Interaction between Electrically and Acoustically Evoked Responses in the Cochlea of the Guinea Pig

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Interaction between Electrically and Acoustically Evoked Responses in the Cochlea of the Guinea Pig

Wisselwerking tussen Elektrisch en Akoestisch Opgewekte Responsies in de Cochlea van de Cavia

(met een samenvatting in het Nederlands)

Proefschrift

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Contents

"...I received a shock in the head, and some moments after I began to hear a sound, or rather noise in the ears, which I cannot well define: it was a kind of crackling with shocks, as if some paste or tenacious matter had been boiling..."

First written account of electrically evoked auditory sensations from Alessandro Volta (1800), not long after inventing the electrolytic cell. Translation from Fan-Gang Zeng (2004).

Volta A. 1800 On the electricity excited by mere contact of conducting substances of different kinds. R. Soc. Philos. Trans. 90, 403-431.

Zeng, F.G. 2004. Auditory prostheses: past, present, and future. In: Zeng, F.G., Popper, A.N., Fay, R.R., (Eds.), Cochlear implants. Auditory prostheses and electric hearing. Springer-Verlag, New York.

General introduction 1

1.1. Introduction

At present, the preferred treatment for severe to profound hearing loss is cochlear implantation. Cochlear implants bypass the damaged sensory hair cells in the cochlea and directly stimulate the auditory nerve by electrical current pulses. Since the introduction of the first single-electrode cochlear implant, technology of implant systems has greatly improved. Nowadays, cochlear implant users can achieve excellent speech understanding and may even be able to talk on the phone. Due to the remarkable performance in terms of speech understanding, criteria for candidacy for implantation are broadened. As a consequence, growing numbers of cochlear implant users have considerable residual low-frequency hearing in the implanted ear. This development has raised the question how electrically and acoustically evoked responses in the cochlea interact. The work presented in this thesis was conducted to investigate this electro-acoustic interaction.

This introductory chapter introduces the reader to the field of electrical stimulation of the cochlea and provides an overview of the recent developments that have led to the concept of combined electro-acoustic stimulation. First, an introduction on normal acoustic hearing will be provided (1.1). Thereafter the electrophysiological methods used in this thesis will be highlighted (1.2). After providing a background on hearing impairment (1.3) the cochlear implant will be introduced (1.4), followed by the concept and advantages of electro-acoustic stimulation (1.5). The following sections deal with the effects of acoustic stimulation on electric hearing (1.6) and effects of electrical stimulation on acoustic hearing (1.7). Last, the aim and outline of this thesis will be provided (1.8).

1.2. Acoustic hearing

The sense of hearing plays an important role in social and cultural communication. People lacking the ability to hear are restricted in their ability to communicate and may be more vulnerable to injury from sources outside their field of view. Physical sound consists of airborne pressure differences travelling away from the source. Objects that generate sound put air molecules in motion that will start vibrating with the same frequency as the source. Perceptual pitch of sound is related to the frequency of the vibration, while loudness is determined by the amplitude of the pressure differences (Mather, 2006).

Sounds are processed in the inner ear, the sensory end organ of hearing. Sound reaches the inner ear via the outer and middle ear (Fig. 1.1). The outer ear is composed of an auricle (pinna) and external auditory canal (meatus externa). The auricle is involved in directional hearing by affecting sound transmission to the middle ear in a frequency-specific manner. The middle ear contains the eardrum (tympanic membrane) and ossicles (malleus, incus and stapes). This system acts as an efficient transducer of airborne sound to liquid pressure

differences in the inner ear, the cochlea. The stapes connects to the cochlea via a flexible membrane, the oval window. The cochlea is responsible for transduction of mechanical energy into electric signals which can be transmitted to the auditory nerve and ultimately to the central nervous system (Mather, 2006).

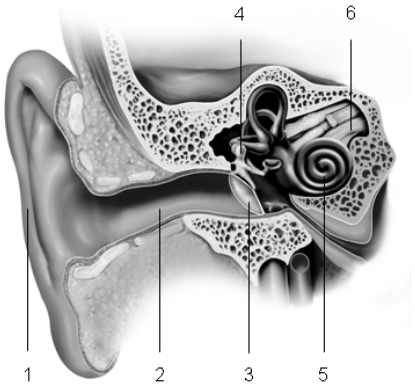

Fig. 1.1. Schematic of the anatomy of the human ear. The outer ear consists of the pinna (1) and the external ear canal (2). The middle ear contains the tympanic membrane (3) and the ossicle chain (4) and the inner ear holds the cochlea (5). The auditory nerve (6) is also shown.
Figure reprinted with permission from MED-EL (Germany).

The cochlea (Latin for "snail") is a coiled tube (Fig. 1.2A and front cover) with a length of 34 mm in humans. This tube is divided in three partitions, or scalae: scala tympani, scala vestibuli and scala media. At their apical end, scala vestibuli and scala tympani are connected by the helicotrema. Basally, the scala vestibuli ends in a flexible membrane, called the oval window. The base of the scala tympani also ends in a membranous structure, the round window. Both scalae are filled with perilymph, a fluid with a composition similar to cerebrospinal fluid. Airborne vibrations are transferred by the stapes into the scala vestibuli via the oval window. Due to the incompressibility of fluids, these pressure differences are transmitted to the scala tympani and are accommodated by the round window. Scala tympani and scala verstibuli are separated along their length by the scala media. The boundary between scala tympani and scala media is formed by the basilar membrane which supports the sensory hair cells that ultimately transduce mechanical energy into electrical signals. Vibrations in the scala tympani evoke displacements of the basilar membrane that take the form of waves travelling from the base of the cochlea (where they originate) to the apical end. The properties of the basilar membrane gradually change from narrow and stiff at the base, to wide and flexible at the apex. Due to these properties, the basilar membrane shows a maximal displacement in response to sound that is frequency-dependent.

The basal part of the basilar membrane responds maximally to high-frequency vibrations, and the apical part to low-frequency vibrations. Hence, the basilar membrane essentially acts as a frequency-to-place converter and decomposes complex sounds into their separate frequency components. The frequency at a given location that evokes the largest response is called the characteristic frequency (CF). Below or above its CF, amplitudes decrease. The amplitude-frequency relation graphically representing this phenomenon is called a tuning curve. The tip of the curve represents the CF, while frequencies below and above this CF result in reduced amplitudes. The presence of a place-dependent gradient of high to low CFs is referred to as a tonotopic organization (Mather, 2006).

The basilar membrane supports the organ of Corti (Fig. 1.2B) which contains the sensory hair cells. Hair cells owe their name to the protrusions at their apical side, called stereocilia. There are four rows of hair cells, each containing approximately 4000 cells in humans. The inner row of cells near the cochlear spiral (modiolus), are referred to as inner hair cells (IHCs), the three outer rows as outer hair cells (OHCs). Apically, hair cells are covered by a fairly rigid, gelatinous structure, the tectorial membrane. The location of the attachment of the tectorial membrane to the lining supporting structures slightly differs from that of the basilar membrane (Fig. 1.2B). Due to the differing sites of attachment, movements of the basilar membrane cause a displacement relative to the tectorial membrane and thereby generate a shearing motion on the stereocilia. Stereocilia subsequently convert these mechanical movements into electrical signals. This transduction process is mediated by mechanoreceptor channels associated with the stereocilia. Stereocilia differ in size and are orderly placed from small to large on hair cells. Movement of stereocilia in the direction of the larger stereocilia depolarizes hair cells, while movement to the opposite direction hyperpolarizes hair cells.

