Age-related declines in auditory temporal processing

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Summary

The auditory processing of complex acoustic environments prominently declines with age. Age-related auditory dysfunction is typically not compensated by traditional hearing aids, because aging is often not only associated with increased peripheral auditory thresholds but also with declines in central auditory processing. These declines are evident from the prominent inability of aged subjects with normal hearing to isolate single sound sources from complex mixtures of sounds and the observation that markers of temporal processing deteriorate in aged humans and experimental animals, independent of peripheral thresholds. The present study investigates neural correlates of temporal processing in the young adult and the aged auditory system.

A crucial feature of auditory temporal processing that underlies analysis of simple and complex sound signals is selectivity of auditory neurons to temporal sound parameters, such as sound duration and inter sound intervals. Neuronal selectivity to temporal parameters is installed by a balanced and timed interplay of excitatory and inhibitory inputs. In support of an age-related decline in temporal processing, the balance of excitatory and inhibitory neurotransmitters and their receptors is disrupted in brain tissue of aged animals.

In the intact auditory system, neuronal temporal tuning is particularly versatile at the inferior colliculus (IC), a large midbrain nucleus that receives convergent excitatory and

inhibitory input from virtually all lower stations of the auditory processing. To evaluate temporal processing in the young adult and the aged auditory system, neuronal sensitivity to temporal parameters of noise burst sequences at the IC of young adult and aged experimental animals was compared using extracellular single cell recordings. Temporal selectivity to sound parameters was significantly decreased in neuronal responses from aged relative to young adult animals. This decrease in selectivity resulted in a decrease in heterogeneity of neuronal responses on the population level and a decreased efficiency in encoding of speech signals. This redundant representation of sound features may hamper the discrimination of sound signals.

Altered selectivity of neurons from aged animals to sound sequences resulted from altered sensitivity to the temporal context of stimulation. Therefore, in the second part of this study, the role of altered temporal context sensitivity in complex stimulation was investigated. Temporal release from masking was probed in young adult and aged subjects using a masked word discrimination task. In this task, the most informative part of the word for discrimination was adjusted to coincide with a transient level decrease in the masking signal (i.e. a single masker modulation). When subjected to the word discrimination task, young adult subjects benefited significantly from the masker modulation. In contrast, the benefit of masker modulation was diminished for aged subjects. In experimental animals, a possible neuronal correlate of this decline in temporal processing is reported: Neuronal response precision was severely diminished in IC neurons from aged animals, when the target word was preceded by a masking signal.

The results from this study add further significance to the hypothesis that declines in temporal processing underlie age-related auditory dysfunction and they underscore the importance of temporal context sensitivity in this matter. Because natural soundscapes generally comprise ongoing sounds and temporal coherence is one of the major cues for the analysis of complex acoustic scenes, temporal context sensitivity is of immense importance to auditory processing. Further study will be required to fully understand the roles of altered context sensitivity in complex acoustic scenes. The study of further aspects of auditory context sensitivity in the processing of complex acoustic scenes and age-related auditory dysfunction provides an interesting challenge for future research in the field.

I

Introduction

Declines in sensory functions are among the most common pathologies in the elderly and cause considerable limitations in everyday life. Sensory aging has, until recently, exclusively been attributed to sensory receptor dysfunction. However, there is mounting evidence that central processing plays a significant part in age-related sensory dysfunction. In the auditory system, psychophysical studies suggest declines in spatial and temporal processing of sound (Gordon-Salant and Fitzgibbons, 1993; Snell, 1997; Strouse et al., 1998; Ross et al., 2007).

The most prevalent symptom of age-related presbycusis is an inability to efficiently isolate single sound sources from complex mixtures of sounds. The separation of sound mixtures into streams of different origin is absolutely vital in ecological soundscapes: it underlies assessment of potential danger and appropriate response planning, and is of course essential to acoustic communication.

The analysis of natural soundscapes represents one of the most challenging tasks our auditory system performs. Rather than being defined by static features, natural sounds are characterized by their spectrotemporal dynamics. Consequently, auditory objects generally comprise a set of features that is defined by its variation over time. Information in speech, for example, is conveyed by temporal fluctuations of signal amplitude, the sound of a violin is characterized by temporal modulations of signal frequency and

melodies arise from sequences of harmonic complexes. The temporal dynamics of sounds are of even higher complexity in natural soundscapes, because sound sources and receivers may be moving through the environment. In addition, in echoic environments spectro-, and spatiotemporal modulations are obscured by reverberation.

As a result of the inherently dynamic nature of sounds, the auditory system operates on multiple time scales. On the one hand, to analyse basic spectral and spatial features of sounds, neural processing is highly precise and temporally acute. In this line, the auditory brainstem contains highly specialized structures that allow computation of sound location from microsecond differences in the arrival of sound at the two ears (for review: Grothe, 2003). On the other hand, because the significance of sound features is encoded in temporal dynamics, the analysis of natural sounds requires sensitivity to stimulus context that spans time scales from a few ms (e.g. to analyse spectrotemporal modulations in speech signals) up to hours or even years (to encode behavioral relevance of sounds). Accordingly, neurons of higher auditory structures such as the auditory midbrain and the auditory cortex exhibit sensitivities to the short-term and long-term history of stimulation (for review: Nelken et al., 2003). The effect of the temporal context of the stimulation on neuronal discharge is called temporal context sensitivity (Malone et al., 2002).

Auditory processing along the auditory pathway

Auditory processing starts as sound waves impinge on the ears, are filtered by pinna structure and funneled into the ear canal. At the outer ear, sound waves ignite a sequence of movements of specialized structures that ultimatively results in frequency decomposition of incoming sounds at the cochlea (Von Békésy, 1960). Frequency decomposition of traveling waves along the basilar membrane is achieved by gradually decreasing stiffness and increasing width from the base to the apex of the cochlea. Localized deflections of the basilar membrane cause graded electrochemical potentials in a localized population of hair cells that are transferred to type 1 spiral ganglion neurons, which transduce action potential responses along their axons (the auditory nerve (AN)). Due to this organization, every AN fibre expresses maximum sensitivity to a certain “best” frequency (BF) depending on the AN fibre’s origin on the basilar membranes. The

frequency-to-place relationship created at the cochlea is preserved along the auditory pathway and underlies all further steps of auditory processing. From here AN fibres protrude to the cochlear nucleus (CN), the first of several auditory processing stations in the brainstem (De No, 1933; Warr, 1966). In the auditory brainstem nuclei, basic features of incoming sounds are extracted in parallel. Output of most of these nuclei converges at the auditory midbrain, in the inferior colliculus. The present study is mainly concerned with neuronal processing at the inferior colliculus, therefore, to gain an understanding of the inferior colliculus, a subset of brainstem nuclei, their connection patterns and their possible functional roles are introduced in the following text.

The CN is the first obligatory station of neural processing in the auditory pathway, from here axonal projections protrude to higher auditory nuclei (fig. 1.1). At CN input, single AN fibres, send collaterals to several morphologically distinct types of CN neurons. Because every AN fibre is most sensitive to a certain frequency, its collaterals create sheets of isofrequency regions in the CN (Webster, 1971). The set of CN neurons innervated by a single AN fibre transforms AN-input according to biophysical and synaptic specializations to create an output tailored to the set of nuclei each neuron type projects to (Rose et al., 1959; Brawer et al., 1974; Rhode et al., 1983b; Rhode et al., 1983a). In this way, each CN neuron within a single isofrequency sheet extracts separate sound features from the incoming action potential train, which are transduced to downstream nuclei for further processing.

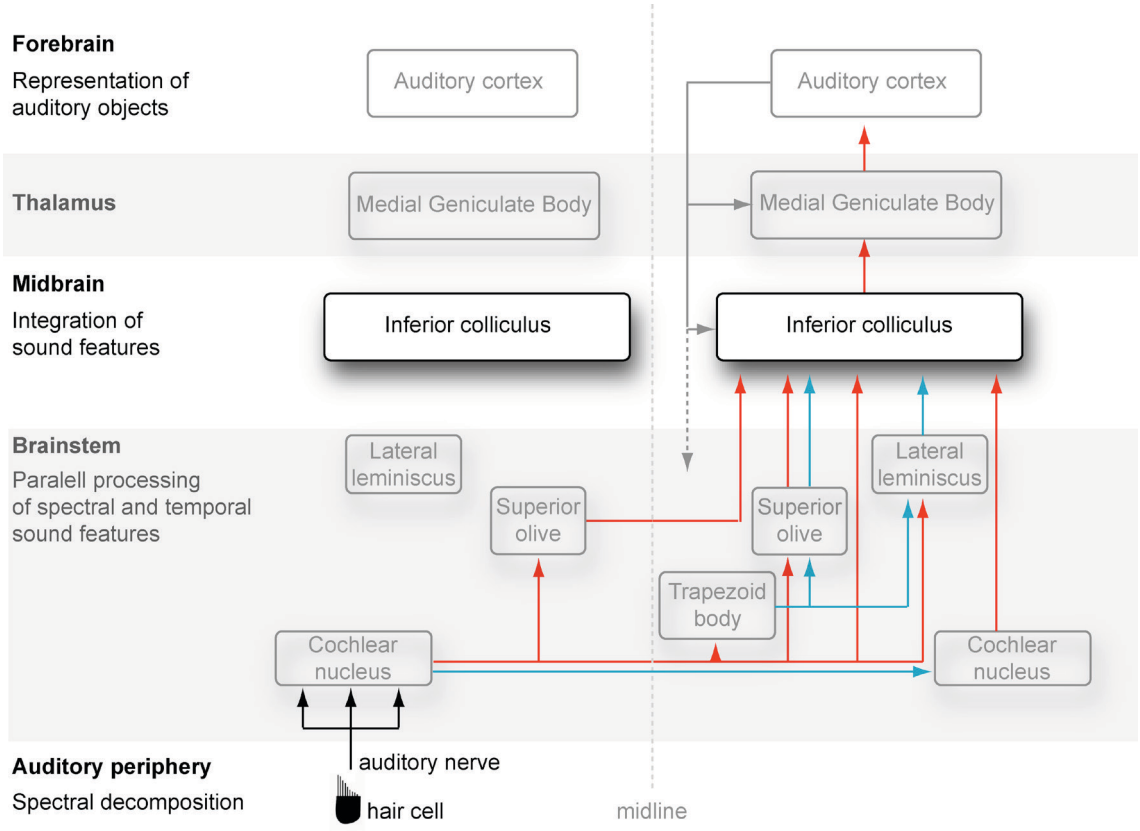

Figure 1.1 Simplified schematic of the auditory pathway.

Incoming sound waves are transformed into electric potentials at the hair cells. Trains of discharge are transduced along the auditory nerve to the cochlear nucleus (CN). From here axons project to the opposite CN to make inhibitory (blue) connections, to the auditory brainstem to make excitatory (red) synaptic connections with nuclei of the superior olive and the lateral leminiscus and to the auditory midbrain to synapse on neurons of the inferior colliculus. Projections from virtually all brainstem nuclei converge on the IC. The IC projects via the auditory thalamus to the auditory cortex. In addition to the ascending, there is also a descending auditory pathway (gray).

Axonal projections from CN neurons on both sides of the brain make synaptic connections with nuclei of the superior olivary complex (SOC) on both sides of the brain (Smith et al., 1991; Smith et al., 1993). Excitatory projections travel to the ipsilateral medial and lateral superior olive (MSO and LSO), the contralateral MSO and the contralateral medial nucleus of the trapezoid body (MNTB). The medial trapezoid body (MNTB) contains extremely fast and reliable synapses to convert excitatory input from the CN to inhibitory output. These inhibitory projections travel the ipsilateral MSO and LSO (Moore and Caspary, 1983; Friauf and Ostwald, 1988; Tsuchitani, 1988a, b). Based on amplitude and timing differences of these inputs from the two ears, MSO and LSO extract sound location in a frequency specific manner (Boudreau and Tsuchitani, 1968; Irvine, 1986; Pecka et al.,

2008). Although the superior paraolivary nucleus (SPON) receives binaural excitatory input from the CN and inhibitory input from the ipsilateral MNTB, it contains mainly monaural neurons that respond to the offset of sound. These neurons have been proposed to play an important part in the temporal processing of sound (Kuwada and Batra, 1999; Behrend et al., 2002; Kulesza et al., 2003). Projections from MSO, LSO and SPON converge at the auditory midbrain in the IC. Output of LSO and MSO to IC is both excitatory and inhibitory (Elverland, 1978; Adams, 1979; Glendenning and Masterton, 1983; Saint Marie et al., 1989); output of the SPON to the IC is primarily inhibitory (Schofield, 1991; Kulesza and Berrebi, 2000; Saldana and Berrebi, 2000; Saldana et al., 2009).

The dorsal NLL (DNLL) receives, in addition to direct input from the contralateral CN, input from the SOC (Glendenning et al., 1981) and has been proposed to enhance representation of sound localization cues computed in the SOC (Pecka et al., 2010). The ventral NLL (VNLL) receives excitatory input from contralateral CN and inhibitory input from the ipsilateral MNTB (Warr, 1969). VNLL neurons, respond with high temporal precision to the onset of sounds, and are probably also important for temporal processing (Covey and Casseday, 1991). Projections from NLL also terminate in the IC. Output of the VNLL and DNLL to the IC is inhibitory (Adams and Mugnaini, 1984; Vater et al., 1997). In addition, CN sends direct excitatory and inhibitory projections to the IC (Adams, 1979, 1983; Cant and Benson, 2003).

In summary, each brainstem nucleus serves to extract separate basic sound features from its inputs (CN, SPON, MSO and LSO) or to enhance the representation of these features (DNLL, VNLL). The excitatory and inhibitory projections from monaural and binaural auditory brainstem pathways converge at the inferior colliculus.

The inferior colliculus can be subdivided into three major divisions, the dorsal cortex, the lateral cortex and the central nucleus (Morest and Oliver, 1984). Lateral and dorsal cortices of the inferior colliculus exhibit rather complex responses to auditory stimuli and are strongly influenced by multisensory (Aitkin et al., 1978; Aitkin et al., 1981; Li and Mizuno, 1997a, b) and descending cortical inputs (FitzPatrick and Imig, 1978; Saldana et al., 1996; Winer et al., 1998). Research in this study was focussed on the largest division of the inferior colliculus, the central nucleus. In the central nucleus of the inferior

colliculus (IC) tonotopic organisation from the cochlea is preserved. Neurons most sensitive to lower frequencies are located in the dorso-lateral, neurons most sensitive to higher frequencies are located in the ventro-medial part of the IC (FitzPatrick, 1975; Casseday and Covey, 1992; Saldana and Merchan, 1992). IC neurons are morphologically of two types: stellate and principal disc-shaped cells. Disc-shaped cells extend along the isofrequency axis, whereas stellate cells are organized perpendicular to the frequency laminae (Oliver and Morest, 1984). As mentioned above, the IC receives projections from virtually all lower brainstem nuclei and from the opposite IC via commissural connections. The IC is thought to perform short-term integration over its synaptic inputs and projects via the medial geniculate body in the auditory thalamus to the auditory cortex (AC).

The auditory cortex is thought to operate on midbrain representation of sound features to facilitate grouping of sounds from a common source (Nelken et al., 2003). Higher auditory centers are then thought to assign more abstract features, such as source identity and source location, to these auditory objects (Fritz et al., 2010; Shamma and Micheyl, 2010; Lee and Middlebrooks, 2011).

In addition to the ascending auditory system a system of descending projections influences neuronal processing at all levels of the auditory pathway. Descending projections from AC to IC, for example, serve to add long term stimulus context to short-term sound feature representation at the IC (Yan and Suga, 1998; Suga et al., 2002; Suga and Ma, 2003; Bajo et al., 2010).

Temporal processing and context sensitivity in the inferior colliculus

The IC lacks the structure function relationship found at lower stations of the auditory pathway. The convergence of projections from virtually all lower brainstem nuclei on common targets in the IC lead to spectro- and spatiotemporal integration of sound features (Koch and Grothe, 2000; Malone and Semple, 2001; Wenstrup and Leroy, 2001; Malone et al., 2002; Portfors, 2004). IC neurons have been reported to be sensitive to various acoustic features; they modulate discharge, for example, in response to changes in direction and velocity of frequency modulations (Woolley and Casseday, 2005; Andoni et al., 2007), frequency of amplitude-modulations (AMs) (Langner and Schreiner, 1988; Krishna and Semple, 2000; Joris et al., 2004), and sound duration (Casseday et al., 1994; Brand et al., 2000; Perez-Gonzalez et al., 2006). Response sensitivity of IC neurons has been shown to depend critically on balanced excitation and inhibition in the IC and at lower stations of the auditory pathway (Irvine and Gago, 1990; Faingold et al., 1991; Vater et al., 1992; Burger and Pollak, 1998, 2001; LeBeau et al., 2001) and to be a function of stimulus context (Malone and Semple, 2001; Malone et al., 2002). IC neurons are sensitive to spatial (Malone et al., 2002; Dahmen et al., 2010), spectral (Mittmann and Wenstrup, 1995; Malone and Semple, 2001; Nelson and Young, 2010) and temporal (Mittmann and Wenstrup, 1995; Nelson et al., 2009) stimulus context.

Context sensitivity in hearing is often demonstrated using simple backward or forward masking stimuli. In backward masking, psychophysical detection of a probe is deteriorated by a succeeding masker (Elliott, 1971). In a subset of IC neurons, discharge to preceding signals is suppressed by subsequent maskers at specific interstimulus intervals (Carney and Yin, 1989) and suppression may last up to 25 ms (Park and Pollak, 1993; Pollak and Park, 1993; Covey et al., 1996). The underlying mechanism may involve onset inhibition from the ipsilateral VNLL (Covey and Casseday, 1999). In forward masking, a preceding masker deteriorates perception of a subsequent probe (Elliott, 1971). Again, a subset of IC neurons suppresses discharge in response to a tone if the tone is preceded by a masking signal (Nelson and Erulkar, 1963; Faingold et al., 1991; Pollak and Park, 1993; Torterolo et al., 1995; Kuwada et al., 1997), and suppression may last up to 100 ms (Covey et al., 1996; Nelson et al., 2009). Persistent or offset inhibition from DNLL or SPON neurons has been proposed to underlie forward suppression (Kuwada

and Batra, 1999; Burger and Pollak, 2001; Kulesza et al., 2003). Forward masking thresholds at the IC are significantly increased relative to those of AN fibres (Relkin and Turner, 1988; Nelson et al., 2009). Forward masking thresholds of IC neurons match psychophysical forward masking thresholds, whereas those of AN fibres are superior to psychophysical forward masking thresholds (Jesteadt et al., 1982; Relkin and Turner, 1988; Nelson et al., 2009). This implies that the auditory system fosters temporal masking of sounds by converging excitatory and inhibitory inputs on IC neurons.

