was initiated by applying an i.m. injection of ketamine (30 mg/kg; Sanofi-Ceva, Düsseldorf, Germany). To prevent saliva secretion during anesthesia, a single dose of atropine sulfate (0.065 mg/kg; B Braun AG, Tuttlingen, Germany) was administered intra-peritoneal. As an analgesic, the owls received a single i.m. injection of buprenorphine (Temgesic, 0.06 mg/kg; Essex Pharma, Munich, Germany).

Before the owls could be used in electrophysiological experiments, a metal head plate had to be fixed onto the skull under anesthesia. The procedure has been described in detail before (Vonderschen and Wagner, 2009). The posterior edge of the head plate served as a stereotactic coordinate for the rostro-caudal as well as the medio-lateral axis during experiments. Above the desired position, a craniotomy was made to allow for penetrations with the electrodes. The craniotomy was sealed with petroleum jelly (Vaseline) during experiments and after terminating the experiment it was covered with antibacterial ointment (Nebacetin, Astellas Pharma, Munich, Germany) and dental cement (Paladur, Hereaus Kulzer, Wertheim, Germany). Anesthesia during experiments was maintained by alternating injections of diazepam and ketamine as required. After terminating the experiment, the owls received a second injection of buprenorphine before the skull was sutured. The wound was treated with antibacterial ointment (Nebacetin). The birds were then removed from the setup and brought to a recovery box where they were provided with food and could be easily monitored. The next day, when the owls were fully recovered and showed no signs of discomfort, they were returned to their aviary. Each owl was used more than once.

5.2.2 Signal generation and data acquisition

All experiments were conducted in a soundproof anechoic chamber (IAC 403A; Industrial Acoustic, Niederkrüchten, Germany). All acoustic signals consisted of 100 ms long dichotic noise or pure tone signals with 5 ms on-and offset cosine ramps. Visual C++ software (Microsoft, Redmond, WA, USA) in combination with Brainware (Tucker-Davis Technologies [TDT], Alachua, FL, USA) on a personal computer (PC) was used to generate the signals. A digital-to-analog (DA) converter (DA 34, System II, TDT9) with a sampling rate of 50 kHz was utilized for DA conversion of the signals. DA-converted signals could then be individually attenuated for the left and right channel (PA4, TDT) before they were transmitted to the anti-aliasing filter (FT6, TDT). Finally, the signals were power-amplified (Yamaha AX 590) and send to calibrated ear phones (Sony MDR.E831LP). Recordings were done with epoxylite tungsten microelectrodes (12-18 MΩ; FHC; Bowdoin-
ham, ME, USA). The recorded electrophysiological signals were pre-amplified (M. Walsh Electronics, Pasadena, CA, USA), impedance matched, amplified and filtered (50 Hz notch filter, band-pass filtering 0.3-5.0 kHz), analog-to-digital converted (25 kHz, AD1; TDT) and finally read into a PC. Online pre-analysis and spike sorting of the recorded signals was done with Brainware. Final data analysis was done off-line with self-written Matlab routines (The MathWorks, Natick, MA, USA) and GraphPad Prism (La Jolla, CA, USA).

5.2.3 Stimulation protocol for physiological characterization of the recording site

All recordings were made from the central nucleus of the inferior colliculus (ICC). Coarse targeting of the ICC was achieved by known stereotactic coordinates in reference to the posterior edge of the head plate (~8-11 mm caudal, ~2-7 mm lateral, 12-17 mm below brain surface). Broadband noise (0.1-20 kHz) or tonal signals were used for stimulation. All cells were initially characterized by their responses to interaural time differences (ITD) with varying step size (“ITD tuning curve”, negative ITDs indicate left ear leading), interaural level differences (ILD, “ILD tuning curve”, step size 4 dB spanning from -20 to +20 dB, negative ILDs refer to signals being louder in the left ear), frequency (“isolevel frequency response function” or “frequency tuning curve” (FTC), 0.2 to 9.2 kHz, step size 0.5 kHz) and level (“rate vs. level (RLF) function”, binaural and monaural paradigms, average binaural level at best ITD and best ILD between 0 and 72 dB attenuation). These stimuli were presented with the rate of 1 Hz in random order and stimuli were repeated five to ten times. These recordings were used to assign a response as originating from ICCcore or ICCls. The final classification into neurons being recorded from the ICCcore or the neighboring ICCls was based on a comparison of the neuron’s response criteria with established physiological criteria for both ICCcore and ICCls (Wagner et al., 2007, see supplementary material) in reference to the medial shell of the ICC (ICCms) and the external nucleus of the inferior colliculus (ICX). Briefly, both ICCcore and ICCls show a cyclic ITD-tuning when stimulated with broadband noise with no suppression of the side peaks in comparison to the main peak as observed in ICX. Neurons in ICCms show no tuning to ITD. Responses to ILD are flat in ICCcore whereas they are sigmoid in ICCls favoring contralateral ILDs. ILD tuning curves in ICX show a single peak. Whereas FTCs in ICX are typically broad and represent frequencies higher than 3.5 kHz, FTCs in both ICCcore and ICCls show a clear tonotopy from low to high frequencies with increasing recording depth. Frequency tuning width in these two nuclei is typically in the range of 1/3 of an octave at frequencies >1.5 kHz. Stimuli from both ears excite neurons in ICCcore (EE property) whereas ICCls neurons can only be excited by contralateral stimulation (EI property). Neurons in ICX cannot be excited by monaural stimulation (00-property).
5.2.4 Stimulation protocol to test for adaptation

After the initial characterization of the units, more detailed recordings were performed. Monaural FTCs were obtained at higher resolution (0.1 – 0.2 kHz step size) for the ipsi- and contralateral side when units in ICCcore were recorded and only for the contralateral side when the units were classified as originating from ICCLs. The best frequency (BF) eliciting maximum spike rate was determined online (Figure 13A-F). Afterwards a monaural RLF was recorded using the previously identified monaural BF. Five stimulus levels corresponding to 90%, 70%, 50%, 30% and 10% of the maximum firing rate in the monaural RLF were determined online as well (Figure 13G-L). These stimulus levels served as the masker levels for the following two stimulus paradigms to quantify adaptation (for more details see next section). Two-tone stimuli were used to investigate the effect of adaptation in narrowband neurons of the inferior colliculus. In both paradigms, the level of the first stimulus (also called the masker) always corresponded to one these five levels (10% to 90%, hereafter termed ‘masker level’) taken from the monaural RLF (Figure 13-F).

Figure 13: Exemplary tuning curves. A-C: Binaural frequency tuning curves recorded at the unit’s best ITD and ILD. Best frequencies (BF) were 0.4 kHz (A), 1.7 kHz (B) and 5.44 kHz (C). D-F: Monaural frequency tuning curves of the same units recorded at higher resolution. D: A low frequency tuning curve of a single unit recorded in ICCcore with BF and BF 50 of 0.4 kHz. E: Contralateral frequency tuning curve of a multi unit recorded in ICCcore. BF of this particular unit was 1.6 kHz and BF 50 was 1.73 kHz. F: Frequency tuning curve of a multi unit with a medium BF of 5.8 kHz (BF 50: 5.44 kHz) recorded in ICCcore. G-L: Corresponding monaural rate-level functions (RLF) recorded with the monaural BF. G: The non-saturating RLF of this unit spanned almost the entire range of levels tested. H: Monaural RLF of unit O14P12U445 showed similar dynamic range than unit O72P17U065 (G). I: The RLF of unit O21P14U076 was narrower compared to G and H and saturated for attenuations lower than 16 dB attenuation. Levels corresponding to 10%-90% of the dynamic range were determined online and are indicated by gray arrows. J-L: Corresponding raster plots of the monaural RLFs shown in G-I. Each black dot represents the occurrence of a spike. Note the increased number of spikes during the period of stimulation (400-500 ms) and the reduced spontaneous activity after the stimulus has been terminated.
5.2.4.1 Stimulus paradigms to quantify adaptation: 2nd level tuning

In the first stimulus paradigm, two stimuli were presented at the neurons monaural BF with an inter-stimulus-interval (ISI) of 0 ms. While the level of the masker and the ISI were held constant, the level of the second stimulus (the probe) was increased from an equal level as the masker to 25 dB louder than the masker (Figure 14A-C). The step size of level increment was 5 dB. This stimulus paradigm was termed 2nd level tuning. The stimuli were presented at the rate of 1 Hz in random order with 10 repetitions for each stimulus parameter (2nd level or probe).

![Figure 14: Exemplary 2nd level tunings. A-C: Raster plots of the same units as in Figure 13. Black dots indicate the occurrence of spikes. The masker was presented after 400 ms and lasted for 100 ms. The probe was presented from 500 ms to 600 ms. The stimulus level corresponded to 50% of the saturating RLF. The gray shaded area indicates the unit's responses to the masker, whereas the responses to the probe are indicated by the transparent boxes. The level of the probe was depicted on the ordinate. Note, that the responses of the units to the masker remained rather constant whereas the responses to the probe strongly depended on the probe level. The number of spikes at the probe level of 0 dB was generally reduced for the response to the probe, whereas the number of spikes to the probe increased with increasing probe level. D-F: Calculated response ratios for the corresponding 2nd level tunings.](image)

5.2.4.2 Stimulus paradigms to quantify adaptation: Inter-stimulus interval (ISI) tuning

In the second stimulus paradigm, the stimulus level of both masker and probe were held constant so that the two stimuli were presented at the same level corresponding to one of the five levels taken from the monaural RLF (10% - 90%). Instead, the inter-stimulus interval (ISI) between stimulus offset of the masker and stimulus onset of the probe was varied between 25 ms and 1600 ms on a log2 scale. This stimulus paradigm was termed ISI tuning and the sweep duration was prolonged from one second to three seconds (Figure 15A-C). Stimuli were presented at the rate of 0.33 Hz and ten sweeps for each stimulus parameter (ISI) were recorded.
Figure 15: Exemplary ISI tunings. 

A–C: Raster plots of the same units as in Figure 13 recorded at 50% masker level. Black dots indicate the occurrence of spikes. The masker was presented after 400 ms and lasted for 100 ms. The probe was presented at ISIs increasing from 25 ms to 1600 ms. The gray shaded area indicates the unit's responses to the masker. The ISI was depicted on the ordinate and the level of both stimuli was equal. 

D–F: Calculated response ratios for the corresponding ISI tunings.

5.2.5 Data analysis

5.2.5.1 General data analysis

Primarily, several general response characteristics of a unit were determined: 1) the average spontaneous discharge rate was calculated from the spiking activity in the 400 ms period preceding the stimulus and 2) the average response rate was the response rate during stimulation minus the spontaneous rate. The response rate was calculated for an interval of 100 ms after stimulus onset. To compensate for the unit's response latency, an algorithm of Friedman and Priebe (Friedman and Priebe, 1998; 1999) was applied as previously described in Vonderschen and Wagner (2009).

Frequency tuning curves were analyzed as reported earlier (Singheiser et al., 2010a). Briefly, the BF – which is the frequency eliciting maximum response in an FTC - was determined. Additionally, the width at half height of the FTC (W<sub>50</sub>) was calculated and W<sub>50</sub> corresponds to the range of frequencies over which the discharge rate of the unit corresponded to 50% of the maximum discharge rate (Pena et al., 2001; Wagner et al., 2002). To reduce a bias in sampling resolution of the BF, the center frequency (BF<sub>50</sub>) at half height of the FTC was calculated as well.

The stimulus- or masker levels corresponding to 10%, 30%, 50%, 70% and 90% of the saturating response of the monaural RLF were determined audio-visually online during the recording sessions. Hereby, the stimulus levels in dB attenuation were read out for threshold as well as for saturation of the firing rate. The maximum and minimum spike rates for these levels were then defined. Based in these response rates, the masker levels corresponding to 10% to 90% of the maximum response were determined accordingly. The dynamic range was calcul-
lated by subtracting the stimulus level [dB attenuation] complying with 90% of the RLF from the stimulus level [dB attenuation] corresponding to 10% of the RLF.

5.2.5.1.1 Spontaneous ratio

The spontaneous ratio (SR) of the monaural RLFs was computed for each of the five masker levels to investigate whether there existed a post-stimulatory adaptation that depended on the level of stimulation:

\[ SR = \frac{R_{\text{post}}}{R_{\text{pre}}} \]  

(Eq. 3)

The spike rate of a 50 ms post-stimulatory time window \(R_{\text{post}}\) was divided by the spike rate of a 50 ms time window that preceded the stimulus \(R_{\text{pre}}\). Whereas the pre-stimulatory time window encountered the spontaneous activity just before the stimulus was presented, the post-stimulatory window was shifted by twice the unit’s latency to ensure that no stimulus-evoked spikes were taken into account.

To quantitatively analyze adaptation, several measures were calculated that allow for a comparison of the recorded data with datasets from different nuclei and model systems. Hereby, two different types of adaptation will be investigated: response adaptation and spike-frequency adaptation.

5.2.5.2 Response adaptation

Response adaptation focused on the general coding properties of ICC neurons to respond to two subsequent stimuli as introduced in the 2\(^{nd}\) level and the ISI tuning. Hereby, the responses to the masker served as the response criterion and the responses to the probes were analyzed with respect to the responses for the masker.

5.2.5.2.1 Response ratio

First, the response ratio (RR) for each stimulus parameter (2\(^{nd}\) level or ISI) was calculated. This value is the quotient of the unit’s response rate to a given probe \(R_p\) divided by the mean response rate to all masker responses \(R_m\):

\[ RR = \frac{R_p}{R_m} \]  

(Eq. 4)

A pair wise calculation of the response ratio led to a high variability in the resulting response ratios, since each stimulus parameter was repeated only ten times. By taking the mean ratio of all responses to the masker, the variability in the response ratio could be reduced since the value of the denominator was based on 60 sweeps in the 2\(^{nd}\) level tuning and 70 sweeps in the ISI tuning. Response ratios <1 denotes a reduction in response rate to the probe and thus are an indication for response adaptation. Exemplary response ratios are shown in Figure 14D-F (2\(^{nd}\) level tuning) and Figure 15D-F (ISI tuning).
5.2.5.2.2 Masking ratio

To allow a comparison of the data recorded in the barn owl’s ICC with other studies on response adaptation, a further analysis was introduced for the results of the ISI tuning. In contrast to the response ratio, the responses to the masker were excluded in the calculation of the masking ratio (Figure 16). The idea behind this analysis was to investigate the temporal influence of the masker on the responses to the probe. It was assumed, that the influence of the masker becomes less effective with an increasing ISI between masker and probe. After a certain amount of time, the response to the probe should be independent of the masker and could be used as a reference stimulus to quantify the temporal effects of forward masking. In the ISI tuning, the responses to the probe after an ISI of 1600 ms was used as the reference value and the masking ratio (MR) was calculated by dividing the response rate to each probe tone (Rₚ) by the response rate of the reference probe (Rᵣᵣ):

\[
MR = \frac{R_p}{R_{rᵣ}} \quad \text{(Eq. 5)}
\]

A change in the median masking ratio of ±20% was determined as the criterion for stimulus facilitation or suppression (Finlayson, 1999). The time course of recovery from suppression was quantified as a function of the ISI using a single exponential approximation fitted to the data

\[
y(ISI) = R_x + R_0 \cdot e^{-\frac{ISI}{\tau_r}}, \quad \text{(Eq. 6)}
\]

where \(R_x\) is the asymptotic response magnitude and \(R_0\) is the difference from steady state to the initial peak. The time constant of recovery is given by \(\tau_r\).

Figure 16: Computation of the masking ratio. A: Exemplary raster plot. B: Scheme of A demonstrates the computation of the masking ratio. The responses to the masker are shown in light gray, whereas the responses to the probe are depicted in dark gray. The responses to the probe tone at an ISI of 1600 ms were taken as reference value.
5.2.5.3 Spike-frequency adaptation

SFA examines the temporal progression and the amount of the reduction of a neuron’s response to ongoing stimulation. The temporal decay of the spike rate was quantified by the time constant $\tau$ whereas the amount of reduction in the spike rate was quantified by the adaptation ratio.

5.2.5.3.1 Time constant $\tau$

The time constant $\tau$ is a good estimate for the temporal decay of neuronal responses and allows a comparison of the temporal dynamics of SFA across nuclei and model systems. To compute the time constants, single exponential functions (Eq. 5) were fitted to the PST-histograms of the responses to masker and probe in the 2nd level and ISI tunings (Figure 17).

$$R(t) = R_\infty + R_0 e^{-t/\tau} \quad (Eq. 7)$$

Since neurons generally tend to respond with fewer spikes to pure tone stimulation in comparison to noise stimuli, the PST histograms were smoothed using bin sizes of 5 ms to reliably fit the time constant to the data. The time constant $\tau$ was computed separately for the responses to both masker and probe. Time constants longer than 100 ms were classified as tonic since they exceeded the stimulation period of 100 ms.

![Figure 17: Computation of the time constant $\tau$ and the adaptation ratio.](image)

The time constant was computed using single exponential decay functions. The adaptation ratio was determined by dividing the mean response rate at steady state for the period of 80-90 ms of stimulus presentation (bin size 5 ms) by the maximum response rate in a 5ms bin.

5.2.5.3.2 Adaptation ratio

The adaptation ratio was calculated to quantify the amount of SFA of particular units to their responses to both masker and probe. SFA is characterized by an initially high response rate at stimulus onset in the peri-stimulus time histogram (PSTH) that gradually decreases to a steady state with ongoing stimulation (Figure 17). The amount of SFA was defined by the quotient of the response rate at steady state (bins of 5 ms from 80-90 ms of the response to the stimulus) divided by the response rate in the maximum bin (bin size 5 ms) in the PSTH (Figure 17). The smaller the adaptation ratio the larger the resulting SFA in the unit’s response to a certain stimulus. This definition is similar to what has been called percent adaptation by Crumling and Saunders (2007). Whereas tonic units have adaptation ratios close to one, phasic or phasic-tonic units have adaptation ratios close to zero.
5.3 Results

For this project, 100 units in the ICC of seven barn owls were recorded. Thirty-nine units (18 single and 21 multi units) were located in ICCond, whereas 61 units (26 single units and 35 multi units) were collected in ICCls. Recordings with stimulation from both ears were available for many units in ICCond so that a maximum of 120 responses obtained with monaural stimulation could be analyzed. All multi units showed homogenous spike forms and generally, the responses of single and multi units were similar and showed no significant differences in the tuning parameters that were analyzed. For this reason, the responses of single and multi units were pooled for the analyses. Similar observations were made for important tuning characteristics like frequency and dynamic range between ICCond and ICCls: the data from these nuclei were pooled as well. As in the previous chapter on stereausis, I will first describe the general response properties of the units that were recorded and then focus on the results concerning both response- and spike-frequency adaptation in the two stimulus paradigms.

5.3.1 General response properties – frequency tuning

To analyze the impact of tonal double-stimulation on adaptation of narrowband neurons in the ICC of barn owls, it was necessary to precisely determine the BF and the frequency tuning width of each unit. This was done by first recording FTCs that tested almost the entire audible spectrum of barn owls (Figure 13.A-C). More detailed FTCs were recorded afterwards by decreasing the sampling size from 0.5 kHz to 0.1-0.2 kHz. Additionally, not only binaural FTCs were recorded but also monaural FTCs to test for differences in BF. Exemplary monaural FTCs are shown in Figure 13.D-F.

Comparable to the results in chapter 4.3.3, no significant differences between BF and BF50 were observed for this project as well (binaural: P=0.4604; ipsi: P=0.912 (both paired t-tests); contra: P=0.964 (Wilcoxon matched-pairs test)). Additionally, the coefficients of determination between BF and BF50 were high (binaural: R²: 0.963; ipsi: R²: 0.995; contra: R²: 0.995), the slopes of the linear regressions were close to 1 (see inset in Figure 18.A) and did not differ significantly (ANCOVA, P=0.42). When the relationship of frequency tuning width W50 and BF50 was analyzed, it turned out that only in the binaural paradigm an increase in W50 could be observed for increasing BF50. The slopes of the linear regressions for both monaural paradigms did not differ significantly from zero (ANCOVA; ipsi: P=0.054; contra: P=0.261; see Figure 18.B). W50 was significantly broader for the binaural paradigm than for both monaural paradigms (Kruskal-Wallis and Dunn’s post-test; P<0.0001).
Figure 18: Frequency tuning in ICC. **A:** Comparison of binaural (black squares), ipsilateral (dark gray diamonds) and contralateral (light gray circles) FTCs. BF_{50} was plotted as a function of BF. FTCs were highly symmetrical across the entire frequency spectrum for the binaural and monaural stimulus paradigms as indicated by high coefficients of determination (R^2 binaural: 0.963; R^2 ipsi: 0.995 and R^2 contra: 0.995). The slopes of the linear regressions did not differ significantly as well (ANCOVA, P=0.4195).

**B:** Relationship of frequency tuning width W_{50} and BF_{50}. For the binaural paradigm, W_{50} increased with increasing BF_{50} whereas no significant increase could be found for the monaural paradigms (ANCOVA, P<0.05). The slopes of the linear regressions in **A** and **B** are given in the inset. **C:** Distribution of BF_{50} for the binaural and the monaural stimulus paradigms (bin size 1.0 kHz; black: binaural; dark gray: ipsilateral; light gray: contralateral). **D:** Relationship of BF_{50} and recording depth. BF_{50} increased as the electrode was lowered into the brain.