It is thought that the stereociliar mechanoreceptors consist of mechanically gated non-selective cation channels. In rest, the chance that these channels are open (the open-probability) is small and current flow into hair cells is limited. Deflection of stereocilia in the direction of the large stereocilia increases open-probability, while deflection to the opposite side increases the number of channels in the closed state. As a result, hair cells generate biphasic responses oscillating around the resting potential when sound is perceived. Most charge transfer across stereocilia upon channel opening is mediated by K^+, because this is the most abundant cation in endolymph. In addition, Ca^{2+} and Na^+ ions may also be involved (Pickles, 2008). This mechanoreceptor system is extremely sensitive and displacements of stereocilia by as little as 0.3 nm is sufficient to alter receptor potentials (Mather, 2006). These minute movements are on the atomic scale and correspond approximately to the diameter of a large atom such as that of mercury.

Depolarization of IHCs leads to release of the neurotransmitter glutamate, which subsequently travels across the hair cell synapse to the dendrites of the

nerve cells of the cochlear nerve (nervus acousticus, or nerve VIII). The cell bodies of the auditory nerve cells are located in Rosenthal's canal (Fig. 1.2C) which spirals alongside the modiolus. Therefore, auditory nerve cells are referred to as spiral ganglion cells (SGCs). A single hair cell is connected to 10 - 20 SGCs, while a single SGC never receives input from more than one IHC. Tuning characteristics of SGCs are therefore similar to those of IHCs (Pickles, 2008).

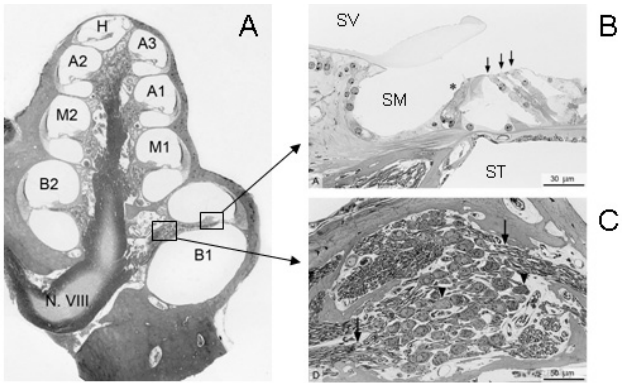

Fig. 1.2. Histological sections of the cochlea of the guinea pig. (A) Low-magification overview of a guinea pig cochlea sectioned through the midmodiolar plane. Details of the organ of Corti (B) and Rosenthal's canal (C) of the basal turn are shown at higher magnification. Arrows in the organ of Corti indicate outer hair cells (OHCs) and the asterisk shows the inner hair cell (IHC). Arrowheads in Rosenthal's canal indicate nerve fibers and arrows indicate spiral ganglion cells.

IHCs are innervated by afferents conveying information from periphery to the central nervous system, while OHCs are innervated mainly by efferents and receive information from the central nervous system (Mather, 2006). Hence, OHCs are probably not involved in encoding sound stimuli and are thought to have a more regulatory role. OHCs have been shown to contract upon depolarization (Brownell et al., 1985) and unlike IHCs, their longest stereocilia are tightly anchored in the tectorial membrane (Pickles, 2008). These characteristics allow OHCs to affect basilar membrane movement in response to initial basilar membrane displacements. OHCs are therefore thought to be involved in the active amplification of basilar membrane movements, thereby increasing the sensitivity of the cochlea to sound. In addition, OHCs are thought to improve the relatively crudely tuned properties of the basilar membrane by sharpening the tuning curves. Efferent innervation of OHCs is probably necessary to regulate the input gain delivered by the OHCs (Mather, 2006).

After activation, SGCs generate action potentials that travel through their axons (the auditory nerve fibers) via the auditory nerve to the auditory brainstem. Besides the "place code" of frequency due to the tonotopic organization of the

cochlea, frequency information is also represented by a temporal code in the auditory nerve (Pickles, 2008). Auditory nerve fibers have the tendency to fire during a specific phase of a tonal sinusoidal input, referred to as phase locking. This phenomenon is probably related to the movement of hair cell stereocilia and phase-specific release of glutamate in the synaptic cleft. Especially at low frequencies (<1 kHz) this phenomenon can be particularly strong (Palmer and Russell, 1986). Despite the fact that a single fiber may respond on average only once every hundred cycles or so, synchronous firing of multiple fibers during a particular phase can provide frequency cues to the auditory system (Pickles, 2008).

Besides providing frequency (pitch) cues, auditory nerve fibers also convey information of sound level (loudness) by increasing their firing rate according to stimulus input level. Firing rate is governed by the amount of glutamate released in the synaptic cleft. Repeated firing of auditory nerve fibers is limited by their refractory period. During the absolute refractory period (~0.3 ms), fibers cannot respond to stimulation irrespective of stimulus level. During the following relative refractory period of several milliseconds, fibers respond to stimuli only when presented at supra-threshold levels (Miller et al., 2001).

The auditory nerve fibers join in the acoustic nerve and project to the cochlear nucleus (CN), the first of a series of auditory brainstem nuclei. The CN is thought to integrate information of the frequency composition and temporal features of sound ("what" processing). The CN projects bilaterally to the superior olive (SO) and to the inferior colliculus (IC). Bilateral input to the SO and IC allow these nuclei to process the directionality of sound ("where" processing). The SO projects to the IC and the IC projects to the medial geniculate nucleus (MG). IC and MG process sound in the frequency and temporal domain ("what" processing). MG neurons finally project to the primary auditory cortex located in Heschl's gyrus for higher order processing of sound. The tonotopy of the cochlea is conserved all the way up to the level of the primary auditory cortex (Mather, 2006; Pickles, 2008).

1.3. Measuring the functionality of the auditory system

Functionality of the auditory system can be tested by subjective (psychophysical) methods and by objective measures. Psychophysical tests rely on behavioural responses, while objective measures make use of physiological responses of the auditory system.

Widely clinically applied psychophysical tests are pure tone and speech audiometry. Pure-tone audiometry determines the perceptive threshold of a range of frequencies. A tone audiogram provides information on the presence, severity and frequency-dependence of hearing loss. The difference between audiograms based on air and bone conduction allows differentiation between conductive and

sensorineural hearing loss (see next section). A speech audiogram estimates threshold levels of speech understanding and is indicative of the severity of functional impairment (Newman and Sandridge, 2006). Functioning of the auditory system can also be assessed by objective measures, such as the acoustically evoked potentials (AEPs). AEPs represent synchronous electrical neural activity in the auditory system and result in potentials that can be recorded with electrodes. Detection thresholds are lowered by amplifying the signal. Signal-to-noise ratios are improved by repetitive stimulus presentation and time-synchronous averaging techniques. AEPs are clinically applied and also widely used in fundamental research. AEP recordings can be helpful for determining hearing sensitivity and for otoneurologic assessment (Newman and Sandridge, 2006).

Electrocochleography (ECochG) measures the neural responses of the cochlea to acoustic stimuli and is based on the gross activity of large numbers of receptor and nerve cells. The ECochG potential (Fig. 1.3) consists of three responses: cochlear microphonics (CM), compound action potential (CAP) and summating potential (SP).

The CM is an alternating current response that more or less follows the waveform of the acoustic stimulus. The CM is derived mainly from OHCs and its spatial localization corresponds to the travelling wave. When the transducer channels open upon arrival of the travelling wave, current is drawn away from the scala media, making it less positive. After channel closure, the scala media becomes more positive. Biphasic potential changes are therefore generated as described earlier. The SP is a direct current shift also generated by hair cells. The polarity of this baseline shift varies with stimulus frequency and level (Pickles, 2008).