Complex temporal sequences of excitation and inhibition create complex response selectivity of IC neurons. Rather than being exclusively sensitive to individual sound parameters, IC neurons are also sensitive to the short-term temporal relationship of sound elements (Portfors and Wenstrup, 1999; Malone and Semple, 2001; Wenstrup and Leroy, 2001; Malone et al., 2002). To illustrate short-term context sensitivity of IC neurons, duration selectivity as a function of interstimulus interval (colour coded) is shown in figure 1.2 (similar observations are published: Faure et al., 2003; Krebs et al., 2008; Khouri et al., 2011). The neuron presented in the left panel of figure 1.2 A exhibits bandpass selectivity for sound duration if sound pulses are presented at interstimulus intervals of 4 to 16 ms; if sound pulses are presented at longer or shorter intervals duration selectivity deteriorates. Similarly, neurons presented in middle and right panel of figure 1.2 A exhibit long pass duration selectivity only if sounds are presented at intervals ranging from 32 ms to 128 ms and 16 ms to 128 ms, respectively. At shorter interpulse intervals duration selectivity changes to allpass (middle panel) and shortpass (right panel). As illustrated in figure 1.2 B, short-term context sensitivity of duration tuning results from temporal interaction of excitatory and inhibitory inputs (Casseday et al., 1994; Covey and Casseday, 1999; Faure et al., 2003). The neuron presented in figure 1.2 B faithfully responds to every presentation of a 64 ms sound pulse if the sound pulse is presented at 128 ms intervals. At progressively shorter interpulse intervals, only the first sound pulse elicits neuronal discharge, discharge to successive pulses is suppressed. In summary, parameter selectivity of inferior collicular neurons is shaped by temporal stimulus context.

Sensitivity to stimulus context is of immense importance to the processing of natural soundscapes, which are generally characterized by ongoing sounds. Furthermore,

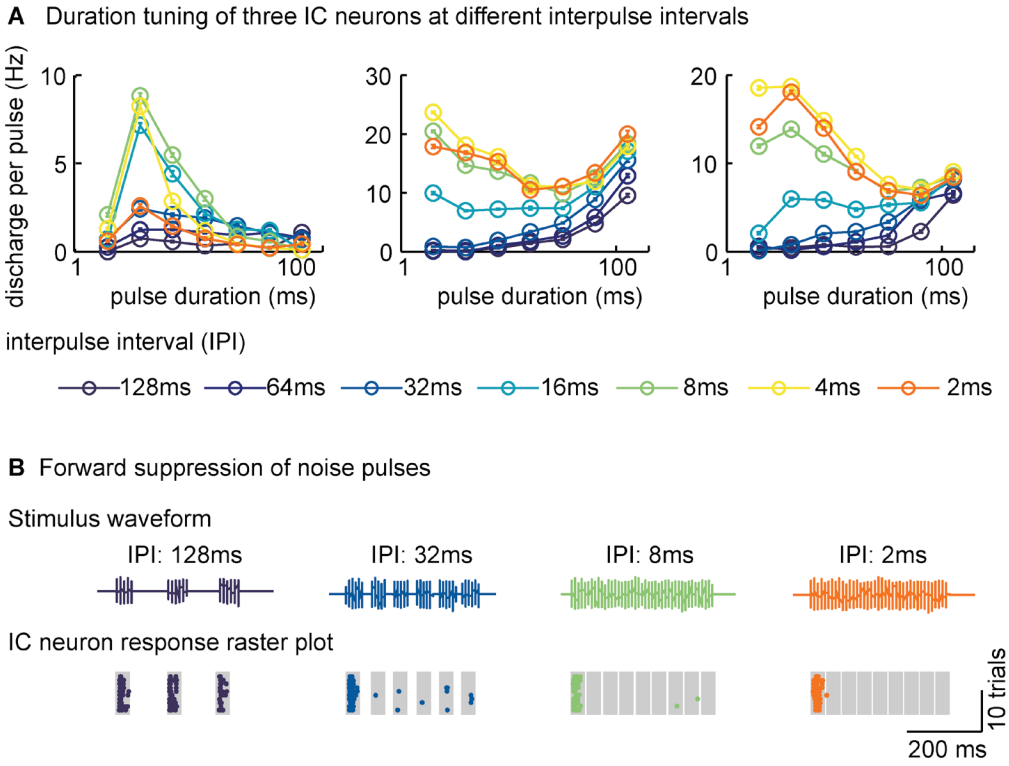

Figure 1.2 Duration selectivity as a function of stimulus context.

(A) Duration tuning curves of three IC neurons. Stimuli of durations specified at the x-axis were presented at different intervals. Interpulse intervals are colour coded. Note that for all three neurons, duration tuning is a function of interpulse interval. (B) Stimulus waveform and response raster plot of an IC neuron. The neuron responds to every 64 ms pulse if pulses are presented at 128 ms intervals. At progressively shorter intervals, response to subsequent pulses is suppressed. Gray rectangles indicate sound pulses. Data unpublished.

perception of the acoustic environment is dependent on the temporal relationship of sound elements. In this line, adaptive mechanisms have been shown to contribute to signal analysis. For example, ongoing stimulation at different sound levels shifts rate-level functions of IC neurons to optimise coding of most probable levels (Dean et al., 2005) and IC neurons adapt their coding of sound location to stimulus statistics (Dahmen et al., 2010). Moreover preceding stimulation has been shown to sharpen frequency tuning (Wang et al., 2007) and to enhance detection of subsequent probes in IC neurons if masker is wideband and probes are narrowband (Nelson and Young, 2010). These adaptive coding mechanisms potentially facilitate signal detection in mixtures of sounds.

Converging inputs from lower and higher auditory centers produce differential response selectivities in inferior collicular neurons. Differential response selectivity of IC neurons leads to heterogeneity in the IC neuron population. Although redundant response patterns of the neural population can help to overcome stochastic responses of individual neurons (due to overrepresentation of the stimulus), overlapping representation of stimulus features has been shown to hamper stimulus discrimination (Nadal and Parga, 1994). Moreover, heterogeneity of receptive fields increases information encoded in population activity (fig. 1.3) (Fisher information; Shamir and Sompolinsky, 2006; Chelaru and Dragoi, 2008). Depending on the desired outcome, different encoding strategies may therefore be utilized at different levels of the brain. Accordingly, it is assumed that sensory systems move from redundant encoding in the periphery to an efficient, heterogeneous encoding in higher level processes (Atick, 1992). Evidence for this organization comes from other sensory modalities, e. g. taste (Rolls and Treves, 1990) and vision (Rolls and Tovee, 1995). For the auditory system, Holmstrom et al. (2010) reported heterogeneous receptive fields at the level of the IC and hypothesized that the IC plays a critical role in the efficient encoding of auditory information by facilitating the discrimination of behaviourally-relevant sounds. Across the population of IC neurons sound discrimination is facilitated by complex selectivity, which increasing heterogeneity. A recent study in the IC reports that heterogeneity of neuronal responses lead to a decrease in signal correlations over the IC population relative to lower auditory nuclei. This could increase the encoding efficiency for natural stimuli (Holmstrom et al., 2010).

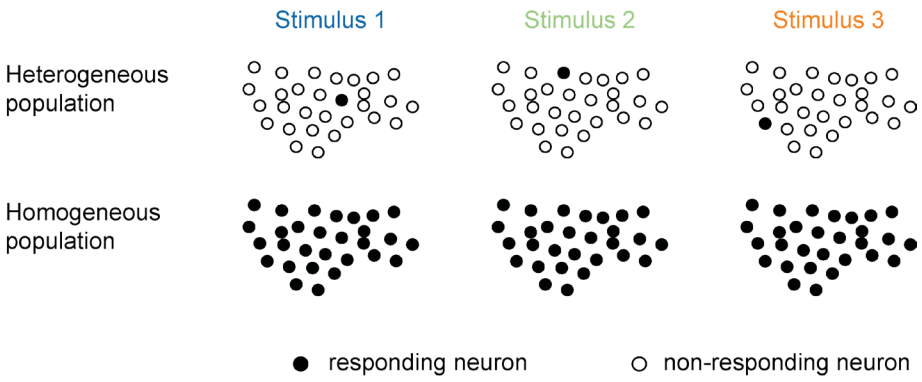

Figure 1.3 Heterogeneous and homogeneous populations.

In a heterogeneous population, neurons differ in their response selectivity. Stimuli evoke response in only one neuron, and each stimulus evokes response in a different neuron. Population activity consequently differs depending on the stimulus. In contrast, in a homogeneous population, neurons share common response properties. In this scheme, stimulus 1,2 and 3 evoke responses in all neurons. Population activity does not differ as a function of stimulus.

Age related alterations in auditory processing

Auditory peripheral sensitivity prominently declines with age; over 70 % of Germans above 60 years of age show significant increases in pure tone thresholds (Hesse, 2005). Moreover, a growing body of evidence suggests that age-related declines in central auditory processing occur in addition or independent of a decline in peripheral thresholds (Gordon-Salant and Fitzgibbons, 1993; Strouse et al., 1998; Gifford et al., 2007; Ross et al., 2007), making presbycusis one of the most common pathologies among the elderly.

Under laboratory conditions, severe declines in masked speech recognition with age have been reported (Frisina and Frisina, 1997). Moreover, in masked speech recognition tasks, aged participants show only negligible benefits in performance if signal to noise ratio is transiently improved (masker modulation; Gustafsson and Arlinger, 1994; Dubno et al., 2002). In contrast, adding masker modulation induces a pronounced increase in speech recognition for young adult subjects (Miller, 1947). Interestingly, the decline in modulation unmasking for aged subjects has been associated with increased forward masking (Gifford et al., 2007).

Because information in speech is conveyed primarily by temporal fluctuations of signal amplitude, it has been proposed that declines in performance of aged subjects in speech in noise tasks are caused by alterations in auditory temporal processing (Gordon-Salant and Fitzgibbons, 1993). Accordingly, for aged humans the duration of the minimal detectable gap in noise, forward masking thresholds and minimal resolvable angle in sound localization are larger (Snell, 1997; Strouse et al., 1998; Abel et al., 2000; Ross et al., 2007). Increases in the duration of the minimal detectable gap and of forward masking thresholds with age have also been reported for experimental animals (Barsz et al., 2002; Hamann et al., 2004; Gleich et al., 2007).

As explained in the previous chapters, there is mounting evidence that auditory temporal processing relies on a balanced and timed interplay of excitation and inhibition (Grothe, 1994; Covey and Casseday, 1999; Pecka et al., 2008). In line with the hypothesis that temporal processing is altered in aged animals, pre- and postsynaptic levels of inhibitory neurotransmitters and their respective receptors are significantly reduced in brain tissue from aged animals (Banay-Schwartz et al., 1989; Milbrandt et al., 1994; Milbrandt et al.,

1996; Willott et al., 1997; Milbrandt et al., 2000), and receptor subunit compositions are changed (Milbrandt et al., 1997; Krenning et al., 1998). These changes potentially disrupt the balance of excitation and inhibition in aged brain tissue. Furthermore, recent studies on the visual system showed orientation and direction selectivity of primary cortical neurons to progressively decay with age (Schmolesky et al., 2000). This decay correlates with downregulation of the inhibitory neurotransmitter system (Leventhal et al., 2003).

Selectivity to amplitude modulations is installed by a balanced and timed interplay of excitation and inhibition (Grothe, 1994). In accordance with decreased synaptic weight of inhibitory inputs, studies on IC responses to sinusoidal amplitude-modulated stimuli in the IC of aged rats (Shaddock Palombi et al., 2001) and mice (Walton et al., 2002) report altered tuning to AM parameters.

Aims of this study

In this study, I aimed to add to the understanding of age-related changes in temporal processing. I approached this problem (1) in psychophysical experiments involving young adult and aged human participants and (2) in electrophysiological recordings in an animal model (Mongolian gerbils, *meriones unguiculatus*). The gerbil is an ideal model for auditory research because, unlike mice and rats, it has excellent low frequency hearing and gerbil audiograms are similar to humans (Ryan, 1976).

The versatile temporal tuning patterns of auditory midbrain neurons make the IC a prime target to study age related declines in temporal processing. Given reduced levels of inhibitory neurotransmitters in the aged auditory system (Banay-Schwartz et al., 1989; Milbrandt et al., 1994; Milbrandt et al., 1996; Willott et al., 1997; Milbrandt et al., 2000; Burianova et al., 2009), IC response selectivity is likely to deteriorate in the aged auditory system. To evaluate sensitivity of IC neurons to temporal stimulus parameters in the aged relative to the young adult auditory system, I performed electrophysiological recordings from the IC of young adult and aged gerbils. In the first project (detailled in chapter III), I report on altered selectivity of IC neurons from aged animals to temporally modulated signals.

Altered temporal selectivity reported for IC neurons from aged animals (chapter III) can

be thought of as altered sensitivity to short-term stimulus context. With the second project (chapter IV), I aimed to evaluate consequences of altered context sensitivity on the processing of complex sounds, using a combined psychophysical and electrophysiological approach. Young adult and aged subjects were asked to discriminate words embedded in modulated masker. In this task, the most informative part of the words was adjusted to coincide with the masker modulation, such that the most informative part of the word occurred simultaneously with the quiet period in masker. Whereas young adult subjects benefited significantly from the transient fluctuation in the masking signal, the benefit was negligible for aged subjects. The single masker modulation allowed me to directly probe the effect of the preceding masking signal on the neuronal processing of the probe (the word). Recordings from gerbil IC revealed decreased response precision to the probe signal during masker modulation in aged animals. This suggests that altered context sensitivity in aged animals deteriorates temporal release from masking, which is probably a crucial property of the auditory system, when multiple sound sources are simultaneously active.

II

Materials and Methods

Age-related alterations in temporal processing were assessed in psychophysical experiments with young adult (21-28 years) and aged (58-65 years) human subjects. Neural correlates of age-related declines in auditory temporal processing were investigated in electrophysiological experiments with young adult (3 month) and aged Mongolian gerbils (3 years).

Acoustic stimuli

Psychophysics

Speech stimuli. To psychophysically assess temporal processing in the auditory system a set of 35 German words spoken by the same male German native speaker was used in a masked word discrimination task. Words were subdivided into groups of five rhyming words each. Words within rhyme groups differed solely in their first consonant (e.g. nag, mag, rag, jag, tag) (fig. 2.2 B). To avoid discrimination of words due to differences in fundamental frequency, fundamental frequency of all words was set to 100 Hz using STRAIGHT software (Kawahara et al., 1999). Speech stimuli were presented at 55 dB SPL.

Masker. Words were masked with a speech spectrum noise that was refreshed for each trial in a pseudo-random manner. Masker signals were presented at signal to noise ratios (SNRs) from -15 to 6 dB in 3 dB steps

Masker Modulation. Masker modulation consisted of a single short temporal gap and rise and fall times. Structure and duration of the masker modulation were chosen to match the most frequent modulation in Fastl noise. Fastl noise is a masker developed for speech audiometry that mimics the temporal and spectral characteristics of speech (Fastl, 1987) (fig. 2.2 A to C). Modulations in 60 s of Fastl noise were most frequently of 35 ± 5 ms duration at their faintest point (fig. 2.2 B). To obtain masker modulation structure, Fastl noise modulations of the most frequent duration were rectified and averaged (fig. 2.2 C). The average envelope was fitted with two 2-order polynomials. The resulting structure was 120 ms in duration and was used for masker modulation. The timing of the masker modulation was chosen to coincide with the first letter of each word.

Stimulus presentation. Acoustic stimuli were built in MatLab (MathWorks, Massachusetts) converted to analogue signals (RX6, Tucker Davis Technologies (TDT) System 3, Florida) and delivered to headphones (HDA 200, Sennheiser, Germany) by TDT System3 hardware. The input to the headphones was corrected to have a flat frequency response between 400 Hz and 12 kHz.

Electrophysiology

Tone stimuli. To acquire frequency response areas of single neurons, pure tone stimuli of 100 ms duration (rise-fall times were automatically adjusted to avoid spectral artifacts) at various frequencies and sound pressure levels were presented to the animals. Frequency range and sound pressure level were adjusted to cover the excitatory receptive fields of the neurons.

Pulse matrix. To obtain neuronal temporal receptive fields of single neurons, 49 sequences of broadband noise bursts gated with symmetrical trapezoid functions, with each sequences having constant width and repetition rate, were presented to the animals. Although sinusoidal amplitude-modulated stimuli (SAM) are commonly used to analyse neuronal temporal processing, I chose to infer indicators for temporal selectivity from rate responses to a matrix of noise burst sequences (pulse matrix, PM). The reason is that IC neurons are not exclusively sensitive to modulation frequency but are also sensitive to pulse duration, pause duration and rise time of the stimulus (Krebs et al., 2008). These parameters cannot be independently varied using SAM stimuli. In the PM, I

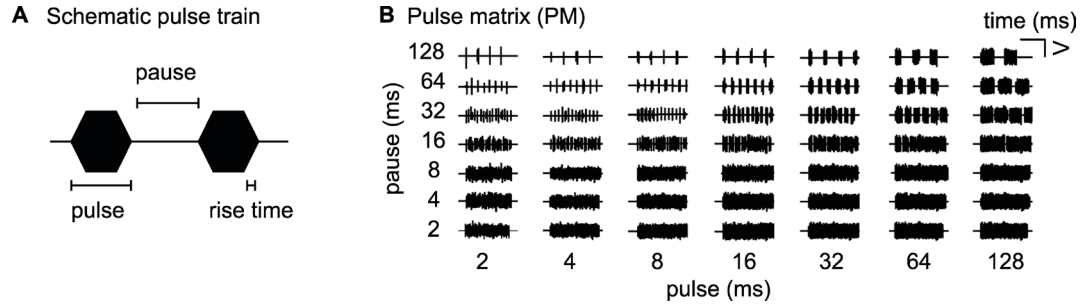

Figure 2.1 Pulse Matrix.

(A) Schematic of a pulse train consisting of two trapezoid pulses with 1 ms rise and fall times each. Pulse duration is defined as the entire duration of the pulse including rise- and fall-time; pause duration is defined as the entire silent interval between pulses. (B) Pulse matrix. The pulse matrix consists of 49 trains of trapezoid broadband noise pulses. Pulse and pause duration span from 2 ms to 128 ms in logarithmic intervals. Pulse trains are 512 ms to 640 ms long.

independently varied the duration of noise pulses and the duration of silent intervals between pulses (pause) in seven logarithmic steps from 2 to 128 ms, creating a matrix of 49 stimuli of different combinations of pause and pulse duration (fig. 2.1 B). Rise and fall times of trapezoidal noise pulses lasted 1 ms each (fig. 2.1 A).