BF_{50} values were in the range of 0.24 kHz to 7.9 kHz for the binaural paradigm, 0.38-6.78 kHz for the ipsi- and 0.33-8.6 kHz for the contralateral paradigm (Figure 18C). No significant differences in BF_{50} were observed for the three stimulus paradigms tested (Kruskal-Wallis test, P=0.679). BF_{50} furthermore increased with increasing recording depth (Figure 18D). Although the data were taken from several penetrations in some owls, the trend was clearly visible and the coefficients of determination were generally high (owl 14: R^2 0.236; owl Furie: R^2 0.45; owl Giny: R^2 0.601; owl Haensel: R^2 0.34; owl Ole: R^2 0.769; owl X-Mas: R^2 0.045; owl Zhaki: R^2 0.933).
5.3.2 General response properties – rate-level-functions

Since one goal of the project was also to investigate the influence of the masker level on adaptation, the recordings were carried out at five levels that corresponded to 10%, 30%, 50%, 70% and 90% of the unit’s dynamic range. As may be seen in Figure 13G-I, the dynamic ranges were different between units. In some units, the dynamic range covered almost the entire range of stimulus levels tested (Figure 13H) whereas in other units the dynamic range was rather small spanning only twenty or thirty decibels (Figure 13I). The threshold level for dynamics ranges was variable as shown in Figure 19A, C. The same applied for response saturation, where an additional increase in the stimulus level could not enhance the neuron’s response rate (Figure 13G-I). The mean values for the stimulus levels that corresponded to individual masker levels of the dynamic range are shown in Figure 19A (ICCcore ipsi: light gray; ICCcore contra: black; ICCls contra: dark gray). The slopes of the linear regressions did not differ significantly between the three stimulus paradigms (ANCOVA, P=0.322) and the coefficients of determination were high as well (ICCcore contra: R²: 0.978, ICCcore ipsi: R²: 0.995; ICCls contra: R²: 0.984). Since statistical differences in the mean stimulus levels were not consistent between stimulus paradigms (for ICCcore contra vs. ICCls contra significant differences were only found for 10% and 30 % of the RLF, Mann-Whitney test, P<0.05, the same hold true for a comparison of ICCcore contra vs. ICCcore ipsi), the datasets were pooled to compute a single dynamic range as shown in Figure 19B. The mean stimulus level (±SD in dB attenuation) was plotted as a function of the masker level. As may be seen by the large standard deviations, the masker levels differed largely for individual neurons.

A further characteristic of RLFs is the dynamic range indicating the sensitivity of a neuron to a change in stimulus level. For a comparison of the dynamic range, which was defined as the interval of stimulus levels between 10% and 90% of the saturating response, the values for the contralateral stimulus paradigms of ICCcore and ICCls were pooled since no consistent statistical differences could be found between both sub-nuclei. The analysis further revealed no significant differences between ipsi- and contralateral stimulation for the dynamic ranges (Mann-Whitney test, P=0.14). The median for the contralateral paradigm (30 dB) was lower than for the ipsilateral paradigm (38 dB), but the interquartile range was comparable in both paradigms. The same also applied for the maxima (60 dB for both paradigms), whereas the minima differed between ipsi- and contralateral stimulation (ipsi: 20 dB; contra: 12 dB, Figure 19C). The histogram in Figure 19D gives a more detailed view on the distribution of the dynamic ranges for ipsi- and contralateral stimulation. In both stimulus paradigms, most units had dynamic ranges that covered between 20 and 50 decibels and they were not correlated with BF50 in either paradigm (ipsi: R²: 0.08; contra: R²<0.002, Figure 19E).
Figure 19: Rate-level functions in ICC. **A:** Mean (±SD) values for stimulus levels [dB attenuation] that corresponded to 10% - 90% of the dynamic range of the monaural RLFs recorded at the unit’s BF. The slopes of the linear regressions for ICCcore contra (black), ICCcore ipsi (light gray) and ICCls contra (dark gray) did not differ significantly (ANCOVA, P=0.322). **B:** Mean (±SD) stimulus levels corresponding to 10% - 90% of the dynamic range when ipsi- and contralateral values were pooled. **C:** Box-and-whisker plots of the dynamic range for ipsi- and contralateral RLFs. The median of the dynamic range for contralateral RLFs was lower than for the ipsilateral RLFs but the dynamic range between both paradigms did not differ significantly. This was indicated by the interquartile ranges as well as a Mann-Whitney test (P=0.14). **D:** Histogram for the dynamic ranges that were observed for ipsi- and contralateral stimulation. The dynamic range of most units covered 20 dB to 50 dB (bin size 10 dB). **E:** Relationship of dynamic range and BF50. As may be seen, the dynamic range did not depend on the unit’s BF50. The coefficients of determination were low for both stimulus paradigms (see inset).

### 5.3.2.1 Spontaneous ratios

After the basic characteristics of the monaural RLFs were analyzed, the effect of the masker level on adaptation was investigated more closely. As mentioned in chapter 5.2.5.1.1, the spontaneous ratio was computed for the individual masker levels. As may be seen in Figure 20, the spontaneous ratios strongly depended on the level of the masker. For high masker levels (Figure 20A, B), more than 60% percent of the neurons showed post-stimulatory adaptation indicated by SRs <1. When the level of the masker was reduced the number of neurons showing post-stimulatory adaptation continuously decreased to 35% for 10% masker level. This trend could be confirmed by plotting the medians for the spontaneous ratio as a function of the masker level (Figure 20F): the spontaneous ratios increased with decreasing masker level (R²: 0.65). A Mann-Whitney test approved this trend, since the spontaneous ratios for 10% masker level were significantly higher than those for 90% masker level (P=0.0249) indicating higher spontaneous activity after stimulus termination (compare Figure 13J-L). However, when the spontaneous ratios were analyzed by a Kruskal-Wallis test, no significant differences were found (P=0.1656) arguing for a smooth increase of the spontaneous ratio with decreasing stimulus level.
Figure 20: Spontaneous ratio. The spontaneous ratios (SR) were computed for each masker level (A: 90%; B: 70%; C: 50%; D: 30%; E: 10%) and grouped in bins (bin size = 1). Units with SRs <1 fired less spikes after the stimulus had been terminated than spontaneously before the stimulus was presented indicating post-stimulatory adaptation. This adaptation strongly depended on the masker level. For 90% masker level, 74% of the units had SRs that were smaller than 1, whereas for fainter masker levels the percentage of neurons that showed post-stimulatory adaptation decreased to 35%. F: Median SRs as a function of masker level. Median SRs increased with decreasing masker level showing less post-stimulatory adaptation for faint stimulus levels.
5.3.3 Response adaptation in ICC neurons

In the next chapters, I will focus on the analyses of adaptation in the barn owl’s ICC induced by tonal double-stimulation. At first, the results of response adaptation are presented. Response adaptation describes the response properties of neurons to the probe in reference to the masker. The results were quantified by the response ratio in both the 2nd level and ISI tuning as well as by the masking ratio in the ISI tuning only. At first, I will focus on the 2nd level tuning before the data for the ISI tuning are presented.

5.3.3.1 Response ratios in the 2nd level tuning

When the response ratios for the 2nd level tuning were analyzed, a dramatic effect of the masker’s stimulus level could be observed (Figure 21). In Figure 21A-E, the response ratios are shown as a function of the increase in the level of the probe for all units that could be recorded at the corresponding masker levels: 90% (A), 70% (B), 50% (C), 30% (D) and 10% (E). Each gray line represents one unit. The dashed black line marks the response ratio resembling unity: here the response rate to the probe was equal to the response rate of the masker. As may be seen, the variability in the response rate increased with decreasing masker level (Figure 21A-E). At high masker levels (Figure 21A), the response ratios for most units clustered below unity and even an increase in the stimulus level of the probe could not drive the unit’s responses to response ratios larger than unity. With decreasing masker levels, especially for 50% (Figure 21C) to 10% (Figure 21E), the response ratios continuously increased with increasing stimulus level of the probe. This was also expressed in the numbers of the response ratios on the ordinate, which grew with decreasing masker level.

The objective description of the response ratios was quantified in Figure 21F-J. Similar to Figure 21A-E, the response ratios were displayed as a function of the probe’s stimulus level. The black dots in each subplot denote the mean (±SEM) response ratio for each probe level. The slopes and coefficients of determination for the linear regressions are listed in each subplot as well. For the highest masker level (Figure 21F), an increase in the probe level could not drive the units to similar response rates for the responses to both the masker and the probe. In contrast, with increasing level of the probe, mean response ratios decreased and fell below the value for equally loud stimuli (probe level of 0 dB). This result was confirmed by the negative slope of the linear regression as well a statistical analysis where it was tested, whether the response ratios were significantly smaller than unity (one-sample t-tests and Wilcoxon signed-rank tests). At 90% masker level, this was the case for all probe levels (Figure 21F; one-sample t-tests, all P<0.05). However, no significant differences in the response ratio between 0 dB and 25 dB probe level could be found (Wilcoxon matched-pairs test, P=0.109). With decreasing level of the masker (Figure 21G-J), the results changed dramatically. At a masker level of 70% (Figure 21G), the response ratios increased slightly with ascending level of the probe, which may be seen in the shallow slope of the linear regression (see inset in Figure 21G). Additionally, all response ratios were closer to unity and only when the masker and the probe had the same stimulus level, a significant reduction of the response ratio was observed (see also Figure 22A). However, an increase in the probe level of 25 dB could not enhance the response ratio to unity although the response ratio for this probe level was significantly larger than the response ratio for 0 dB probe level (Wilcoxon matched-pairs test, P=0.0007).
Figure 21: Response ratios in the 2nd level tuning. A–E summarizes the response ratios as a function of increase in the stimulus level of the probe tone for the five masker levels (A: 90%; B: 70%; C: 50%; D: 30% and E: 10%). Each gray line represents one unit recorded at that particular masker level. The dashed black line in each subplot resembles the response ratio of unity where no differences in the response rate to both masker and probe occurred. Note the different scales for the ordinate in A–E. The response ratios increased systematically with decreasing level of the masker. F–J: Mean (±SEM) response ratios are displayed as a function of the stimulus level for the probe. The dashed line again represents unity in the response rate of the masker and the probe tone. Note the differences in the slopes of the linear regressions for the five masker levels. Significant differences in the response ratios deviating from unity are indicated by asterisks (one-sample t-tests, P<0.05: *; P<0.01: **; P<0.001: ***).
With ongoing reduction of the masker level (Figure 21H-J), the response ratios increased almost linearly with increasing level of the probe. The coefficients of determination for the linear regressions were very high (>0.9 for masker levels ≤50%; Figure 21H-J). As the level of the masker decreased, the slopes of the linear regressions became steeper with increasing probe level (Figure 23B). Additionally, with further increasing the level of the probe, the response ratios became significantly larger than unity indicating that the neurons were able to respond to an increase in the probe level. Hereby, the response ratios for 20 dB and 25 dB probe level were significantly larger than those at 15 dB for masker levels ≤50% (Wilcoxon matched-pairs test, all P<0.05). At 10% masker level, the response ratios between 20 dB and 25 dB probe level differed significantly as well (Wilcoxon matched-pairs test, P=0.0458). For all masker levels except 90%, only those responses to the probe were significantly reduced in comparison to unity where both masker and probe had the same stimulus level (Figure 22A). Furthermore, the response ratios at 0 dB probe level decreased as a function of descending masker level from about 0.8 at 90% masker level to 0.55 at 10% masker level indicating stronger response adaptation for lower stimulus levels (Figure 22A). This decrease was significant (Mann-Whitney test, P<0.0001). Figure 22B displays the increasing steepness of the slopes for the linear regressions as a function of the masker level.

**Figure 22:** Relationship of response ratio and masker level. **A:** The mean response ratios for the probe stimuli of 0 dB are shown as a function of the masker level. The responses ratios decreased with decreasing masker level and differed significantly between 90% and 10% masker level (Mann-Whitney test, P<0.0001). **B:** Comparison of the slopes [1/dB] for the linear regressions shown in Figure 21F-J. With decreasing masker level the slopes of the linear regressions increased and became steeper indicating a higher ability of the neurons to code an increase in the probe level.
The next step in the analyses was to compute the compensation for the response ratio. The compensation corresponded to an increase in the stimulus level of the probe that was required to achieve unity in the response ratio (Figure 23A, B).

Figure 23: Compensation of the response ratios in the 2nd level tuning. A: Calculated response ratios are plotted as a function of the masker level. The response ratios (mean ±SEM) were taken from Figure 21F-J and color-coded due to their masker level (90%: black to 10%: light gray). The starting point of each of the five curves was set to the mean masker level [dB attenuation] that corresponded to 10% - 90% of the RLF (rightmost dot for each color-coded line, which corresponds to a probe level of 0 dB). The following response ratios are plotted for stimulus levels that increased in steps of 5 dB according to the step size in the 2nd level tuning. The dotted line marks the response ratio of unity. Note that the stimulus level is given in dB attenuation. B: Computed increase of the probe level that was required for compensation of the response ratios. Data were taken from linear regressions in Figure 21F-J.

The mean (±SEM) response ratios for each masker level (10% - 90%) were computed and plotted as a function of the probe level (Figure 23A). The rightmost dot in each curve therefore represents the response ratio for the stimulus condition where masker and probe had the same level (probe level of 0 dB). These starting points were plotted at the mean stimulus level that corresponded to each of the five masker levels taken from monaural RLFs. As may be seen in Figure 23A, the response ratio for the masker level corresponding to 10% (light gray dot) was smaller than the response ratio corresponding to 90% masker level (black dot). By increasing the level of the probe tone in increments of five decibels, the change of the response ratios depended on the stimulus level of the masker. When the level of the masker was high (black curve), the response ratios progressively decreased with increasing probe levels and did not cross the threshold of unity. When the level of the masker was low, the slopes of the response ratios became steeper (light gray curves; see also Figure 22B). The response ratios increased with increasing level of the probe tone and the threshold of unity was crossed when the level of the probe was increased by only a few decibels. Figure 23B displays the results of the computed increase of the probe level that was required to compensate for the response ratio and thus to release the unit from response adaptation. It was obvious that this release depended dramatically on the level of the masker. When the masker level was set to 90%, no compensation of the response ratio could be achieved. For a masker level corresponding to 70% of the RLF, the probe level must have been increased by about 33 dB to release the units from response adaptation. This would exceed the range of probe levels tested, therefore it remains unclear how reliable this value would be. By further decreasing the masker level down to 50% - 10% of the RLF, the neurons became more sensitive to a change in the level of the probe tone. An increase of about 5-7 dB was sufficient to overcome response adaptation. Table 1 displays a comparison of the computed increase for the probe level that would have been required to equal the response between masker and probe. The compensations for the probe
were computed with three different functions. As may be seen in Table 1, the results were comparable for the different fits and the units thus seemed to be very sensitive to small changes in the probe level. Their state of response adaptation seemed to play a relevant role in the coding capabilities to tonal double-stimulation.

**Table 1: Compensation in the 2nd level tuning.** Comparison of the computed increase for the probe level to achieve compensation in the 2nd level tuning. Three different fits were applied, which were similar in their computed increase of the probe level.

<table>
<thead>
<tr>
<th>Masker level [% RLF]</th>
<th>Linear fit</th>
<th>Boltzmann fit</th>
<th>Polynomial fit 2nd order</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>70</td>
<td>33.57</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>50</td>
<td>6.85</td>
<td>7.15</td>
<td>6.41</td>
</tr>
<tr>
<td>30</td>
<td>5.22</td>
<td>5.7</td>
<td>4.74</td>
</tr>
<tr>
<td>10</td>
<td>4.98</td>
<td>5.78</td>
<td>4.71</td>
</tr>
</tbody>
</table>

5.3.3.1.1 **Response ratios in octave bands**

In a last analysis, it was investigated whether the sensitivity of ICC neurons to small changes in the level of the probe was a common effect independent of the unit’s BF or whether units tuned to different BFs displayed varying sensitivities. Therefore, the BF of each unit was binned into octave bands corresponding to 1-2 kHz, 2-4 kHz and 4-8 kHz and the compensation for response ratio was computed for these octave bands as well.

The trends described for the pooled analysis were confirmed in octave band binned units as well. For high masker levels (90% and 70% of the RLF, Figure 24A,B), the units were generally not able to compensate the adaptation of the probe tone by an increase of the probe tone’s level. The response ratios were typically smaller than unity and did not differ significantly between neurons having different BFs as indicated by similar slopes of the linear regressions. Here, no significant differences could be found for 90% (ANCOVA, P=0.209) and 70% masker level (ANCOVA, P=0.163). For lower masker levels (50% to 10% masker level, Figure 24C-E), the sensitivity described before (Figure 21) was similar for all octave bands although significant differences in the slopes of the linear regressions could be found for 50% (ANCOVA, P=0.0004) and 10% of the RLF (ANCOVA, P=0.006). However, the increase in the level of the probe that would have been required to release the neurons from adaptation was comparable for all frequency bands and did not differ much (± 2 dB) from the results of the pooled analysis for the linear regressions (Table 2). Only for 50% masker level, the required increase of the probe level for the highest octave band was almost twice as large than for the pooled dataset and the lower octave bands. In contrast, at 30% masker level, the units in the lowest octave band required a computed increase in probe tone level of less than 1 dB, whereas the higher octave bands required similar increments in the levels of the probe.
Figure 24: Relationship of response ratio and BF. Differences in response ratios are displayed as a function of the probe level for octave band binned neurons. The response ratios (mean ±SD) of the units shown in Figure 21 were binned according to their BF (1-2 kHz: light gray; 2-4 kHz: gray; 4-8 kHz: black). A: 90% masker level, B: 70% masker level, C: 50% masker level, D: 30% masker level and E: 10% masker level. The recovery from adaptation for the octave band binned neurons was comparable to the pooled dataset. The slopes and the coefficients of determination are given in the insets. Significant differences (ANCOVA) between the slopes of the linear regression are indicated by asterisks (P<0.01: **; P<0.001: ***). The dashed line marks the response ratio of unity.

Table 2: Comparison of response compensation in the 2nd level tuning. The required increase in the level of the probe tone for the pooled dataset was compared to the same units that were binned into octave bands according to their BF. The required increase in the probe level was similar across all frequencies.

<table>
<thead>
<tr>
<th>Masker level [% RLF]</th>
<th>Pooled units [dB]</th>
<th>1-2 kHz [dB]</th>
<th>2-4 kHz [dB]</th>
<th>4-8 kHz [dB]</th>
</tr>
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<tbody>
<tr>
<td>50</td>
<td>6.85</td>
<td>4.37</td>
<td>4.96</td>
<td>10.62</td>
</tr>
<tr>
<td>30</td>
<td>5.22</td>
<td>0.41</td>
<td>6.07</td>
<td>6.1</td>
</tr>
<tr>
<td>10</td>
<td>4.98</td>
<td>6.15</td>
<td>5.72</td>
<td>4.19</td>
</tr>
</tbody>
</table>
The data presented in this chapter indicate that the neurons in the barn owl’s ICC were very sensitive to small changes in the stimulus level irrespective of the unit’s BF50. For high masker levels that exceed 70% of the unit’s RLF, the neurons could not compensate the adaption of a previously presented masking stimulus. On average, an increase of 5 – 7 dB in the level of the probe tone was sufficient to release the neurons from response adaptation.

5.3.3.2 Response ratios in the ISI tuning

In contrast to the analyses of the response ratios in the 2nd level tuning (Figure 21), the results of the response ratios in the ISI tuning were less obvious and the stimulus level of the masker seemed of minor relevance in this stimulus paradigm (Figure 25). As may be seen, the curves for the response ratios ran mostly in parallel to unity and no apparent influence of the masker level on the response ratios could be observed (Figure 25A-E). This was also indicated by rather similar scales for the response ratios on the ordinate at all masker levels (Figure 25A-E).

The objective evidence was confirmed by the quantitative analyses of the data. A significant reduction in the responses to the probe in reference to the masker was found for ISIs shorter than 800 ms in most extreme cases (one-sample t-tests, P<0.05; Figure 25F-J) but for most masker levels, this reduction was only significant for ISIs ≤100 ms (Figure 25F, I and J). The suppression of the responses to the probe therefore was less influenced by the masker level in the ISI- than in the 2nd level tuning. When the response ratios for the shortest ISI of 25 ms were analyzed as a function of the stimulus level (Figure 26A), no systematic effect of the masker level on the response ratios was found. Here, no significant differences could be observed between 90% and 10% masker level (Mann-Whitney test, P=0.1692). Analog effects were also detected for representative ISIs of 100 ms, 400 ms and 1600 ms (Figure 26B-D). For these ISIs, the masker level did not systematically affect the response ratios. However, when the ISI was increased, the response ratios shifted closer to unity (Figure 26A-D). Since no consistent effects of the masker level on the response ratios could be observed, the response ratios for particular ISIs were pooled across all masker levels (Figure 26E). Statistical analyses confirmed significant reductions of the response ratios in reference to unity for ISIs up to 400 ms (one-sample t-tests, P<0.05). For the same dataset, a double exponential approximation was computed as well. The resulting recovery time constants had a fast component of 3.028 ms and a slow component of 299.2 ms (Figure 26F). The latter recovery from adaptation was in the range of the recovery from suppression using significant reductions of the response ratio (400 ms). This recovery was much slower than the recovery computed with single exponential approximations applied for individual masker levels. Here, the recovery time constants were shorter than 17 ms for all masker levels (Figure 25F-J). For 70% masker level (Figure 25G) it was not possible to fit a single exponential function to the data. When the compensation for the response ratio was computed with linear regressions (Figure 25F-J), the resulting ISIs were longer than one second (Figure 26G).
Figure 25: Response ratios in the ISI tuning. A-E summarizes the response ratios as a function of the ISI between masker and probe for the five masker levels (A: 90%; B: 70%; C: 50%; D: 30% and E: 10%). Each gray line represents a unit recorded at that particular masker level. The dashed black line in each subplot represents unity for the response rates for the masker and the probe. Note the similar scales in A-E. The response ratios increased slightly with decreasing level of the masker. F-J: Mean (±SEM) response ratios as a function of the ISI between masker and probe. The dashed line again represents unity in the response rate to the masker and the probe tone. Note the similarity in the slopes of the linear regressions (black line) for the five masker levels. The gray solid line in F-J shows the single exponential fit to the data indicating a recovery of the response ratios as a function of ISI. Significant differences of the response ratios deviating from 1 are indicated by asterisks (one-sample t-tests; P<0.05: *; P<0.01: **; P<0.001: ***).
Therefore, the compensation with linear regressions was much slower than the results described previously for the recovery time constants (Figure 25F-J). The recovery from adaptation thus acted either on extremely short time scales of only a few milliseconds when single exponential fits were applied or on really slow time scales (longer than one second) when the recovery was computed by linear regressions. Intermediate recovery periods were achieved by pooling the response ratios across all masker levels: analyses based solely on a significant reduction of the response ratio yielded a recovery interval of 400 ms (Figure 26E) that was comparable to the slow component of the recovery time constant using a double exponential approximation (300 ms; Figure 26F).