CAP responses represent synchronized auditory nerve fiber activity. CAP waveforms typically consist of two negative deflections (N_1 and N_2) at the beginning and sometimes also at the end of the stimulus. N_1 generally appears 1 ms after CM onset, N_2 after 2 ms. N_1 is generated by highly synchronous firing of auditory nerve fibers at stimulus onset. N_2 is thought to arise from a synchronous second firing of nerve fibers. For high-frequency stimuli, CAP responses reflect activity of high-CF auditory nerve fibers. At low frequencies however, fibers with low CFs contribute most to the CAP only at low stimulus levels. At high sound levels these CAPs become dominated by high-CF fibers due to a basal-ward spread of the travelling wave on the basilar membrane (Pickles, 2008).

CAPs evoked at low frequencies reveal so-called frequency-following responses. This phenomenon is caused by the phase-locking properties of auditory nerve fibers, as described earlier (Pickles, 2008). Phase-locking disappears at high frequencies, probably due to decreased alternating current responses related to the capacitance of hair cells. Furthermore, increased direct current responses at high stimulus frequencies cause the firing rate of auditory nerve fibers to become less dependent on alternating current responses (Palmer and Russell, 1986).

Another widely used AEP is the auditory brainstem response (ABR). The

ABR consists of a series of vertex-positive peaks (Fig. 1.4) that are thought to represent different neural sources (Newman and Sandridge, 2006). For the guinea pig, the first positive peak (P_I) and negative peak likely represents auditory nerve activity (i.e., the CAP). Later peaks probably represent different brainstem nuclei. P_{II} is believed to arise from the ipsilateral CN, P_{III} from the contralateral SO, P_{IV} from the bilateral response in regions lateral to the SO, and P_V from the IC (Wada and Starr, 1989; Newman and Sandridge, 2006).

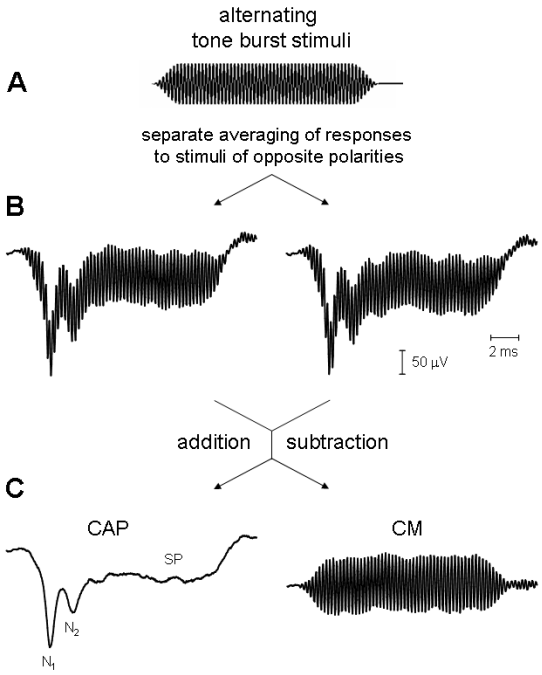

Fig. 1.3. Example ECochG recordings in a normal-hearing guinea pig (Ela49) recorded from the apex of the cochlea. Tonal stimuli consisted of tone bursts with a frequency of 8 kHz presented at 80 dB SPL (A). Stimuli of opposite polarity alternated and ECochG recordings were separately averaged (B). Adding and division by 2 (i.e. the average) of the ECochG response yields the compound action potential (CAP) of the auditory nerve. Subtraction and division by 2 yields the cochlear microphonic (CM) consisting mainly of outer hair cell responses. First and second negative peaks (N_1 and N_2) and summating potential (SP) in the CAP signal are indicated. Trace onsets correspond to acoustic stimulus onset.

1.4. Hearing impairment

Hearing impairment can result from defects anywhere along the auditory pathway and can be roughly divided in conductive and sensorineural hearing losses.

Conductive hearing loss is associated with dysfunction of mechanical structures of the outer and middle ear. Sensorineural hearing loss results from impaired functioning of neural structures in the cochlea, auditory nerve, or central auditory system (Mather, 2006).

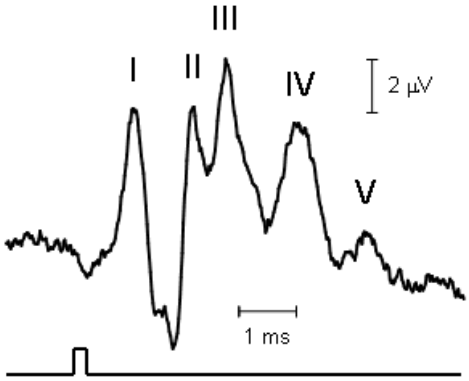

Fig. 1.4. Example auditory brainstem response (ABR) recording in a normal-hearing guinea pig (Ame15). Transcranial screw electrodes were used for recording and were placed 1 cm caudal from bregma (active) and 2 cm rostral from bregma (reference). Stimuli consisted of alternating clicks (monophasic 40 µs) at 77 dB peSPL and responses were separately averaged. Addition of these responses (and division by 2) reduced the stimulus artifact and yielded the final ABR recording. Postitive peaks (indicated with roman numerals) are used to determine peak latency, and amplitudes can be determined relative to an adjacent negative peak.

Conductive hearing loss can be caused by obstruction of the auditory meatus, damage to the tympanic membrane, or impeded functioning of the ossicles. Damage to the tympanic membrane can occur due to infection of the middle ear cavity (otitis media). Impeded functioning of the ossicles can occur after fluid build-up in the middle ear during an otitis media, or due to calcification (otosclerosis). Conductive hearing loss can often be successfully treated by medical treatment. Possible interventions are antibiotics to fight the infection, surgical relief of the impaired movement of the ossicle chain, or acoustic amplification with hearing aids to compensate for the loss in conduction efficiency (Mather, 2006).

The great majority of cases of sensorineural deafness are related to the cochlea, and specifically to damaged, degenerated or absent hair cells (Wilson, 2004). Hair cells are delicate structures and are easily damaged by (1) loud sound, (2) ototoxic drugs, (3) infection, (4) metabolic disturbance, (5) allergy, (6) genetic disorders and (7) age. Long-term loss of IHCs can result in a secondary degeneration of SGCs (Xu et al., 1993). Mammalian hair cells (and SGCs) do not regenerate once lost (Versnel et al., 2007) and sensorineural deafness is a permanent hearing impairment. As a consequence, ageing is a common cause

of sensorineural hearing loss. Age-related loss of hair cells is characterized by a gradual degeneration, starting at the base of the cochlea and progressing to the apex (Mather, 2006). Basal hair cells are typically also more vulnerable to ototoxins, such as aminoglycoside antibiotics (Brummett et al., 1979) and cytostatics (Hamers et al., 2003).

Cochlear damage can result in perceptual changes, including raised thresholds and broader frequency tuning. Raised thresholds due to a loss of OHCs may not be satisfactorily solved by acoustic amplification as with conductive hearing loss. Even after compensating for the raised thresholds, sounds may still be perceived unclear and distorted because of broadened frequency tuning of individual nerve fibers by impaired OHC functioning. Broader tuning impairs the ability of listeners to segregate frequency components in complex signals. This impaired frequency resolution impairs speech understanding, especially in noisy environments (Mather, 2006).

The preferred method of treatment for severe hearing loss is acoustic amplification. However, when hearing loss is worse (i.e., severe to profound), other modes of stimulation are necessary to restore functional hearing (Gantz et al., 2005 and Fig. 1.6).