Speech stimuli used in chapter III. To evaluate encoding efficiency of complex natural stimuli, eight snippets from German sentences taken from a clinical test (Oldenburger Satztest, sample sentences kindly provided by Dr. Birger Kollmeier) were used in chapter III. All words were spoken by the same male German native speaker. Speech stimuli were presented at 20 dB above noise threshold.

Speech stimuli and masking signals used in chapter IV. To stimulate IC neurons, a stimulus paradigm was used that was nearly the same as in psychophysical experiments (see above). The only changes were (1) to reduce the number of words in the electrophysiology paradigm to 25 per signal to noise ratio and masking condition (continuous or modulated) in order to limit recording time per cell, and (2) to adjust signal presentation level to 20 dB above single neuron threshold.

Stimulus presentation. Acoustic stimuli were built in MatLab converted to analogue signals (RX6) and delivered to loudspeakers (ER-2, Etymotic Research, Illinois) by TDT System3 hardware. The input to the electrostatic speakers and the headphones was corrected to have a flat frequency response between 400 Hz and 12 kHz. The output of

the loudspeakers was fed to the ear canal in a sealed system. Prior to each experiment, frequency response and energy in frequency bands was verified using calibrated probe tube microphones (ER10B+, Etymotic Research, Illinois) connected to the computer for analysis.

Experimental procedures

Psychophysical experiments

All psychophysical experiments were performed in a sound attenuated chamber using headphones (see above). Signal presentation was dichotic.

Temporal word discrimination threshold. In this task the most informative part of each of the 35 target words for the word discrimination task was identified psychophysically. Because the task required discriminating rhyming words that exclusively differed in their first letter, the first letter of each word was the most informative part for discrimination. To psychophysically identify the end of the first letter, 9 subjects were asked to adjust a slider to the time point at which the first letter of each word could no longer be perceived. After slider position was adjusted, the word, cut at the position of the slider, was played back to the subject. Subjects could then either approve or readjust slider position. This procedure was repeated 6 times for each word and each participant. Approved slider positions were collected and compared across participants (results for one example rhyme group are shown in fig. 2.2 D). To anchor masker modulation in the masked word discrimination task, slider positions were averaged over repetitions and subjects.

Word discrimination in quiet background. To control for possible differences in task handling and speech recognition abilities between young and aged participants, subjects were asked to identify each of the 35 words five times in quiet in a 5-alternative forced-choice task (5-AFC).

Word discrimination in noise. Target stimuli were presented embedded in masker signals with SNRs from -15 to 6 dB in 3 dB steps. Target level was maintained at 55 dB SPL throughout the experiment; masker level was varied in pseudo-random order. For each masker level, the first word was preceded by 10 s of masker to allow subjects to adapt to

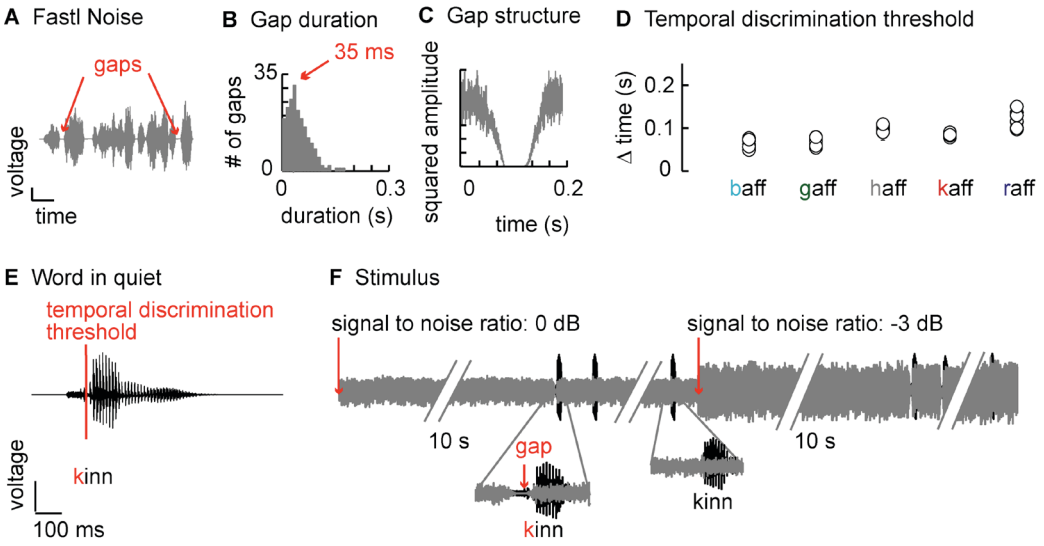

Figure 2.2 Schematic of word discrimination paradigm.

(A) Fastl Noise. A fluctuating noise that simulates the average spectral and temporal features of fluent speech (Fastl, 1987). (B) Most frequent gap duration in Fastl Noise. Gap duration was measured by a threshold criterion. (C) Mean envelope of gaps of 35 ± 5 ms duration. Mean envelope was fitted with 2 polynomials and was used to envelope masker modulation. (D) Perceived end of consonant for rhyme group 7. Circles represents mean perceived end of first consonant ± standard deviation of one subject. (E) Stimulus waveform at 0 dB and -3 dB SNR. Word discrimination sequence is preceded by 10 s of masker to allow subjects to adapt to masker level. Each word is presented twice during each SNR, once embedded in continuous and once embedded in modulated masker. Depiction of stimulus is interrupted by for space constraints.

the new masker presentation level (fig. 2.2 E). During each session every word was presented twice, once with a modulated masker and once with a continuous masker, in each masker level condition (fig. 2.2 E, insets). After each word, subjects were asked to choose the perceived word from the five words from the respective rhyme group displayed on a touch-screen. Subject responses were analysed off line.

Participants. Temporal word discrimination threshold was measured based on responses from nine young subjects (23 to 28 years). Data for this test was collected in six sessions à 45 min. Word discrimination performance was measured based on responses from eleven young (21 to 26 years) and ten aged subjects (58 to 65 years). Data for the word discrimination task was collected in nine sessions à 1 h. Subjects were paid an hourly rate for their efforts.

Electrophysiological Experiments

Animals. Action potentials in response to auditory stimuli were recorded in vivo from single IC neurons of 29 young adult (ca. 3 month) and 20 aged (ca. 3 years) Mongolian gerbils of both sexes. All experiments were approved by the German Tierschutzgesetz (Regierung Oberbayern; 55.2-1-54-2531-57-05).

Anesthesia. To induce anesthesia, a ketamine/xylazine mixture was applied. Ketamine is a dissociative anesthetic and combines analgesic and hypnotic effects; it acts on N-methyl-D-aspertate (NMDA) receptors for glutamate, the main excitatory neurotransmitter in the central nervous system (Anis et al., 1983; Thomson et al., 1985). Xylazine has sedative, analgesic and muscle relaxant properties. It acts on α_2-adrenergic receptors in the nervous system (Docherty and Starke, 1982).

Surgical procedures. The surgical procedures applied in this study were described in detail previously (Siveke et al., 2006) and were essentially the same for young adult and aged gerbils. Briefly, gerbils were anesthetized by an initial intraperitoneal injection (0.5 ml per 100 g body weight) of a physiological NaCl solution (Ringer solution) containing ketamine in 20 % v/v and xylazine in 2 % v/v concentration. During surgery a dose of 0.05 ml of the ketamine/xylazine mixture was applied subcutaneously every 30 min. During recording animals were injected continuously with anesthesia via an automatic pump (801 Syringe Pump, Univentor, Spain) at a pump rate of 1.7 to 3 µl per min, depending on body weight. Animal body temperature was monitored, and if necessary adjusted using a thermostatically controlled heating pad. Prior to recording, skin and tissue covering the upper part of the skull were carefully removed. A small metal rod was mounted on the frontal part of the skull in order to secure the head of the animal in a custom-made stereotactic device during recordings (Schuller et al., 1986).

After surgery, the animal was transferred into a sound attenuating chamber and the animals head was fixed in the stereotactic device. A craniotomy and a durotomy were performed at 1.3 to 2.6 mm lateral to the midline and 0.5 to 0.8 mm caudal to the bregmoid axis. After recording the animal was euthanized by injection of 1 ml of 20 mg/ml Pentobarbital in Ringer solution. The position of the electrode was marked by a lesion induced by a sinusoidal current of 20 kHz and 10 µA for 90 s. The brain was removed from the head and was fixed in 4 % paraformaldehyde for two days. The brain

was then transferred to 30 % sucrose and was stored at 4 °C for 2 days. Subsequently, the brain was placed in tissue-freezing medium, frozen solid, and cut in a standard plane for sections. Transverse 45 μm sections were cut in a cryostat at -21 °C. The sections were then Nissl-stained, and recording sites were verified via light microscopy.

Neural Recordings. Tungsten electrodes, of 3 to 10 MΩ impedance, were mounted in a seven electrode system (Thomas Recordings, Germany). A custom made glass tube with an inner diameter of ~300 μm at the tip was attached to the shaft probe of the multi-electrode to reduce the inter-electrode distances to approximately 80 to 300 μm. After craniotomy and durotomy, the multielectrode array was introduced in the brain at 85° to 90° angles relative to brain surface and electrodes were advanced to the central IC, starting approximately 2 mm below the brain surface. Electrodes were moved individually to isolate signals from single units. Action potentials were conventionally amplified, converted to digital signals (RX5, TDT) and fed to a computer running Brainware (Jan Schnupp, University of Oxford UK, for TDT). For each cell, spike waveforms were identified initially during the experiment and were verified off-line by spike-sorting analysis (MClust, free-ware spike sorting by David Redish, available at redishlab.neuroscience.umn.edu/MClust/MClust.html).

General recording protocol. Animals were presented with white noise bursts of 100 ms duration with 5 ms linear rise and fall times at repetition rates of 4 Hz, while electrodes were advanced through the IC. When a single unit was encountered, stimulation was switched to pure tone stimuli of 100 ms duration (rise-fall time adjusted to avoid spectral artifacts) at various frequencies and sound pressure levels. From the acquired frequency response areas best frequency (BF, frequency the neuron was most sensitive to), threshold (lowest sound pressure level to elicit neuronal discharge or to suppress spontaneous activity) and temporal response types (described in the results section) were determined. Neurons were clustered into temporal response type classes based on the temporal pattern of their response to a pure tone at BF 20 dB above threshold.

Recording protocol chapter III. 95 aged and 92 young adult neurons were recorded during presentation with the pulse matrix (PM, described above). The PM was presented to the animal at 20 dB above noise threshold.

In order to classify neurons in relation to their response pattern to the PM stimuli, cells were stimulated with 9 repetitions of 250 ms trapezoid broadband frozen noise bursts at 20 dB above noise threshold and sorted according to their temporal response pattern (described below and in chapter III) off-line. Repetition rate of noise pulses was 1.3 Hz.

57 young adult and 47 aged IC neurons were additionally stimulated with eight speech snippets taken from a clinical test at 20 dB above noise threshold. From these recordings the first 125 ms were used for analysis. This time window corresponds to the first one to two letters from each of the eight snippets (specifically: Br, Ta, St, Ni, K, Pe, Ul, Do).

Presentation order of stimuli was randomized (interleaved). Responses to ≥ 9 repetitions of each stimulus were recorded.

Recording protocol chapter IV. 74 neurons from young adult and 72 neurons from aged animals were recorded during presentation of five repetitions of 25 of the words used in the psychophysical test at 20 dB above pure tone threshold, (1) in unmasked condition and (2) in masked condition. In the masked condition the continuous masker was (as in the psychophysical task) randomly either modulated or continuous during word presentation (fig. 2.2 E). Each word was presented five times in random order for each masking condition and each SNR from -9 dB to 9 dB in 3 dB steps. As in the psychophysical task, after switching masker levels, neurons were allowed to adapt to masker level for 10 s before the first word was presented. Neural responses were analysed off-line.

Data analysis and statistics

Psychophysics

Psychometric functions. Psychometric functions were calculated based on mean performance of each subject under each condition over 5 repetitions (1 repetition per day). Each set of mean data points for SNRs from -15 to 6 dB was fitted with a sigmoid function for each subject.

$$y(x) = \frac{a}{e^{-\lambda(x-b)}} + c$$

Formula 1. Sigmoid function.

For a, b, c and λ initial values were defined and these values were optimized using the MatLab build-in "*fminsearch*" function.

Modulation unmasking. To compare modulation unmasking in young and aged subjects, difference in classification success between modulated and continuous conditions was calculated for each trial and each subject. Effect of masker modulation on classification success was compared using multivariant analysis of variance (MANOVA).

Electrophysiology

Temporal response types. Response type classes were sparse, onset, primary-like, sustained, late sustained (including long-latency sustained and built-up neurons) and offset (including offset and inhibitory neurons) (described in the result section, table 1 and fig. 3.1). Response types were identified from responses to ten repetitions of pure tones at best frequency and to nine repetitions of 250 ms frozen noise bursts. Classification was performed using a MatLab function dependent on the onset component (first 50 ms), the ongoing component of the response (50 ms for tones and 200 ms for noise) and activity after sound offset (20 ms after sound offset to 150 ms after sound offset for tones and 20 ms to 500 ms after sound offset for noise). For post-stimulus-time histograms (PSTH), spikes were binned in 5 ms bins.

The MatLab built-in bootstrapping ("*bootstrp"*) function was used to obtain significance values for differences observed in the distributions of temporal response patterns between neurons from young adult and aged animals. This routine drew 5000 sub-samples from the actual samples of neurons. From the distribution of sub-samples of neurons from young adult animals obtained from *bootstrp*, the probability to obtain the observed value from the aged distribution by chance was calculated for each temporal response type.

PM receptive fields. PM receptive fields are defined as the relation between the temporal frequency of action potentials evoked during stimulus presentation, and pulse and pause duration of the stimulus pulse train.

Temporal selectivity index. An index of temporal selectivity (TSI) was calculated from PM receptive fields as follows:

$$\mathrm{TSI} = \frac{\max(\mathrm{rPM}) - \min(\mathrm{rPM})}{\max(\mathrm{rPM})}.$$

Formula 2. Temporal selectivity index.

rPM is the discharge rate evoked by the PM. The index assumes a value of one if the cell's discharge rate is modulated to zero in response to any pulse train in the PM (and the neurons response is therefore perfectly selective); and assumes a value of zero if the cell's discharge rate is constant over the PM space (and the neurons response is therefore unselective).

Pearson correlation coefficient. As a measure of similarity, Pearson correlation coefficients (r) were calculated between PM receptive fields of every possible pair of cells.

$$r_{ij} = \frac{E\left(E\left(x_i - Ex_i\right) \cdot \left(x_j - Ex_j\right)\right)}{\sqrt{E\left(\left(x_i - Ex_i\right) \cdot \left(x_i - Ex_i\right)\right) \cdot E\left(\left(x_j - Ex_j\right) \cdot \left(x_j - Ex_j\right)\right)}}$$

Formula 3. Pearson correlation coefficient.

E signifies the expected value or mean. x_i and x_j signify cell1 and cell2 in a pair of cells.

Principal component analysis. Principal components (PCs), and eigenvalues of the covariance matrix (the variance explained) were computed based on PM receptive fields of single neurons. The mean of each PM receptive fields was subtracted prior to analysis. To compare influence of PCs on individual PM receptive fields, distributions of weights (the coordinates for the representation of each individual PM receptive field in PC space) were analysed first separately for PC one, two and three. Secondly, to illustrate the change in shape of PM receptive fields between young adult and aged animals, neurons were clustered based on their coordinates in three dimensional PC space, using the MatLab built-in *"kmeans"* clustering algorithm. The algorithm yielded robust clusters using Euclidean distances: for each neuron the mean distance to neurons within its assigned cluster was much smaller than the distances to neurons assigned to different clusters. Separation of cluster centroids was tested using MANOVA.

Spike distance metric. Trains of action potentials in response to human speech were analysed in terms of dissimilarity. To achieve this, a decoding paradigm was employed. To decode a response evoked by one trial of one stimulus from the set it was removed from the set and a distance metric was employed to infer the stimulus that evoked it. The metric allows computation of the average distance from the picked response to all of the responses evoked by a given stimulus, except itself. The picked response was then classified as evoked by the stimulus for which the average distance to the picked response was minimal. Instances of correct classification of responses to a certain stimulus were counted and divided by the number of stimulus presentations to obtain classification success.

Different metrics have been proposed to compute the distance between responses (Victor and Purpura, 1996; van Rossum, 2001). In this study, distances between responses were calculated based on the metric proposed by Victor and colleagues (Victor and Purpura, 1996; software available at http://neuroanalysis.org/toolkit). The metric measures the distance between two responses as the overall cost of the set of operations required to transform one response into the other. Possible operations include the insertion of a spike, the deletion of a spike, and the time-shift of a spike. The metric can therefore be used to evaluate the distance between responses at different time scales.

Details of the implementation of the metric can be found in Victor and Purpura (1996) and Aronov (2003).

Decoding assumptions chapter III. In chapter III distances between spike trains were calculated based on spike counts.

To decode speech snippets from populations of neurons, distances were calculated for each cell separately and distance matrices were then summed over cells prior to decoding.

To quantify heterogeneity of encoding patterns, (1) for each neuron the percent correct classifications achieved when decoding of the set of eight speech snippets was based on single neuron responses, (2) for every possible pair of neurons (within each population) the percent correct classifications achieved when decoding was based on responses from a pair of neurons was computed. The increase in percent correct classification of speech snippets that paired encoding caused for each neuron (the difference in percent correct classification of speech snippets between classification based on responses from one neuron, and classification based on responses from two neurons) was then measured.

Decoding assumptions chapter IV. In this chapter, a 10 ms resolution was chosen to evaluate distances between trains of action potentials. This temporal resolution yielded the highest classification success for neurons from both young adult and aged animals (data not shown). To evaluate encoding of speech in spike trains across masker levels, spike trains obtained in response to masked words were compared to responses obtained during word presentation in quiet background. To quantify differences between modulated and continuous masking conditions, word discrimination performance was compared using a bootstrapping procedure. For this purpose trains of action potentials obtained during presentation of modulated and continuous maskers were randomized, and the probability to observe by chance a difference in classification success equal or larger than the difference between classification success of words masked by a modulated and by a continuous masker was calculated. Unmasking was assumed significant if $p < 0.01$.

Discharge in response to masker. Discharge in response to masker was measured during an 80 ms window between word presentations (for illustration fig. 4.4 A).

Discharge in response to target. Discharge in response to target was measured during the time frame of the silent gap in masker modulation in all masker conditions: (1) quiet, (2) modulated and (3) continuous.