Figure 26: Response ratios and recovery from adaptation in the ISI tuning. A-E: The response ratios for four ISIs are plotted as a function of the masker level (A: 25 ms; B: 100 ms; C: 400 ms and D: 1600 ms). The response ratios increased as a function of the increasing ISI comparable for all masker levels. E: Pooled responses ratios across all masker levels. The mean (±SEM) responses ratios were significantly reduced in reference to unity for ISIs up to 400 ms (one-sample t-tests, all P<0.05; significance levels are indicated as follows: P<0.05: *; P<0.01: **; P<0.001: ***). F: Double exponential recovery function computed for the pooled response ratios across all masker levels. The recovery time constant of the slow component was 299.2 ms. G: The computed ISI required for compensation in the response ratio was plotted as a function of the masker level. The data were taken from Figure 25F-J using linear regressions.
Figure 27 summarizes the relationship of masker level and response ratio required for compensation in the two stimulus paradigms. In the 2nd level tuning (Figure 27A), the response to the probe depended strongly on the decreasing masker level since the response ratios became larger for increasing probe levels. Therefore the slopes of the linear regressions increased as well (Figure 21F-J). Whereas the slope of the linear regression was negative for 90% masker level (Figure 21F and Figure 27A) indicating an ongoing suppression of the response to the probe, the slopes of the linear regressions became steeper for decreasing masker levels. Response adaptation evoked by the masker could be compensated by an increase of the stimulus level of the probe tone (Figure 21G-J). The increase of the computed stimulus level required for the probe to compensate response adaptation decreased therefore as well (Table 1).

**Figure 27: Comparison of 2nd level and ISI tuning.** 

A: The slopes (mean ±SEM) of the linear regression computed for the 2nd level tuning were taken from Figure 21F-J and are displayed as a function of the stimulus level of the masker. Note that the slopes became steeper with decreasing level of the masker. B: The slopes (mean ±SEM) of the linear regressions computed for the ISI tuning were taken from Figure 25F-J. Only a slight increase in the steepness of the slope could be observed for stimulus levels decreasing from 90% to 50%. For lower masker levels, the steepness of the slope saturated. Note the different scales on the ordinates for A and B. Figure A has been shown in Figure 22B before.

In contrast to the 2nd level tuning, the results of the ISI tuning were less evident concerning the relationship of stimulus compensation and masker level. Similar to the 2nd level tuning (Figure 27A), the slopes of the linear regressions increased with decreasing masker level in the ISI tuning (Figure 27B). But the change in the steepness of the slopes was much smaller for the ISI tuning than for the 2nd level tuning for corresponding masker levels (compare Figure 27A, B). Furthermore, in the 2nd level tuning, the slopes continuously increased for decreasing masker levels (Figure 27A), whereas in the ISI tuning the slopes saturated for masker levels lower than 50% of the RLF (Figure 27B).

In summary, the analyses of the response ratio revealed substantial differences of tonal double-stimulation varying either in the level of the probe tone (2nd level tuning) or the ISI between masker and probe in the responses of ICC neurons. Whereas in the 2nd level tuning, a clear influence of the masker level was found, this effect was less pronounced in the ISI tuning, where the stimulus level of the masker seemed of minor relevance. Instead, the ISIs ≥25 ms had a strong impact on the responses to the probes. ICC neurons were found to be very sensitive to small changes in the level of the probe stimulus and the overall level of the masker furthermore influenced this sensitivity. In contrast, the ISI tuning revealed a relative insensitivity of ICC neurons to the overall stimulus level, if masker and probe tone were separated by an interval of at least 25 ms. Nonetheless, the effect of the masker could hamper the responses to the probes for periods of 300 ms to 400 ms.
5.3.3.3 Masking ratio

The results of the masking ratio are displayed in Figure 28 for the five stimulus levels of the masker. In Figure 28A-E, the pooled datasets of the masking ratio revealed a similarity to the progression of the response ratios in the ISI tuning (Figure 25A-E). The masking ratio of most units was rather insensitive to changes in the inter-stimulus interval and ran mostly in parallel to unity (Figure 28A-E). Additionally, no obvious changes in the progression of the masking ratio were observed for the different masker levels. Nonetheless, a quantitative analysis of the results revealed some effects of the masker level and the ISI on the masking ratio (Figure 28F-J).

According to Finlayson (1999) a change in the masking ratio of -20% was defined as suppression. For the highest masker level of 90% (Figure 28F), surprisingly no suppression of the response rate between the probes and the reference value was found. With a further decrease of the masker level (Figure 28G-J), the suppression of the response rates for the probe tones increased systematically towards longer ISIs: 25 ms (70% masker level, Figure 28G); 50 ms (50% and 30% masker level, Figure 28H, I) and 100 ms (10% masker level, Figure 28J). Here, the suppression of the probes was clearly influenced by the level of the masker. However, the results of the statistical analyses (Wilcoxon signed-rank tests) were somewhat different from the definition of suppression and no consistent effect of the masker level on the statistical deviation from unity could be observed (see asterisks in Figure 28F-J). When the data were fitted with a straight line to compute the ISI required for compensation of the masking ratio (black lines in Figure 28F-J), the ISIs were generally longer than 800 ms and increased with decreasing level of the masker as may be seen in Figure 28F-J (90%: 908 ms; 70%: 1131 ms; 50%: 2637 ms; 30%: 1056 ms; 10% 1425 ms). Applying a single exponential approximation (gray curves in Figure 28F-J) yielded much shorter recovery periods than those for the linear regression and here, the recovery time constants displayed a systematic increase from about 6 ms (90% masker level, Figure 28) to 10.26 ms (10% masker level, Figure 28J).

For the masking ratios pooled across all stimulus levels, the recovery time constant of the single exponential approximation was in the same range as the recovery functions of the unpooled stimulus levels (recovery time constant: 8.142 ms). A double exponential recovery function as shown for the pooled response ratio (Figure 26F) could not be computed for the pooled masking ratio. In summary, a systematic effect of the masker and its level on the responses to the probes could only be observed for short ISIs.
Figure 28: Masking ratios in the ISI tuning. A–E summarizes the masking ratio as a function of the increase in the ISI between the probes for ISIs ≤800 ms and the reference value at an ISI of 1600 ms for the five masker levels tested (A: 90%; B: 70%; C: 50%; D: 30% and E: 10%). Each gray line represents a particular unit. The dotted black line in each subplot resembles the masking ratio of unity where no differences in the response to the probe and the reference value occurred. The masking ratios at all stimulus levels of the masker ran rather in parallel to the masking ratio of unity for all ISIs. F–J: Median masking ratios as a function of the ISI. The dashed black line again represents response unity to the probes and the reference value. Note that the masking ratio was smaller than unity for all ISIs at the five masker levels tested. The slopes of the linear regressions (black lines) became slightly steeper with decreasing masker level whereas the time constants increased (gray lines). Significant differences of the masking ratios deviating from unity are indicated by asterisks (Wilcoxon signed-rank tests; significance level P<0.05: *; P<0.01: **; P<0.001: ***).
5.3.3.4 Comparison of response ratio and masking ratio

Since the ISI tuning was used for the computation of the response ratio (Figure 25) and the masking ratio (Figure 28), it was interesting to compare the results of both analyses. The required ISI to compensate response adaptation in the response ratio decreased with decreasing stimulus level of the masker from about 3.4 s at 90% to 1.3 s at 70% masker level and remained relatively constant for all lower stimulus levels (Table 3). An opposite effect could be observed for the masking ratio (Table 3), where an increase of the required ISI was found for decreasing masker levels. Interestingly, the computed ISIs remained rather constant for lower masker levels (70% - 10%, Table 3) and the differences were in the range of 88 ms to 260 ms (Table 3). However, the ISIs required for compensation of response adaptation were similar in both the response and masking ratio and generally lasted longer than one second.

Table 3: Compensation in the ISI tuning. The ISIs that were required to compensate for the response or masking ratio were computed with linear regressions. The ISIs between both analyses were similar and lasted longer than one second.

<table>
<thead>
<tr>
<th>Masker level [% RLF]</th>
<th>Response ratio [ms]</th>
<th>Masking ratio [ms]</th>
<th>Difference response – masking ratio [ms]</th>
</tr>
</thead>
<tbody>
<tr>
<td>90%</td>
<td>3410</td>
<td>908</td>
<td>2502</td>
</tr>
<tr>
<td>70%</td>
<td>1264</td>
<td>1131</td>
<td>133</td>
</tr>
<tr>
<td>50%</td>
<td>1233</td>
<td>2637</td>
<td>-1404</td>
</tr>
<tr>
<td>30%</td>
<td>1315</td>
<td>1056</td>
<td>259</td>
</tr>
<tr>
<td>10%</td>
<td>1513</td>
<td>1425</td>
<td>88</td>
</tr>
</tbody>
</table>

The results for the recovery time constants for individual masker levels were similar between the response and masking ratio (Table 4) as well. Here, an increase in the recovery time constant was found for decreasing masker levels in both analyses. Generally, the recovery time constants were slightly shorter for the masking ratio than for the response ratio. The time constants for the response ratio increased from 7 ms (90% masker level) to 16 ms (30% masker level, Table 4). The shortening of the time constants to 5.7 ms for 10% masker level was somewhat contradictory. For the masking ratio, an increase of the time constants could be observed from 6.17 ms at 90% masker level to about 10.3 ms for 30% and 10% masker level (Table 4). However, no comparison could be made for the pooled ratios, since a double exponential approximation could not be computed for the masking ratio.
Table 4: Comparison of the recovery time constants. Single exponential approximations were applied to compute the recovery time constants. The time constants for the response ratios were slightly longer than those for the masking ratio.

<table>
<thead>
<tr>
<th>Masker level [% RLF]</th>
<th>Response ratio [ms]</th>
<th>Masking ratio [ms]</th>
<th>Difference response – masking ratio [ms]</th>
</tr>
</thead>
<tbody>
<tr>
<td>90%</td>
<td>7.22</td>
<td>6.17</td>
<td>1.05</td>
</tr>
<tr>
<td>70%</td>
<td>---</td>
<td>8.28</td>
<td>---</td>
</tr>
<tr>
<td>50%</td>
<td>9.9</td>
<td>7.02</td>
<td>2.88</td>
</tr>
<tr>
<td>30%</td>
<td>16.08</td>
<td>10.34</td>
<td>5.74</td>
</tr>
<tr>
<td>10%</td>
<td>5.71</td>
<td>10.26</td>
<td>-4.55</td>
</tr>
</tbody>
</table>

The recovery from response adaptation for the probe stimuli as a linear process thus was much slower than for single exponential recovery functions in both the response- and masking ratio (Table 3 and Table 4). Based on these differences it would mean that the neurons in ICC act either on rather fast time scales as indicated by the recovery time constants shorter than 20 ms or on rather slow time scales as indicated by the linear regressions that yielded compensation intervals of longer than one second. To assess these differences, it might be useful to compare the recovery intervals with the statistical analyses of both the response ratio (Figure 25F-J) and masking ratio (Figure 28F-J). For the response ratios in Figure 25F-J, a significant reduction of the responses to the probe could be observed for ISIs ≤200 ms (one-sample t-tests, P<0.05). Similar observations were made for the masking ratios (Figure 28F-J, Wilcoxon signed-rank tests, P<0.05). When the results of the pooled datasets were compared, the results were again comparable for the response- and masking ratio. The analysis of the pooled response ratios in the ISI tuning revealed a significant reduction in the responses to the probe for ISIs up to 400 ms (Figure 26E). In the masking ratio, the responses for the probe stimuli in relation to the reference stimulus were significantly reduced even for ISIs of 800 ms (Wilcoxon signed-rank tests, all P<0.0035; data not shown). This difference in the recovery of the masking ratio might be related to the low number of sweeps the computation was based on. In contrast to the response ratio, where the response on the masker was determined by 70 sweeps, only 10 sweeps could be used in the computation of the masking ratio. Nonetheless, the impact of the masker affected the responses to the probes for a post-masker period of at least 300 ms to 400 ms in both analyses.
5.3.4 Spike-frequency adaptation in ICC neurons

Similar to the chapter on response adaptation, I will describe the results for SFA in both the 2nd level and the ISI tuning. For each paradigm, I will present the data obtained for the pooled responses to the masker before the results for the probe stimuli are compared to the pooled masker responses. Finally, I will investigate SFA in both paradigms in pair wise analyses for masker-probe interactions.

5.3.4.1 Time constants and adaptation ratios for pooled masker responses in the 2nd level tuning

Typically, the responses of the neurons were not constant during stimulation. The responses showed higher activity at stimulus onset that declined to a plateau during ongoing stimulation (Figure 29B, C). This decline in the rate of spikes is termed spike-frequency adaptation and was quantified by the two measures of the adaptation time constant $\tau$ and the adaptation ratio. The response of unit O21U066 in Figure 29A decayed with a time constant of more than 100 ms. The response type therefore was regarded as tonic since the time constant exceeded the period of stimulation. In contrast, the time constants of the units O14U403 and O21U076 showed primary-like response types with time constants between 20.2 and 3.6 ms, respectively (Figure 29B, C). It was now first analyzed whether there was a difference between the response types of all units if the analysis was based on individual responses for each masker at a given probe level or between the response types when all responses to the masker were pooled. This analysis was performed due to the fact that the computation of time constants and adaptation ratios strongly depends on the number of spikes. For a pair wise analysis, the computation was based on ten sweeps that were recorded for each masker-probe combination whereas for the pooled masker responses, the PSTH was computed from 60 sweeps in the 2nd level tuning. For 50% masker level, the number of tonic units as judged by the time constant was 29% for the unpooled units. This number decreased only slightly to 27% for the pooled masker responses. Thus, the two analyses yielded similar numbers of tonically responding units. Comparable results were also found for the four other masker levels. For this reason, the pooled dataset will be used first to describe the results of time constant and adaptation ratio.

![Figure 29: PSTHs for pooled masker responses.](image)

A-C: The stimulus duration of the masker is denoted by the black bar above the PSTH. The black solid lines denote the computed single-exponential fits. A: A tonic response to the masker ($\tau$: 50.4 s; AR: 0.5357). B: Phasic-tonic response with a short time constant of 20.2 ms and an adaptation ratio of 0.2875. C: Phasic-tonic response with a rapid time constant of 3.6 ms and an adaptation ratio of 0.117.
The percentage of tonically responding units depended on the stimulus level of the masker (Figure 30A-E): at 10% masker level, about 22% of the units responded tonically to the stimulus. This percentage increased to 32% at 90% stimulus level. The time constants for the units shown in Figure 29B, C were clearly below 100 ms: 20.2 ms for unit O14U493 and 3.6 ms for unit O21U076. The responses did not decay to zero with ongoing stimulation but ended in a plateau. These units responded in a phasic-tonic or primary-like fashion. This response behavior was observed in a majority of the units (Figure 30A-E) and was caused by spike-frequency adaptation (SFA). This was furthermore quantified by the distribution of the time constants and the adaptation ratios (Figure 30): The median time constants for phasic-tonic units varied between 7.2 ms at 10% and 15.1 ms at 50% masker level with a trend to an increase of the time constant from low to high masker levels. This was confirmed by a statistical analysis for all time constants shorter than 100 ms. A Kruskal-Wallis test revealed a significant difference in the medians for the time constants (P<0.0001). On a more closer look, Dunn’s post-tests detected significant differences for the combinations 90% vs. 30%, 90% vs. 10%, 50% vs. 30% and 50% vs. 10% (all P<0.05).

No significant differences in the time constants for all masker levels could be found between units recorded in ICCcore and ICCls.

The relation between the maximum peak and the tonic plateau of the neuron’s response to the stimulus was quantitatively measured by the adaptation ratio. The analyses of the adaptation ratios were based on all units irrespective of the duration of the time constant and thus their response type unless otherwise noted. Theoretically, all tonic units should have adaptation ratios close to 1 if the responses would not fluctuate in time. But as may be seen in Figure 29A, the response showed a high variability of spikes resulting in an adaptation ratio of 0.54 for that particular unit. The medians for the adaptation ratios scattered between 0.25 at 10% masker level and 0.4 at 70% masker level (Figure 30F-J). The median tonic plateau thus was between 25% and 40% of the maximum response. Although the adaptation ratios covered the whole possible range from 0 to 1 (Figure 30F-J), the distributions of the adaptation ratios for masker levels between 10% and 50% of the saturating response were clearly skewed towards lower values. This might indicate that many units had a low tonic response in reference to the peak discharge rate, which was also reflected in the low median adaptation ratios. On the other hand, the plateau was proportionally higher than 50% of the maximum response in the PSTH of many units for the two highest masker levels (Figure 30F, G). A Kruskal-Wallis test revealed significant differences between the medians obtained for the five masker levels (P<0.0001). Dunn’s post-tests demonstrated highly significant differences between the medians for the adaptation ratio between 10% masker level and those obtained at 70% and 90% masker level, respectively (P<0.0001). When the adaptation ratios of only those units were analyzed that had time constants shorter than 100 ms, significant differences were found between 90% and 30% masker level as well as between 70% and 30% masker level (Kruskal-Wallis and Dunn’s post-tests, P=0.0018). Unlike for the time constants, significant differences in the adaptation ratio could be found between units recorded in ICCcore and ICCls at the masker levels of 90% (t-test, P=0.0261) and 50% (Mann-Whitney test, P=0.0482).
Figure 30: Masker time constants and adaptation ratios in the 2nd level tuning. A-E: Histograms for the time constants of the responses to the masker at stimulus levels from 90% (A) to 10% (E). With decreasing masker level the median time constants decreased from 11.64 ms to 7.19 ms. F-J: Histograms of the corresponding adaptation ratios. The median adaptation ratios decreased from 0.37 at 90% masker level (F) to 0.25 at 10% masker level (J).

Since both time constants and adaptation ratios depended on the masker level, it was also tested whether there was a correlation between the two measures at different masker levels. Generally, a linear relation could explain less than 5% of the variability. For time constants that were in the range of 5 ms and 100 ms, the coefficients of determination scattered between 0% for 90% masker level and 19% for 50% masker level.

In summary, the majority of ICC neurons adapted very fast to a level of about 30% of the maximum onset response in the PSTH and especially the time constants co-varied with the stimulus level of the masker.

5.3.4.2 SFA for the probe responses in the 2nd level tuning

After it could be shown that the stimulus level of the masker had substantial impact on the time constants and adaptation ratios of ICC units, it was now analyzed how both the level of the masker as well as the level of the probe affected the responses to the probes. This will be first demonstrated for all units recorded at a masker level corresponding to 50% of the saturating RLF since the responses at this masker level were most stable (see Figure 36A and Figure 38A). For example, unit O14U403 responded primary-like to the masker but tonically to the probe (Figure 31A). The number of units responding tonically to the masker increased from 27% to 54% for tonic responses to the probe. This difference in tonically responding units between the masker and the probe was highly significant (Wilcoxon matched-pairs test, P=0.0004). The unit shown in Figure 31B responded in a phasic-tonic manner to both the masker and the probe. This occurred in 46% of the cases.
To test whether the probe level had an influence on the temporal dynamics of the responses, those units that had time constants below 100 ms were analyzed more closely. These units showed a reduction in the ongoing response from a phasic onset peak to a lower-level tonic response in the analysis interval. This for example was illustrated in Figure 31B where the time constant of the response to the probe (16.2 ms) was longer than the time constant to the masker (3.6 ms). At 50% masker level, 73% of the responses to the masker had time constants below 100 ms (Figure 30C). For the responses to the probes at the same stimulus level (0 dB probe level; Figure 32A), only 46% of the units responded primary-like to the probe. Although the percentage of primary-like responses between masker and probe decreased dramatically, the median time constants for primary-like units were in the same range (15 ms for the masker and 16 ms for the probe). If the responses of only those neurons that responded primary-like to the masker were compared with the responses to the probe at 0 dB probe level, the time constants for the masker were significantly shorter than those for the probe (Wilcoxon matched-pairs test, $P<0.0001$). With an increase of the probe level, the number of tonically responding units decreased only marginal and the median time constants below 100 ms for the responses to the probe decreased slightly as a function of the probe level: At 0 dB probe level, 46% of all units had time constants below 100 ms (Figure 32A) whereas at 25 dB probe level 55% of the units had time constants shorter than 100 ms (Figure 32F). However, no significant differences for the time constants shorter than 100 ms were found within all probe levels at 50% masker level (Kruskal-Wallis test, $P=0.6589$).
Figure 32: Probe time constants and adaptation ratios at 50% masker level. A-F: Histograms for the time constants to the different probe levels from 0 dB (A) to 25 dB (F) louder than the masker. The number of units tested (N), the percentages of units having time constants shorter or longer than 100 ms as well as the median time constants are given in the inset. G-L: Corresponding adaptation ratios for the same units and the six probe levels. The number of units analyzed and the median adaptation ratios are listed as well. The difference in the number of analyzed units between the histograms for the time constants and adaptation ratios was due to a failure of computing time constants in some PSTHs.