1.5. The cochlear implant

The method of choice for treatment of severe to profound sensorineural hearing loss is cochlear implantation. A cochlear implant (CI) bypasses the damaged hair cells in the cochlea and directly stimulates the auditory nerve electrically (Wilson, 2004). At present, more than 120,000 people have been implanted worldwide (Fallon et al., 2009). The history of electrical stimulation of the cochlea was reviewed by Simmons (1966) and Zeng (2004b). The following section dealing with this matter draws especially on their work.

The discovery that electric stimulation can directly evoke auditory sensations is credited to the Italian scientist Alessandro Volta in 1800 (Volta, 1800; Zeng, 2004b). Shortly after inventing the electrolytic cell in Paris in 1790, Volta started experimenting with the effects of electrical stimulation on the senses. In one experiment he inserted two metal rods in his ears and connected them to the poles of a battery containing 30 - 40 cells, which approximately equalled a 50-Volt battery (Simmons, 1966). After closing the circuit he observed: "At the moment when the circuit was completed, I received a shock in the head, and some moments after I began to hear a sound, or rather noise in the ears, which I cannot well define: it was a kind of crackling with shocks, as if some paste or tenacious matter had been boiling… The disagreeable sensation, which I believe might be dangerous because of the shock in the brain, prevented me from repeating this experiment" (Volta, 1800; Zeng, 2004b). In 1801, Ritter repeated this experiment, though with about 5 times as many cells. Not surprisingly, his

report also included disagreeable cerebral side effects (reviewed by Simmons, 1966). These side effects probably discouraged further research, and during more than a century reports on electrical stimulation of the auditory system appeared only sporadically. Most of these studies called attention to the fact that a sound sensation could be produced (Simmons, 1966).

Using more advanced technology, Stevens and colleagues started examining the underlying mechanisms of electrically evoked auditory sensations (Stevens, 1937; Stevens and Jones, 1939) and identified three possible mechanisms underlying "electrophonic hearing" (Clark Jones et al., 1940). First, the observation that sinusoidal stimulation could evoke a hearing sensation with a perceived pitch corresponding to the electric frequency was ascribed to mechanical activation of hair cells (now referred to as the electrophonic response). Second, the sensation of a tonal sound with a pitch twice that of the electrical sinusoid stimulus was attributed to the conversion of electric stimuli into sound stimuli by the tympanic membrane. Last, noise-like sounds with steep loudness growth functions were attributed to direct neural activation. The first auditory sensations in a deaf patient by electrical stimulation were reported around the same time in 1935.

The modern era of cochlear implants did not emerge before 1957, when Djourno and Eyries successfully electrically stimulated the first deaf subject in Paris. Though it was probably the cochlear nucleus rather than the auditory nerve that was stimulated (reviewed by Eisen, 2003), their success initiated the intensive research in the 1960's and 70's to restore hearing. In this period human and animal models of electric hearing were developed and the fundamental problems of electric hearing were uncovered (e.g. Moxon, 1971). These early observations are still recognized as the fundamental limitations of the cochlear implant today, and include the narrow dynamic range, steep loudness growth, broader tuning, and the limited temporal pitch that can be achieved by electric stimulation (Zeng, 2004b).

In 1984 the first clinically approved cochlear implant (House-3M) was developed by House (California) and the 3M company (Minnesota). This single-electrode implant had several hundred users and was mainly functional as an aid for lip-reading and sound awareness. Subsequently the Ineraid (Symbion) device with six electrodes was developed in Utah and also had several hundred users. In Belgium and France the Laura and Digisonic and MX20 were developed which could operate at 15 channels (Zeng, 2004b).

Modern implants have dramatically improved in terms of both hardware and software compared to the first single-electrode implants. Contemporary CIs can substantially improve speech understanding and some CI users may even be able to talk on the phone (Zeng, 2004b). Modern implant systems consist of a number of essential components. A microphone converts sound into an electrical signal. This signal is fed to a speech processor for conversion into a set of stimuli that is ultimately sent to the electrode array. To bridge the transcutaneous passage

from speech processor to electrode array, the signal is encoded into a radio signal that is transmitted from an external coil to an internal (implanted) receiving coil. After decoding this signal into an appropriate set of electric stimuli it is sent to the intracochlear electrode array (Fig. 1.5). Processor and microphone are generally housed together in a standard "behind-the-ear" unit. The external and internal coils are held together by magnets.

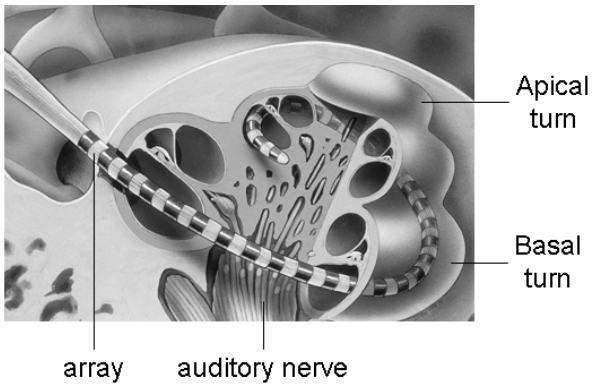

Fig. 1.5. Schematic of a cochlear implant electrode array. The array enters enters the cochlea in the basal turn and is advanced through the scala tympani to more apical parts. Electrical stimuli excite nerve fibers of the auditory nerve.
© Ed Zilberts (By courtesy of Cochlear Ltd.)

The receiver-stimulator package is surgically embedded in a recession drilled in the temporal bone. The ground (reference) electrode is implanted remote from the cochlea, generally in the temporalis muscle. Some systems use a metal band around the receiver-stimulator package as a ground electrode. The electrode array is inserted into the scala tympani of the cochlea via the round window, or through a cochleostomy drilled near the round window. Cochlear implant systems take advantage of the tonotopy of the cochlea by conveying electric stimuli representing high acoustic frequencies to basal electrodes, and signals representing low frequencies to apical electrodes. At present there are three major cochlear implant manufacturers: Med-El Corporation in Austria, Advanced Bionics Corporation in the U.S. (Clarion device), and Cochlear Corporation in Australia (Nucleus device). Electrode design, processing strategies and signal transmission techniques differ between cochlear implant companies and between systems (Zeng, 2004b).

With regard to processing strategies, today's implant systems deploy advanced software to process and encode sound. A commonly applied processing strategy is the CIS (continuous interleaved sampling) strategy (Wilson et al., 1991). CIS coding is based on amplitude-modulation of trains of symmetric biphasic pulses presented at a constant pulse rate of about 1000 pulse/s (pps) at

each electrode. Modulation frequencies and depths depend on the input sound. Basically, the signal from the microphone is fed to a bank of band-pass filters with different cut-off frequencies. Each filter provides input for one electrode. High-pass filters drive the basal electrodes and low-pass filters the apical electrodes. The envelope of each filter is extracted by an envelope detector, which is subsequently used to modulate the amplitude of the pulse train. Pulse trains are presented in a non-overlapping (interleaved) way between nearby electrodes to minimize channel-channel interaction. Simultaneously applied current pulses would add up by vector summation, increasing current spread and decreasing electrode-specificity (Wilson, 2004).

Despite the overall excellent performance on standard audiology tests such as speech understanding in quiet, individual scores may differ greatly between implanted subjects and can range from 0% to 100%. This variability can depend on the software (processing) and hardware (electrode array) of the particular implant system, though most of the variance is explained by subject-related variance. This variance is possibly related to cochlear status (in particular the extent of neural degeneration in the cochlea), integrity of the central auditory system, and cognitive functioning of the subject (Zeng, 2004b). Degeneration of the auditory nerve is seen after long-term sensorineural hearing loss, which is thought to be caused by a loss of neurotrophic support of SGCs from IHCs. (Leake and Rebscher, 2004; Agterberg, 2009).