Precision of response and latency of first spike. To obtain estimates of temporal precision and reliability of neuronal responses, a method similar to the one introduced by Berry and coworkers (1997) was employed. The method was first introduced for the visual system and is described in detail elsewhere (Berry et al., 1997; Berry and Meister, 1998). Briefly, because sensory neurons generally show multiple peaks in their PSTH and temporal parameters of firing during these events may differ within cells, neuronal response trains were divided into sequences of multiple firing events. Temporal parameters of each firing event were then calculated separately. To obtain a consistent demarcation of firing events, boundaries of firing events were drawn at minima (v) in the PSTH. Minima were required to be significantly lower than neighboring maxima (p_1 and p_2), such that $\sqrt{p_1 \cdot p_2} / v \geq 0.1$. Prior to minima identification, each PSTH was smoothed with a Gaussian filter of width σ, equal to the time scale of modulation in the respective discharge rate. Time scale of modulation in discharge rate was determined from the shuffled autocorrelation function. Discharge precision measures within firing events were based on absolute spike times, they were therefore not sensitive to the choice of smoothing (Berry et al., 1997).

Temporal precision of firing events is defined to be the standard deviation of the timing of the first spike in each firing event. Response precision or **entrainment** over firing events is defined to be

$$et = \frac{\mathrm{var}\left(s_i - Es_i\right)}{Es_i}.$$

Formula 4. Entrainment.

E signifies expected value or mean, s_i signifies number of action potentials per repetition.

Latency of the first spike is defined to be the mean time of the first spike during the temporal gap in the masker modulation.

III

Impaired Temporal Selectivity in the Inferior Colliculus of Aged Mongolian Gerbils

In this chapter, in vivo extracellular recordings from young adult (3 ± 1 month) and aged (39 ± 4 month) gerbils were performed to identify a neural correlate of deteriorated temporal processing in the aged auditory system. Using temporally modulated stimuli, neuronal responses of neurons from young adult and aged animals were compared in terms of selectivity and sensitivity of discharge rate to temporal stimulus parameters and in terms of dynamic range of discharge rate modulation over the stimulus space. Complex natural stimuli were employed to estimate consequences of altered temporal selectivity and sensitivity for encoding of complex signals in neuronal populations. This work represents the first electrophysiological evidence of an age-related decline in neuronal temporal selectivity and in population coding efficiency in response to complex natural sounds.

Basic neuronal response characteristics in the inferior colliculus

In order to compare the neural representation of the temporal parameters of the auditory stimulus IC extracellular action potentials from 133 IC neurons from 15 young adult and 112 IC neurons from 11 aged Mongolian gerbils were recorded. Prior to analysis of the representation of temporal stimulus parameters in these cells, neurons from young adult and aged animals were characterized and compared in terms of best frequency (BF), single neuron thresholds, temporal discharge patterns evoked by acoustic stimulation with pure tones and broadband noise, and spontaneous activity.

Best frequency and threshold distributions

BFs of cells from young adult and aged animals ranged from approximately 2 to 12 kHz (The upper frequency limit was due to the sound system and does not reflect the upper limit of audible frequencies for gerbils). Neurons with BFs above 6 kHz seemed slightly overrepresented in the population of neurons from aged animals. However, the difference in median of both populations was not statistically significant (Wilcoxon rank sum test; p=0.1).

Single neuron thresholds were significantly elevated in the population of older compared to younger animals (median young adult 53 dB SPL; median aged 64 dB SPL; $p=1\cdot10^{-5}$ Wilcoxon rank-sum test). To test whether, this severely altered frequency tuning, the width of frequency tuning functions 10 dB above threshold (Q_{10dB}) was measured. Q_{10dB} values did not differ significantly between young adult and aged animals (Wilcoxon rank sum test p=0.2), allowing for the following direct comparison of the neuronal responses.

Temporal response patterns and spontaneous activity

Spontaneous activity was measured over 7 s for 89 neurons in young adult and 103 neurons in aged animals. 61 % (55/89) of young adult neurons and 52 % (53/103) of aged neurons showed spontaneous discharge rates ranging from 0.1 to 40 Hz. Distributions of spontaneous activity did not differ significantly between aged and young adult animals (Wilcoxon rank sum test p=0.7).

To obtain a characterization of the distribution of temporal discharge patterns in populations of neurons from young adult and from aged animals, neurons were sorted into eight classes based on response patterns to (1) a 100 ms pure tone at BF 20 dB above pure tone threshold and (2) a 250 ms broadband noise pulse 20 dB above noise threshold. Neurons were clustered based on their onset component (first 50 ms of stimulation), ongoing component (remaining time of stimulation, 50 ms for tones and 200 ms for noise) and offset component (the first 20 to 150 ms after stimulus offset for tones and 20 to 500 ms for noise; classification scheme and representative neurons from every class are shown in table 1 and fig. 3.1). Separate classes were defined for primary-like and sustained neurons that showed spontaneous activity (sub and plb), because the ratio of driven rate to spontaneous activity influenced responses to the pulse matrix (see fig. 3.6).

Populations of neurons from both age groups showed similar distribution of temporal discharge patterns in response to pure tones (differences were not statistically significant; bootstrapping, explained in Material and Methods and table 2). In response to stimulation with 250 ms broadband noise pulses, distributions of temporal response patterns differed between populations (table 2). In response to noise pulses (1) 10 % (9/87) of the young adult but only 2 % (2/94) of the aged population were not driven or only sparsely driven (table 2; $p=1\cdot10^{-3}$ bootstrapping). (2) The number of primary-like responders with and without spontaneous activity increased from 24 % (21/87) of young adult to 39 % (37/94) of aged neurons (table 2; $p=6\cdot10^{-4}$ bootstrapping). There was no obvious relation between the temporal spike pattern of a neuron in response to pure tones and its temporal spike pattern in response to broadband noise.

Most spontaneously active neurons recovered spontaneous activity during the 500 ms silent interval between noise burst repetitions (24/29 neurons in young adult animals;

39/46 neurons in aged animals). In most spontaneously active neurons from young adult animals that recovered spontaneous activity during the silent interval, after stimulus offset a dip in firing rate below spontaneous levels that persisted for around 150 ms was observed. The exceptions to this rule were six of the seven offset responders and neurons with very low spontaneous activity (< 0.5 Hz). In contrast, in aged animals, beside all offset neurons, eight primary-like neurons discharged within 150 ms after stimulus offset (fig. 3.1 B, F and G) with discharge rate > 3 Hz. This change in discharge behavior following noise pulses may influence processing of temporal sequences of information e.g. the detection of temporal gaps, forward masking and AM processing.

Table 1. Temporal response patterns: classification scheme.

Temporal response pattern	Response components		
	Onset (first 50 ms of stim.)	**ongoing (remaining stim. time*)**	**Offset (from stim. offset**)**
Sparse	< 2 Hz		≤ mean(on,ongoing)
Onset	> 2.5 Hz	< 1.5 Hz	not specified***
Primary-like	> 1.5·ongoing	> 1.5 Hz	0 Hz
Sustained	> 2 Hz & ≥ 1.5·ongoing	> 2 Hz & ≤ 2·onset	0 Hz
Primary-like spontaneous	> 1.5·ongoing	> 1.5 Hz & > offset+std(offset)	> 0 Hz
Sustained spontaneous	> 2 Hz & ≥ 1.5·ongoing	> 2 Hz & ≤ 2·onset	> 0 Hz
Long-latency/built-up	not specified	> 2·onset	not specified***
Offset/inhibitory	< offset – std(offset) or no activity		> 0 Hz

* remaining stimulus time: 60 to 110 ms for pure tone stimulation and 60 to 260 ms for noise stimulation.

** from stimulus offset: activity from 120 ms to 250 ms from stimulus onset for pure tone (pulse duration 100 ms) and 270 to 750 ms for noise (pulse duration 250 ms) stimulation.

*** onset and late sustained neurons were not subdivided into spontaneously not active and active cells because only 2/7 onset cells and 2/5 late sustained cells in young; and 1/5 onset cells and 5/10 late sustained neurons in aged animals showed spontaneous activity.

Table 2. Distribution of temporal response patterns.

		sparse	**onset**	**primary-like**	**sus-tained**	**primary-like spont.**	**sustained spont.**	**late sustained**	**offset**
tones	**young (n=95)**	1 %	10 %	17 %	39 %	5 %	17 %	10 %	1 %
	aged (n=77)	0 %	10 %	15 %	31 %	5 %	24 %	14 %	0 %
noise	**young (n=87)**	10 %	8 %	14 %	21 %	10 %	24 %	6 %	7 %
	aged (n=94)	2 %	5 %	21 %	19 %	18 %	21 %	8 %	5 %

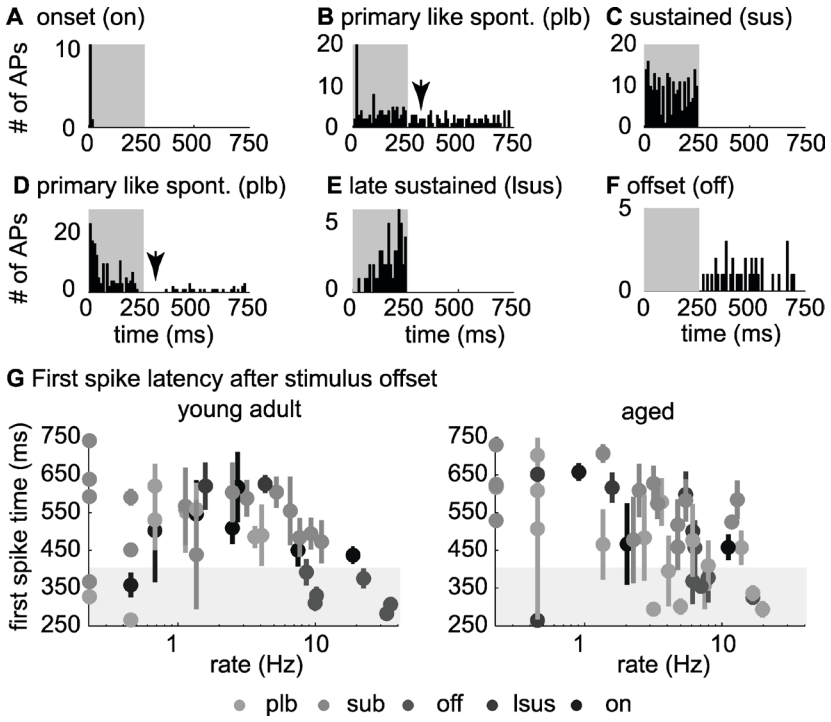

Figure 3.1 Types of neuronal response patterns evoked by broadband noise.

(A-F) show PSTHs (bin width 5 ms) of selected neurons from six temporal response types in response to 250 ms broadband noise pulses. Gray shaded areas indicate the duration of the stimulus. All neurons were recorded from aged animals. (A) Onset neuron. (B) Spontaneously active primary-like neuron. Note that this neuron (in contrast to the neuron presented in D) does not show a dip in discharge after stimulus offset (arrows in B and D). This behavior was exclusively observed for aged neurons. (C) Sustained neuron. (D) Spontaneously active primary-like neuron. Note that this neuron (in contrast B) shows a dip in discharge after stimulus offset (arrows). (E) Late sustained/build-up neuron. (F) Offset/inhibitory neuron. For details on temporal response types see text and table 1.

(G) Absolute time of first action potential after stimulus offset (mean over repetitions ± std) versus rate of discharge between stimuli for spontaneously active neurons. Response types of neurons are coded in colours: Onset (on), primary-like (plb), sustained (sub), late sustained (lsus), offset (off), sparse (spa) cells that showed spontaneous activity. Gray shaded areas: first 150 ms after stimulus offset.

Neuronal responses to pulse train stimulation

Next, neuronal selectivity to temporal modulations of stimulus amplitude was evaluated. I recorded from 95 IC neurons from aged and 92 IC neurons from young adult animals, while stimulating the contralateral ear with a matrix of trapezoid noise pulses of varying pulse and pause duration (pulse matrix, PM; explained in Materials and Methods and fig. 2.1). Because there is evidence that the temporal code for AM parameters in the auditory brainstem is, by and large, converted to a rate code in the IC (Langner and Schreiner, 1988; Langner, 1992; Frisina, 2001; Joris et al., 2004), analysis was focused on discharge rate.

In figure 3.2, response patterns (raster plots) and PM receptive fields of two example neurons from young adult (fig. 3.2 A ,C and E) and two example neurons from aged (fig. 3.2 B, D and F) animals are presented. The two neurons shown in figure 3.2 A, C (young adult neuron) and B, D (aged neuron) were offset responders. Both neurons were driven to their highest discharge rates by pulse trains composed of long pause and short pulse durations. The neuron from the young adult animal was not (fig. 3.2 A and C), but the aged neuron was weakly (fig. 3.2 B and D) driven by pulse trains composed of short pulse and short pause durations. In figure 3.2 E (young adult) and F (aged) two sustained neurons are presented. Both neurons showed spontaneous activity. However, the cell from the young adult group ceased discharging after sound offset for 0.17 ± 0.02 s and then recovered spontaneous activity, whereas the cell from the aged group did not cease to discharge after sound offset but immediately recovered spontaneous activity. The young adult neuron was driven maximally by pulse trains composed of long pulse and short pause durations or short pulse and long pause durations (fig. 3.2 E). This cell did not discharge when stimulated with sounds of short to medium pulses in combination with pauses of medium length. In contrast, the neuron from an aged animal was driven by every single pulse train in the PM. Only small modulations in discharge rate are apparent from this neuron's PM receptive field (fig. 3.2 F). In this set of examples, neurons recorded from young adult animals responded to only a subset of temporal modulations from the PM. In contrast, neurons recorded from aged animals responded to every pulse train they were presented with. This suggests that neurons from aged animals might be less selective to pulse-pause combinations than neurons from young adult animals.

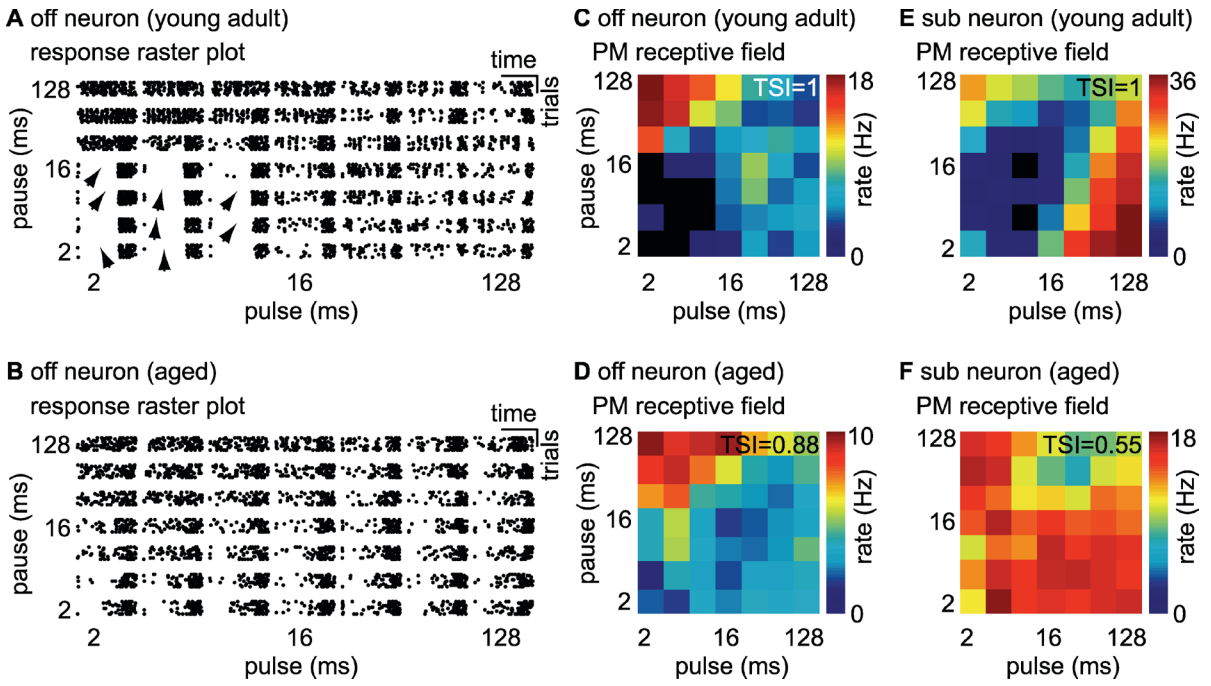

Figure 3.2 Single neuron responses to pulse matrix.

(A) Raster plot of an offset neuron from a young adult animal. Stimuli that suppressed response are indicated by arrows. (B) Raster plot of an offset neuron (off) from an aged animal. Discharge of this neuron was not suppressed by any stimulus in the set. (C) 3-D rate function (PM receptive field) of the offset neuron presented in A (young adult). Each square in the surface plot represents the average rate over 9 repetitions of the pulse train. Discharge rate is colour coded (black: no discharge; dark red: maximum discharge). Selectivity index (TSI) in the right upper corner gives the difference between maximum and minimum discharge rate divided by the maximum discharge rate of the neuron in response to the pulse matrix. (D) PM receptive field for the offset neuron presented in B (aged). (E) PM receptive field for a sustained spontaneous (sub) neuron from a young adult animal. (F) PM receptive field for a sustained spontaneous (sub) neuron from an aged animal (TSI < 1).

Neuronal temporal selectivity to sound pulse and pause duration

To test whether the observed decrease in selectivity to temporal modulations of neurons from aged animals was stable over the population (1) the relative range of discharge rate modulation (using a temporal selectivity index), and (2) the width of PM receptive fields was compared across populations. The temporal selectivity index TSI was defined as the difference between the cell-specific maximum in discharge rate evoked by the PM and the minimum discharge rate evoked by the PM, divided by the maximum discharge rate. The index consequently assumes a value of one if the cell's discharge rate is modulated to zero in response to any pulse train in the PM (and the neuron's response is therefore

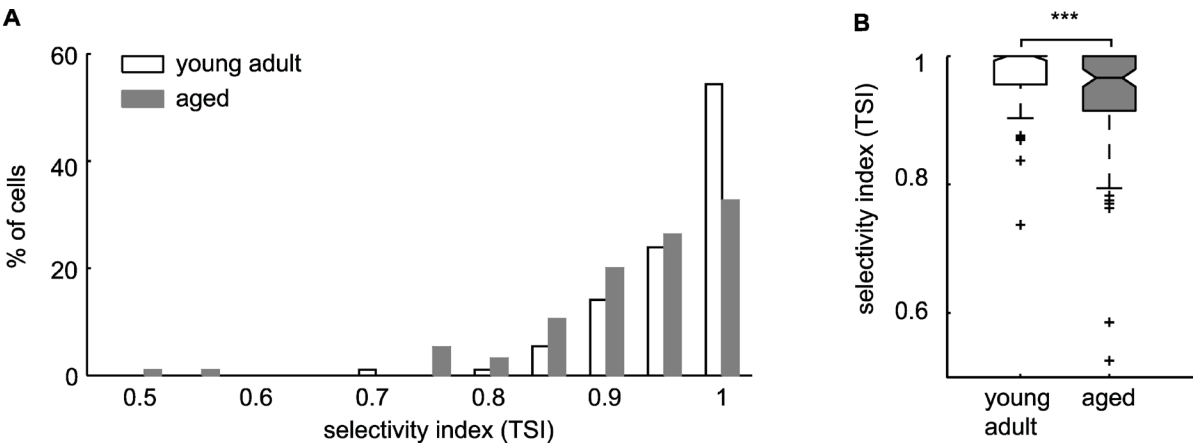

Figure 3.3 Decrease in temporal selectivity in aged animals.