The adaptation ratios for the responses to the probe did not depend on the level of the probe. The median adaptation ratios scattered between 0.21 and 0.25 dB for all probe levels (Figure 32G-L). As may be seen in Figure 32G-L, the majority of neurons had adaptation ratios that were shifted towards smaller values for all probe levels indicating strong SFA in the PSTHs. Furthermore, for probe levels higher than 15 dB (Figure 32J-L), no adaptation ratios larger than 0.95 were found, whereas at lower probe levels these large adaptation ratios occurred indicating tonic-like responses (Figure 32G-I). No significant differences between the adaptation ratios at different probe levels could be found either for the entire population of neurons or for only those units that had time constants shorter than 100 ms (Kruskal-Wallis tests, P=0.7815 and P=0.9623, respectively).

So far only the responses to the probes at 50% masker level have been described, the following analyses compare the responses to the probes to their corresponding responses to the masker (Figure 33). The cumulative probability distributions for the time constants of primary-like units revealed a clear effect of the masker level on the responses to the probes: For the two extreme masker levels (90% and 10%), the distributions for the time constants were widely scattered (Figure 33A, E), whereas at 50% masker level the distribution was very similar for all probe levels in comparison to the masker (Figure 33C). Furthermore, the cumulative probability distributions of the probes (gray curves) shifted in reference to the masker (black dashed curve) as a function of the masker level. For the highest masker level of 90% (Figure 33A), the curves for the probe stimuli were generally shifted left of the masker for time constants longer than 25 ms. This trend gradually reversed with decreasing masker levels. At 10% (Figure 33E), the cumulative probability distributions for the probes were shifted right of
the masker indicating longer time constants for the probes in reference to the masker. Comparing only those responses, that had time constants shorter than 100 ms either for the masker or for individual probe levels at that specific masker level, it turned out that the time constants of primary-like responses were generally not significantly different between masker and probe for masker levels of 10% to 50% (Kruskal-Wallis tests, P>0.05; Figure 33C-E). For masker levels of 90% and 70%, significant differences in the time constants of primary-like units were detected between the responses to masker and probe (Kruskal-Wallis tests and Dunn’s post-tests: P=0.0441 and P=0.0386, respectively; Figure 33A, B). Thus, if neurons responded phasic-tonically to a stimulus, the time constants of these units were generally not significantly different between the responses to either the masker or to the probe when the stimulus level was in the lower half of the RLF. Once neurons adapt with primary-like responses, they seem to adapt irrespective of their current state of adaptation.

However, the results were completely different, when the responses to the probe were investigated in reference to their corresponding response to the masker. This was investigated more closely for pooled masker responses that had time constants shorter than 100 ms and their matched probe response at either 0 dB or 25 dB probe level: When both stimuli had the same stimulus level, the time constants between masker and probe differed significantly for all masker levels (Wilcoxon matched-pairs tests, all P<0.0001). The time constants for the probe stimuli were longer than those for the masker. When the probe level was 25 dB louder than the masker level, the results were similar to 0 dB probe level with the only exception found for 90% masker level. Here, no significant difference between masker and probe could be observed (Wilcoxon matched-pairs test; P=0.133) in contrast to masker levels of 70% - 10%, where the time constants differed significantly (Wilcoxon matched-pairs tests, P<0.0001). Concerning the medians of the time constants for the different probe stimuli at individual masker levels, no common trend could be found: for 90% masker level, the median time constants increased slightly with increasing probe level (8.04 ms for 0 dB and 11.07 ms for 25 dB). This observation was more extreme at 30% masker level. Here, the median time constants increased from 11.47 ms at 0 dB probe level to 24.27 ms at 25 dB. Conflicting observations were made for the three other masker levels, where the time constants decreased with increasing probe level. The most extreme case was found for 70% masker level, where the median time constant decreased from 27.49 ms at 0 dB to 7.29 ms at 25 dB probe level. However, the median time constants for the six probe levels generally did not differ significantly within individual masker levels (Kruskal-Wallis tests, all P>0.09) either for the entire population of units or only primary-like units. The only exception for significant differences between the time constants for the probe was found for primary-like responses at 70% masker level (Kruskal-Wallis test, P=0.0239).

When the response types were analyzed, it was found that more units responded in a phasic-tonic than in a tonic manner if the level of the probe was increased (data not shown). The effect was most dramatic at 90% masker level: When masker and probe had the same stimulus level, only 44% of the units responded phasic-tonically to the probe. At 25 dB probe level, 66% of the units had time constants shorter than 100 ms. Comparable effects were found for the other masker levels as well but the effect became less obvious. For example, at 10% masker level, 50.44% of the units had time constants shorter than 100 ms for 0 dB probe level. An increase in the probe level to 25 dB louder than the masker yielded 53% of the units responding phasic-tonically to the probe.
The analyses of the adaptation ratios for the probe tones at different masker levels revealed a more homogeneous distribution (Figure 33F-J) than the analysis of the time constants (Figure 33A-E). The adaptation ratios for the probe stimuli were generally smaller than those for the masker at all stimulus levels indicating stronger SFA for the responses to the probe than for the masker. The gray curves for the probe stimuli were shifted left of the dashed line resembling the masker in Figure 33F-J. This was the case for all probe levels in comparison to the masker and confirmed by statistical analyses (Kruskal-Wallis tests, all P<0.05). It was additionally verified more closely by paired analyses between the masker and individual probe responses (Wilcoxon matched-pairs tests, all P<0.05). The only exceptions between masker and probe were found for probe levels of 25 dB at 30% and 10% masker level as well as 20 dB probe level at 10% masker level (Wilcoxon matched-pairs tests, P>0.05). As for the adaptation ratios at 50% masker level, no significant differences in the adaptation ratio for the probes could be found within the other stimulus levels as well (Kruskal-Wallis tests, P>0.05).

Figure 33: Cumulative probability distributions for the probe responses in the 2nd level tuning. A-E: Cumulative probability distributions for the time constants of only primary-like probe responses recorded for masker levels of 90% (A) to 10% (E). At 90% masker level, the distribution of the time constants for the probes (gray lines) was slightly left of the masker (black dashed line) whereas the result was reversed at 10% masker level. F-J: Cumulative probability distributions for the adaptation ratios. Here, the adaptation ratios of all probes were generally lower than those of the masker for all masker levels.

Figure 34 summarizes the relationship of time constants and masker level for the pooled masker responses as well as the responses to the probes at 50% masker level. As may be seen in Figure 34A, the time constants of primary-like responses to the masker decreased significantly with decreasing masker level (Kruskal-Wallis test, P<0.0001) from about 12 ms at 90% masker level to about 7 ms at 30% and 10% masker level. However, the longest time constant was found at 50% masker level. Significant differences indicated by Dunn’s post-tests are indicated by asterisks in Figure 34A. In contrast to the time constants for the responses to the masker, the time constants for the responses to the probes decreased with increasing probe level (Figure 34B). Starting at
0 dB probe level, the median time constants decreased almost linearly from 15 ms to 10 ms at 15 dB probe level. A further increase of the stimulus level of the probe up to 25 dB did not result in an ongoing shortening of the time constants.

A comparison of the relationship between stimulus level and adaptation ratio again revealed different results for the responses to the masker and the probe (Figure 34C, D). Whereas the median adaptation ratios decreased significantly with decreasing stimulus level for the responses to the masker (Kruskal-Wallis test and Dunn’s post-test, P<0.0001; Figure 34C), the median adaptation ratios remained stable for the responses to the probes for all probe levels (Figure 34D).

Figure 34: Summary of the time constants and adaptation ratios in the 2nd level tuning. A: Median time constants for primary-like units are plotted as a function of the masker level for all units with time constants shorter than 100 ms. The time constants decreased significantly with decreasing masker level (Kruskal-Wallis test, P<0.0001). B: Median time constants for the response to the probe at 50% masker level. The median time constants decreased with increasing probe level but without any statistical significance (Kruskal-Wallis test, P=0.6589). C: Median adaptation ratios showed a significant decrease as a function of the masker level (Kruskal-Wallis test, P<0.0001). D: Adaptation ratios for the probes at 50% masker level. The adaptation ratios did not change significantly with increasing probe levels (Kruskal-Wallis test, P=0.78). The slopes as well as the coefficients of determination for the linear regressions are given in the insets. Number of asterisks denotes significance level (P<0.05: *; P<0.01: **; P<0.001: ***).
5.3.4.3 Pair wise masker-probe interactions in the 2nd level tuning

Since the previous analyses were based on pooled responses for the masker and focused on units having time constants shorter than 100 ms, it was now analyzed how the probes were directly affected by the masker in pair wise analyses. For the time constants, no restrictions in their duration (primary-like or tonic) were made neither for the responses to the masker nor for the responses to the probe.

Generally, the median time constants for the responses to the probe were longer than the median time constants for the responses to the masker in this pair wise analysis (Figure 35). This was the case for all probe levels with the only exceptions observed at 90% masker level and probe levels ≥15 dB (Figure 35A). Here, the median time constants for the probe responses were slightly shorter than those for the masker. However, no significant differences in the median time constants could be found neither between the responses to the masker and probe (Wilcoxon matched-pairs test, all P>0.25) nor within the responses to the masker or the probe (Kruskal-Wallis tests, P=0.99 and P=0.10, respectively). With decreasing stimulus level of the masker, the time constants for the responses to the probe were consistently longer than those for the responses to the masker (Figure 35B-E). At 70% masker level (Figure 35B), significant differences in the time constants between the responses to the masker and probe were found for probe levels of 5 dB (Wilcoxon matched-pairs test, P=0.006) and 15 dB (Wilcoxon matched-pairs test, P>0.0001). At 50% masker level (Figure 35C), all time constants for the responses to the probe tones were significantly longer than those for the masker (Wilcoxon matched-pairs tests, 0 dB: P=0.0004; 5 dB: P=0.03; 10 dB: P<0.0001; 15 dB: P=0.0003; 20 dB: P<0.0001 and 25 dB: P=0.0016). For decreasing masker levels, the trend of longer time constants for the probe tone responses was confirmed. At 30% masker level (Figure 35D), significant differences (Wilcoxon matched-pairs tests) were found for probe levels of 15 dB (P=0.009), 20 dB (P<0.0001) and 25 dB (P=0.0047). At 10% masker level (Figure 35E), the median time constants for all probe responses were again significantly longer than those for the masker (Wilcoxon matched-pairs tests, 0 dB: P=0.0161; 5 dB: P=0.0285; 10 dB: P<0.0001; 15 dB: P=0.0024; 20 dB: P<0.0001 and 25 dB: P<0.0001).

Figure 35: Time constants in the 2nd level tuning. The median time constants for the responses to the probe were shown as a function of the median time constants for the responses to the masker (A: 90%, B: 70%, C: 50%, D: 30% and E: 10%). The time constants are color-coded for each probe level and significant differences between the time constants to the masker and probe were indicated by asterisks (see inset; P<0.05: *, P<0.01: **, P<0.001: ***). Note the different scales of the ordinate for A-C and D-E.
Apart from a systematic shift towards shorter time constants for the responses to the masker as a function of decreasing masker level (Figure 35A-E), there was a dramatic decline in the duration of the time constants for the probe responses at different masker levels. Whereas the median time constants for high masker levels (90% - 50% of the RLF, Figure 35A-C) were in the range of 1000 ms to 30000 ms in the most extreme cases, they were much shorter (below 100 ms) for lower masker levels (30% and 10% of the RLF, Figure 35D-E).

Figure 36 displays the decrease of the median time constants for the responses to the masker (Figure 36A) and the probe (Figure 36B) as a function of decreasing masker level. For the masker, the median time constants at 10% masker level were significantly shorter than those at 90% masker level for most probe levels (Mann-Whitney tests, 0 dB: P=0.008; 10 dB: P=0.0053; 15 dB: P=0.122; 20 dB: P=0.0004 and 25 dB: P=0.0279). A similar trend was observed for the median time constants for the responses to the probe, which mostly decreased with decreasing stimulus level of the masker as well (Figure 36B). However, for a probe level of 25 dB, the median time constant rather increased with decreasing masker level. Furthermore, no significant differences in the median time constants of the responses to the probes could be found between 90% and 10% masker level (Mann-Whitney tests, all P>0.26) indicating a large variability of the time constants in the responses to the probe. The slopes of the linear regressions shown in Figure 36A, B are listed in Table 5. No significant differences were observed between the linear regressions of the responses to the masker whereas the slopes of the linear regressions for the responses to the probes differed significantly (ANCOVA, P=0.4478 and P=0.0087, respectively).

Figure 36: Relationship of time constant and masker level in the 2nd level tuning. Median time constants for the responses to the masker (A) and the probe (B) are shown as a function of the stimulus level. A: The time constants for the masker decreased systematically with decreasing stimulus level for all probe levels tested. Asterisks denote significant differences in the median time constants between 90% and 10% masker level (Mann-Whitney tests; P<0.05: *, P<0.01: **, P<0.001: ***). B: The time constants of the responses to the probe generally decreased with decreasing probe level, but no significant differences in the median time constants could be found between 90% and 10% masker level. Curved lines represent linear fits on a log scale. Note the differences in the scales of the ordinates in A and B.
Table 5: Linear regressions for the time constants in the 2nd level tuning. The slopes were calculated from the linear regressions displayed in Figure 36A,B.

<table>
<thead>
<tr>
<th>2nd level</th>
<th>Slopes of linear regressions to the masker</th>
<th>Slopes of linear regressions to the probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 dB</td>
<td>y=0.5048x+10.57</td>
<td>y=254x-3483</td>
</tr>
<tr>
<td>5 dB</td>
<td>y=0.1732x+16.52</td>
<td>y=336.8x-7453</td>
</tr>
<tr>
<td>10 dB</td>
<td>y=0.4657x+6.313</td>
<td>y=7.415x-124</td>
</tr>
<tr>
<td>15 dB</td>
<td>y=0.2852x+7.294</td>
<td>y=133.2x-1234</td>
</tr>
<tr>
<td>20 dB</td>
<td>y=0.4283x+2.157</td>
<td>y=133.5x-1219</td>
</tr>
<tr>
<td>25 dB</td>
<td>y=0.1773x+11.37</td>
<td>y=-0.2362x+63.44</td>
</tr>
</tbody>
</table>

In contrast to the time constants, the adaptation ratios in the 2nd level tuning did not change much for the different masker levels (Figure 37). The median adaptation ratios for the responses to the masker showed a decrease from about 0.25 to 0.3 at higher masker levels (Figure 37A-C) to adaptation ratios between 0.1 and 0.2 for lower masker levels (Figure 37D, E). A similar observation was made for the responses to the probe shown on the ordinates in Figure 37. Here again, adaptation ratios for higher masker levels (Figure 37A-C) were slightly larger than those for lower masker levels irrespective of the stimulus level of the probe (Figure 37D, E). A pairwise comparison of the adaptation ratios between the responses to masker and probe led to two significant differences (Wilcoxon matched-pairs tests), which both occurred at probe levels of 25 dB: At 30% (P=0.029; Figure 37D) and 10% masker level (P=0.004; Figure 37E), respectively. For the remaining masker-probe pairs, no systematic effect of the masker on the adaptations ratios for the probe was observed.

Figure 37: Adaptation ratios in the 2nd level tuning. The median adaptation ratios for the responses to the probe are displayed as a function of the adaptation ratio for the responses to the masker (A: 90%; B: 70%; C: 50%; D: 30% and E: 10%). The adaptation ratios are color-coded for each probe level from 0 db to 25 db and decreased slightly with decreasing stimulus level. Significant differences in the adaptation ratio between the responses to the masker and the probe are indicated by asterisks (P<0.05: *; P<0.01: **).
When the adaptation ratios were investigated separately for the responses to the masker and the probe (Figure 38A, B), a reduction of the adaptation ratio as a function of the masker level was obvious for all masker responses as well as for most probe responses indicating stronger SFA for fainter stimuli. For the responses to the masker, adaptation ratios at 10% masker level were significantly lower than those at 90% masker level for five out of six probe levels (Mann-Whitney tests, 0 dB: P<0.0001; 5 dB: P=0.0376; 10 dB: P<0.0001; 15 dB: P=0.0254 and 25 dB: P<0.0001). However, for 20 dB probe level, a trend towards a significant reduction could be observed (Mann-Whitney test, P=0.0523).

![Figure 38: Relationship of adaptation ratio and masker level in the 2nd level tuning. A: A significant reduction of the adaptation ratio with decreasing masker level could be observed for the responses to the masker for most probe levels (see inset). B: The adaptation ratios for the probes were significantly reduced only for probe levels ≤15 dB (see inset) between 90% and 10% masker level. The number of asterisks denotes significance level (P<0.05: *; P<0.01: **; P<0.001: ***).

The adaptation ratios for the responses to the probe tones revealed a similar outcome than the results for the responses to the masker (compare Figure 38A, B). For the probes, a reduction in the response ratio could be observed for decreasing masker levels between 90% and 10% as well. But these reductions were only significant (Mann-Whitney tests) for probe levels ≤15 dB (see inset in Figure 38B; 0 dB: P=0.0052; 5 dB: 0.0108; 10 dB: P=0.0011 and 15 dB: P=0.0124). For probe levels of 20 dB and 25 dB, P values corresponded to P=0.9833 and P=0.1398, respectively. Table 6 summarizes the slopes of the linear regressions computed for the adaptation ratios in the 2nd level tuning (Figure 38A, B). The slopes of the linear regressions did not differ significantly (ANCOVA) neither for the responses to the masker (P=0.2215) nor for the response to the probe (P=0.0966).
Table 6: Linear regressions for the adaptation ratios in the 2nd level tuning. The slopes were calculated from the linear regressions displayed in Figure 43A, B and did not differ significantly either for the responses to the masker or the responses to the probe.

<table>
<thead>
<tr>
<th>2nd level</th>
<th>Slopes of linear regressions to the masker</th>
<th>Slopes of linear regressions to the probe</th>
</tr>
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<td>0 dB</td>
<td>$y=0.0022x+0.0784$</td>
<td>$y=0.0018x+0.1096$</td>
</tr>
<tr>
<td>5 dB</td>
<td>$y=0.0009x+0.1649$</td>
<td>$y=0.0013x+0.1542$</td>
</tr>
<tr>
<td>10 dB</td>
<td>$y=0.0015x+0.1371$</td>
<td>$y=0.0022x+0.1014$</td>
</tr>
<tr>
<td>15 dB</td>
<td>$y=0.0013x+0.1411$</td>
<td>$y=0.0013x+0.1456$</td>
</tr>
<tr>
<td>20 dB</td>
<td>$y=0.0011x+0.1592$</td>
<td>$y=-0.0001x+0.2153$</td>
</tr>
<tr>
<td>25 dB</td>
<td>$y=0.0024x+0.0624$</td>
<td>$y=-0.0008x+0.1917$</td>
</tr>
</tbody>
</table>

In summary, SFA of ICC neurons was strongly influenced by the stimulus level of the masker in the 2nd level tuning. Not only the percentage of neurons responding primary-like to the masker depended on the stimulus level but also the duration of the time constants shortened as a function of a decreasing masker level. The same relationship could be observed for the adaptation ratios where the amount of SFA was stronger for lower masker levels. SFA of the responses to the probes depended not only on the stimulus level of the masker but also on the stimulus level of the probe. The time constants and adaptation ratios for the responses to the probes were strongly influenced by the preceding masker, especially when the stimulus level of the probe was equal to the stimulus level of the masker.

5.3.4.4 Time constants and adaptation ratios for the pooled masker responses in the ISI tuning

The time constants and adaptation ratios in the ISI tuning were analyzed according to the results of the 2nd level tuning. First, the pooled responses to the masker will be described before the responses to the probes are introduced. As in the 2nd level tuning, SFA and response types of the neurons depended on the masker level (Figure 39A-E). For a masker level of 90%, around 54% of the recorded units responded in a primary-like manner to the masker (Figure 39A). With decreasing masker level, the percentage of this response type increased to about 70% for all neurons at 30% (Figure 39D) and 10% masker level (Figure 39E). The only exception was found for a masker level of 70% (Figure 39B), where about 80% of all units responded phasic-tonically to the masker. Although more units showed a phasic-tonic response with decreasing masker level, the median time constants for this response type were relative stable around 10 ms. Statistical analyses revealed no significant differences of the time constants between the masker levels either for the entire population of neurons (Kruskal-Wallis test, $P=0.1805$) or only those units that responded primary-like to the masker (Kruskal-Wallis test, $P=0.7877$).

The analyses of the adaptation ratios revealed only small changes for decreasing masker levels (Figure 39F-J). At 90% masker level, the median adaptation ratio was 0.32 (Figure 39F) and the distribution of the adaptation ratios was rather broad. Similar results were found for masker levels of 70% (Figure 39G) to 30% (Figure 39I). Here, the median adaptation ratios scattered around 0.3 indicating that the response at steady state was adapted by about 70% in comparison to the onset peak. Only for stimuli close to threshold, the median adapta-
tion ratio for pooled masker responses dropped to 0.21 (Figure 39J) indicating stronger SFA in the PSTH of the responses to the masker. The histogram was skewed towards lower values for these responses. Adaptation ratios differed significantly between all masker levels (Kruskal-Wallis test; P=0.0397 and Dunn’s post-test, P<0.05 for a comparison between 90% and 10% masker level).

Figure 39: Masker time constants and adaptation ratios in the ISI tuning. The median time constants for all stimulus levels were computed for the pooled responses to the masker (A: 90%; B: 70%; C: 50%; D: 30% and E: 10% masker level). With decreasing masker level, the percentage of units responding in a phasic-tonic manner to the stimulus increased from 54.32% at 90% masker level (A) to 73.03% at 10% masker level (E). However, the median time constants remained stable around 10 ms for all masker levels. F-J: Adaptation ratios displayed for the masker levels. A change in median adaptation ratio could not be observed for the masker levels spanning from 90% to 30% (F-J). At 10% masker level, the median adaptation ratio dropped from about 0.3 to 0.21 (J).