One of the major limitations of electric hearing is the limited pitch percept of CI users (Zeng, 2004a). Pitch perception improves speech understanding in realistic (noisy) environments by aiding in the separation of relevant stimuli from irrelevant background noise (Drennan et al., 2007). Pitch perception also improves the esthetical quality of complex sounds such as music (Galvin et al., 2009). Pitch perception can be based on place cues and temporal cues. Place cues in CI users are relatively poor due to the limited number of electrodes and the limited ability of electrodes to stimulate a discrete population of neurons (Zeng, 2004b). Temporal cues are also less effective in implant users compared to normal-hearing listeners. While normal-hearing listeners are able to discriminate 1 – 2 Hz pitch differences at frequencies around 100 Hz, CI users require a difference of 10 - 20 pulses per second (pps) when a train of around 100 pps is used. Furthermore, the upper limit of pitch discrimination in most implanted subjects is ~300 pps (Zeng, 2002), while this is about twice as high in normal-hearing listeners (Carlyon and Deeks, 2002). Hence, CIs are unable to provide pure tone percept, let alone the sensation of harmonics and music.

A second issue of present-day implant systems is the lack of fine structure in the electric signal. Most processing strategies present acoustic envelopes and discard the fine structure of the original acoustic signal (Zeng, 2004a). Discarding the fine structure does not negatively affect speech understanding, probably because of the limitations of CI users to use temporal cues (Zeng, 2002). However, the absence of fine structure information reduces pitch percept, speech

understanding in noise, speaker identification, and it may also negatively affect the understanding of tonal languages (Moore, 2008).

1.6. Electro-acoustical stimulation

Despite the limitations of electric hearing, CI users have a remarkably good performance in terms of speech understanding in quiet. While the first single-electrode implants were mainly functional as an aid for lip reading and sound awareness, contemporary implants are capable of substantially increasing sentence recognition scores and some CI users are even able to talk on the phone (Zeng, 2004b). These developments have resulted in a gradual broadening of the clinical criteria for candidacy for implantation (Cohen, 2004).

A rather large subpopulation of hearing impaired people has a hearing loss characterized by an audiometric pattern referred to as a "steeply sloping" high-frequency hearing loss (Fig. 1.6). This type of hearing impairment is a common pattern of adult sensorineural hearing loss. These subjects can have substantial residual hearing at frequencies below 1 kHz (50 dB loss or less) and little or no hearing above that frequency (70 dB loss or more). Due to the severity of high-frequency loss, acoustic amplification of these frequencies is usually not helpful in these cases. Without the high-frequency percept, consonants are not properly perceived and speech understanding is greatly impaired. Recognition of monosyllabic words in quiet in this population typically is below 15% correct. These subjects can expect a higher level of speech reception with a cochlear implant than with a well-fitted hearing aid, and are now widely regarded as implant candidates (Wilson et al., 2003). These observations have led to the suggestion that, in such cases, electrical stimulation of the basal part of the cochlea might be complemented with acoustical stimulation of the apical part.

The concept of combined electro-acoustical stimulation (EAS) was first introduced by Von Ilberg. He implanted a patient with residual low-frequency hearing and provided the subject with a conventional hearing aid in the implanted ear. He showed that the implanted subject was able to successfully integrate electric and acoustic stimulation. Moreover, the subject had excellent speech understanding, especially in a noisy environment (Von Ilberg et al., 1999). This report triggered intensive research on the possibilities and advantages of EAS. Subsequent studies confirmed the successful integration of electric and acoustic hearing (Gantz and Turner, 2003) and residual hearing was shown to be particularly beneficial in difficult tasks relying on pitch perception, such as speech understanding in noise and melody recognition (Gantz et al., 2005; Fraysse et al., 2006; Gstoettner et al., 2008; Turner et al., 2008b; Lenarz et al., 2009). EAS has proven to be especially beneficial for speech understanding in competing-talker ("babble") background noise, a notoriously difficult situation for CI users (Gantz et al., 2005; Turner et al., 2008a).

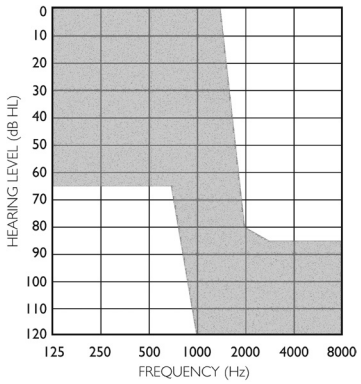

Fig. 1.6. Audiometric hearing range of potential candidates for electro-acoustic stimulation in the form of an audiogram. Tonal frequencies are arranged along the horizontal axis and the y-axis indicates the hearing loss in dB. 0 dB corresponds to normal hearing sensitivity. Figure reprinted with permission from MED-EL (Germany).

The promising results of EAS has led to the development of "hybrid implants" (Fig. 1.7) that combine a CI with a conventional hearing aid in a single device (Gantz and Turner, 2004; Hochmair et al., 2006).
EAS in the same ear requires that residual low-frequency hearing is preserved after implantation. This can be achieved by a relatively shallow inserting depth of the electrode array and the use of various surgical techniques to make the initial drilling and subsequent insertion as atraumatic as possible ("soft surgery") (Adunka et al., 2004b; Gstoettner et al., 2004; Gantz et al., 2005; James et al., 2005).

Besides "soft surgery" techniques, short electrode arrays have been developed that do not penetrate the acoustically sensitive apical parts of the cochlea. An example of such a short electrode is the Iowa/Nucleus Hybrid "S" cochlear implant (Fig. 1.7A) that has a 10 mm insertion depth with 6 channels in the distal 6 mm (Gantz and Turner, 2003). These short electrodes might also minimize interaction between electrical and acoustical stimulation by spatially segregating both stimulus modalities. As an alternative, thinner and more flexible EAS electrode arrays have been designed with an intermediate insertion depth and a normal number of electrode contacts such as the Nucleus Hybrid L electrode (16 mm, 22 contacts) (Lenarz et al., 2006), or with a regular insertion depth, such as the Med-El FlexEAS electrode (~25 mm, 12 contacts, Fig. 1.7C, Adunka et al., 2004a).

The use of arrays with regular insertion depths can be preferable above the use of short arrays, since low-frequency hearing might be lost after implantation due to insertion trauma (Gstoettner et al., 2008), progressive hearing loss (presbyacusis) (Yao et al., 2006), or sudden deafness syndrome (Gantz et al., 2009). Alternatively, the CI user might prefer electrical stimulation above EAS

(Gstoettner et al., 2008). In either case, the profoundly deaf subject might be left with a suboptimal implant (Fitzgerald et al., 2008). Studies from the Iowa group, however, indicate that short implant (10 mm) EAS users can achieve very high speech recognition scores with ES only (Reiss et al., 2008). In this regard, it is interesting to note that 10 mm implant users with functional acoustic hearing up to 750 Hz achieved high speech recognition scores when the full complement of acoustic frequencies from 750 Hz and above were delivered to the implant. Possibly prolonged use of a short electrode array enabled these subjects to use a highly compressed and shifted frequency map, corresponding to the observed change in pitch perception over time associated with individual electrodes (Reiss et al., 2007).