(A) Distribution of selectivity indices (TSIs) of neurons from young adult (white) and aged (gray) animals. More neurons from young adult animals were unresponsive to a subset of pulse trains (TSI = 1) and more neurons from aged animals responded with > 6 % (TSI < 0.94) of their maximum discharge rate to every pulse train from the pulse matrix. (B) Box plot of selectivity indices. Temporal selectivity was significantly lower for aged neurons (Wilcoxon rank sum test p = 8·10-4).

highly selective); and assumes a value of zero if the cell's discharge rate is constant over the PM space (and the neuron's response is therefore unselective). The young adult cells displayed in figure 3.2 C and E modulated their discharge to zero in response to a subset of pulse trains and their TSIs are therefore 1. In contrast, discharge rates of aged cells shown in figure 3.2 D and F were modulated to at most 22 % and 45 % of their respective maxima in driven rate, resulting in TSIs of 0.88 and 0.55, respectively.

Indeed, the distribution of selectivity indices for the entire population of neurons from aged animals was significantly shifted towards lower values relative to selectivity indices of neurons from young adult animals (fig. 3.3 A and B; Wilcoxon rank-sum test $p= 8{\cdot}10^{-4}$). The observed shift in distributions in aged animals resulted (1) from a decreased number of highly selective cells (31/95 neurons in aged vs. 50/92 neurons in young adult animals with TSI=1) and (2) from an increase in the number of cells that responded with 6 % of their maximum discharge rate to least favorable pulse trains (cells with a TSI < 0.94). This difference was evident for cells of all temporal response patterns. However, it was particularly salient for cells that showed primary-like responses while presented with broadband noise and were spontaneously active, and for offset neurons.

Although the functional and computational role of the IC is not yet understood, it is

widely accepted that many IC cells exhibit non-linearities in their response profiles (e. g. non-monotonic rate level functions, SAM band-pass tuning). The selective suppression of responses to a subset of pulse trains observed here represents such a non-linearity, which will be referred to as “suppression”. Suppression in this context does not necessarily mean suppression as a consequence of direct inhibition but as a consequence of computations at some level that might have involved inhibition.

Having observed a decreased number of highly selective cells in aged animals, I was prompted to ask whether pulse trains that were suppressive to the remaining highly selective cells in aged animals were consistent in their temporal parameters to pulse trains that were suppressive to highly selective cells in young adult animals. In order to test this, I computed the frequency with which responses to each pulse train were suppressed in the aged and in the young adult population. In neurons from both populations, pulse trains that were composed of long pulses and short pauses were more likely to elicit a response than pulse trains composed of short durations and long pauses (fig. 3.4 A and B). However, when the probability distributions for the two populations were subtracted, it became apparent that long pulse durations (> 16 ms) were even less suppressive to aged neurons than to young adult neurons (fig. 3.4 C). Pulse trains composed of short durations (< 16 ms) and short pauses (< 8 ms) were also more likely to be suppressive to young adult compared to aged neurons. Pulse trains composed of short pulses (< 16 ms) and long pauses (> 4 ms) were more likely to be suppressive in neurons in aged animals (Wilcoxon ranksum test $p=0.04$). Taken together, trains of long pulses independent of pause duration and trains of short pulses and short pauses were less likely to be suppressive to neurons from aged animals than to neurons from young adult animals. Furthermore, although overall fewer neurons in aged animals were suppressed by any pulse train from the PM, more aged neurons than young adult neurons were suppressed by pulse trains with short pulse and long pause durations. It is interesting to note that while the set of pulse trains that is more likely suppressive to neurons from young animals comprises pulse trains that feed sound energy into the system over a long period of time, the set of pulse trains that is more likely suppressive to neurons from aged animals feeds energy only transiently. The loss of complete suppression in aged animals is therefore highly dependent on the temporal dynamics of the stimulus.

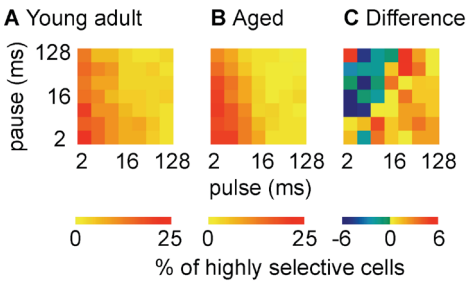

Figure 3.4 Suppressive effect of pulse trains on neurons from young adult and aged animals.

(A) Probability of each pulse train from the pulse matrix to be suppressive neurons from young adult animals. Each square in the surface plot depicts the percentage of cells the pulse train of the indicated pulse and pause duration was suppressive to. Percentage is colour coded. (B) Probability of each pulse train from the pulse matrix to be suppressive to neurons from aged animals. (C) Difference between A and B. Pulse trains of short pulse duration and long pause duration were more likely to suppress discharges in aged animals (Wilcoxon rank sum test p=0.04).

Besides a decrease of highly selective neurons in aged animals, an increase of neurons that responded with a minimum rate to the PM that was > 6 % of the maximum discharge rate to the PM was observed. Since TSI measures the relative dynamic range of discharge rates across all pulse trains from the PM, it is possible that aged neurons compensate for the loss of selectivity by increasing their maximum discharge rate and thereby maintain their absolute dynamic range of discharge rates. However, maximum discharge rates were invariant, while minimum discharge rates were significantly elevated in aged animals (Wilcoxon rank sum test p= 0.046). Furthermore, if maximum and minimum discharge rates are considered in relation to TSI, a significant increase in maximum and minimum discharge rates with decreasing TSI in young adult animals is evident (maximum: r=-0.5, $p=5\cdot10^{-7}$; minimum r=-0.9, $p=1.8\cdot10^{-29}$; fig. 3.5 A), whereas, for neurons from aged animals, maximum discharge rates were invariant over TSI (r=0.05, p=0.6; fig. 3.5 A) and minimum rates increased with decreasing TSI (r=-0.8, $p=1\cdot10^{-22}$; fig. 3.5 A). Although there was no change in absolute dynamic range of discharge rate between young adult and aged animals, the relation of absolute dynamic range of discharge rate and TSI was significantly altered: young adult neurons with low TSI modulated their discharge rate over a larger absolute dynamic range in response to the PM than aged neurons with low TSI (fig. 3.5 B).

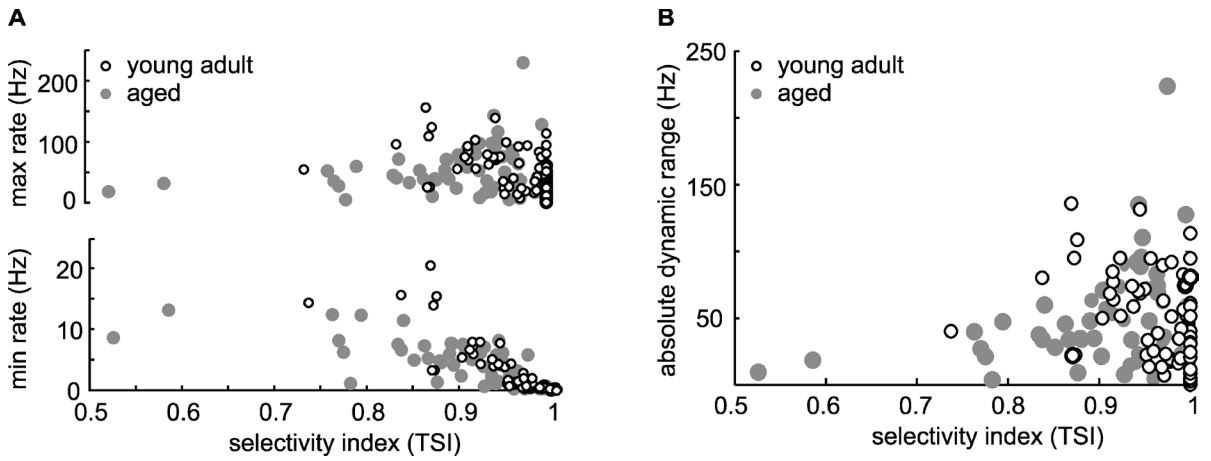

Figure 3.5 Decreased dynamic range of discharge to pulse matrix in aged neurons with low selectivity indices.

(A) Maximum (upper panel) and minimum (lower panel) discharge rates in response to pulse matrix (PM) in relation to the selectivity index (TSI). Maximum and minimum discharge rates in response to the PM increased with decreasing TSI for neurons from young adult animals (maximum: r=-0.5, $p=5\cdot10^{-7}$; minimum r=-0.9, $p=1.8\cdot10^{-29}$), but maximum discharge rate remained constant and minimum discharge rate increased with decreasing TSI in aged animals (maximum: r=0.05, p=0.6; minimum: r=-0.8, $p=1\cdot10^{-22}$). (B) Absolute dynamic range of discharge rate in response to the PM for neurons from young adult (white) and neurons from aged (gray) animals. Neurons with low TSI from aged animals showed smaller dynamic ranges than neurons with low TSI from young adult animals (Correlation between TSI and absolute dynamic range for young adult animals: r=-0.43, $p=2\cdot10^{-5}$ and for aged animals: r=0.03, p=0.7).

The observation that minimum discharge rate to the PM increased but maximum discharge rate was invariant in aged relative to young adult animals and that cells with low TSI modulated their discharge rate over a smaller absolute dynamic range in response to the PM suggests that PM receptive fields are less sharply tuned in aged than in young adult animals. Response rate functions to amplitude modulations are classically analysed using a threshold criterion (Langner and Schreiner, 1988). Type and width of receptive fields for each neuron are then defined based on AM-parameters that elicit a discharge rate that exceeds the chosen threshold. If a threshold criterion of up to 10 % was chosen, a significant increase in receptive field width in the population from aged animals relative to the young adult population was observed (Wilcoxon rank sum test p=0.04). As for the suppressive pulse trains, I asked whether pulse trains that elicited a discharge that exceeded threshold were comparable for both populations. For each response type, I calculated the probability that a certain pulse train would drive a cell from this group to a discharge that exceeded threshold. As can be inferred from the resulting 2-D histograms (fig. 3.6), tuning is largely consistent within response types (dark red areas). Neurons from

aged and from young adult animals shared preferences for similar pulse trains. However, because the relative range of discharge rates was compressed in aged animals, neurons from all response types showed less selectivity to pulse trains from the PM. Note that spontaneous activity was not subtracted for this measure of selectivity. Subtracting spontaneous activity would mask the effect of the decrease in pulse train selectivity, because effects of pulse train stimulation on spontaneous activity differed between young adult and aged animals. While 30 % of neurons from the young adult population were significantly suppressed below spontaneous activity (14/48 neurons suppressed overall 346/2352 pulse trains below spontaneous activity) in response to pulse train stimulation, only 10 % of neurons from aged animals suppressed significantly below spontaneous activity (8/87 neurons suppressed 128/4214 pulse trains below spontaneous activity). Moreover, young adult neurons suppressed their responses to significantly lower discharge rates below spontaneous activity (Wilcoxon ranksum test $p= 2 \cdot 10^{-6}$). The increase in receptive field width was particularly pronounced for primary-like and sustained neurons that showed spontaneous activity (plb and sub fig. 3.6). From the group of spontaneously active neurons, neurons that discharged shortly (within 150 ms) after stimulus offset (fig 3.1 B and G) had particularly wide receptive fields (for five of seven neurons, discharge rate in response to every pulse train from the PM exceeded threshold).

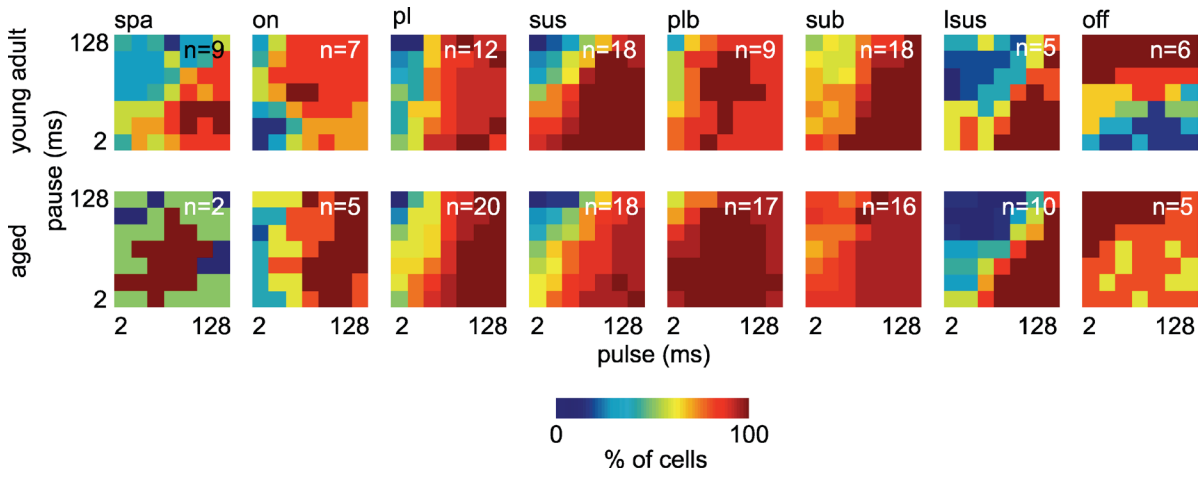

Figure 3.6 Increased width of PM receptive fields in aged animals.

Neurons were grouped according to neuronal response type: spa (sparse); on (onset); sus (sustained); pl (primary-like spontaneous); sub (sustained spontaneous); plb (primary-like spontaneous); lsus (late sustained/built-up); off (offset/inhibitory). Surface plots of 2-D histograms summarize numbers of neurons of each response type that responded with a rate > 10 % of their maximum discharge to the indicated pulse trains. The upper panel shows neurons from young adult, the lower panel neurons from aged animals. Dark blue signifies that the corresponding pulse train was very unlikely to elicit a response > 10 % of maximum discharge, dark red that the corresponding pulse train elicited a response > 10 % of maximum discharge in all neurons tested. Note that dark red areas are particularly large for plb, sub and off neurons from aged animals. Over all neurons recorded, the number of pulse trains per neuron that elicit a discharge rate >10 % of the maximum discharge rate was significantly higher in aged animals (Wilcoxon rank sum test $p = 0.04$).

Preference in discharge for sound pulse and pause duration

In the preceding paragraphs, I have shown that neurons from aged animals hardly modulate their responses significantly below spontaneous activity and that the number and pattern of pulse trains that are suppressive to IC neurons differs between young adult and aged animals. These changes in temporal processing not only imply a decrease in temporal selectivity, but also a change in preference for temporal parameters (which corresponds to a change in shape of receptive fields) in neurons from aged animals. In order to test whether receptive field shape was altered in aged relative to young adult animals, principal component analysis (PCA) on both PM receptive field populations was performed. PCA performs linear transformations on multidimensional data, generating a new set of variables, called *principal components* (PCs). Because all the PCs are orthogonal to each other (there is no redundant information) the set of PCs explains the

variance in the data ideally with fewer variables than the original data. When analysing PM receptive fields of neurons from young adult and aged animals separately, I found that for sustained neurons (sus) from aged animals 90 % of the variance was explained by the first and only 10 % by the second PC (fig. 3.7 A). In contrast, for sustained neurons from young adult animals, 50 % of the variance of PM receptive fields was explained by the first, 30 % by the second and 10 % by the third PC (fig. 3.7 A). The difference in variance explained indicates that PM receptive fields of sustained neurons from young adult animals may be more variable in shape than those from aged animals. In order to test this, PCA was performed on the joint population of PM receptive fields from young adult and aged animals. As with the young adult and aged animals separately, for the joint population of PM receptive fields, the most influential component represented a shift in discharge rate for long pulse and short pause durations, the second PC represented a scaling of the preference for pulse trains of short pulse and short pause durations and the third PC represented a shift towards short to medium pulse and long pause durations (fig. 3.7 B left panel). The representation of the original PM receptive fields in principal component space is reflected in the neuron specific weights for each PC. As expected, weights for PC three were significantly more variable in young adult than in aged animals (fig. 3.7 B right panel; Ansari-Bradley test $p=0.02$). To understand the influence of PC one, two and three on individual PM receptive fields, neurons were sorted into clusters based on their representations in PC space (detailled in Materials and Methods). Neurons formed three robust clusters: the mean distance of each neuron to the centroid of its assigned cluster (0.04 ± 0.007) was two orders of magnitude smaller than the mean distance between centroids of different clusters (1.4 ± 0.4) and cluster centroids were significantly different (MANOVA $p=1 \cdot 10^{-7}$). The first cluster consisted of neurons showing a preference for short pulse and short pause durations and, in addition, a duty cycle preference (fig. 3.7 C left upper panel). The second cluster consisted of neurons showing a strong duty cycle preference (fig. 3.7 C right upper panel). This cluster was mostly dominated by neurons from aged animals (13/18 neurons from aged and 8/19 neurons from young adult animals). The third cluster consisted of neurons showing a strong preference for short pulse and short pause durations (fig. 3.7 C left lower panel). This cluster was clearly dominated by neurons from young adult animals (5/19 neurons from young adult and 1/18 neurons from aged animals). Taken together, a clear

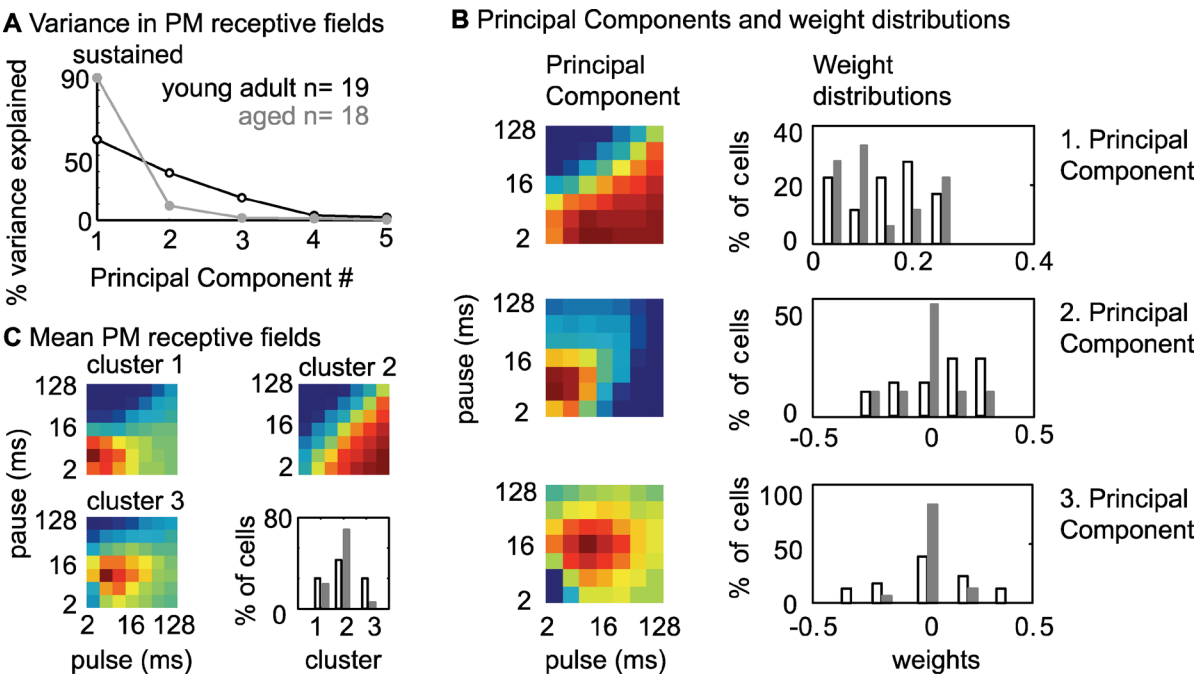

Figure 3.7 Strong duty cycle preference in PM receptive fields of sustained neurons from aged animals.