5.3.4.5 SFA for the probe responses in the ISI tuning

In the ISI tuning, the responses to the probes were relatively constant for all stimulus levels as may be seen in the cumulative probability distributions (Figure 40). Comparable to the 2nd level tuning (Figure 33), the distributions for the time constants scattered more for masker levels of 90% (Figure 40A) and 10% (Figure 40E) than for 50% masker level (Figure 40C). Nonetheless, when the responses to the probes were analyzed as a function of the masker level, no significant differences were found either for the time constants or the adaptation ratios between masker and probe (Kruskal-Wallis tests, all P>0.05). But note, that the analyses of the time constants in this paragraph were only computed for those units that responded phasic-tonically to the masker or the probe. Thus, if neurons showed a primary-like response to the probe, the time constants did not depend on the ISI or the masker level and the neurons again responded irrespective of their state of adaptation. The responses to the probes however differed in some cases, when the statistical analyses were performed as a function of the inter-stimulus interval: Significant differences were generally found for shorter ISIs (≤400 ms) than for longer ISIs (800 ms - 1600 ms). For the shortest ISI of 25 ms, no significant differences could be detected for the time constants but for the adaptation ratios: the median adaptation ratio at 10% masker level was significantly lower.
stants but for the adaptation ratios: the median adaptation ratio at 10% masker level was significantly lower than those at 90%, 70% and 50% masker level (Kruskal-Wallis test and Dunn’s post-tests, P=0.0057). Whereas the time constants and adaptation ratios did not differ significantly for an ISI of 50 ms, significant differences were found for the time constants at ISIs of 100 ms between 90% masker level and masker levels ≤50%, respectively (Kruskal-Wallis test and Dunn’s post-tests, P=0.0005 for the entire population and P=0.0458 for primary-like units only). For ISIs of 200 ms, 400 ms, 800 ms and 1600 ms, no significant differences in the responses to the probe tones could be found for all masker levels, when the time constants of primary-like units were analyzed (Kruskal-Wallis tests, all P>0.05). When the time constants irrespective of the unit’s response type for ISIs ≥200 ms were investigated, the only significant difference was found for an ISI of 400 ms (Kruskal-Wallis test, P=0.0355). Here, a post-test revealed that the median time constant at 90% masker level (58.07 ms) was significantly longer than the median time constant at 10% masker level (18.08 ms). The adaptation ratios of the responses to the probes showed no significant differences for the varying stimulus levels at ISIs of 800 ms and 1600 ms (Kruskal-Wallis tests, all P>0.05). For ISIs of 200 ms and 400 ms, the adaptation ratios differed significantly between the masker levels (Kruskal-Wallis tests, P=0.0209 and P=0.0371, respectively). In summary, the time constants of primary-like responses in the ISI tuning did not differ significantly within the probe responses or in comparison to the masker for all masker levels (Figure 40A-E). Similar observations were made for the adaptation ratios (Figure 40F-J). When the probe responses were analyzed as a function of the ISI, some significant differences were found for the time constants and adaptation ratios especially for ISIs ≤400 ms but without any consistent trend.

Figure 40: Cumulative probabilities of time constants and adaptation ratios in the ISI tuning. The cumulative distributions for the time constants of the probes are compared to the masker (A: 90%; B: 70%; C: 50%; D: 30% and E: 10%). The distributions for the probes are shown in gray whereas the masker is represented by the dotted black line. Note the scatter in the distributions at 90 (A) and 10% (E) masker level. F-J: Adaptation ratios for the responses to the probe stimuli in the ISI tuning. The cumulative probability distributions for the probes were above the adaptation ratio of the masker for all stimulus levels and thus on average smaller.
It was furthermore investigated how the pooled masker responses directly influenced the responses to the probe stimuli. For the time constants, generally no significant differences were detected between the responses to the masker and the probe (Kruskal-Wallis tests, all P>0.05). However, some statistically relevant differences were found between the time constants of the masker in reference to the time constants of the responses to the probe either for the shortest ISI of 25 ms or the longest ISI of 1600 ms. When the time constants for the entire population of neurons were analyzed, significant differences between masker and probe occurred for masker levels of 90% and 50% at 25 ms ISI (Mann-Whitney tests, P=0.0255 and P=0.0058, respectively). The time constants for the pooled responses to the masker were longer than the time constants for the probe. No significant responses were observed between the pooled responses to the masker and the probe at an ISI of 1600 ms as well as for a comparison between the probe responses of 25 ms or 1600 ms (Mann-Whitney and Kruskal-Wallis tests, all P>0.05). Similar results were obtained, when the time constants of only those units were compared, that showed a phasic-tonic response to either the masker or the probe. Here, significant differences were found for the responses to the masker and the probe for masker levels of 70% and 50% at 25 ms ISI (Mann-Whitney tests, P=0.0486 and P=0.0084, respectively) and between the masker and the probe for an ISI of 1600 ms at 10% masker level (Mann-Whitney test, P=0.0491). Again, the time constants for the responses to the masker were longer than the time constants for the responses to the probe. No significant differences were found for a comparison of the time constants for the probe tones between the ISIs of 25 ms and 1600 ms. This might indicate masker-induced suppression for the probe responses indicating stronger SFA at shorter ISIs.

In contrast to the analyses of the time constants, the comparison of the adaptation ratios between the pooled responses to the masker and the probes revealed significant differences for all masker levels (Figure 40F-J; Kruskal-Wallis tests): The adaptation ratios for the responses to the masker were significantly higher than the adaptation ratios for the probes (90%: P=0.0028; 70%: P=0.0205; 50%: P=0.0023; 30%: P=0.0035 and 10%: P=0.0002) indicating less SFA for the responses to the masker. More detailed analyses using Dunn’s post-tests revealed a broad variety of differences between the adaptation ratios for the masker and the probes at individual masker levels. Whereas the differences in the responses were significant for all masker-probe combinations at 10% masker level (Figure 40J; Dunn’s post-tests, all P<0.05), for most masker-probe combinations at 30% masker level (Figure 40I; Dunn’s post-tests, all P<0.05 except for the ISIs of 25 ms and 100 ms) as well as 90% masker level (Figure 40F; Dunn’s post-tests, all P<0.05 except for 200 ms) only few differences for masker-probe combinations were found for masker levels of 50% (Figure 40H, Dunn’s post-tests, P<0.05 for ISIs of 100 ms, 200 ms and 800 ms) and 70% (Figure 40G, Dunn’s post-test, P<0.05 for an ISI of 50 ms). Confirmed by the statistical analyses, the adaptation ratios for the responses to the probes differed significantly from the responses to the masker for most stimulus levels. This was most extreme for the lowest as well as the highest masker level of 10% and 90%, respectively.

Finally, the time constants and adaptation ratios of the responses to the masker are displayed as a function of the masker level (Figure 41A, C). The median time constants of the primary-like units decreased only slightly with decreasing masker level and the time constants were in the range of 10 ms without any significant difference (Figure 41A, Kruskal-Wallis test, P=0.7877). The median adaptation ratios showed also a decrease with decreasing masker level that was significantly different between 90% and 10% masker level (Kruskal-Wallis and Dunn’s post-test, P=0.0397; Figure 41C). Generally, the tonic response plateau was between 20% and 40% of the maximum response rate in the phasic response onset. The adaptation properties of the responses to the probe
stimuli at 50% masker level are shown for the time constants (Figure 41B) and adaptation ratios as well (Figure 41D). As has been mentioned earlier, the median time constants between the response to the masker and the response to the probe at an ISI of 25 ms differed significantly for 50% masker level (Mann-Whitney test, P=0.0084). Furthermore, the median time constants for the responses to the probe for ISIs below 100 ms were shorter (5 ms to 8 ms) than the median time constant for the masker at 50% masker level. Only for ISIs longer than 200 ms, the median time constants for the probe (10 ms to 12 ms) increased and were in the range of the time constant for the response to the masker (10 ms). The median adaptation ratios for the responses to the probes at 50% masker level were in the range of 0.125 to 0.25 (Figure 41D) and thus below the median adaptation ratio for the response to the masker at 50% (0.313, Figure 41B). As mentioned before, the adaptation ratios at ISIs of 100 ms, 200 ms and 800 ms were significantly lower than the adaptation ratio for the corresponding response to the masker. No systematic increase of the adaptation ratio could be found with increasing ISIs as indicated by the very shallow slope of the linear regression (y=0.00001x+13740).

Figure 41: Summary of the time constants and adaptation ratios in the ISI tuning. A: Median time constants are plotted as a function of the masker level for all units responding in a phasic-tonic manner to the masker. Only a slight decrease of the time constants could be observed for decreasing masker levels. B: Median time constants for the responses to the probe at 50% masker level. Here, the median time constant increased slightly with increasing interval between the offset of the masker and onset of the probe. C: Median adaptation ratios for the masker decreased significantly as a function of the masker level (Kruskal-Wallis test, P=0.0397). D: Adaptation ratios for the probe at 50% masker level. The adaptation ratios did not change significantly with increasing ISIs. The slopes as well as the coefficients of determination for the linear regressions are given in the insets. Curved lines in B and D show the linear regressions on a log2 scale. The significance level is indicated by an asterisk (P<0.05: *).
5.3.4.6 Pair wise masker−probe interactions in the ISI tuning

When the time constants for masker and probe were analyzed pair wise for the entire population of neurons in the ISI tuning, the results were different from those in the 2nd level tuning (compare Figure 35 with Figure 42). Generally, the median time constants for all masker levels were in the same range and shorter than 100 ms for both the responses to the masker and the probe (Figure 42) although the time constants were broadly scattered for 90% masker level (Figure 42A). Neither a systematic shift towards longer time constants for the responses to the probe nor any significant differences for the time constants between the responses to the masker or the probe were observed for all masker levels (Wilcoxon matched-pairs test, all P>0.05). Thus, the results were different from the results of the 2nd level tuning (Figure 35) where the time constants for the probes were strongly influenced by the masker. This effect might result from the different ISIs used in both paradigms. Whereas the ISI was set to 0 ms in the 2nd level tuning, it was extended to at least 25 ms in the ISI tuning.

A further analysis of the time constants in the ISI tuning revealed similarities to the time constants recorded in the 2nd level tuning (Figure 36). When only the time constants for the responses to the masker were analyzed, a decrease of the median time constants could be found for decreasing masker levels. However, a significant shortening of the time constants could only be observed for those stimuli, where the ISI between masker and probe was short (≤200 ms; Figure 43A). This finding was somewhat contradictory, since the ISI had no influence on the responses to the masker. When the responses to the probe tones were analyzed (Figure 43B), a consistent reduction of the time constants could not be observed. A significant (Mann-Whitney tests) shortening of the time constants with decreasing masker levels could be found for the ISIs of 100 ms (P=0.0003), 400 ms (P=0.0043) and 1600 ms (P=0.0018). However, the scatter of median time constants was broader for the responses to the probe (Figure 43B) than to the masker (Figure 43A) and the median time constants were slightly longer for the responses to the probe. No significant differences in the median time constants between masker and probe were observed for all ISIs at 90% and 10% masker level (Wilcoxon matched-pairs tests, all P>0.25 and all P>0.18, respectively).

Figure 42: Time constants in the ISI tuning. The median time constants for the responses to the probe are displayed as a function of the median time constants for the responses to the masker (A: 90%, B: 70%, C: 50%, D: 30% and E: 10%). The time constants are color-coded for each ISI between masker and probe (see inset). No significant differences between the time constants of the responses to the masker and probe were found.
Figure 43: Relationship of time constant and masker level in the ISI tuning. Median time constants for the responses to the masker (A) and the probe (B) are displayed as a function of the masker level. A: The time constants decreased slightly with decreasing stimulus level. Significant differences (Mann-Whitney tests) in the time constants between 90% and 10% masker level occurred for ISIs ≥200 ms. B: For probe responses with ISIs ≥100 ms, the time constants decreased with decreasing masker level. Significant differences are indicated by asterisks (P<0.05: *; P<0.01: **; P<0.001: ***). A trend towards a significant shortening of the time constants with decreasing stimulus level were observed for stimuli with longer ISIs between masker and probe.

The comparison of the slopes (Table 7) for the linear regressions displayed in Figure 43A, B revealed significant differences (ANCOVA) in the responses to the masker (P=0.0213) and the probes (P=0.0208) as well.

Table 7: Linear regressions for the time constants in the ISI tuning. The slopes were calculated from the linear regressions displayed in Figure 43A, B and differed significantly for the masker and the probe.

<table>
<thead>
<tr>
<th>ISI</th>
<th>Slopes of linear regressions to the masker</th>
<th>Slopes of linear regressions to the probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 ms</td>
<td>y=0.4618x+8.568</td>
<td>y=-0.1132x+33.9</td>
</tr>
<tr>
<td>50 ms</td>
<td>y=0.4279x+11.48</td>
<td>y=-0.0942x+32.07</td>
</tr>
<tr>
<td>100 ms</td>
<td>y=0.3272x+24.81</td>
<td>y=0.6482x+2.744</td>
</tr>
<tr>
<td>200 ms</td>
<td>y=0.2089x+19.36</td>
<td>y=0.0778x+24.67</td>
</tr>
<tr>
<td>400 ms</td>
<td>y=0.0103x+32.81</td>
<td>y=0.5222x+6.885</td>
</tr>
<tr>
<td>800 ms</td>
<td>y=0.2272x+18.1</td>
<td>y=0.0286x+30.08</td>
</tr>
<tr>
<td>1600 ms</td>
<td>y=0.0478x+24.21</td>
<td>y=0.6335x+4.885</td>
</tr>
</tbody>
</table>
According to the analyses of the adaptation ratios in the 2nd level tuning (Figure 37), the adaptation ratios in the ISI tuning did not change significantly for decreasing masker levels (Figure 44). Median adaptation ratios for the responses to the masker were mostly scattered between 0.1 and 0.25 and decreased only slightly with decreasing masker level (Figure 44A-E). A comparable observation was made for the adaptation ratios for the responses to the probes. Furthermore, no significant differences in the median adaptation ratios were found between the responses to masker and probe for any ISI (Wilcoxon matched-pairs test, all P>0.05).

Figure 44: Adaptation ratios in the ISI tuning. The median adaptation ratios for the responses to the probe are displayed as a function of the median adaptation ratio for the responses to the masker. Adaptation ratios were separated for the five masker levels (A: 90%; B: 70%; C: 50%; D: 30% and E: 10%) and color-coded for individual ISIs. No significant differences in the median adaptation ratios between the responses to the masker and probe could be found (Wilcoxon matched-pairs tests, all P>0.05).

When the effect of the masker level on the adaptation ratio was investigated for the responses to both masker (Figure 45A) and probe (Figure 45B), it turned out that the median adaptation ratios were widely distributed and no systematic effect of the masker level on the adaptation ratio could be observed. Additionally, no significant reductions in the adaptation ratio occurred for masker levels of 90% and 10% (Mann-Whitney tests, P>0.05). When a pair wise analysis was performed for those neurons, where the ISI tuning could be tested at all masker levels, no systematic effect of the masker level on the adaptation ratio was detected as well (Wilcoxon matched-pairs test, P>0.05): only for the ISI of 25 ms, the adaptation ratio of the masker showed a significant reduction (Wilcoxon matched-pairs test, P=0.0213). For the responses to the probes, the adaptation ratio at the ISI of 100 ms was significantly reduced between 90% and 10% masker level as well (Wilcoxon matched-pairs test, P=0.0497).
Figure 45: Relationship of adaptation ratio and masker level in the ISI tuning. A: Median adaptation ratios for the masker generally did not change for decreasing stimulus levels and no significant reductions of the adaptation ratios between 90% and 10% masker level were observed (Mann-Whitney tests, all P>0.05). B: The adaptation ratios for the probes did not systematically change with decreasing masker levels as well. A decrease was found for the shortest ISI of 25 ms as well as for ISIs of 800 and 1600 ms, but without any statistically significance.

The computed slopes for the linear regressions displayed in Figure 45A, B are summarized in Table 8. As for the 2nd level tuning (Table 6), no significant differences (ANCOVA) in the steepness of the slopes could be observed for the linear regressions to the masker (P=0.2589) or the probe (P=0.7865) in the ISI tuning as well.

Table 8: Linear regressions for the adaptation ratios in the ISI tuning. The slopes of the linear regressions were computed from the dataset in Figure 45A, B.

<table>
<thead>
<tr>
<th>ISI</th>
<th>Slopes of linear regressions to the masker</th>
<th>Slopes of linear regressions to the probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 ms</td>
<td>y=0.0004x+0.1321</td>
<td>y=-0.0015x+0.065</td>
</tr>
<tr>
<td>50 ms</td>
<td>y=0.0002x+0.1491</td>
<td>y=-0.0002x+0.1486</td>
</tr>
<tr>
<td>100 ms</td>
<td>y=0x+0.1734</td>
<td>y=-0.0001x+0.1594</td>
</tr>
<tr>
<td>200 ms</td>
<td>y=0.0008x+0.1038</td>
<td>y=0.0002x+0.1383</td>
</tr>
<tr>
<td>400 ms</td>
<td>y=0.0001x+0.1948</td>
<td>y=0.0008x+0.115</td>
</tr>
<tr>
<td>800 ms</td>
<td>y=0.0019x+0.3346</td>
<td>y=0.0011x+0.873</td>
</tr>
<tr>
<td>1600 ms</td>
<td>y=0.0004x+0.1375</td>
<td>y=0.0008x+0.1171</td>
</tr>
</tbody>
</table>

In the ISI tuning, SFA for the responses to the masker showed a correlation with the stimulus level. Again, both the time constants and the adaptation ratios decreased with decreasing masker level and the results were similar to those obtained in the 2nd level tuning. Concerning SFA for the responses to the probes, the time constants were less variable than those shown in the 2nd level tuning. In the ISI tuning, the time constants between masker and probe were in the same range, which might be caused by a minimum ISI of 25 ms. The adaptation ratios between masker and probe were only marginally influenced by the masker and were not significantly different between the responses to the masker and the probe.
5.3.5 Comparison of \( \tau \) and AR between the 2\textsuperscript{nd} level and ISI tuning

It was finally analyzed, whether the time constants and adaptation ratios differed between the 2\textsuperscript{nd} level and the ISI tuning. Since the responses to the masker were unbiased by a preceding stimulus, the results should be similar in both stimulus paradigms and only depend on the masker level. Figure 46 summarizes the result of the comparison of the adaptation properties in both stimulus paradigms. The median time constants decreased in both stimulus paradigms as a function of the masker level for the entire population of neurons (Figure 46A, B). For the highest masker level, the median time constants for the entire population were in the range of 40 ms to 50 ms. For 70\% to 30\% masker level, the time constants decreased only slightly in the 2\textsuperscript{nd} level tuning and remained constant (around 25 ms) in the ISI tuning. The shortest time constants were found for 10\% masker level and scattered between 8 ms and 15 ms in both stimulus paradigms. When the median time constants of the whole population of neurons was analyzed (Figure 46A), no significant differences between the 2\textsuperscript{nd} level and ISI tuning could be observed (Mann-Whitney tests, all \( P>0.05 \)), although the time constants tended to be shorter in the 2\textsuperscript{nd} level than in the ISI tuning. The decrease was best fitted by single exponential decays that were not significantly different for both stimulus paradigms (ANCOVA, \( P>0.05 \)). Based on the temporal decay of SFA, neurons in the barn owl’s ICC show short-term adaptation (time constants of a few tens of milliseconds). The median time constants for the primary-like response types in the 2\textsuperscript{nd} level and the ISI tuning (Figure 46B) also decreased as a function of the masker level. However, this decrease was minimal and the time constants were in the range of 7 ms to 16 ms. Unlike to the analysis of all time constants (Figure 46A), where shorter time constants were generally found in the 2\textsuperscript{nd} level tuning, a change was observed in this analysis: at higher masker levels (90\% to 50\%), the time constants were shorter in the ISI tuning, whereas for lower masker levels, the time constants were shorter in the 2\textsuperscript{nd} level tuning. Due to these very short time constants, SFA of primary-like neurons can be regarded as rapid. Whereas no significant differences were observed for the slopes of the linear regressions (ANCOVA, \( P=0.2998 \)), a significant difference of the time constants was found for the masker level of 30\% between the 2\textsuperscript{nd} level and ISI tuning (Mann-Whitney test, \( P=0.0351 \)). Although the time constants did not differ significantly in both stimulus paradigms, it was finally analyzed whether the neurons displayed a difference in their temporal SFA to both stimulus paradigms. Therefore, the time constants of the pooled masker responses those neurons were compared, that were tested in both the 2\textsuperscript{nd} level as well as the ISI tuning. For 90\% of the masker level, 52.5\% of the units had either time constants shorter than 100 ms or longer than 100 ms in both tunings, whereas 47.5\% of the units had different time constants in both tuning types. Thus, SFA was variable between both tunings for individual units. With decreasing masker level, the response behavior was more stable between the 2\textsuperscript{nd} level and ISI tuning and generally, more than 65\% of the recorded units responded with comparable time constants in both stimulus paradigms. For example at 50\% masker level, 54\% of the units had time constants shorter than 100 ms in both stimulus paradigms, whereas 14.6\% of the units had time constants longer than 100 ms. At this masker level, about 32\% of the units displayed a different temporal decay in both stimulus paradigms, whereas 68\% of the units showed no difference. Most stable responses were observed for 10\% masker level. Here, 69\% of the units had time constants below 100 ms in both tunings, whereas 14\% of the neurons responded with time constants longer than 100 ms. Only a minority of the units showed different response types at this stimulus level (17\%), whereas the majority of 83\% responded either primary-like or tonic to the stimulus.
The comparison of the adaptation ratios (Figure 46C) revealed a similar outcome as the analysis for the time constants: the adaptation ratios decreased as a function of the masker level in both stimulus paradigms. For 90% masker level, the median adaptation ratios were in the range of 0.3 to 0.4 and deceased to median adaptation ratios of 0.2 to 0.3 for 10% masker level. Here, the adaptation ratios were generally lower in the ISI than in the 2nd level tuning. A significant difference in the adaptation ratios between 2nd level and ISI tuning was only found for a masker level of 70% (Mann-Whitney test, P=0.0129). No significant differences could be detected in the slopes of the linear regressions (ANCOVA, P=0.3591).