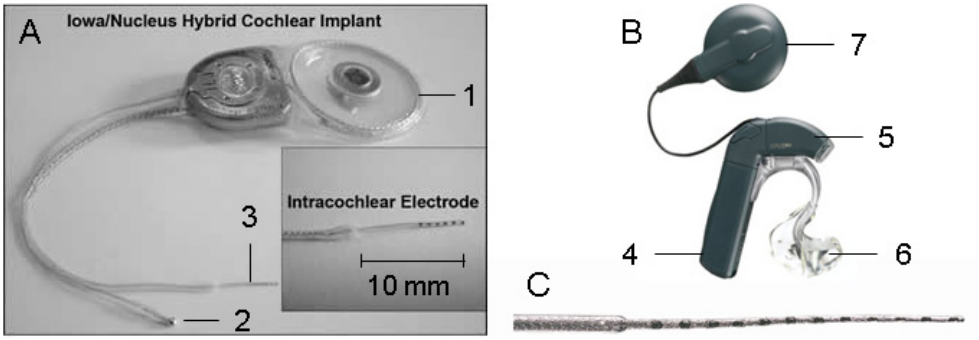

Fig. 1.7. Hybrid implant systems. (A) Internal parts of the Iowa/Nucleus Hybrid "S" implant showing the coil (1), ground electrode (2), intracochlear electrode array (3), and an enlarged view of the array (inset). The implanted part of the implant is 10 mm in length with 6 electrodes placed in the tip (scale bar). (B) External parts of the MED-EL hybrid implant system consisting of a battery-fed (4) DUET speech processor (5) that codes frequencies of high and low frequencies. Low-frequency signals are fed to the acoustic hearing aid (6) for amplification. High-frequency signals are transmitted via the external coil (7) to the electrode array. (C) FlexEAS electrode array. The implanted part is 32 mm and is depicted on approximately the same scale as the short electrode in (A).
Figure (A) was reprinted from Turner et al. (2008a) and kindly provided by the Journal of Rehabilitation Research & Development (public domain).
Figures (B, C) were reprinted with permission from MED-EL (Germany).

In all, EAS seems a promising method for treatment of severely hearing-impaired people with residual low-frequency hearing, and clearly has advantages over electrical stimulation (ES) and acoustical stimulation (AS) alone in this subpopulation. However, there is considerable variability in the performance between EAS users (Gantz et al., 2009). Furthermore, some EAS candidates prefer to use ES, although receiving benefit from additional AS (Lenarz et al., 2009), and some CI users even report a detrimental effect of residual hearing on

sound quality (Gstoettner et al., 2008). In addition, a minority of EAS candidates end up with post-operative speech understanding scores below pre-operative scores with AS (Luetje et al., 2007; Gantz et al., 2009). Besides proven predictors of post-operative performance such as the amount of residual hearing, age of implantation, duration of deafness and etiology (Rubinstein et al., 1999; Gantz et al., 2009; Lenarz et al., 2009), interaction between electrically and acoustically evoked responses might play a role in this EAS users.

1.7. Effects of hair cell activity on electrically evoked responses

Since the pioneering studies of Stevens and colleagues (Clark Jones et al., 1940), studies on electrically evoked auditory-nerve responses (Fig. 1.8) have mostly been performed on deafened cochleas. The reason for using cochleas devoid of hair cells is two-fold. First, since criteria for cochlear implantation have only recently been relaxed, most implant users have cochleas that have virtually no residual hair cells. Second, response properties of auditory nerve fibers are relatively uniform when no functional hair cells are present, while fibers in normal cochleas can show complex response patterns (Abbas and Miller, 2004). The concept of EAS and introduction of hybrid implants has, however, led to renewed interest in electrically evoked responses in the acoustically sensitive cochlea.

In deafened cochleas, auditory nerve fibers show short-latency responses of ~0.5 ms. These so-called α-responses are evoked by direct electrical activation of neural elements (Moxon, 1971; Van den Honert and Stypulkowski, 1984). Fibers in cochleas with functional hair cells show two additional modes of firing based on response latency. The α-response has a long latency of 2 – 5 ms and is thought to have an electrophonic origin involving electro-mechanical transduction followed by basilar membrane movements and normal mechanical activation of IHCs. The long latency may be explained by the intermediate steps between stimulation and fiber activity, involving basilar membrane movement and synaptic activity (Moxon, 1971). A possible site for electro-mechanical transduction is the OHC (Hubbard and Mountain, 1983), which has been shown to physically contract in response to electric stimulation (Brownell et al., 1985; Ashmore, 1987). This electromotile response can in turn evoke basilar membrane movements (Reuter et al., 1992; Nuttall and Ren, 1995). Another response only observed in normal-hearing cochleas has a latency of ~1 ms, intermediate to that of the α- and β-response. This δ-response is attributed to a direct depolarization of IHCs, or to an indirect mechanical activation of IHCs (e.g. by a direct action on the stereocilia) (Van den Honert and Stypulkowski, 1984).

Recent work on electro-acoustic interaction by Abbas and colleagues has focussed on the effect of hair cell activity on electrically evoked responses. By reversibly inactivating hair cells with furosemide, it was shown that the presence

of hair cells (i.e., without acoustical stimulation) decreased electrically evoked compound action potential (eCAP) amplitude (Hu et al., 2003). This phenomenon was explained by the fact that the presence of functional hair cells can increase random activity of auditory nerve fibers due to spontaneous hair cell activity (Liberman and Dodds, 1984). Spontaneous activity in the auditory nerve may reduce firing synchrony and can therefore lead to smaller eCAP responses, since eCAPs represent highly synchronized firing activity of nerve fibers.

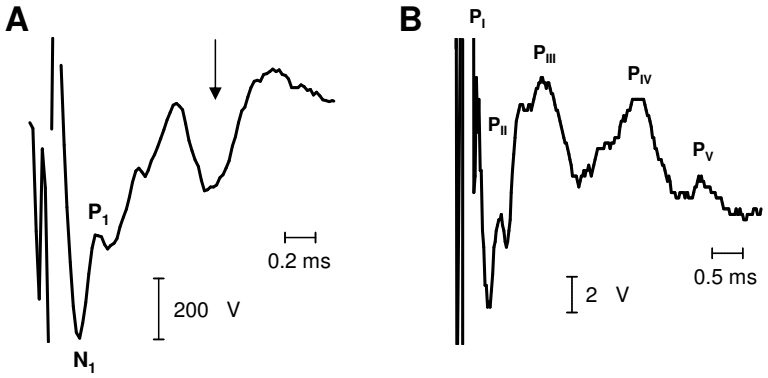

Fig. 1.8. Example recordings of an (A) electrically evoked compound action potential (eCAP) and (B) electrically evoked auditory brainstem response (eABR) in a normal-hearing guinea pig (Ame32). Electric stimuli were delivered by a monopolar intracochlear stimulation electrode in the base of the cochlea using "positive-first" biphasic pulses (40 µs/phase) of 900 µA. An extracochlear electrode on the apex was used for eCAP recording. Transcranial screws served as eABR recording electrodes and were placed 1 cm caudal from bregma (active) and 2 cm rostral from bregma. The first negative (N_1) and positive peak (P_1) of the eCAP are indicated, as well as the different positive eABR peaks (P_I-P_V).

It was subsequently shown that acoustical stimulation also suppressed eCAPs (Nourski et al., 2005, 2007). These effects were explained on the single-fiber level by a decreased synchrony of firing when electric stimuli were presented during acoustic stimulation (Miller et al., 2009). Furthermore, absolute firing rates of auditory nerve fibers were higher during EAS than during ES or AS alone, but lower than would be expected on the basis of simply adding the separate responses (Miller et al., 2009). This latter phenomenon was also found in the inferior colliculus when multineuronal firing rates to EAS were compared to firing rates evoked with ES and AS (Vollmer et al., 2010).