(A) Variance in PM receptive fields of sustained neurons from young adult and aged animals explained by principal components (PCs). PCs were computed from PM receptive fields of young adult and aged animals separately. (B) First, second and third PC of PM receptive fields of sustained neurons from young adult and aged animals. In all the 3-D-Plots, dark red depicts strong responses, dark blue weak responses. Weights for the third PC tend to cluster around zero for aged but are spread to positive and negative values for young adult animals (Ansari-Bradley test p=0.02) (C) Mean PM receptive fields of neurons from young adult and aged animals. Neurons were clustered based on their representation in three dimensional principal component space (weights). Note that cluster two comprises neurons that showed a strong preference in discharge for short duty cycles and was mostly dominated by neurons from aged animals. Cluster three comprised neurons that showed a clear preference for pulse trains with short to medium pulse and pause durations. This cluster was dominated by sustained neurons from young adult animals.

preference for short pulse and pause durations seemed to be lost in sustained neurons from aged animals. Instead 72 % of neurons showed wide receptive fields with a preference for pulse trains with short duty cycles. This corresponds to a change in shape of PM receptive fields in favour of pulse trains with long pulse and short pause durations and therefore an unselective response for a neuron of sustained response type.

Neurons from other response type classes did not show significant changes in PM receptive field variety when analysed with PCA.

Similarity of temporal receptive fields

Over the populations of IC neurons from young adult and aged animals, a decrease in temporal selectivity and a decrease in variety of shape of temporal receptive fields should amount to an increase in similarity of temporal receptive fields within the population of IC neurons from aged animals. In order to quantitatively analyse the similarity of PM receptive fields, PM receptive fields of every possible pair of cells within both populations were correlated. If PM receptive fields of a pair of neurons congruently vary with temporal parameters, correlation of this pair of neurons is strong and positive (correlation around 1); if, on the other hand, PM receptive fields of a pair of neurons vary opposite to each other, correlation of this pair of neurons is strong and negative (correlation around -1). In figure 3.8 A, a pair of neurons from young adult animals is presented (fig. 3.8 A left panel: lsus and fig. 3.8 A right panel: off) whose PM receptive fields ran nearly opposite to each other (r=-0.76). Distributions of pair wise correlation coefficients (r) of all neurons from young adult and from aged animals are plotted in figure 3.8 B. Compared to the distribution of r of pairs of neurons from young adult animals, the distribution of r of neurons from aged animals showed a trend to more positive values. In particular, strong and medium negative correlations were diminished and weak and strong positive correlations were increased in the aged population of cells (Wilcoxon ranksum test $p=9\cdot10^{-8}$). This indicates an increased similarity of aged neurons' responses to the PM.

Taken together, an increase in signal correlations (and therefore similarity) in response to the PM was observed in the sample of neurons from aged animals. This increase in signal correlations was due to a reduced number of sparse responders and an increased similarity of PM receptive fields in terms of PM receptive field shape and width of sustained responders, primary-like responders and off responders both within and across response types. The population of aged neurons was, in other words, less heterogeneous in its preference for temporal modulations than the sample of young adult neurons.

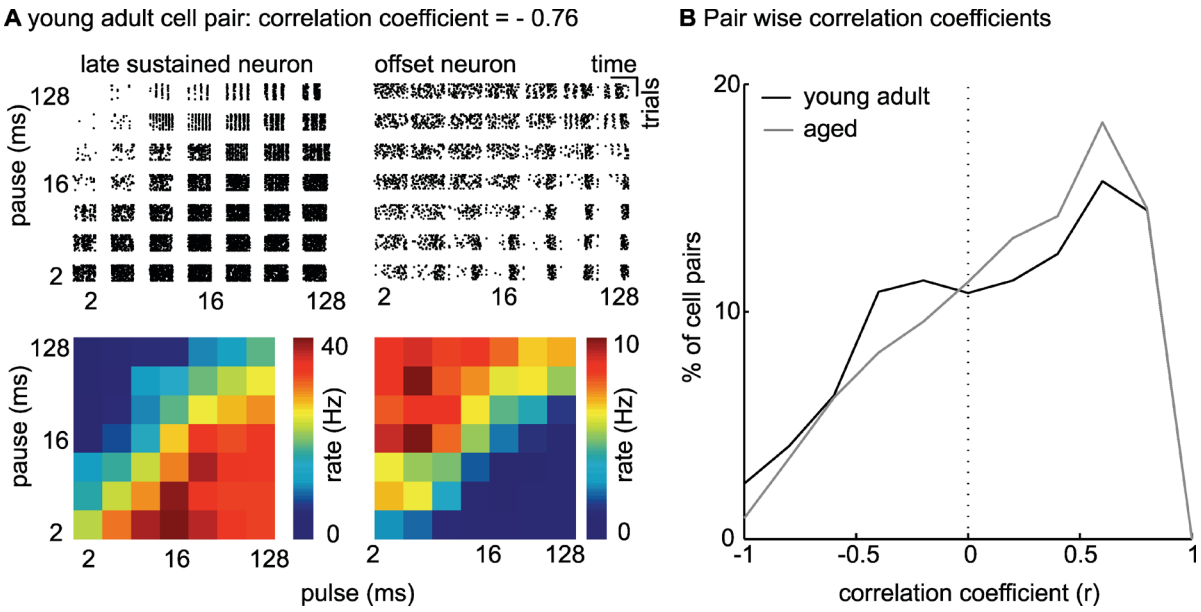

Figure 3.8 Increase in strong positive correlations between pairs of neurons from aged animals.

(A) Raster plots and PM receptive fields of two example neurons from a young adult animal. Neuronal response types were late sustained (left panel) and offset (right panel). The sustained neuron was well driven by pulse trains of long pulse and short pause durations, whereas the offset neuron was hardly driven by these stimuli. Receptive fields of these two neurons were therefore of nearly opposite directions (correlation coefficient r=-0.76). (B) Probability distribution of r of all possible pairs of neurons from young adult (black) and aged (gray) animals. Significantly less pairs of neurons from aged animals show high negative correlations (Wilcoxon rank sum test p=1.8·10-4).

Encoding of speech

The temporal parameters that varied across pulse trains in the PM were pulse and pause duration. Pulse and pause duration, along with rise/fall times and the dependent parameters duty cycle and modulation frequency, define amplitude modulations of natural communication signals. I assume that if receptive fields for sound pulse and pause duration are less heterogeneous across neurons from aged animals, this set of neurons will be less efficient in representing complex natural AM-signals like those occurring in speech. In order to test this young adult and aged animals were presented with eight different German speech snippets taken from a clinical test, while recording single unit responses from 57 neurons from young adult and 47 neurons from aged animals. For analysis the first 125 ms were cut from neuronal responses to these sentences (fig. 3.9 A). To quantified how well speech snippets can be separated based on rate responses of single neurons, the set of speech snippets was decoded in terms of dissimilarity of rate responses using a spike train distance metric (Victor and Purpura, 1996). The set of eight speech snippets was decoded equally well from single neuron responses from young adult and aged animals (distributions of percent correct did not differ significantly between young adult and aged animals; Wilcoxon rank-sum test $p=0.9$). Based on the observation that neurons from aged animals were less selective to temporally modulated sounds and therefore heterogeneity of responses was reduced, I hypothesized that, although single neurons from aged animals encoded this set of eight speech snippets reliably, joint encoding was less efficient for aged than for young adult animals. To test this, (1) for each neuron the percent correct classification of speech snippets based on the single neuron response, and (2) for each possible pair of neurons (within each population) the percent correct classification based on paired single neuron responses was calculated (fig. 3.9 B). The increase in percent correct classification of speech snippets (benefit) was then measured based on the paired response, relative to the percent correct classification of speech snippets based on separate responses (arrows, fig. 3.9 B). If benefits of joint encoding were compared across populations it became apparent that, for young adult animals, the benefit of taking a second cell into account was significantly greater than for aged animals (Wilcoxon rank sum test $p=7\cdot10^{-13}$; fig. 3.9 C). This indicates that although rate responses of neurons from aged animals to this set of

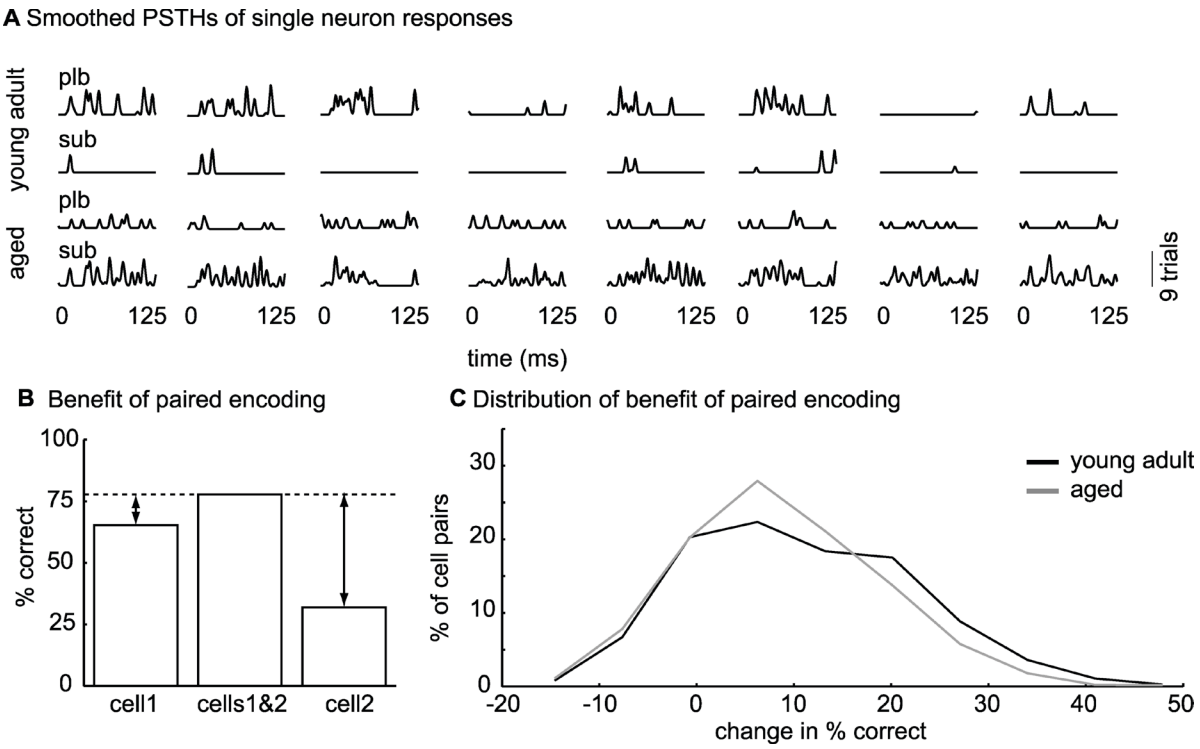

Figure 3.9 Decrease in benefit of joined encoding of speech for pairs of neurons from aged animals.

(A) Smoothed PSTHs of neuronal responses to 125 ms speech snippets from one primary-like spontaneous (plb) and one sustained spontaneous (sub) neuron from a young adult animal (upper two panels) and from an aged animal (lower two panels), respectively. (B) Neuronal responses to eight speech snippets were decoded based on dissimilarity of rate responses using a distance metric. Benefit of pair wise encoding was defined as the difference between classification success of every possible pair of neuron (cell1&2) and each neuron separately (cell1 and cell2). Arrows indicate benefit of paired encoding. (C) Probability distribution of benefit of paired encoding for neurons from young adult (black) and aged (gray) animals. Benefit of paired encoding was significantly larger for neurons from young adult than for neurons from aged animals (Wilcoxon rank sum test p=7·10-13).

speech snippets were still dissimilar enough to decode the speech snippets correctly, neurons from aged animals responded to more similar temporal features, which significantly decreased the benefit of joint encoding based on discharge rate.

Taken together, data presented in this chapter provide evidence for a significantly altered representation of temporal parameters in the IC of aged gerbils. IC neurons in aged animals showed significantly reduced selectivity and variety in tuning to the duration of sound pulses and pauses. The reduction in selectivity and variety led on the population level to a decrease in heterogeneity of temporal receptive fields and resulted in inefficient encoding of natural stimuli.

IV

Declined Temporal Acuity in the Aged Auditory System of Humans and Mongolian Gerbils

Altered selectivity to sequences of sound pulses indicates altered sensitivity to the temporal context of the stimulus in aged animals. Ongoing stimulation is therefore likely to differentially affect neurons in the young adult and aged auditory system.

To investigate consequences of altered context sensitivity on the processing of complex sounds, modulation unmasking was probed (1) psychophysically and (2) electrophysiologically. (1) 11 young adult (21 to 26 years) and 10 elderly (58 to 65 years) participants performed a word discrimination task. Words were masked either by a continuous or by a temporally modulated masker. (2) During extracellular recordings from the IC, the stimulus used in psychophysical experiments was presented to 14 young adult (3.5 ± 0.5 month) and 9 aged (41 ± 4 month) anesthetized gerbils.

Modulation unmasking of speech in young and aged human subjects

To investigate processing of complex acoustic signals during masker modulation in the aged auditory system, performance from young adult and elderly humans in a word discrimination task was compared.

First, to ensure that speech understanding and task handling abilities were similar in both age groups, word discrimination performance in quiet was tested in a 5-AFC paradigm. In quiet background there was no difference in word discrimination performance between age groups; young adult and aged participants discriminated words with equal classification success in quiet (fig. 4.1 B; ttest $p >> 0.05$).

When a masker was added, word discrimination performance declined for all subjects with decreasing signal to noise ratio (SNR) (fig. 4.1 A). For young adult participants, masker modulation yielded a significant increase in word discrimination performance over a wide range of signal to noise ratios, as evident from psychometric functions (fig. 4.1 A left panels). Although some aged subjects also benefited from masker modulation over some signal to noise ratios (fig. 4.1 right panels), release from masking was significantly smaller for aged subjects (fig. 4.1 B; MANOVA $*p < 0.05$). Thus, aged subjects, when under continuous acoustic stimulation, are unable to efficiently use information presented during transient level fluctuations in the masking signal. These results are in line with results from previous studies reporting age-related declines in modulation unmasking of speech signals (Gustafsson and Arlinger, 1994; Dubno et al., 2002; Gifford et al., 2007).

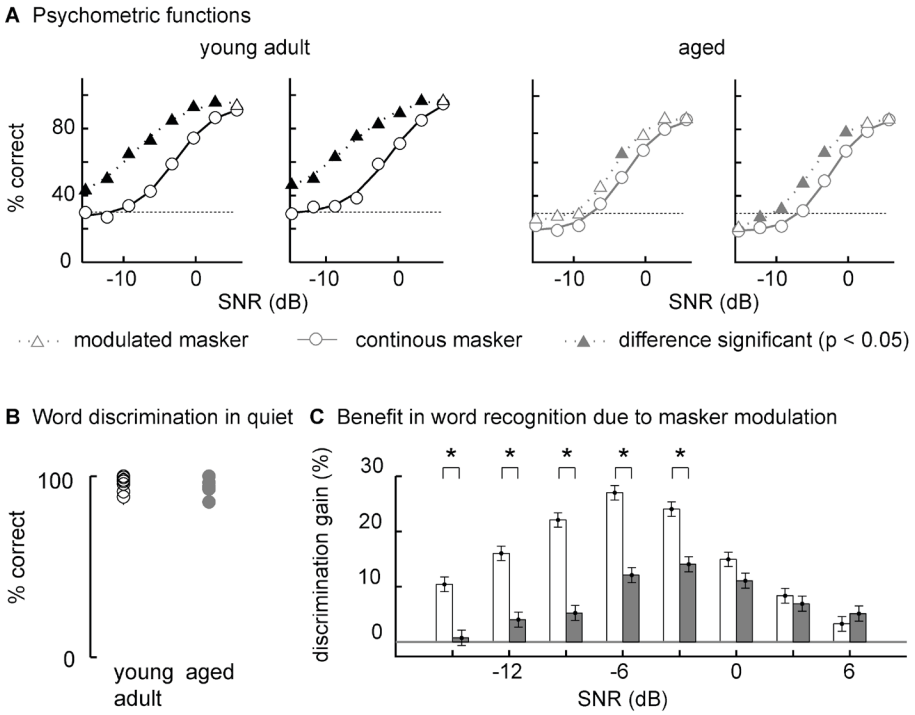

Figure 4.1 Modulation unmasking in young adult and aged human subjects.

(A) Example psychometric functions from two young adult and two aged subjects. Percent correct identified words are plotted vs. SNR. Solid lines represent word discrimination performance in continuous masker, dashed lines word discrimination in modulated masker. Benefit in word discrimination performance due to masker modulation is larger in young adult than in aged subjects. (B) Word discrimination performance for young adult (n=11) and aged (n=10) subjects in quiet background. Word discrimination performance did not differ between age groups (ttest $p >> 0.5$). (C) Modulation unmasking is significantly larger for young adult than for aged subjects for SNRs from -3 to -15 dB (MANOVA $*p < 0.05$). Bars represent mean difference between discrimination performance in modulated masker and discrimination performance in continuous masker ± standard error in young adult (white) and aged humans (gray).