Figure 46: Comparison of $\tau$ and AR between the 2nd level and ISI tuning. A: The median time constants for the 2nd level (black) and ISI (gray) tuning are displayed as a function of the masker level. B: Comparison of the median time constants for primary-like responses in both tunings. C: Median adaptation ratios in the 2nd level and ISI tuning. For the linear regressions in B and C, the slopes of the regressions as well as the coefficients of determination are given in the insets.

The results of the unpooled time constants and adaptation ratios for the masker revealed some differences in reference to the results of the pooled responses to the masker (Figure 46). Comparing the time constants of the responses to the masker and the probe revealed consistent similarities in both the 2nd level and ISI tuning. In both tunings, the time constants for the responses to the masker decreased as a function of decreasing masker level (Figure 36A and Figure 43A). Additionally, the time constants had similar durations for the five masker levels in both tunings. At 90% of the RLF, median time constants were in the range of 25 ms to 60 ms in the 2nd level tuning (Figure 36A) and between 25 ms and 65 ms in the ISI tuning (Figure 43A). No significant differences of the time constants could be observed between the 2nd level and ISI tuning (Mann-Whitney test, P=0.63). The same held true for 70% masker level, where no significant differences could be found (Mann-Whitney test, P=0.76). However, for masker levels between 50% and 10% of the RLF, the time constants were significantly shorter for the 2nd level than the ISI tuning (Mann-Whitney tests, 50%: P=0.0281; 30%: P=0.0067 and 10%: P=0.041). These differences of the unpooled responses in comparison to the pooled responses to the masker might be due to the number of spikes used for the computation of the time constant and adaptation ratio since the unpooled computations were based on 10 sweeps whereas the pooled responses were based on sixty sweeps in the 2nd level tuning and seventy sweeps in the ISI tuning. The differences of the time constants and adaptation
ratios of the unpooled data might be caused by variability of the resulting PST histograms. Since the variability in the responses to the probe tones was strongly influenced by the level of the masker on the one hand and the stimulus paradigm (either 2nd level or ISI tuning) on the other hand, a direct comparison of the responses to the probe will be highly speculative. Therefore, I will discuss the results of these two stimulus paradigms in more detail in the next chapter.

5.4 Discussion

Two different types of adaptation were investigated in the barn owl’s ICC using tonal double-stimulation at different stimulus levels in two distinct stimulus paradigms. The amount of response adaptation as well as spike-frequency adaptation strongly depended on both the level of stimulation as well as the interval between masker and probe. Before I will discuss the results of adaptation I shall first relate the results of the basic tuning properties to literature.

5.4.1 Frequency tuning

The frequency tuning curves recorded in ICC (Figure 13A-F) were in accordance with previously published observations for the barn owl (Wagner et al., 2002; Wagner et al., 2007; Singheiser et al., 2010a). Frequency tuning curves were highly symmetric across the entire frequency range (Figure 18A) and BF and BF$_{50}$ did not differ significantly as well (Pena et al., 2001; Singheiser et al., 2010a). The tuning curves were typically primary-like as found in the IC of mammals (Kuwada et al., 1984; Semple and Kitzes, 1985; Nuding et al., 1999; Langner et al., 2002; Yan et al., 2005) and half maximum frequency tuning width (W$_{50}$) increased with increasing BF$_{50}$ at least for the binaural paradigm (Figure 18B, Pena et al., 2001; Wagner et al., 2002). W$_{50}$ was generally below 2.5 kHz for BFs of 7 kHz to 8 kHz, which was also in accordance to frequency tuning curves recorded either in NL (Pena et al., 2001) or the ICCcore (Wagner et al., 2002) of the barn owl. Like in the project on stereausis, monaural frequency tuning curves were significantly narrower than binaural frequency tuning curves. This was true for BFs below 3 kHz but also for frequencies covering the entire audible spectrum of the barn owl. Comparable observations have been previously described for IC neurons in mammals as well (Kuwada et al., 1984; Semple and Kitzes, 1985).
5.4.2 Rate-level functions and spontaneous ratios

Generally, the units showed monotonic rate-level functions (Figure 13G-L). However, the units displayed a large variability concerning threshold, dynamic range and saturation (Figure 19B). Comparable observations have been made by Wagner et al. (2002) for monaural RLFs recorded in the barn owl’s ICCcore (their Figure 9). They also observed no significant differences concerning the dynamic range or the thresholds between ipsi- and contralateral stimulation (Wagner et al., 2002). This was in accordance to this study: significant differences were neither found for the dynamic ranges between contralateral stimulation in ICCcore or ICCls nor between the pooled contralateral dynamic ranges and the ipsilateral dynamic ranges (Figure 19C). However, some differences were found for the stimulus levels at 10% and 30% of the dynamic range between ICCcore and ICCls in this study. But since no consistent differences occurred for the entire dynamic range, the data were pooled and a following analysis for the linear regressions of the mean stimulus levels as a function of the masker level yielded no significant differences in their slopes (Figure 19A; ANCOVA, \( P=0.322 \)). Therefore, the data presented in this thesis agree with data published for the ICCcore of the barn owl (Wagner et al., 2002).

The dynamic ranges that were recorded covered between 12 and 60 decibels (median ipsi: 38 dB; median contra: 30 dB; Figure 19D) without significant differences between ipsi- and contralateral stimulation. Similar dynamic ranges were found for other nuclei in the auditory system of birds as well. In the barn owl’s auditory nerve, total dynamic ranges covered between 27 dB to 77 dB with a mean of about 49 dB (Köppl and Yates, 1999). Dynamic ranges in the barn owl’s NM and NL depended on the best ITD and varied between 21 and 26 dB in NL for best and worst ITDs, respectively. Monaural RLFs also recorded in the barn owl’s NL had dynamic ranges of around 25 dB for ipsi- and contralateral stimulation (Pena et al., 1996) and thus were narrower than those recorded in ICC. In the cochlear nuclei of the chicken, dynamic ranges spanned 15 dB to 65 dB (average 44 dB) in NA and 15 dB to 55 dB (average 38 dB) in NM (Warchol and Dallos, 1990). The dynamic ranges recorded in the auditory system of mammals typically covered a matchable range in peripheral and central auditory nuclei: rate-level functions showed either a monotonic or non-monotonic shape that encompassed 30 dB to 50 dB (Palmer and Evans, 1982; Ehret and Merzenich, 1988; Eggermont, 1989; Sutter and Schreiner, 1995). Additionally, as has been shown in Figure 19E, the dynamic range did not depend on the unit’s best frequency. This also has been shown by Köppl and Yates (1999), where the dynamic range did not correlate with CF in the AN of the barn owl.

In a last analysis, the spontaneous ratio for the dynamic range was investigated. As may be seen in Figure 20, the post-stimulatory suppression strongly depended on the masker level. The louder the level of the stimulus the less spontaneous spikes occurred in a period of 50 ms after the stimulus ended. The post-stimulatory suppression increased as a function of increasing stimulus level. Unfortunately, this has never been quantified in other nuclei of birds and mammals as well. But as may be seen in the publication of Wagner and colleagues (2002), this post-stimulatory suppression was also present in their RLFs and strongly depended on the stimulus level (their Figure 9). Especially the raster plots in their Figures 9a and 9d show striking similarities to the raster plots of the RLFs recorded in this study (Figure 13J-L): The post-stimulatory suppression strongly decreased with increasing stimulus level for ipsi- and contralateral stimulation. Spontaneous activity and post-stimulatory suppression might therefore also play an important role in the coding of stimulus parameters, since this effect can also be observed not only in rate-level functions but also in ITD or frequency tuning curves (Wagner et al., 2002).
5.4.3 Response adaptation quantified by the response ratio

Concerning response adaptation and suppression of the responses to the probes in reference to the masker, large differences were observed between the two stimulus paradigms (2nd level and ISI tuning) as well as for different levels of the masker. In the 2nd level tuning, the responses to the probe were significantly reduced in comparison to the response to the masker when both stimuli had the same level. This could be shown by response ratios smaller than unity (Figure 21F-J). Moreover, the response ratios significantly decreased with decreasing masker level (Figure 22A). However, this effect could not be observed for the ISI tuning where the level of the probe and the masker had the same level as well (Figure 25F-J, Figure 26A). For the masker levels of 70% and 10%, the response ratio at the shortest ISI was not significantly reduced in comparison to unity. This discrepancy between both tunings might be caused by the differences in the inter-stimulus interval: Whereas in the 2nd level tuning, the ISI was set to 0 ms, it was 25 ms in the ISI tuning. The presentation of the two stimuli immediately after each other without any ISI might be the reason for stronger response adaptation in the 2nd level tuning in comparison to the ISI tuning. This was statistically confirmed by Mann-Whitney tests (P<0.05) for the masker levels of 10% to 70% of the RLF. In a different study on adaptation in the barn owl’s IC, Gutfreund and Knudsen (2006) also demonstrated that response adaptation for the probe depended on the ISI. For ICCls units, a significant reduction in the response to the probe could be observed for their shortest ISI of 40 ms, when both stimuli had the same duration. Additionally, they tested the effect of changing the duration of the masker and varying the ISI between masker and probe. Their results indicated, that a duration of 50 ms for the masker and an ISI of 10 ms between masker and probe resulted in a significant response reduction to the probe (their Figures 5C-F and 6C-F). Decreasing the duration of the masker from 50 ms to 30 ms and thereby increasing the ISI from 10 to 30 ms resulted in a reduced response to the probe that was significantly not different from the response to the masker. Thus, the critical interval for adaptation might be in the range of 10 ms to 30 ms. However, their probe stimulus was always 10 dB fainter than the masker and thus a certain response reduction to the probe is to be expected. Nonetheless, the results of both studies showed a strong impact of the ISI on response adaptation to a probe tone in reference to a masker that lasts longer than 30 milliseconds.

A stimulus design as presented in the 2nd level tuning has not been tested in other studies so far. The results clearly showed the ability of probe tones to overcome response adaptation in ICC neurons in reference to a masker stimulus with increasing probe levels (Figure 21 and Figure 23). But this ability was not identical for all masker levels and showed a clear dependency on the stimulus level of the preceding masker (Figure 23). For stimulus levels that corresponded to the lower half of the dynamic range (10% to 50%), a probe level increase of approximately 5 dB to 7 dB was sufficient to release the units from adaptation (Table 1). For higher masker levels (70% and 90%), a compensation of the response ratio was not possible even by increasing the probe level by 25 dB (Figure 23 and Table 1). Furthermore, an increase of the probe level up to 25 dB louder than the masker led to a reduction of the response ratios for a masker level of 90% (Figure 21F and Figure 23A). The high sensitivity for masker levels around 50% was expected since the sensitivity of neurons is highest at the steepest slope of the RLF. However, this enhanced sensitivity to small changes in the stimulus level even for lower parts of the dynamic range might be devoted to the overall hearing sensitivity and the ecological niche of barn owls. As a bird of prey that mainly relies on auditory cues like the faint rustling of a mouse on the ground, the entire auditory perception of the barn owl is specialized to detect faint changes of auditory signals (Payne, 1962; 1971; Konishi, 1973b; 1973a). The results described for the entire population of recorded units was also
confirmed when the units were binned into three octave bands (Figure 24). The computed increase of the probe level required to compensate for the response ratio was similar for the octave bands in comparison to the pooled units (Table 2), although the compensation for the octave band of 4-8 kHz required an increase of about 10 dB in comparison to 7 dB for the pooled units. The similarity of the response ratios across all frequencies might be explained by the representation of the entire frequency spectrum within the ICC of barn owls (Wagner et al., 1987; Wagner et al., 2002; Wagner et al., 2007) and emphasize its function as a ‘relay nucleus’ that transmits auditory information to both the midbrain and forebrain pathway (Arthur, 2005; Pérez and Peña, 2006; Vonderschen and Wagner, 2009; Pérez et al., 2009).

The results for the ISI tuning were less apparent than the results for the 2nd level tuning concerning the response ratios (compare Figure 21 and Figure 25). Whereas the response ratios in the 2nd level tuning strongly depend on the level of both the masker and the probe no such clear dependency was found in the ISI tuning. A significant reduction of the response ratio was generally found for ISIs shorter than 100 ms but without a clear relation to the masker level (Figure 25). Whereas the response ratios were significantly reduced for ISIs ≤800 ms at 50% masker level (Figure 25H), significant reductions for shorter ISIs were found for most other masker levels (mostly ≤100 ms; Figure 25F, I-J). The recovery from response adaptation therefore was slowest for that region of the dynamic range of the RLF where best coding properties would be expected. If the ISI required to cross the threshold of unity was computed by a linear regression, the recovery from response adaptation would be rather slow. Compensation for response adaptation would take longer than 1 s for masker levels of 10% to 70% (Figure 26B). For 90% masker level, the computed ISI would even be longer than 3 s and therefore exceeded the range of ISIs tested. It remains doubtful, whether this would reflect a true interval. However, by comparing the response ratios as a function of the ISI (Figure 25), it was obvious that the computed response ratios ran mostly in parallel to unity and the linear regressions thus were not very steep yielding long ISIs required for compensation. Especially in comparison to the 2nd level tuning, the slopes of the linear regressions showed large differences in their steepness (Figure 27). By taking into account the significant deviations of the response ratios being smaller than unity, the results of the pooled probe responses confirmed response adaptation for ISIs up to 400 ms (Figure 26E). This was much longer than the time window described by Gutfreund and Knudsen (2006): For ICCs neurons, a significant response reduction to the probe could be observed for an ISI of 40 ms. For an ISI of 80 ms, the responses were still reduced in comparison to the control but the reduction was not significant. Longer lasting adaptation was found for ICX neurons, which also showed significant response adaptation to ISIs longer than 320 ms (Gutfreund and Knudsen, 2006). Therefore, the temporal suppression observed in the current thesis resembled more the results of ICX neurons as measured by Gutfreund and Knudsen (2006).

Since the neurons recorded for this study sometimes showed a strong variability in their unaffected responses to the masker, the response ratios were computed by dividing the response of the probe by the mean of all masker responses. In comparison to the pair wise analyses of the response ratios, this averaging across 60 or 70 sweeps led to much more stable response ratios and allowed robust computation of the time constants and adaptation ratios as well. This variability of ICC neurons to the masker responses was puzzling since in previous studies, the responses of ICC neurons showed less variability than for example NL neurons (Christianson and Pena, 2006). The difference in the responses of ICC neurons might be explained by the stimuli used: Whereas Christianson and Pena used a binaural broadband stimulus, the neurons in the current study were excited with
monaural pure tone stimuli and therefore less neural spiking activity is expected that might lead to a greater variability in the overall response.

5.4.3.1 Recovery from response adaptation in the ISI tuning

To describe the recovery from response adaptation, single exponential approximations were computed as a function of the ISI (Figure 25). The resulting time recovery constants were shorter than 17 ms for all ISIs. No clear relation of the recovery time constants with different masker levels was observed. The recovery time constants were between 6 and 7 ms for 10% and 90% masker level and 16 ms for 30% masker level. This recovery was faster than the one determined by a linear regression. By pooling the response ratios across all masker levels, an increased recovery time constant was obtained. A double exponential approximation yielded a fast component of 3 ms and a slow component of 300 ms that best described the recovery for long ISIs (Figure 26). In a study of adaptation in the IC of the guinea pig using interaural phase disparities (Ingham and McAlpine, 2004) recovery time constants of about 225 ms were found. The recovery time constants thus were well comparable to the ICC of the barn owl.

5.4.4 Response adaptation quantified by the masking ratio

The responses of the masking ratio were analyzed in a similar way as for the response ratio in the ISI tuning. The difference between both analyses was that for the masking ratio only the responses to the probe stimuli were taken into account and the responses to the masker were omitted. The results of the masking ratio (Figure 28) were comparable to those of the response ratios (Figure 25). The responses to the probe that were presented shortly after the masker showed a substantial reduction in the masking ratio as a function of the masker level. According to the definition of Finlayson (1999), a suppression or response adaptation was considered as a change of the masking ratio by 20%. This was only the case for ISIs ≤100 ms. Furthermore, a clear trend of adaptation was found as a function of the masker level: With decreasing masker level, adaptation to the probe tone increased for ISIs of 25 ms at 70% masker level to ISIs of 100 ms at 10% masker level. However, the general responses showed only a moderate suppression in the reference to the probe tone since the maximum masking ratios were reduced by about 30% in comparison to unity (Figure 28). In comparison to the results presented by Finlayson (1999), the masking ratios for the owl were much smaller than those recorded to pure tone stimulation in the inferior colliculus of the rat. He found changes relative to the probe of 30% to 100% for ISIs around 30 ms (Finlayson, 1999). This difference in the masking ratios between owl and rat might be explained by the different stimulus parameters used in both studies. Whereas the relation of masker-probe duration was 200/30 ms for the experiments conducted by Finlayson (1999) the relation was equal for both stimuli in this study (100 ms for masker and probe, respectively). Due to the differences in the stimulus duration between both studies and the large variety in the masking ratios, the recovery time constants showed substantial differences as well. Whereas the time constants for the owl were below 15 ms (6.17 ms at 90% to 10.26 ms at 10% masker level) and thus similar to the recovery time constants found for the response ratios at all masker levels, the recovery time constants for the rat were much longer (mean: 271.4 ms; Finlayson, 1999). Both studies showed an insensitivity of recovery time constants to changes in the masker level.
5.4.5 Comparison of the recovery from response adaptation

The temporal recovery functions found in the IC of two different species of mammals yielded comparable temporal response windows for neurons to recover from response adaptation although different methods were applied. Ingham and McAlpine (2004) observed recovery functions in the range of 225 ms for the guinea pig and Finlayson (1999) found slightly longer recovery time constants for the rat (270 ms). The slow component of the recovery time constant (300 ms) computed for the pooled responses in the barn owl’s ICC thus was well comparable to the data obtained in mammals (Figure 26F). Temporal recovery windows found in the ICX of barn owls (Gutfreund and Knudsen, 2006) were also in the same range (320 ms) although no exponential recovery functions were applied. Judging the recovery period of ICC neurons by a significant reduction of the response rate to the probe in reference to the masker yielded short recovery periods on medium time scales between 50 ms and 100 ms for some masker levels. When the response ratios were again pooled across all masker levels, then the responses to the masker could significantly suppress the responses to the probe for about 400 ms. This was also comparable to the recovery time windows of Ingham and McAlpine (2004) as well as those of Finlayson (1999). But despite the good agreement of the recovery periods with data from the IC in mammals, the responses obtained in the current study were longer than those obtained by Gutfreund and Knudsen for the ICCIs of the barn owl. They found a reduction in the response to the probe only for ISIs up to 80 ms whereas the reduction in ICX neurons lasted for about 320 ms and thus was comparable for the recovery periods presented previously.

Recovery time constants of different brainstem nuclei in the auditory system of mammals showed a tendency towards recovery time constants longer than 100 ms recorded with different stimulus paradigms. Recovery time constants determined for the auditory nerve neurophonic (ANN) in the cat showed a rapid (16.2 ±9.8 ms) and a slow (125 ±50.1 ms) component depending on the masker level (Chimento and Schreiner, 1990). For the cochlear nucleus of chinchillas, recovery time constants were in the range of 200 ms to 370 ms (260-290 ms for primary-like neurons) with no systematic variation of CF (Boettcher et al., 1990). In the guinea pig, recovery time constants for neurons recorded in the cochlear nucleus were fast (34 ms) and full recovery was complete within 200 ms (Bleeck et al., 2006). For the rat, the recovery time constants were around 100 ms for neurons of the superior olive and between 70 ms to 100 ms for the IC, respectively (Finlayson and Adam, 1996; Finlayson, 2002). For the IC, no correlation of the recovery time constants with either the BF or the threshold could be observed (Finlayson and Adam, 1996; Finlayson, 2002).

In summary, the recovery time constants presented in this study as well as in previous studies varied considerably not only between birds and mammals, but also between studies in the same nuclei for different species of mammals. These differences might be due to various analyses the recovery functions were based on as well by the usage of different ISIs between masker offset and probe onset. Whereas the shortest ISI for this study was set to 25 ms, other studies were carried out with the shortest ISI of 1 ms (Ingham and McAlpine, 2004; Bleeck et al., 2006) or 2 ms (Finlayson, 1999). Despite of the heterogeneous results from previous studies, the recovery period obtained for ICC neurons in the barn owl was in general agreement with data from literature. The responses to a masker stimulus could suppress the responses to a probe stimulus with the same level for periods between 300 ms and 400 ms.
5.4.6 Spike-frequency adaptation of the responses to the masker

The time constants and adaptation ratios computed for the responses to the masker in both the 2nd level and the ISI tuning were similar and showed no consistent significant differences between the two stimulus paradigms (Figure 46). Whereas the time constants for the units responding only in a primary-like manner significantly decreased between 90% and 10% stimulus level for the responses to the masker in the 2nd level tuning, no significant differences could be observed for the time constants in the ISI tuning (Figure 46B). The median time constants for primary-like units were in the range of 8 ms to 16 ms for both tunings. When the time constants were analyzed for the entire population, a decrease of the time constants with decreasing masker level could be observed as well (Figure 46A). For a high masker level, the median time constants were in the range of 40 ms to 45 ms, for 50% masker level the median time constants clustered between 20 ms and 25 ms whereas the median time constants were between 10 ms and 20 ms close to threshold (10% masker level). Depending on the response type of the unit, a dependency of the time constants with stimulus level was obvious (Figure 46A) whereas for primary-like units, the median time constants were stable between 8 ms and 16 ms (Figure 46B). The response behavior as judged by the time constants of the neurons was stable for both stimulus paradigms when the level was ≤70% of the RLF. For these levels, more than 65% of the units displayed comparable time constants (either longer or shorter than 100 ms) in both the 2nd level and ISI tunings whereas for 90% masker level, about 50% of the units different time constants to the pooled masker responses in both stimulus paradigms.