These studies indicate that functional hair cells may reduce and desynchronize auditory nerve activity. Hence, hair cell activity decreases effectiveness of electrical stimulation, which might reflect detrimental effects of hair cells on electric hearing. However, moderate desynchronization of auditory

nerve fiber activity is believed to actually increase electric hearing performance (Zeng et al., 2000; Chatterjee and Robert, 2001).

1.8. Effects of electrical stimulation on acoustically evoked responses

Early reports on the effects of electrical stimulation on acoustically evoked responses made use of direct current, and demonstrated that the CAP and CM could be facilitated or suppressed, depending on the polarity of the electric stimulus (Tasaki and Fernandez, 1951, 1952). These effects were later shown to be paralleled on the single-fiber level. It was shown that tone-evoked single-fiber discharges either increased or decreased depending on the polarity of the applied direct current (Konishi et al., 1970), or on the phase of the applied 5–30 Hz sinusoidal current (Teas et al., 1970).

Studies on the effects of pulsatile electrical stimulation of the cochlea showed that CAPs were suppressed when the acoustic stimulus was presented within ~4 ms after the electrical masker pulse (Norris et al., 1977), likely due to refractory mechanisms. A recent report describing multineuronal recordings in the IC, agrees with these findings and showed pronounced suppression of acoustically evoked responses by electrical pulses when presented shortly before, or during a tone burst (Vollmer et al., 2010). In contrast, studies by Simmons et al. (1978) and Ball (1982) showed that CAPs were increased in amplitude after a period (5 – 120 min) of intraneural stimulation with biphasic pulses at a low stimulus rate (50 - 100 pps). Since CAP amplitudes were also increased on the contralateral side, the phenomenon of CAP potentiation was explained by central effects mediated by efferents.

Other studies focussed on hair-cell mediated effects and revealed that (ipsilateral) acoustically evoked auditory nerve responses were suppressed by sinusoidal currents delivered to the cochlea (McAnally et al., 1993). Refractory mechanisms at the level of the auditory nerve were excluded by presenting acoustical probe stimuli 10 ms after the electric masker stimulus. Intervals this long are thought to exclude effects of neural adaptation by refractoriness (Stypulkowski and Van den Honert, 1984). Peripheral adaptation at the level of hair cells can last hundreds of milliseconds (Smith et al., 1983) and can be mediated by a variety of processes such as neurotransmitter depletion (Sumner et al., 2002). The authors showed that CAP suppression was tuned to the frequency of the sinusoidal current. This tuning of suppression could be observed at frequencies as low as 2 kHz, despite the fact that the electrical stimulus was delivered to the high-frequency region of the cochlea (McAnally et al., 1993). These findings were explained by the assumption that electrical stimulation generated travelling waves that migrated apically in the cochlea to that region where the CF of the basilar membrane matched the frequency of the sinusoidal current stimulus. These assumptions

agreed with earlier results from Moxon. He found that sinusoidal current applied at the base of the cochlea excited fibers maximally when the electric frequency matched the fibers' CF. He also explained these findings by electro-mechanical processes in the cochlea (Moxon, 1971). A later study confirmed these findings by showing that suppression of electrophonically evoked CAPs by sinusoidal current stimuli was tuned to the frequency of the acoustic masker (Kirk and Yates, 1994). Pulsatile stimuli were also shown to suppress tone-evoked CAPs by electrophonic mechanisms (McAnally and Clark, 1994). Suppression of acoustically evoked CAPs was maximal when acoustic frequency corresponded to a maximum in the frequency spectrum of the pulsatile stimulus. These findings suggested that each frequency component in the pulsatile stimulus evoked a mechanical response travelling to the place in the cochlea with the corresponding CF (McAnally et al., 1997). Direct evidence for electro-mechanical processes in the cochlea was obtained by direct measurements of electrically evoked movements of the basilar membrane (Nuttall and Ren, 1995) and these observations were strengthened by modelling studies (Xue et al., 1995).

In all, electrical stimulation results in suppression of acoustically evoked responses, though sporadic enhancing effects are also reported. Suppression can be mediated by refractory effects due to direct electrical activation of the auditory nerve, or by hair-cell mediated effects involving electro-mechanical transduction processes.

1.9. Aim and outline of this thesis

Because of the growing population of EAS users and the ongoing broadening of the audiometric inclusion criteria for cochlear implantation, it becomes increasingly more important to gain insight in how electrically and acoustically evoked responses interact in the cochlea. The main objective of this thesis is to characterize cochlear responses to EAS. The most important research questions address how ES interacts with auditory nerve responses to AS, and how AS interacts with auditory nerve responses to ES. In order to answer these questions we present the results of electrophysiological recordings of cochlear potentials in the guinea pig, in which responses to EAS are compared with those evoked by AS or ES alone.

Chapter 2 starts with a description of the evaluation of our animal model with regard to the anesthetic regime that was used. All experiments described in this thesis were performed in guinea pigs under general anesthesia using the volatile anesthetic isoflurane evaporated in a mixture of nitrous oxide and oxygen. Anesthesia was essential due to the invasive surgery needed to access the cochlea. Effects of isoflurane and nitrous oxide on the CAP, one of the main response parameters in this thesis, have not been investigated before.

Chapter 3 describes the effects of electrical stimulation on acoustically

evoked CAPs in normal-hearing guinea pigs using extracochlear stimulation electrodes on the base of the cochlea. The electric stimuli used mimicked those of present-day implants, and consisted of short biphasic pulse trains. The results of these experiments provide data on the effects of electric pulse trains on tone-evoked CAPs in healthy, untreated cochleas using minimally invasive techniques.

In chapter 4 we further investigate the effects of electrical stimulation on acoustically evoked CAPs. In this study an attempt was made to better approach the situation in human EAS users. First, we developed a guinea pig model of high-frequency hearing loss. We describe this model functionally by means of CAP threshold increases, and histologically by means of hair cell and spiral ganglion cell losses. Second, current stimuli were not delivered extracochlearly as in chapter 3, but presented via an intracochlear platinum wire electrode in the base of the cochlea to mimic a cochlear implant electrode. Of particular interest in this chapter is the question whether low-frequency evoked CAPs are affected by electrical stimulation delivered in the base of the cochlea.

In chapter 5 we make an attempt to unravel the mechanism behind the suppression of CAPs by electrical stimulation. In cochleas with functional hair cells, electric stimuli can directly excite neural elements, or they can evoke auditory-nerve responses by hair-cell mediated mechanisms (i.e., electrophonic activity). In this chapter efforts are made to separate CAP suppression evoked by direct electrical neural activation from suppression by electrophonics. Furthermore, electrophonic CAPs are described in terms of dependence on hair cell and SGC loss in animals with high-frequency hearing loss.

Chapter 6 describes the results from experiments in which the effects of the presence of hair cells on eCAPs were investigated. For these experiments we adopted the guinea pig model of high-frequency hearing loss. First, the amplitude of eCAPs is related to hair cell and SGC loss in the absence of acoustic stimuli. Thereafter the effects of acoustical stimulation on eCAPs are investigated. Effects of noise are related to the extent of hearing loss. In addition, effects of noise on electrically evoked activity in the auditory brainstem are described by means of electrically evoked ABR recordings.

Chapter 7 provides a summary and a general discussion of the results and concludes with the clinical implications of this work.