Modulation unmasking in single neurons from the inferior colliculus

The decline in modulation unmasking observed psychophysically in humans has been proposed to be due to a decrease in the temporal acuity of the aged auditory system (Gifford et al., 2009). An age-related decline in temporal processing has also been reported psychophysically and electrophysiologically in the auditory midbrain of aged Mongolian gerbils (Barsz et al., 2002; Hamann et al., 2004; Gleich et al., 2007; Khouri et al., 2011). To investigate if the deficit in modulation unmasking of natural signals is represented in the aged auditory midbrain, extracellular recordings from the IC of young adult and aged anesthetized gerbils during monaural presentation with the word discrimination paradigm were performed. Responses to the word discrimination paradigm in quiet and at least one SNR were recorded from 74 neurons from young adult and 72 neurons from aged animals. The majority of neurons recorded from gerbil IC expressed reliable responses to certain features of the speech stimulus and a subset of

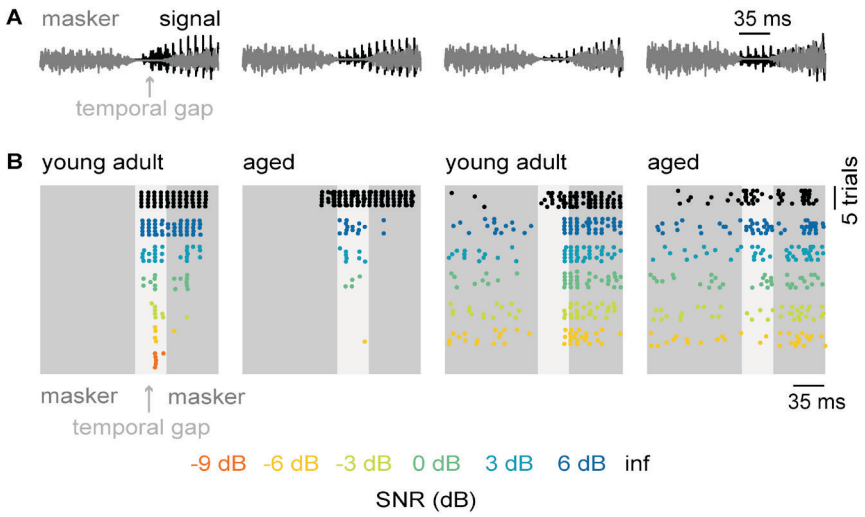

Figure 4.2 IC neuronal responses to speech in modulated masker.

(A) Stimulation waveform that evoked responses shown in (B). Position of stimulus waveforms and raster plots are vertically aligned. (B) Response raster plots of two neurons from young adult and two neurons from aged animals to words in modulated masker at different SNRs. SNRs are colour coded. Dark gray fields represent masker, light gray bars represent gap in modulated masker. Note that responses from young adult animals are more precise and tend to have larger response latencies during masker modulation.

neurons partly preserved this pattern over SNRs (fig 4.2). In figure 4.2 two neurons from aged and two neurons from young adult animals are presented. Neurons in the two left panels do not respond to masker, response to masker of neurons shown in the two right panels increase in vigor with increasing masker level.

In order to quantify similarity of neuronal temporal response patterns to speech signals across masker levels, a spike train distance metric was employed (Victor and Purpura, 1996). Based on this metric, words were decoded from neuronal response patterns, with classification success well above chance level in quiet background (63/74 in young adult and 61/72 in aged animals) and classification success of words in quiet did not differ between age groups (ttest $p >> 0.05$). If words were masked with a continuous or modulated masker, the majority of IC neurons from young adult and aged animals preserved their temporal response pattern to words in quiet (at 6 dB SNR: 41/63 neurons from young adult and 49/61 cells from aged animals classified above chance level in either modulated or continuous masking condition). For 19 % of these neurons from young adult and 10 % of these neurons from aged animals, decoding of words was more successful if the masker was modulated than if the masker was continuous (modulation unmasking neurons fig. 4.3 A; 8/41 in young and 5/49 in old; bootstrapping $p < 0.01$). For 2/41 neurons from young adult and 3/49 neurons from aged animals, masker modulation caused a deterioration of response to words relative to response to words in quiet (bootstrapping $p < 0.01$). For the remaining fraction of neurons, masker modulation neither improved nor diminished classification success relative to continuous masker (23/41 in young adult and 32/49 in aged neurons). Strikingly, masked word discrimination was highest if words were decoded from modulation unmasking neurons and masked word discrimination was lowest if words were decoded based on responses of neurons that showed no unmasking (MANOVA $p < 0.05$; fig. 4.3 B). Taken together, a subset of IC neurons encoded words more reliably if the masking signal was modulated than if the masking signal was continuous. Moreover, neuronal word discrimination performance was highest if decoding of words was based on responses of neurons from this modulation unmasking group. Based on the assumption that the behavioral performance reflects discrimination of neurons having the greatest acuity (Skottun et al., 2001) and behavioral performance is similar for humans and gerbils, these data may represent a

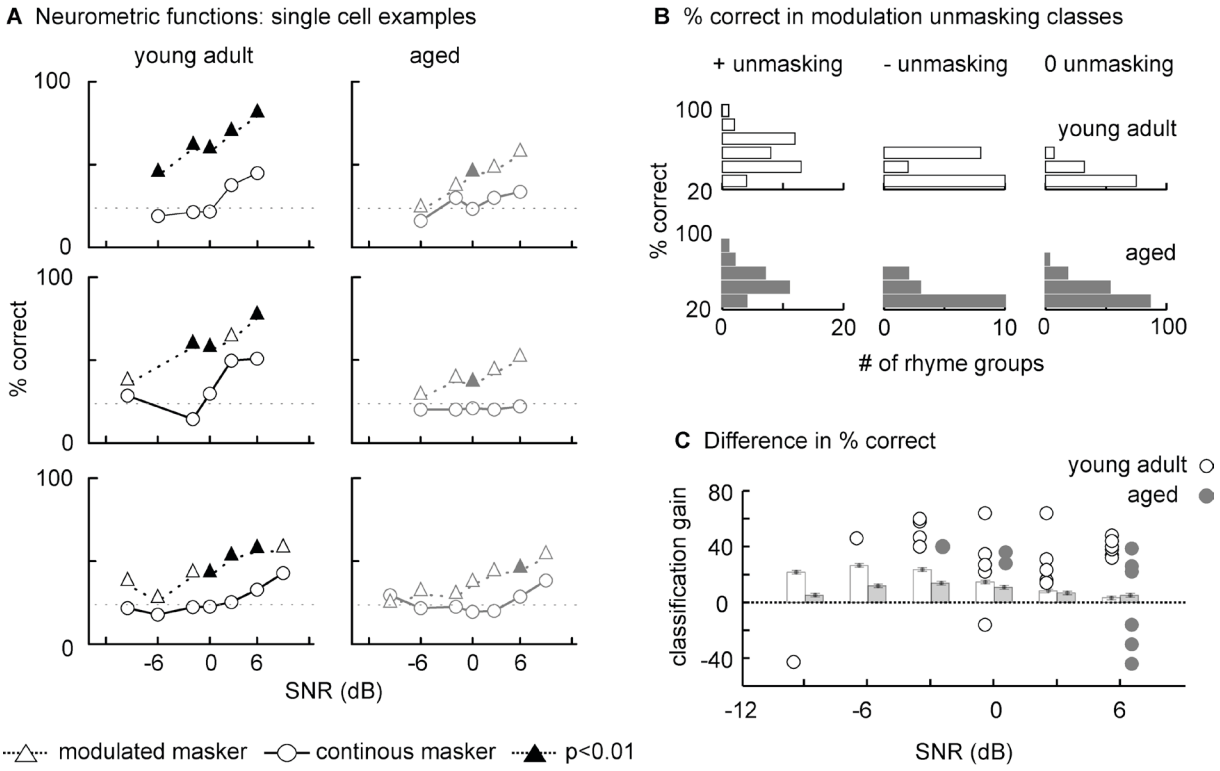

Figure 4.3 Modulation unmasking in single IC neurons.

(A) Neurometric functions for three neurons from young adult and aged animals. Significant unmasking is indicated by filled symbols (bootstrapping $p < 0.01$). (B) Distribution of percent correct classification success as a function of modulation unmasking. For neurons from young adult and aged animals, classification success of words was larger if decoded from modulation unmasking neurons (+ unmasking; left panel) than in neurons that did not show unmasking (0 unmasking; right panel; MANOVA $p < 0.01$). (C) Modulation unmasking. Each circle represents difference in classification success of words between modulated and continuous masking condition for modulation unmasking neurons (+/-) from young adult (white) and aged (gray) (bootstrapping $p < 0.01$). Masker modulation was larger in neurons from young adult than in neurons from aged animals. Bars represent release from masking obtained from human psychophysics (see fig. 4.1 C).

neural correlate of modulation unmasking observed in psychophysics.

To quantify modulation unmasking as a function of age, I compared the benefit of masker modulation in modulation unmasking neurons from young adult and aged animals. In line with the psychophysical experiments, modulation unmasking was larger in neurons from young adult animals than in neurons from aged animals (fig. 4.3 A and C).

Neuronal response precision during masker modulation

Having found a possible neural correlate for this specific form of modulation unmasking, I was prompted to ask whether I could identify parameters of neuronal responses that correlated with modulation unmasking and that deteriorated with age.

I analysed (1) discharge rate in response to masker and (2) discharge rate in response to words during temporal gap in masker modulation (analysis windows are exemplified in figure 4.4 A). In general, mean discharge rate in response to masker increased with increasing masker level. Discharge in response to masker was stronger in modulation unmasking neurons from young adult than in modulation unmasking neurons from aged animals (fig. 4.4 B; filled circles, $p < 0.05$ ttest). Discharges in response to words during temporal gap decreased with increasing masker level. Again, this decrease was smaller in modulation unmasking neurons from young adult than in modulation unmasking neurons from aged animals (fig. 4.4 C; filled circles, $p < 0.05$ ttest).

To evaluate response precision during temporal gap in masker modulation, (1) latency of first spike, (2) temporal precision and (3) entrainment of action potentials were analysed. Independent of modulation unmasking behavior and age, response precision during masker modulation decreased over SNRs (fig. 4.4 E and F). Decrease in response precision was accompanied by an increase in mean latency of response during masker modulation (fig. 4.4 D). Strikingly, precision of responses to target during masker modulation was significantly smaller in modulation unmasking neurons from aged animals than in modulation unmasking neurons from young adult animals (note that an increase in entrainment corresponds to a decrease in response precision; fig. 4.4 E and F; filled circles, $p < 0.05$ ttest). Moreover, modulation unmasking neurons from aged animals showed decreased response latencies relative to gap onset for SNRs from 0 dB to -6 dB.

Taken together, these results provide evidence that the processing of complex acoustic signals during transient improvements in SNR is less precise in the aged than in the young adult gerbil auditory system. This decreased response precision may potentially underlie the decreased temporal masking release that was evident in psychophysical experiments with aged human subjects.

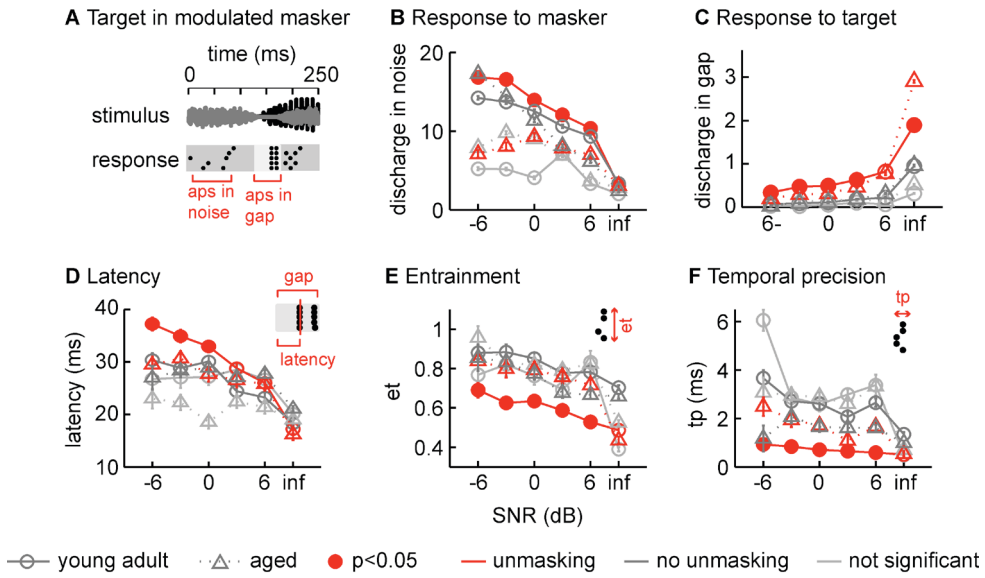

Figure 4.4 Neuronal discharge and precision in response to speech in modulated masker.

(A) Schematic of stimulus and raster plot of response from a young adult neuron. (B) Discharge in response to masker for modulation unmasking neurons (red), neurons that did not show unmasking (dark gray) and neurons that did not respond reliably to masked words (light gray) from young adult (circles) and aged (triangles) animals. Symbols represent mean discharge rate, horizontal bars indicate standard error (filled circles; $p < 0.05$ ttest). (C) Discharge rate in response to words during masker modulation. (D) Latency of response to target during masker modulation. (D) Response precision during masker modulation. Note that an increase in entrainment corresponds to a decrease in response precision. et: entrainment. (E) Temporal precision of response to target during masker modulation. tp: temporal precision.

VI

Discussion

This work provides important insight into auditory temporal processing in the aged and the young adult auditory system. The first part of the present study (chapter III) describes age-related alterations in the neural processing of synthetic sound sequences and altered coding of complex natural signals. Selectivity and variety of tuning to temporal parameters of noise pulse trains were significantly reduced in neurons from aged animals. Reduced selectivity and variety led to a decrease in heterogeneity of temporal receptive fields on the population level and resulted in inefficient encoding of natural stimuli.

Reduced selectivity of neuronal responses to temporal pulse train parameters can be thought of as altered sensitivity of IC neurons to temporal stimulus context. In the second project (chapter IV), I investigated consequences of altered context sensitivity in the aged auditory system on processing of speech during masker level fluctuations. I showed that aged humans are unable to efficiently use transient improvements of signal to noise ratios for signal extraction. On the neuronal level, altered context sensitivity decreased response precision in neurons from aged animals during masker modulation.

Technical considerations

Because I compared responses of neurons from aged animals to neurons from young adult animals, it is important to ensure that (1) anaesthetic condition in young adult and aged animals was similar and (2) that I recorded from comparable populations of neurons in both age groups.

(1) Because levels of inhibitory neurotransmitters are reportedly reduced in the aged auditory system (Caspary et al., 1995; Milbrandt et al., 1996; Willott et al., 1997; Milbrandt et al., 2000) and inhibition is crucial to auditory temporal processing (Casseday et al., 1994; Grothe, 1994; Grothe et al., 2001; Pecka et al., 2008), I chose anaesthetic agents that act primarily on excitatory neurotransmission (ketamine/xylazine). I can, of course, not exclude the possibility that anaesthesia induced differential effects on the young adult and the aged excitatory neurotransmitter system. However, data from the present study are largely in line with a previous study performed on only mildly tranquilized animals (Walton et al., 2002), and temporal release from masking reported from anaesthetized animals is comparable to temporal release from masking reported psychophysically from humans. These findings argue against artefacts in the data due to anaesthetic conditions.

(2) In recordings from gerbil IC, I used an array of seven electrodes allowing me to sample the medio-lateral and rostro-caudal extent of the IC during each experiment. Moreover, I recorded from neurons with similar distribution of BFs and temporal patterns in response to pure tones in both age groups. Given that certain temporal response patterns are thought to cluster in certain areas of the IC (Roth et al., 1978) and several neurons were sampled per frequency band, it is unlikely that the differences in response properties reported in the present study were caused by a sampling bias rather than by age related alterations in neural processing.

When comparing pure tone thresholds, I found the median of pure tone thresholds to be increased but the width of frequency tuning at 10 dB above threshold (Q_{10dB}) unchanged. Because one would expect increased width of tuning if outer hair cell fidelity was the reason for increased pure tone thresholds (Ashmore, 1987), there may be, in addition to

alterations in central auditory processing, a conductive component to age-related changes of sound processing.

Release from masking for speech in the gerbil auditory system

In the present study, a combination of human psychophysics and gerbil electrophysiology allowed me to investigate the neural processing underlying temporal release from masking. There are several possible explanations for the rather small fraction of modulation unmasking neurons found in the inferior colliculus of gerbils (8/74 in young adult and 5/72 in aged animals). Because neural processing tends to be optimized for the representation of behaviourally-relevant stimuli (Gehr et al., 2000; Wang et al., 2005; Holmstrom et al., 2010), it is possible that modulation unmasking of speech is less prominent in the gerbil than in the human auditory system. Another possible reason is that although natural speech was used in this study and masker modulation was naturalistic (Fastl, 1987), the stimulation paradigm did not resemble a natural acoustic situation. Modulation unmasking may have been activated in an additional set of neurons if stimulation was binaural and directional information was included in the stimulation paradigm. On the other hand, if a small number of neurons sufficiently represent additional information available during transient level fluctuations, it is not necessarily advantageous to sustain a large number of modulation unmasking neurons.

Although additional mechanisms likely govern speech processing in the human auditory system, processing of masked speech signals in animal models is not irrelevant to understand neuronal processing schemes in humans. Several lines of evidence support the relevance of neural processing of speech in experimental animals to the human auditory system. Firstly, according to hierarchical processing, sound parameters are represented in responses of subcortical neurons regardless of behavioural relevance. Accordingly, important parameters of speech signals are coded in neuronal responses of experimental animals at several stages of the auditory pathway (Watanabe, 1978; Delgutte, 1980; Rhode, 1998). Secondly, recent evidence suggest that natural vocalisations, regardless of behavioural relevance, evoke specialized processing optimized to extract temporal modulations of natural vocalisations in concurrent noise (Lesica and

Grothe, 2008). Thirdly, temporal release from masking is a phenomenon likely to be present in gerbils. Relevant spectrotemporally modulated vocalisations (e.g. from predators) are likely obscured by temporally modulated environmental maskers, making temporal release from masking advantageous for survival. Accordingly, speech signals were encoded reliably in a large portion of IC neuron responses and this representation was preserved, even if noise was added (in 65 % of IC neurons recorded from young adult animals), and a fraction of neurons encoded speech signals more reliably if masker was modulated than if it was continuous. Although additional mechanisms may add to temporal release from masking of speech in humans and temporal release from masking in natural soundscapes, the decline in response precision during masker modulation I report in the IC of aged gerbils is likely to contribute at least partially to an age-related decreased modulation unmasking.