Ingham and McAlpine (2004) found time constants of IPD sensitive neurons in the IC of guinea pigs that were in the range of 50 ms and thus generally longer than those obtained for most masker levels in this study. When Ingham and McAlpine analyzed monaural time constants for the same units, the median time constant was 34.5 ms and thus similar to the monaural time constants measured for the owl. Unfortunately, Ingham and McAlpine did not report the stimulus level for their testing procedures except for the measurements of the frequency tuning curves, where it was varied between 60 dB and 100 dB. This range of stimulus levels would encompass the dynamic range of most neurons recorded in this study and might add some variability to the comparison of the time constants. Furthermore, the stimuli used by Ingham and McAlpine (2004) had a duration of 300 ms, which was three times longer than the duration of the stimuli in this study. Two independent studies focused on the correlation of stimulus duration and adaptation time constants and found contradictory results. In one study, the adaptation of a neuron’s firing rate was adaptable to many time scales and the time constants increased with increasing periods of stimulation between 1 s to 40 s (Fairhall et al., 2001), whereas in a different study using stimulus durations of 5 and 20 s, no significant differences between the time constants were detected (Dean et al., 2008). However, it can be summarized that the time constants measured for the barn owl were shorter than those measured for the guinea pig. Spike-frequency adaptation in the barn owl’s ICC thus can be classified as rapid (time constants of a few ms) or short-term (few tens of ms) adaptation based on the temporal decay (for a classification see introduction of Loquet et al., 2003).

Time constants measured in various mammals and nuclei using different stimuli as well as stimulus durations or stimulus levels yielded a broad variability of temporal dynamics of SFA: Short-term adaptation determined for the ANN in the cat (Chimento and Schreiner, 1990) resulted in rapid adaptation time constants (3 ms to 7 ms) that were invariant of the stimulus level as well as short-term time constants that decreased with increasing stimulus level (10 dB: 166 ms; 20 dB: 83 ms; 30 dB: 73.5 ms). In contrast to the time constants determined for the ANN, time constants measured in the auditory nerve to pure tone stimulation did not vary as a function of
the stimulus level but the temporal decays were in the same range as for the ANN (Chimento and Schreiner, 1991). Slightly contradictory time constants and dependencies were found for the ventral cochlear nucleus of the rat using the recordings of near-field evoked potentials: whereas rapid time constants decreased for increasing stimulus intensities (0.1 ms to 15 ms), short-term time constants were invariant of the stimulus intensity (0.9 ms to 100 ms; Loquet et al., 2003). Recordings in the AN of the gerbil resulted in short-term time constants of about 60 ms whereas the time constants for rapid adaptation were in the range of 1 ms to 10 ms. The latter time constants decreased with increasing stimulus level and both showed a clear dependency on stimulus frequency (Westerman and Smith, 1984). For medial olivocochlear neurons of the guinea pig, time constants of 47 ms were recorded that were independent of the unit’s CF and showed only small correlations with the stimulus level (Brown, 2001). For neurons recorded in the rat’s superior olive using pure tone stimuli, the time constants were below 20 ms and they were not correlated either with BF or threshold of the neuron (Finlayson and Adam, 1996). Furthermore, they did not differ between ipsi- or contralateral stimulation (time constants were below 10 ms for the monaural paradigms; Finlayson and Adam, 1996). Unfortunately, the number of studies dealing with the temporal properties of SFA in birds is sparse. One study investigated time constants in the AN of the chicken using pure tone stimuli at CF about 20 dB above threshold (Crumling and Saunders, 2007). The temporal decay measured was generally in the range of 18 ms and did not depend on the stimulus level. Thus the time constants determined for the neurons in the barn owl’s ICC were generally shorter than those determined for most species with the exception for the monaural time constants recorded in the superior olive of the rat (Finlayson and Adam, 1996) and those for the AN of the chicken (Crumling and Saunders, 2007).

The amount of SFA quantified by the adaptation ratios in the responses to the masker exhibited a clear dependency of SFA and stimulus levels (Figure 46) and no consistent differences were found between the 2nd level and ISI tuning. Generally, the adaptation ratios covered the full range from zero to one. Therefore, in some units, 100% of the maximum discharge rate was adapted at steady state whereas for individual units, almost no adaptation occurred indicating a tonic-like response to the stimulus (Figure 30 and Figure 39). For different masker levels, between 60% (AR: 0.4 at 90% masker level) and 80% (AR: 0.2 at 10% masker level) of the maximum discharge were adapted in the corresponding PST histograms and SFA was stronger for lower stimulus levels. Ingham and McAlpine observed adaptation ratios between 0.24 and 0.6 in IPD sensitive neurons of the guinea pig. Monaural adaptations ratios were again systematically shifted towards lower values and ranged from 0.05 to 0.31 (Ingham and McAlpine, 2004). This range of adaptation magnitude was well in accordance with the range of adaptation ratios for the owl. Medial olivocochlear neurons of the guinea pig exhibited average adaptation ratios of 31% (Brown, 2001) whereas neurons recorded in the superior olive of rats had adaptation ratios between 50% and 60% (Finlayson and Adam, 1996). Comparable values for adaptation magnitude have been also determined in the AN of the chicken for different stimulus levels. Here the tonic rate was adapted by about 50% in reference to the peak discharge rate (Crumling and Saunders, 2007). In contrast to the broad variance of the time constants for different species and nuclei, the adaptation ratios of the same studies showed a more homogeneous distribution. Due to the broad scatter of adaptation ratios (for example Figure 30 and Figure 39 in this study or Figure 4B in the study of Ingham and McAlpine, 2004), the different response types of neurons might be involved and thus could have influenced the amount of SFA. The correlation of decreasing adaptation ratios and decreasing stimulus levels might be impaired by the response properties of the neurons for different sound levels. Whereas for low stimulus levels, only a few spikes were typically fired, a high discharge rate for high stimulus levels will elevate the entire amount of spikes in the PSTH. For a given stimulus, the PSTH to the
masker levels of 10% and 90% might be identical in their shape but differ in the number of spikes between the maximum discharge rate as well as the discharge rate at the plateau of the response. Since the computation of the adaptation ratio is based on the number of spikes at steady state, low-level stimuli might bias the adaptation ratios towards lower values in comparison to high stimulus levels. Besides this methodological reason for varying adaptation ratios also different response properties of neurons might be taken into account. The responses behavior might depend on the stimulus level as well, i.e. onset responses to faint stimuli and primary-like responses to loud stimuli.

5.4.7 Spike-frequency adaptation of the responses to the probe

Although many studies focused on the temporal progression and the magnitude of adaptation for a masker stimulus as well as the recovery from adaptation in reference to the masker, only a few studies focused on the adaptation properties for the probe tones. A common observation for the responses to the probe stimuli was a suppression of the response rate in comparison to the response to the masker. This was the case when the level of the probe was equal to the level of the masker in the 2nd level tuning (Figure 21) or when the ISI between masker and probe was short (Figure 25). Depending on the masker level, an increase in either the probe level or the ISI could compensate for response adaptation and the responses to both stimuli became more similar. It was now interesting to investigate, how SFA properties in the responses to the probe were directly influenced by the masker. Concerning the time constants for primary-like units in the 2nd level tuning, significant differences between the responses to the pooled masker and the probe at 0 dB probe level were found for all masker levels: the time constants to the probe responses were significantly longer than those to the masker. Similar results were found for the comparison of the responses between masker and probe at 25 dB probe level (Figure 33). When the time constants were investigated in a pair wise analysis for all masker levels as well as the six probe levels (Figure 35), the influence of the masker on the probe became obvious. Whereas for the highest masker level of 90% (Figure 35A), no significant differences between the time constants for masker and probe were found a systematic shift towards longer time constants in the responses to the probe for all probe levels could be found with decreasing masker level (Figure 35B-E). The masker thus directly influenced the temporal response properties to the probes. Furthermore, the time constants for the responses to the probe were not only affected by the masker but also by the level of the probe. As may be seen in Figure 34B, the median time constants for primary-like units at 50% masker level decreased slightly with increasing probe level, however without any statistical significance. Similar effects were observed for the other masker levels as well (data not shown). In the 2nd level tuning, not only the level of the masker but also the level of the probe seemed to have a substantial impact on the temporal responses properties of ICC units tuned to different frequencies.

In contrast to the 2nd level tuning, the time constants for the probes in the ISI tuning were less scattered. An overall analysis of the time constants for the pooled masker responses and the probe responses generally yielded no significant differences (Kruskal-Wallis test, P>0.05). But when the time constants of the responses to the masker were compared to the time constants of the probe for the shortest ISI of 25 ms, significant differences between masker and probe occurred at 90% and 50% masker level. The analysis of only primary-like units detected significant differences between masker and probe for 70% and 50% masker level (Figure 40). A pair wise analysis of the time constants of the responses to both masker and probe (Figure 42), revealed that the time con-
stants showed some scatter for 90% masker level (Figure 42A), but without any statistically significant differences. With decreasing masker level the median time constants between masker and probe became more similar and showed no significant differences for all ISIs tested. This was additionally confirmed in Figure 41B where the time constants for the probe at 50% masker level were plotted as a function of the ISI: For primary-like units, the median time constants were in the range of 5 ms to 12 ms and showed only little correlation with increasing ISI. Comparable observations were found for the other masker levels as well (data not shown). In the ISI tuning, the time constants of the probes seemed to be rather invariant of both the ISI and the masker level and adapted irrespective of their current state of adaptation. This hypothesis follows the results of Ingham and McAlpine (2004) who also demonstrated that the time constants of IPD sensitive neurons showed no systematic change with increasing ISIs (their Figure 7B). Additionally, the time constant of the responses to the masker and the probe at different ISIs showed no systematic differences in their temporal decay (their Figure 7A-D) similar to the observations made in this study shown in Figure 41. Conclusively, the time constants for the responses to the probes were strongly affected by a preceding masker when the interval was extremely short like in the 2nd level tuning (0 ms). But not only this short interval, also the level of the masker had substantial impact on the time constants for the responses to the probes in this stimulus combination and resulted in slower SFA for probe responses. Within the probes, the effect of the probe level was only marginal leading to the hypothesis that the time constants of the probes decayed at the same rate irrespective of the state of adaptation. This hypothesis was supported by the ISI tuning, where the interval of 25 ms between masker offset and probe onset was sufficiently long to eliminate the influence of the masker on the temporal decays of the probe.

The adaptation ratios for the probe responses were more homogenous than the time constants for both the 2nd level (Figure 33F-J) and ISI tuning (Figure 40F-J). For the 2nd level tuning, the adaptation ratios for most probe levels were significantly different to the adaptation ratio of the pooled response to the masker at all masker levels. However, no significant differences were found within the population of probe tones at individual masker levels. In contrast to the comparison of the adaptation ratios of the probes with the pooled masker responses, almost no significant differences were found between the pair wise analyses of the adaptation ratios for the 2nd level tuning (Figure 37). Here, significant differences between masker and probe occurred only for probe levels of 25 dB at 30% and 10% masker level. Exactly for these two combinations, no significant differences could be found in the analysis of the probe responses and the pooled masker responses. As mentioned earlier, the computation of the adaptation ratio strongly depends on the response rate at steady state. The pooling of spikes over 60 sweeps as for the pooled masker responses in the 2nd level tuning therefore results in more “valid” histograms for the computation of the adaptation ratio. The differences in the two analyses might be related to this circumstance. When the adaptation ratios at 50% masker level were analyzed as a function of the probe level (Figure 34D), the median adaptation ratios did not change with increasing probe level. Similar trends were observed for higher masker levels (90% and 70%). The peak discharge rate adapted by about 70% - 80% at steady state. In contrast, the adaptation ratios for lower masker levels (30% and 10%) slightly increased with increasing probe level (0.1 at 0 dB to about 0.2 at 25 dB; data not shown). The amount of adaptation for the probe responses in reference to the pooled masker responses was significantly affected in the ISI tuning as well (Figure 40F-J). Here again, the adaptation ratios for the probes were significantly smaller than those for the pooled masker response indicating stronger SFA in the responses to the probes. These differences were most obvious for the masker levels close to threshold and saturation of the RLF. However, as in the 2nd level tuning, no systematic and significant differences could be observed for the adaptation ratios in pair wise masker-probe analyses (Figure
The reason for this difference might be explained by the same reasons as for the 2nd level tuning. Just like in the 2nd level tuning, the adaptation ratios did not change systematically as a function of the stimulus parameter (ISI) of the probe (Figure 41D): The adaptation ratios were rather constant at 50% masker level for all ISIs tested. The same applied for the probe adaptation ratios at the other masker levels (data not shown). The steady state responses of the probes were adapted by 80% to 90% of the peak discharge rate and thus showed stronger SFA than for the pooled masker response at 10% stimulus level (Figure 41C).

In summary, the adaptation ratios for the probe responses were strongly affected by the preceding masker and showed stronger SFA in comparison to the pooled masker responses. In the pair wise analyses of the adaptation ratios between masker and probe, no consistent differences were found. The results thus are ambiguous. Concomitantly, the adaptation ratios were neither affected by the stimulus levels of the probe nor by the interval between masker and probe. Thus, the neurons seemed to display a constant rate of SFA independent of their current state of adaptation for the probe stimuli. In the study of Ingham and McAlpine (2004), the adaptation ratio for the probe response was larger than for the masker (about 0.65 and 0.42, respectively) indicating lower SFA and a more tonic response to the probe (compare their Figure 2). With increasing ISIs between masker and probe, the mean adaptation ratios systematically shifted towards smaller values indicating stronger SFA in the growing response to the probe. This difference between both studies might be explained by the binaural stimulus paradigm of IPD sensitive neurons in contrast to only monaural stimulation in this study. However, when Ingham and McAlpine investigated monaural adaptation properties, adaptation ratios as well as time constants were smaller and shorter than those for the binaural paradigm. Unfortunately, they did not vary the ISI for the monaural stimuli, which makes it difficult to speculate about the change of monaural adaptation properties with increasing ISI. However, the monaural adaptation ratios of Ingham and McAlpine were close to the monaural adaptation ratios measured for the owl at various 2nd levels and ISIs.

5.4.8 The role of adaptation in the computation of auditory space

The response properties of ICC neurons in the midbrain of the barn owl were very sensitive to faint changes in the stimulus level and an increase of about five decibels released the neurons from adaptation at least for stimulus levels being in the lower half of the dynamic range. The fast recovery from adaptation that occurred within time scales below 400 ms also supported a large sensitivity to tonal double-stimulation. As mentioned before, the fast recovery was in accordance with the recovery from adaptation measured by Gutfreund and Knudsen (2006) in the IC of the barn owl. Although this thesis focused only on same-frequency adaptation, Gutfreund and Knudsen also compared same-frequency to different-frequency adaptation in ICCls and ICX. For ICCls neurons, same-frequency adaptation was stronger than across-frequency adaptation but both were less effective in ICCls than in ICX. According to Gutfreund and Knudsen, the stronger adaptation in ICX compared to ICCls could preserve the representation of stimulus location of space-specific neurons and thus enhance or favor the computation of the bimodal map. Since no consistent differences in the adaptation properties of ICCcore and ICCls neurons were found in this study, this might give a hint to the basic underlying computational properties of these neurons. This might be dedicated to the ICC as a relay nucleus, which projects to both the fore- and midbrain pathway where different mechanisms of sound representation are realized (Pérez and Peña, 2006; Vonderschen and Wagner, 2009; Pérez et al., 2009). Similar time constants across the entire frequency
range may be due to the lack of phase-locking to frequencies higher than about 1 kHz in IC and favor constant coding properties across frequencies. Comparable adaptation properties to the probe stimuli for all masker levels in general might serve the basis for across-frequency integration (Takahashi and Konishi, 1986; Fujita and Konishi, 1991) that is required for the elimination of phase-ambiguity and the precise computation of auditory space in ICX neurons.

As has also been shown by Gutfreund and Knudsen (2004), the coding of auditory space in the barn owl’s ICX was differently affected by adaptation: When the ITD of the first stimulus was different from the ITD of the second stimulus, then only a modest adaptation could be observed in ICX neurons. When both stimuli had the same ITD, an enhanced adaptation was found in ICX neurons. The shortest ISI tested by Gutfreund and Knudsen was 50 ms and the shortest ‘silent gap’ had a duration of 10 ms. These adaptation processes therefore also acted on fast time scales that were close to the recovery time scales observed in ICC neurons of the current study. A further neural mechanism that also plays an important role in the encoding of auditory space is the precedence effect. Here, the response to an echo (or lagging sound) is suppressed in reference to a leading sound when the position of space differs between these two stimuli (Keller and Takahashi, 1996; Spitzer et al., 2003; 2004; Nelson and Takahashi, 2008; Nelson and Takahashi, 2010). The adaptation observed in the precedence effect thus was contradictory to the adaptation observed by Gutfreund and Knudsen. Since for the precedence effect, the temporal intervals of the two stimuli were either below 10 ms (Keller and Takahashi, 1996) or even partially overlapping (Spitzer et al., 2004) an even more rapid adaptation mechanism underlying the computation of the precedence effect is to be assumed. This might favor short-term or even rapid time constants as well as fast recovery mechanisms as observed for ICC neurons in the present thesis.

5.4.9 The role of adaptation in sound level coding

In the past decade, a growing number of studies investigated the ability of neurons to adapt their firing rate over a large range of stimulus levels that finally allows the precise encoding of high-probability sounds (Dean et al., 2005; Dean et al., 2008; Kvale and Schreiner, 2008; Nelson et al., 2009; Wen et al., 2009). These studies could clearly demonstrate, that the neurons were able to shift their entire dynamic range according to high probability sound levels and thereby keeping the sensitivity to a variation in the stimulus level constant. Such an effect could not be observed in the current study. A constant increase in the stimulus level of the probe at high masker levels resulted rather in suppression than in an enhancement of the response ratios (compare Figure 21F-G; Figure 23A). The ability of ICC neurons to precisely code an increase in the level of the probe stimuli especially at lower masker levels (Figure 21H-J, Figure 23J) might nevertheless be an indication for an enhanced sensitivity for the encoding of rapid changes of stimulus levels. As has been demonstrated by Dean and colleagues (2008), guinea pig IC neurons were much more sensitive to changes in the stimulus level from low to high (time constant 160 ms) in comparison to changes from high to low (time constant 331 ms). Comparable observations were also made by Kvale and Schreiner (2003) for ICC neurons of the cat. In contrast, dynamic range adaptation in the AN of the cat was weaker in comparison to the IC and not sufficient to ensure a precise coding over the whole dynamic range for stimulus levels above 60 dB SPL (Wen et al., 2009). These findings suggest that the adaptive mechanisms of sound level coding first occur at level of the AN and undergo major changes and optimized tunings in ascending auditory nuclei. Therefore, the sensitivities observed for rapid
changes in the increase of stimulus levels might serve underlying computations required for the detection or localization of either approaching hunters (in the case of the guinea pig) or moving prey (in case of the barn owl).

5.4.10 Mechanisms of adaptation

Since adaptation is a widespread synonym for decreasing response rates in neuronal systems as well as for a suppressed response to subsequent stimuli, many mechanisms might account for adaptation. In general, synaptic depression seems to play a relevant function for adaptation (Wehr and Zador, 2005; Goutman and Glowatzki, 2007). Inhibition is also likely to play a role as has been shown by the spontaneous ratios in the present study (Figure 20) or by Wagner and colleagues (2002). But also intrinsic cellular properties like feedback (potassium or calcium currents) and feed forward (sensory inputs) are involved in adaptation (Gollisch and Herz, 2004). Calcium dependent channels are involved in adaptation and favor distinct response characteristics of neurons as has been shown in a model study (Prescott and Sejnowski, 2008). Whereas calcium activated potassium channels enhanced spike rate coding, voltage gated M-type potassium currents improved spike time coding. Lastly, also the context of stimuli seems to be involved in adaptation processes at all levels of the auditory pathway. A model of the interactions between the inner hair cell and the AN synapse revealed that the dynamic range adaptation originated from a mechanism peripheral to the spike-generation mechanism and thus response history must have been involved (Zilany and Carney, 2010). Similar conclusions were drawn for IC neurons where the adaptation to acoustic motion cues was explained by a mechanism called “adaptation of excitation” (McAlpine et al., 2000; Ingham et al., 2001; McAlpine and Palmer, 2002). For midbrain and forebrain neurons, the response behavior was dependent on the stimulus context as well. In stimulus-specific adaptation, the neuron’s response was enhanced when the stimulus was rare whereas for common stimuli, the response of the neurons showed adaptation that could last for several seconds (Ulanovsky et al., 2004; Reches and Gutfreund, 2008; Anderson et al., 2009; Malmierca et al., 2009; Reches et al., 2010).