Relation to previous studies

Altered representation of temporal modulations in the inferior colliculus of aged animals is consistent with previous observations in rats (Shaddock Palombi et al., 2001), mice (Walton et al., 2002) and gerbils (Boettcher et al., 1996). Boettcher et al. (1996) used auditory brainstem recordings to show altered neural processing of short temporal gaps between two successive noise bursts in aged gerbils. Walton et al. (2002) observed that spike rates at best modulation frequencies of sinusoidal amplitude-modulated (SAM) stimuli were more variable in population of neurons from young adult than from aged animals. Shadduck-Palombi et al. (2001) observed a decrease in SAM-bandpass and an increase in SAM-lowpass tuned neurons in aged animals. In line with these observations, I report decreased selectivity to noise pulse trains in the inferior colliculus of aged animals. I extended these findings to show decreased efficiency in population encoding of complex natural signals.

Results from the temporal release from masking project are in line with previous investigations of temporal release from masking in human psychophysics (Gustafsson and Arlinger, 1994; Dubno et al., 2002; Gifford et al., 2007).

Implications for population coding

The decrease in selectivity of IC responses to temporal modulations results in a decreased heterogeneity of temporal receptive fields of neurons from aged animals. One might argue that a decrease in heterogeneity of the neural population creates an overrepresentation of the stimulus and therefore helps to overcome stochastic responses of individual neurons. However, overlapping representation of stimulus features hampers stimulus discrimination (Nadal and Parga, 1994). It is therefore assumed that different encoding strategies are utilized at different levels of the brain (Atick, 1992). Evidence for this organization comes from other sensory modalities, e. g. taste (Rolls and Treves, 1990) and vision (Rolls and Tovee, 1995). For the auditory system, Holmstrom et al. (2010) reported heterogeneous receptive fields for spectrotemporal modulations at the level of the IC. They hypothesized that the IC plays a critical role in the efficient encoding of auditory information by facilitating the discrimination of behaviourally-relevant sounds (Holmstrom et al., 2010). Across the population of IC neurons sound discrimination is facilitated by complex selectivity, thereby increasing heterogeneity. The results from the present study show that temporal receptive fields of IC neurons from aged animals are less heterogeneous than temporal receptive fields of IC neurons from young adult animals and encoding of a set of natural stimuli is less efficient in the aged population of neurons. I speculate that if a more complex set of stimuli was used, responses of single neurons would be less informative and joint encoding would be more important.

Auditory processing is essentially hierarchical: complex response selectivity at higher levels of the auditory pathway is generated by convergence and nonlinear summation of output from lower level neurons. Therefore, increased redundancy at the IC of aged animals is likely inherited from lower levels of the auditory pathway and is imposed on higher levels of auditory processing. In particular, increased redundancy at the IC indicates declines in the extraction of separate sound features (e.g. sound offset) at lower levels of the auditory pathway. Higher auditory centres operate on representations of basic sound features at lower stations of the auditory pathway. The loss of information at the IC is therefore likely to impair the formation, identification and localisation of auditory objects (Ahissar et al., 2009). If the low-level representation of spectrotemporal sound features is indeed ambiguous, it should be more difficult for the aged auditory

system to discriminate stimuli that share similar low-level features and that are not easily categorized by higher levels of auditory processing due to semantic context (Nahum et al., 2010). This decline in sound discrimination would be even more pronounced if low-level cues are additionally obscured by background noise or reverberations.

Imbalanced neurotransmitter levels

Discharge rate selectivity to temporal sound features can be observed at various levels of the auditory pathway, but is particularly versatile in the IC (Brand et al., 2000; Woolley and Casseday, 2005; Perez-Gonzalez et al., 2006; Krebs et al., 2008). These versatile tuning patterns result from the complex set of synaptic inputs IC cells receive and are therefore dependent on outputs of the various auditory nuclei converging at the IC. The dependence of AM selectivity of single IC neurons on network states is nicely illustrated by the finding that AM selectivity can be modulated by changing sound location (Koch and Grothe, 2000) or by blocking inhibition (Casseday et al., 1994; Fuzessery and Hall, 1996; Casseday et al., 2002). In the present study, I observed a significant decrease in temporal selectivity of IC neurons in aged animals, indicating altered weights of inhibitory and excitatory input in the auditory pathway. In line with the results from the present study, a severe down-regulation of the inhibitory neurotransmitter system has been reported for the aged auditory system (Banay-Schwartz et al., 1989; Milbrandt et al., 1994; Milbrandt et al., 1996; Willott et al., 1997; Milbrandt et al., 2000). Studies of monkey primary visual cortex showed that orientation and direction selectivity of single neurons are decreased and that orientation selectivity of single neurons can be restored by GABA application in aged animals (Schmolesky et al., 2000; Leventhal et al., 2003). Similarly, behavioural gap detection thresholds of aged gerbils matched gap detection thresholds of young adult gerbils when γ-vinyl-GABA was systemically applied (Gleich et al., 2003). In this light, reduced temporal selectivity in the auditory midbrain may be caused by reduced inhibitory neurotransmission.

Responses of aged neurons from the present study differ from responses recorded from young adult neurons during blockade of GABA (Faingold et al., 1991; Vater et al., 1992; Caspary et al., 2002) or glycine (Vater et al., 1992). Besides alterations in temporal tuning,

increases in evoked and spontaneous rates are commonly observed as a consequence of blocking inhibition in the IC. Consistently with Shadduck-Palombi et al. (2002), I did not observe an increase in evoked discharge rate. Indeed, Walton et al. (2002) observed an increase in evoked discharge in aged animals; however this might be the result of stimulation with higher SPL. Neither the present nor previous studies observed increases in spontaneous activity at the IC of aged animals (Walton et al., 2002; Khouri et al., 2011). Studies from primary visual (Schmolesky et al., 2000) and auditory cortex (Hughes et al., 2010) report an upregulation of spontaneous activity. Considering reports on changes in spontaneous activity are consistent within and differ across brain structures in the context of a down-regulation of the inhibitory neurotransmitter system, it seems reasonable to speculate that different levels of spontaneous activity at different levels of the brain result from homeostatic processes in the neural network that are caused by the imbalance of excitation and inhibition. If it is further considered (1) that a severe down-regulation of the inhibitory neurotransmitter system has been reported for the entire auditory system and (2) that the IC receives convergent input from almost all other auditory nuclei, it is unlikely that consequences of a decreased weight of inhibitory synapses in the entire auditory system are identical to the consequences of a blockade of inhibition that is confined to an area in the IC. Moreover, alterations in neurotransmitter systems might not be exclusive to inhibitory neurotransmitter systems, but might also affect the excitatory neurotransmitter systems. A disruption of both, excitatory and inhibitory neurotransmitter systems will equally result in an imbalance of synaptic inputs.

Altered neurotransmitter and receptor levels further affect the dynamic modulation of strength of synaptic inputs. $GABA_B$ receptors, for example, have been shown to modulate release probability of synaptic vesicles (Miller, 1998). Dynamic modulation of synaptic weights underlies, among other computations, compensation for stimulus context at the LSO (Magnusson et al. 2008) and has been suggested to have a similar role at the MSO (Hassfurth et al., 2010). An imbalance of both synaptic inputs and synaptic strength will severely disrupt the computational arrangements for auditory processing.

Temporal context sensitivity

Temporal selectivity of IC neurons was evaluated using sequences of noise bursts. Consequently, the decrease in temporal selectivity observed in aged animals results from altered processing of sound sequences and can be thought of as a change in IC context sensitivity. In this line, PCA analysis revealed increased linearity of neuronal responses in aged animals. Sustained neurons from aged animals responded to every pulse of the train; in contrast, most neurons from young adult animals showed temporal response patterns that where not easily predicted from the stimulus waveform.

The auditory system is sensitive to stimulus history along a number of dimensions and time scales (Malone and Semple, 2001; Malone et al., 2002; Ulanovsky et al., 2003; Ingham and McAlpine, 2004; Nakamoto et al., 2006; Scholl et al., 2008). Short-term context sensitivity may serve to maintain spatial and level tuning functions of neurons at a near the optimal point (Dean et al., 2005; Dahmen et al., 2010) and to emphasize deviance from stimulus regularities (Ulanovsky et al., 2003; Malmierca et al., 2009). In this line, preceding stimulation may suppress (Faingold et al., 1991; Pollak and Park, 1993; Kuwada et al., 1997; Nelson et al., 2009) or facilitate response to subsequent stimulation (Nelson and Young, 2010). Facilitation of responses by preceding stimulation is e.g. evident in neuronal tuning to interstimulus delays (Portfors and Wenstrup, 1999) and in velocity and direction sensitivity to frequency sweeps (Phillips et al., 1985; Heil et al., 1992; Covey and Casseday, 1999). In the present study, I observed a decrease in response precision to speech signals preceded by a masking signal in neurons from aged animals relative to neurons from young adult animals. Decrease in response precision in aged animals was associated with a decrease in latency of discharge after masker offset. Inhibition that is either prolonged or initiated by stimulus offset has been ubiquitously reported in the mammalian auditory system (Nelson and Erulkar, 1963; Aitkin, 1986; Faingold et al., 1991; Grothe et al., 1992; Yang and Pollak, 1994; Pedemonte et al., 1997; Sadagopan and Wang, 2010). The decrease in latency of discharge after masker offset in aged animals may result from altered time course of suppression subsequent to stimulation. Accordingly, I observed fewer neurons that suppressed spontaneous activity after stimulus offset in the ICs of aged than in the ICs of young adult animals (Khouri et al., 2011). It is possible that offset inhibition serves to enhance response precision to

subsequent signals. Decreased latency of discharge may, alternatively, reflect prolonged excitation to masker in neurons from aged animals. In either case, in line with altered context sensitivity of neurons from aged animals, representation of speech during masker modulation is differentially affected by preceding noise in neurons from young adult and aged animals and apparently causes a decrease in response precision during transient level fluctuations in aged animals.

Somewhat contrary to the results from this study, time constants of suppression in two-tone paradigms tend to be prolonged in IC neurons from aged rats (Finalyson et al., 2002). In classical forward masking and two-tone suppression paradigms, spectrotemporally matched masker and probe stimuli are employed. In contrast, in the present paradigm a spectrotemporally modulated probe and a spectrotemporally constant masker are used. Given masker and probe of the present stimulus differ in their spectrotemporal characteristics, it is likely that masker and probe recruit only partially overlapping inputs to IC neurons. Therefore, the pattern of context sensitivity may differ between modulation unmasking and forward suppression. A recent study provides evidence for the notion that auditory context sensitivity is a function of the set of activated inputs: Scholes et al. (2011) demonstrated that the effect of preceding stimulation on cortical responses to probe stimuli is a function of probe frequency. In line with altered context sensitivity in the aged auditory system, Horvath et al. (2009) reported shorter integration times in aged than in young adult human subjects in EEG (electroencephalograms) recordings for a subset of sound sequences.

The efficient coding hypothesis suggests that neurons are able to adapt their responses to stimulation statistics in order to extract maximum relevant information from incoming signals. Lesica and Grothe (2011) demonstrated that IC neurons adapt neural processing of naturalistic vocalisation signals to increase signal to noise ratio if background noise is added. Similar mechanisms are evident from auditory processing in a songbird (Nagel and Doupe, 2006). Given existing evidence of converging synaptic input and spectral integration properties at the inferior colliculus, it is likely that, for a subset of neurons in the intact (young adult) auditory system, inhibitory and excitatory inputs are arranged to optimise processing of natural sounds in concurrent fluctuating noise. Data from the present study and from previous studies suggests that this arrangement (in terms of

strength and/or kinetics of pre-synaptic neurotransmission and postsynaptic processing) differs between young adult and aged animals.

The most common symptom of age-related presbycusis is the failure to efficiently analyse complex acoustic situations. As discussed below, there is mounting evidence that temporal context sensitivity is a crucial neural property to perform this task. In this light, altered context sensitivity of neurons in the aged inferior colliculus is the most important finding in this report. To understand to what extend altered context sensitivity underlies declines in the processing of complex acoustic environments, additional experiments will be necessary. (1) To further investigate prevalence and consequences of altered context sensitivity of auditory neurons in the inferior colliculus, temporal receptive fields should be constructed from responses to naturalistic vocalisations and compared between aged and young adult animals. This approach would add support to the hypothesis, that altered context sensitivity influences processing of naturalistic sounds, and would be informative about the time scale and prevalence of altered context sensitivity in the aged auditory system.

(2) I propose that altered relative timing, strength and kinetics of synaptic inputs to IC neurons may underlie the inefficient analysis of vocalisations. To investigate possible alterations in timing and strength of synaptic inputs, a combined *in vivo* and *in vitro* approach may be suitable. *In vivo* current clamp recordings during stimulation with simple temporal masking sequences should reveal relative timing of excitatory and inhibitory inputs to context sensitive cells at the level of the IC. *In vitro*, a comparison of the strength and the kinetics of inhibitory and excitatory inputs between young adult and aged animals could be performed. Miniature recordings of spontaneous IPSPs (inhibitory post-synaptic potentials) and EPSPs (excitatory post-synaptic potentials) in IC neurons would answer these questions. *In vitro* recordings from adult animals are technically challenging. However, miniature recordings require neither stimulation nor the wash-in and wash-out times of drugs and are therefore comparatively quick. This technique is therefore ideally suited to obtain the large sample size required for statistical comparison of IC neurons from brain slices from young adult and aged animals

Outlook: Context sensitivity in complex acoustic scenes

In the intact auditory system, neurons are sensitive to stimulus context along several dimensions. Therefore, deteriorated context sensitivity is likely to affect a wide range of auditory processing. Among other processes, response adaptation to stimulus context is assumed to underlie efficient representation of incoming sounds and temporal integration of sound information.

Efficient representation of the acoustic environment in the auditory system is achieved by dynamic adaptation of coding regimes to stimulus statistics. Given the ability of the auditory system to tune to the sound level distribution of the local acoustic environment is crucial to detect temporal modulations in noisy environments (Dean et al., 2005), a loss of the capacity to fine-tune coding to environmental level statistics in aged subjects will severely hamper the representation of natural soundscapes. Similarly, the intact, young adult, auditory system adapts its representation of sound location depending on stimulus context (Dahmen et al., 2010). If sensitivity to spatial stimulus context is altered analogue to sensitivity to temporal stimulus context in the aged auditory system, the representation of stimulus space is likely to be less informative in the aged than in the young adult auditory system.

In addition to adjusting neural coding to an optimal range, context sensitivity of auditory neurons is of crucial importance to analyse the temporal relationship between sound elements in complex signals. The temporal relationship of sound elements presents an important cue for sound source separation (Winkler et al., 2009). In this line, the analysis of temporal coherence underlies the auditory system's ability to separate acoustic input into auditory streams (e.g. to separate simultaneously active speakers) (Shamma et al., 2011). For example, sounds of very different frequency composition that start and end at the same time are likely to be perceived as a single event; sounds whose onsets and offsets differ by several tens or hundreds of milliseconds are likely to be assigned to separate sound sources (Darwin, 1997). Neural correlates of this phenomenon have been reported throughout the auditory system, and are most prominent at the AC (Micheyl et al., 2005; Micheyl et al., 2007; Pressnitzer et al., 2008; Elhilali et al., 2009; Bee et al., 2010). Altered temporal integration properties in the aged auditory system are likely to

affect the representation of temporal coherence, potentially disturbing sound source separate.

Context sensitivity of auditory neurons has further been shown to be vital to the selective suppression of directional information in echoes and reverberations (Yang and Pollak, 1994; Burger and Pollak, 2001; Pecka et al., 2007). A possible neural mechanism for echo suppression involves temporal masking at the level of the DNLL (Pecka et al., 2007). Altered synaptic weights of inhibitory inputs in the aged auditory system are likely to also deteriorate temporal masking sequences in binaural processing. Given the ability to suppress echo location is of immense importance to auditory processing in reverberant environments, a deterioration of echo suppression may, again, be detrimental to the analysis of complex acoustic situations.

Stimulus context is crucially important to both efficient coding and analysis of auditory scenes. Altered sensitivity to temporal stimulus context for the aged auditory system, as reported in the present study, is likely to underlie a number of age-related sensory processing deficits. Further study will be required to understand altered temporal context sensitivity and to investigate spatial and spectral context sensitivity of the aged auditory system. To directly link the several aspects of temporal processing and the processing of complex acoustic scenes to provide a comprehensive picture of age-related declines in neural processing and auditory function provides an interesting challenge for further research in the field. Based on a thorough understanding of age-related auditory dysfunction gained from this and future studies, research in this field may ultimatively foster the development of sophisticated hearing aids that should be able to compensate for echoic environments and should be able to selectively augment and attenuate sounds emitted by separate sources.

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About the Manuscript

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List of acronyms and initialisms

AC	auditory cortex
AN	auditory nerve
AM	amplitude-modulations
ANOVA	analysis of variance (statistical test)
AP	action potential
BF	best frequency
ca.	circa
CN	cochlear nucleus
DNLL	dorsal nucleus of the lateral leminiscus
GABA	γ-aminobuturic acid
IC	inferior colliculus
LSO	lateral superior olive
MNTB	medial nucleus of the trapezoid body
MSO	medial superior olive
NLL	nucleus of the lateral leminiscus
NMDA	N-methyl-D-aspertate
PC	principal component
PCA	principal component analysis
PM	pulse matrix
PSTH	post-stimulus-time histogram
r	Pearson correlation coefficient
SAM	sinusoidal amplitude-modulations
SNR	signal to noise ratio
SOC	superior olivary complex
SPON	superior paraolivary nucleus
stim.	stimulus
TSI	Temporal selectivity index
VNLL	ventral nucleus of the lateral leminiscus
5-AFC	five-alternative forced-choice

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Publication List

JOURNAL PUBLICATIONS

Published July 2011 — **Khouri L**, Lesica NA, Grothe B (2011). Impaired Auditory Temporal Selectivity in the Inferior Colliculus of Aged Mongolian Gerbils. **The Journal of Neuroscience.** 31:9958-9970**.**

Published Mar 2006 — **Khouri L**, Bock A, and Ullrich O (2006). Analysis of Rab11 function by RNAi mediated silencing. **European Journal Cell Biology.** 85:1:74.

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Ich versichere, dass die vorliegende Arbeit von mir selbstständig und nur unter der Verwendung der angegebenen Hilfsmittel angefertigt wurde.

München, Oktober 2011

Leila Khouri