5.4.11 Conclusion

The data on adaptation presented in this thesis were well in accordance with other studies on adaptation in the auditory system. The impact of response adaptation strongly depended on two major parameters: the general level of the stimulus as well the ISI between masker and probe. For very short ISIs, the responses to the probes were strongly suppressed but decreased gradually with increasing ISIs. However, the masker’s impact on the probe could last for at least 300 ms to 400 ms until response adaptation could be fully compensated. A much more powerful mechanism to overcome response adaptation was an increase of the stimulus level for the probe tone. On average 5 dB were sufficient for the neurons to respond with similar rates to both the masker and the probe stimulus. Nonetheless, this effect was only efficient for masker levels in the lower half of the saturating RLF. As mentioned earlier, this observed sensitivity for small changes in the stimulus level might be relevant for the detection of prey like a mouse rustling on the ground while searching for food whereas the enhanced response adaptation at higher masker levels might be useful for the suppression of disturbing background noises like the wind. Also a reduced response rate to stimuli that were presented at ISIs shorter than 400 ms might be additive for the detection of beginning movements evoked by a mouse on the ground.
SFA in ICC neurons was variable between the responses to the masker and the probe. Generally, neurons showed rapid or short-term adaptation as judged by the time constants of the responses to the masker and especially rapid adaptation did not depend on the masker level. In contrast to the time constants, the amount of SFA in the responses to the masker quantified by the response ratio decreased with decreasing masker level, indicating stronger spike-frequency adaptation that might be caused by a change in the neuron’s response behavior to the masker stimulus. The temporal decay of the responses to a stimulus of primary-like neurons thus might be inherent to ICC neurons and act independent of the stimulus level for the masker. The probe responses in the 2nd level tuning showed a large variability of time constants that was dependent on the masker level as well, especially for probe stimuli that had the same stimulus level as the masker. In the ISI tuning, this effect was substantially reduced most likely caused by the ISI of 25 ms between masker and probe in contrast to 0 ms ISI in the 2nd level tuning. A further observation also pointing in the same direction was found for the time constants of the responses to the probes with changing stimulus parameters of the probe. Whereas the time constants for primary-like units decreased with increasing probe level in the 2nd level tuning for example at 50% masker level, the time constants remained relative stable for the probe responses with increasing ISIs. Thus, the temporal distribution of stimulus presentation was a fundamental parameter influencing the response properties of ICC neurons and essential differences in the encoding of subsequent stimuli could be observed between ISIs of 0 ms and 25 ms. But the responses of the neurons for the probe stimuli for very short ISIs were not static but could be modulated for example by an increase in the stimulus level of the probe as shown in the 2nd level tuning. Although adapted, the neurons could precisely code the increase in the probe amplitude with high accuracy and comparable parameters of SFA again suggesting that at least the neuron’s response behavior was relatively independent of the current state of adaptation. The comparable observations of response properties to tonal double-stimuli between both sub-nuclei and across all frequencies tested supported the fundamental role of the ICC as ‘the’ relevant auditory midbrain nucleus. Ascending auditory information that is processed either to the forebrain or the midbrain has to pass through ICC. Neurons in ICC encode all relevant auditory features like time and level disparities, intensity information as well as the entire spectrum of frequencies before higher order nuclei perform the computation of auditory space (ICX) or other relevant tasks (forebrain). But to do so, the entire spectrum of auditory information has to be allocated by ICC neurons to allow access of all relevant auditory features. As could be shown by the data on response- and spike-frequency adaptation, ICC neurons were capable to enable this access with similar response properties for subsequent stimuli that varied in the interval or the level with high dynamics across the entire range of stimulus frequencies tested.
Recently, the debate about the neural encoding of auditory space using ITDs has become controversial as a general model applicable to both birds and mammals is missing (McAlpine and Grothe, 2003a; Joris and Yin, 2007; Carr and Schnupp, 2009; Grothe et al., 2010). One reason may be the independent evolution of neural mechanisms suitable for sound localization for over 300 million years (Clack, 1997; Köppl, 2009). One mechanism of how a neuronal sound localization circuit could be implemented in the auditory system was proposed by L. A. Jeffress in 1948. The circuit he proposed requires input neurons, which connect the left and right ears in a binaural nucleus that compares the incoming signals from both sides. Delay lines function as “storage elements” that compensate for a time delay between both ears if the sound source is displaced laterally from the midline. Whenever the external time delay is just compensated by the internal delay as represented by the difference in the length of the two delay lines and the neuron these two delay lines connect to, the simultaneous input is transformed into an maximum firing rate of this coincidence detector neuron. This coding of time information by coincidence detectors corresponds to a transformation of a time code into a place code. By transforming the time code into a place code the neurons are able to signal the position of a sound source in azimuth. This theoretical model of how such a sound localization circuit could be implemented in the brain was functionally confirmed in the auditory system of alligators (Carr et al., 2009) and birds such as barn owls and the chicken (Carr and Konishi, 1988; 1990; Slee et al., 2005; Köppl and Carr, 2008; Slee et al., 2010). This finding for two evolutionary sister groups might give a hint to a common and unique sound localization circuit suitable for all groups of vertebrates. However, the presence of delay lines in the mammalian auditory system has been examined in at least three studies and the results are less convincing in the existence of delay lines than as it has been demonstrated for the barn owl (Smith et al., 1993; Beckius et al., 1999; Karino et al., 2011). The observed delay lines in the mammalian system do not seem to be able to account for the observed physiological delays (Karino et al., 2011). This led to the suggestion that the computational mechanisms required to precisely pinpoint the position of a sound source are fundamentally different between in barn owls and mammals, originating in rather functional limitations. In the following discussion I will review the differences between barn owls and mammals with respect to the encoding of ITDs, and relate the results of my thesis to these functional properties of the barn owl’s auditory system.

### 6.1 ITD coding in barn owls and mammals

The difficulty in the encoding of auditory space is that the brain cannot determine a time difference between both ears *per se*. Instead, it must derive information about the temporal arrival of auditory signals indirectly by another mechanism that allows a precise measurement of time. This is achieved by phase-locking, meaning that neurons fire action potentials in response to a specific phase of the incoming sound wave. For low frequencies, where the period of the tone is long, neurons are well able to phase-lock to a specific phase of the stimulus. In the mammalian auditory system, this ability decays for frequencies above 3 kHz because of the limited capability of neurons to follow rapid phase changes (Palmer and Russel, 1986). Barn owls, in contrast, are able to precisely encode the phase of a sound up to frequencies of 8 kHz or 9 kHz (Köppl, 1997b). This out-
standing coding ability allows these birds to exploit ITDs over their entire frequency range for sound localization in azimuth, whereas mammals are limited to frequencies up to around 1.5 kHz to extract ITD information for azimuthal sound source determination. At higher frequencies, mammals rely on ILDs to perform this task.

For both barn owls and mammals, phase-locked neuronal activation is transmitted in spike trains from the cochlea via the 8th cranial nerve to auditory nuclei in the brainstem where, ultimately, binaural information is compared and integrated. As mentioned earlier, timing information in the barn owl is sent via NM to NL where coincidence detector neurons compare the incoming spike trains (Sullivan and Konishi, 1984; Takahashi and Konishi, 1988b; Köppl, 1997a). ILD information is sent to NA and from there further on to LLDp (Sullivan and Konishi, 1984). In both time and the intensity pathways, the entire range of frequencies is represented (Sullivan and Konishi, 1984; Takahashi et al., 1984). In contrast, the medial and lateral superior olive (MSO and LSO) show a clear distinction in the representation of best frequencies. An overrepresentation of high frequencies was demonstrated in the LSO (Beckius et al., 1999) whereas low frequencies were dominantly recorded in the MSO (Goldberg and Brown, 1969; Yin and Chan, 1990). This separation of frequencies could be well explained by the function of these nuclei to either encode ILDs or ITDs. However, recent studies shed new light on the function of these two nuclei, indicating a less strictly separated dominance of neurons to encode either of the two binaural cues. Low-frequency neurons in the LSO of cats have been shown to be not only sensitive to ILD but also to ITD information (Tollin and Yin, 2005). A tracing study conducted in the same species revealed rather an under- than an overrepresentation of low frequency neurons in the MSO (Karino et al., 2011).

When responses of ITD-sensitive neurons to broadband ITD signals were compared between small rodents like gerbils, chinchillas and guinea pigs on the one hand and barn owls on the other hand, large differences became apparent despite the similar physiological range of detectable ITDs (around 200 µs to 300 µs) (Keller et al., 1998; McAlpine et al., 2001; Poganiatz et al., 2001; Harper and McAlpine, 2004; von Campenhausen and Wagner, 2006; Wagner et al., 2007; Hausmann et al., 2009; Pecka et al., 2010; Lesica et al., 2010; Bremen and Joris, 2011). These differences led to new speculations on how ITDs might be encoded. ITD tuning curves in mammals are generally broad with peaks outside the animal’s physiological ITD-range (McAlpine et al., 2001; Hancock and Delgutte, 2004; Pecka et al., 2008a; Lesica et al., 2010). ITD tuning curves in barn owls are cyclic and the inter-peak distance depends on the BF of the recorded unit for all nuclei upstream to IC (Knudsen, 1983; Knudsen and Knudsen, 1983; Takahashi et al., 1984; Sullivan and Konishi, 1986; Wagner et al., 1987; Takahashi and Konishi, 1988c; Carr and Konishi, 1990; Wagner, 1990; Albeck, 1997; Mazer, 1998; Pena and Konishi, 2000; Pena et al., 2001; Wagner et al., 2002; Carr and Köppl, 2004; Bremen et al., 2006; Christianson and Pena, 2006; Fischer et al., 2007; Wagner et al., 2007; Fischer and Pena, 2009a; Singheiser et al., 2010a). For low frequencies, the peaks may lie outside the physiological range for the barn owl as well, but many ITD tuning curves exhibit peaks close to zero (Wagner et al., 2007; Singheiser et al., 2010a). For high frequencies, more than one peak may fall within this range. This labeled line code in the barn owl creates a Jeffress-like map of ITDs that persists throughout the entire auditory pathway and stands in contrast to the auditory system of mammals (Grothe, 2003; Joris and Yin, 2007; Grothe et al., 2010). As explained before, auditory neurons couple to a given phase of the sound signal. Period duration for the low frequencies mammals rely on is longer relative to the animal’s head diameter, meaning that phases repeat at longer interval than they do at higher frequencies. Consequently, auditory neurons are broadly tuned as expected from the small phase changes that occur for low frequencies. The resulting ITD tuning curves show very broad peaks across the physiological range rather than
displaying a modulation of ITDs within this range. Therefore, it is unlikely that more than one peak will fall within the animal’s physiological range of detectable ITDs. As a consequence of these broad tuning curves, best ITDs close to zero were seldom recorded in mammals making it difficult to localize sound sources.

For this reason, it remained rather speculative, how mammals could successfully localize sound sources. Finally, model data published in 2004 seemed to be sufficient to explain the ITD coding in mammals (Harper and McAlpine, 2004). The authors proposed a two-channel model for the encoding of low frequency ITDs: by shifting the peaks of the ITD tuning curves outside the physiological range, the steepest slopes of the rate ITD functions cross the midline of zero ITD regardless of the neuron’s frequency and best ITD. Furthermore, each brain hemisphere was regarded as a single channel that represents the same range of ITD in the contralateral space. For a precise computation of the azimuthal sound source position, the firing activity at the slopes for each channel has to be compared. But which mechanism could be responsible for shifting the peaks of the ITD tuning curves outside of the physiological range as presupposed by the slope coding model of Harper and McAlpine (2004)? Recent studies discovered a precisely timed inhibition that acts on MSO neurons. When this inhibitory influence was blocked with the glycinergic antagonist strychnine during stimulation with pure tone phase delays, two major effects were be observed: at first, the discharge rate of these neurons increased in an ITD-specific manner compared to control experiments. Second, and most importantly, the peaks of the ITD tuning curves that lay outside the physiological range prior to blocking the inhibition started to move closer to the midline and fell within the physiological range (Brand et al., 2002; Magnusson et al., 2008; Pecka et al., 2008b; Werthat et al., 2008). Additional experiments supported the hypothesis of a population code being responsible for sound localization via ITDs. Behavioral experiments and electrophysiological recordings from the gerbil IC clearly demonstrated that ITDs were not encoded by arrays of individual neurons each coding for a specific ITD throughout auditory space as in the barn owl, but by an entire population of neurons within a hemisphere (Lesica et al., 2010).

Thus, the model presented by Harper and McAlpine (2004) might sufficiently explain the coding of low-frequency ITDs in mammals. When the authors additionally tested the model for the ITD coding in the barn owl, however, some caveats became apparent. While in the low frequency range, the authors implied a two-channel model for the barn owl just as for mammals, localization of high-frequency ITDs was optimally explained by the Jeffress model (Harper and McAlpine, 2004). This assumption of a population code as a universal model for low-frequency ITD sound localization was challenged by Wagner and co-workers (2007) at least for the barn owl since a considerable number of low-frequency neurons showed best ITDs close to zero. This is a finding that is not predicted by the slope code model by Harper and McAlpine (2004). In addition, behavioral data obtained from free-flight experiments with barn owls were inconclusive on whether the lower cut-off frequency was around 2.5 kHz as proposed by the optimality-coding model or at higher frequencies (Singheiser et al., 2010b). A further objection of the optimality-coding model is that it does not seem to be universal even among mammals. Recordings obtained in the MSO and the IC of chinchillas revealed a considerable number of low-frequency neurons with best delays within the physiological range (Bremen and Joris, 2011). Since neither delay lines as proposed by Jeffress (1948) nor the two channel model as suggested by Harper an McAlpine (2004) appear to be sufficient to solely explain the localization of low-frequency ITDs, an alternative model based on frequency mismatches between the two ears was investigated in this thesis. The stereaensis model assumes frequency dependent mismatches originating in the cochlea that vary as a function of the external delay (Shamma et al., 1989;
Bonham and Lewis, 1999). Although data recorded in the AN and IC of the cat supported the stereausis model for mammals (Joris et al., 2006), no evidence for stereausis was found for either the low or high frequency ranges in the barn owl and the model failed to account for sound localization (Pena et al., 2001; Fischer and Pena, 2009a; Singheiser et al., 2010a). This finding for the barn owl approves the opinion of Joris and Yin (2007) that the implementation of a neural sound localization based on a systematic frequency mismatch in relation to ITD seems unlikely.

Two final questions remain unsolved so far: first, is there a general mechanism that is responsible for the encoding of time differences? It seems unlikely, that a single mechanism can account for all experimental findings and the underlying computational algorithms. The independent evolution of sound localization circuits seemed to have resulted in different algorithms, one in sauropsids (birds and reptiles) and another in mammals. The second question asks how the owl can resolve large best ITDs that lie outside their physiological range (Saberi et al., 2001) if stereausis fails as a mechanism for sound localization (Pena et al., 2001; Fischer and Pena, 2009a; Singheiser et al., 2010a) and if the Jeffress model seems inappropriate because the owl will never encounter such large ITDs in its natural environment? Two studies focusing on the neuro-anatomical sound localization circuit in birds reported differences in axon lengths from NM neurons to the ipsi- and contralateral NL. Carr and Konishi (1990) reported differences in axon length of about 1 mm for the owl and Seidl et al. (2010) found differences of more than 1.6 mm for the contralateral axons in the chicken. Thus, coincidence detection within the physiological range solely based on neuronal travel distance would be impossible and underlies the need for additional mechanisms for coincidence detection. As proposed by Carr and Konishi (1990) and demonstrated by Seidl et al. (2010), axon diameter and internode distances are regulated at different positions within individual NM axons to precisely adjust the conduction velocities of ipsi- and contralateral signals to enable coincidence detection in NL neurons. The signal in the ipsilateral part of the sound localization circuit is slowed down, allowing the contralateral signal to catch up and to ultimately ensure the precise encoding of ITDs within the animal’s physiological range. In conclusion, it might be possible that large best ITDs are still encoded by a mechanism resembling the one proposed by Jeffress (1948).

### 6.2 The role of the ICC in sound localization

As mentioned in the previous chapter, the auditory system of the barn owl contains a map of azimuth that persists throughout the ascending pathway up to the level of ICC. Neurons in both the NL and the ICC are tuned to ITD and show a narrow tuning to frequency, which results in cyclic ITD tunings where the inter-peak distance depends on the BF of the particular neurons. Additionally, all peaks in ITD tuning curves are of similar height (Wagner et al., 1987; Carr and Konishi, 1990; Wagner et al., 2002; Bremen et al., 2006; Christianson and Pena, 2006; Wagner et al., 2007; Köppl and Carr, 2008). This similarity in peak discharge rate at integer multiples of the period corresponding to the unit’s BF leads to phase ambiguities. The auditory system therefore cannot precisely determine the position of a sound source at this stage of processing. Phantom sources might be localized as a consequence of the animal’s inability to discriminate between these multiple equivalent encodings (Saberi et al., 1998; Saberi et al., 1999). For unambiguous sound source localization, it is therefore a crucial need to optimize the coding of ITDs, and the auditory system has evolved several mechanisms that contribute to resolving this task. At initial stages of the auditory pathway, noise reduction from NL via LLDa to ICCcore enhances the
validity of ITD coding (Christianson and Pena, 2006; Fischer and Konishi, 2008). Similar output of NL neurons is thus summed over three ascending synapses to produce more reliable ITD tuning curves in ICCcore.

However, simple averaging is insufficient to precisely code for the azimuthal position of a sound source. To achieve this task, phase ambiguity has to be overcome by across-frequency integration (Mazer, 1998). ITD sensitive neurons in ICC are organized in a Jeffress-like representation where the ITD is arranged in columns of best ITD. These columns run perpendicular to the iso-frequency laminae that show a tonotopy from low to high BFs with increasing dorso-ventral penetrations (Takahashi and Konishi, 1986; Wagner et al., 1987; Wagner et al., 2002; Wagner et al., 2007). When stimulated with broadband noise, multiple narrow frequency channels are activated across a single column of ITD that now can precisely encode the azimuthal position by suppressing the side peaks relative to the main peak. This computational step is performed by ICCls neurons that integrate ITDs across several frequency channels before projecting onto neurons in ICX (Knudsen and Konishi, 1978; Knudsen, 1983). Both electrophysiological and behavioral experiments in barn owls have demonstrated the necessity of across-frequency integration over a bandwidth of about 3 kHz to determine azimuthal positions of sound sources emitting high frequency signals (Takahashi and Konishi, 1986; Mazer, 1998; Saberi et al., 1998; Saberi et al., 1999; Pena and Konishi, 2000; Singheiser et al., 2010b). To finally pinpoint the target in three-dimensional space, the computation of ILD as a cue for elevation needs to be integrated as well. ILD is independently processed in the auditory pathway via NA and LLDp until ITD and ILD information converge in ICCls (Sullivan and Konishi, 1984; Adolphs, 1993b; Mogdans and Knudsen, 1994; Takahashi et al., 1995; Köppl and Carr, 2003). Similar to ITD, ILD is integrated in ICCls and projected onto ICX neurons that are now able to compute a receptive field of auditory space (Knudsen and Konishi, 1978). A modeling study was able to reveal how ITD- and ILD-dependent signals were multiplied within narrow frequency channels by a mechanism resembling across-frequency integration (Fischer et al., 2009). These data indicate that ICCls is the first site in the ascending auditory pathway where multiplication occurs.

However, auditory information is not only sent to ICX in the midbrain but also to the forebrain nucleus AAr via NO and Field L (Cohen and Knudsen, 1995; Proctor and Konishi, 1997; Cohen et al., 1998; Cohen and Knudsen, 1998). Unlike brainstem and midbrain nuclei, a map of space in the forebrain is lacking and neurons are broadly tuned to ITD – a representation comparable to that in mammals. In contrast to ICX neurons, units in the forebrain are also sensitive to frequencies below 3 to 4 kHz (Cohen and Knudsen, 1996; 1998; Pérez and Peña, 2006; Vonderschen and Wagner, 2009; Pérez et al., 2009). This low-frequency sensitivity results from projections originating in ICC (Arthur, 2005) – a nucleus that spans the entire frequency range of owls – serving as a mediator for the complex and completely different coding principles to ITD between midbrain and forebrain. Thus, the barn owl’s ICC is the central relay station of auditory signals and gives rise to entirely different coding mechanisms. It therefore can be assumed that the underlying coding principles of ICC neurons barely differ much between both sub-nuclei, which has been shown for the tuning to ITDs and frequency (Takahashi and Konishi, 1986; Wagner, 1990; Fujita and Konishi, 1991; Wagner et al., 2002; Bremen et al., 2006; Wagner et al., 2007). These assumptions are supported by the results of adaptation experiments presented in chapter 5. Frequency tuning curves were similar in both sub-nuclei and monaural RLFs recorded with pure tone stimulation generally varied over large stimulus levels but were on average indifferent between ICCcore and ICCls. When the effect of a masker stimulus was tested for the response properties of a neuron to a second stimulus, the response behavior did likewise not differ between the nuclei. Similar observations were made for the temporal
progression and amount of adaptation in the responses to a masker stimulus. Time constants for primary-like units were in the range of 10 ms indicating rapid spike-frequency adaptation and the response at steady state was on average reduced by about 70% in comparison to the onset peak depending on the stimulus level. Recovery from a masker-induced adaptation could be compensated in both nuclei by an increase of the stimulus level to the probe by about 5 dB for stimulus levels close to threshold up to 50% of the saturating RLF. For higher stimulus levels, adaptation could not be overcome by a further increase of the stimulus level for the probe. This sensitivity of ICC neurons to faint increments of the stimulus level might be required for the auditory detection of moving prey during nocturnal hunting, since barn owls preferentially detect, localize and approach faint sound sources over loud ones (Payne, 1962; 1971; Konishi, 1973a; 1973b; Hausmann et al., 2008; Asadollahi et al., 2010; Singheiser et al., 2010b). Also the relatively fast recovery periods from adaptation of about 300 ms to 400 ms might be relevant for the propagation of auditory information to both the mid- and forebrain pathway since they both originate in ICC.

The results presented in this thesis clearly demonstrated the importance of ICC neurons in the encoding of auditory space. The stereausis model can be excluded as an alternative model for the detection of ITDs that lie outside the physiological range of the barn owl. The amount of adaptation strongly depends on the overall stimulus level but can be overcome by either increasing the level of the probe stimulus or the interval between both stimuli. A balance of excitation and inhibition thus acts on both response and spike frequency adaptation in ICC neurons. Recordings in lower auditory nuclei may be performed in future experiments in order to investigate whether the responses described in this thesis originate in ICC itself or develop in the ascending auditory pathway. Also a blockade of the input neurons located in LLDa projecting to ICCcore might be helpful to exploit the origin and amount of adaptation in ICC. Further investigations will be needed to clarify the computation of auditory space. Simultaneous recordings of neurons that share the same ITD but that are sensitive to different frequencies might give uncovered insights in the parallel computational processes that cannot be recorded with common single-channel electrodes. Multi-channel electrodes with a linear and narrow-spaced arrangement of recordings sites might be a powerful tool to investigate the process of across-frequency integration and the amount of adaptation in the ICC in more detail.
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