Abbreviations

ABR	auditory brainstem response
AS	acoustic stimulation
CAP	compound action potential
CI	cochlear implant
CF	characteristic frequency
CN	cochlear nucleus
CM	cochlear microphonic
ES	electrical stimulation
EAS	electro-acoustical stimulation
IC	inferior colliculus
IHC	inner hair cell
MG	medial geniculate nucleus
OHC	outer hair cell
pps	pulses per second
SGC	spiral ganglion cell
SO	superior olive
SP	summating potential

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Effects of isoflurane on auditory evoked potentials in the cochlea and brainstem of guinea pigs

2

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Abstract

Electrophysiological recordings of the auditory system are commonly performed in deeply anesthetized animals. This study evaluated the effects of various concentrations of the volatile anesthetic isoflurane (1-3%) on the compound action potential (CAP), cochlear microphonic CM) and auditory brainstem response (ABR). Recordings were initiated in the awake, lightly restrained animal. Anesthesia was induced with a single dose of Hypnorm® (fentanyl and fluanisone). After tracheostomy increasing isoflurane concentrations were applied in N_2O/O_2 via controlled ventilation. Data were compared to recordings in the awake animal using repeated measures ANOVA and Dunnett's post hoc test. On average, isoflurane dose-dependently suppressed the amplitude and increased the latency of the CAP. CM amplitude was suppressed. These effects were most profound at high frequencies and were typically significant at isoflurane concentrations of 2.5% and 3%. Amplitude and latency of the second negative peak of the CAP (N_2) were affected to a greater extent compared to the first peak (N_1). On average, isoflurane dose-dependently reduced the amplitude and increased the latency of the ABR. These effects were typically significant at an isoflurane concentration of 2%. Effects on peak IV and V were more pronounced compared to the early peaks I and III.

Keywords: Isoflurane; Guinea pig; Compound action potential; Cochlear microphonics; Auditory brainstem response

2.1. Introduction

Experimental electrophysiological recordings of the auditory system are frequently performed under deep anesthesia. General anesthesia is essential when invasive surgery is needed and can be necessary to prevent movement of the animal to minimize artifacts during recordings. Volatile anesthetics such as isoflurane, can be preferable above injection anesthetics since the anesthetic regime can be regulated precisely and adapted swiftly in answer to an altered physiological status of the animal. This can be especially advantageous in sensitive animals such as guinea pigs, which are notoriously difficult for achieving a safe and effective general anesthesia (Wolfensohn and Lloyd, 1994). Anesthetics, including the volatile anesthetics, affect the physiological status of the animal which has to be taken into account when interpreting electrophysiological data in the anesthetized animal.

The acoustically evoked brainstem response (ABR), compound action potential (CAP) and cochlear microphonic (CM) are routinely recorded in anesthetized animals to assess the functionality of the auditory pathway. With

regard to the ABR, general anesthetics such as barbiturates (Shapiro et al., 1984; Drummond et al., 1985; Church and Shucard, 1987), ketamine (Church and Gritzke, 1987) and the halogenated volatiles (Dubois et al., 1982; Sainz et al., 1987; Santarelli et al., 2003) typically increase its latency, especially of later peaks, without affecting the amplitude. Nitrous oxide (Manninenet al., 1985), and the opioids morphine and fentanyl (Samra et al., 1984, 1985) do not affect the ABR. Effects of general anesthetics on the peripheral auditory system are less well characterized and reported effects are variable. Pentobarbital and ketamine have been reported to increase threshold and latency of the CAP at high stimulus frequencies (Cazals et al., 1980). Pentobarbital has also been shown to reduce CM amplitude (Samara and Tonndorf, 1981). In contrast, a later study examining the effect of various anesthetics including pentobarbital and ketamine showed no effects on either CAP amplitude or latency (Brown et al., 1983). The NMDA antagonist 2-amino-5-phosphonovalerate (a ketamine-like compound) has been shown to suppress the amplitude and increase the latency of CAPs, without affecting CM amplitude (Puel et al., 1991). Finally, benzodiazepines were shown to increase CAP amplitude, but decrease the CM (Velluti and Pedemonte, 1986).

This study evaluated the effects of isoflurane on auditory evoked potentials. Isoflurane is a general inhalation anesthetic that induces sedation, hypnosis, immobility and amnesia. Isoflurane has a broad pharmacological profile and affects many neurotransmitter receptor systems including the GABAergic, glycinergic, acetylcholinergic, serotoninergic and glutamatergic system (reviewed by Eger (2004) and Grasshoff et al. (2005)).

Much attention has focussed on the effects of isoflurane on the auditory cortical system and especially the auditory middle latency response (MLR) has received attention. Numerous studies have shown that isoflurane decreases MLR amplitude and increases MLR latency (e.g. Thornton et al., 1992; Schwender et al., 1997; Leistritz et al., 2002). Effects of isoflurane on the auditory brainstem response (ABR) have also been well documented. Several studies on the effects of isoflurane in humans have shown an increased latency of the late ABR peaks. ABR amplitude was unaffected in these studies (Manninen et al., 1985; Sebel et al., 1986; Lloyd-Thomas et al., 1990). In rats isoflurane has been shown to increase the latency of all ABR peaks including peak I (Santarelli et al., 2003). Since the early peak I of the ABR is thought to represent auditory nerve activity (Legatt, 2002), this indirectly indicates that the auditory nerve response is delayed in rats, but not in humans. Effects of isoflurane on the auditory nerve and cochlear responses using direct CAP recordings have not yet been reported. Isoflurane was shown to suppress the amplitude of evoked otoacoustic emissions, indicating an effect on cochlear hair cells (Ferber-Viart et al., 1998).

In this study we examined the effects of various concentrations of the volatile anesthetic isoflurane using electrocochleography and ABR recordings in guinea pigs. We report effects of isoflurane on the amplitude, threshold and latency of the CAP and ABR, and on the amplitude and threshold of the CM.

2.2. Materials and methods

2.2.1. Animals and surgery

Seven healthy female albino guinea pigs (strain: Dunkin Hartley; supplier: Harlan Laboratories) were used that weighed 300-600 g at the time of recording. Surgical procedures on these animals were approved by the Animal Ethical Committee of the Academic Biomedical Centre of the University of Utrecht under numbers 05.02.021 and 2007.I.02.025. Animals were housed according to the standards of the animal care facility of the University of Utrecht.

Animals were equipped with chronically implanted electrodes for electrocochleography and auditory brainstem recordings. Surgical techniques were previously described (Versnel et al., 2007). Briefly, animals were anesthetized with i.m. injections of 40 mg/kg ketamine (Ketanest-S© 25, Pfizer BV) and either 10 mg/kg xylazine (Sedamun, Eurovet) or 0.5 mg/kg medetomidine (Domitor©, Pfizer). Lidocaine was injected s.c. for local anesthesia. A gold ball electrode attached to a Teflon-insulated stainless steel wire served as CAP recording electrode and was positioned in the round-window niche of the right cochlea. For further technical details we refer to Klis et al. (2000, 2002). Three intracranial stainless steel screws served as ABR electrodes and were placed according to Mitchell et al. (1997). In two animals CAPs could not be recorded. In one animal the contact of the round-window electrode was lost, in the other the connector was dysfunctional. One animal was discarded from the ABR dataset due to bradycardia during ABR recording when applying 3% isoflurane.

2.2.2. Experimental design and anesthetic regime

After surgery, animals were allowed to recover for at least a week. Recordings were started in the awake, lightly restrained animal. The animal was then given a single i.m. injection of 0.1 ml/kg Hypnorm® (Vetapharma: 0.315 mg/ml fentanyl + 10 mg/ml fluanisone). Isoflurane anesthesia was induced with 2% isoflurane (Nicholas Piramal Limited) in a mixture of N_2O:O_2= 2:1 using a mouthcap. The animal was tracheostomized and artificially ventilated (Amsterdam infant ventilator mk3, Hoekloos) with isoflurane and N_2O:O_2= 2:1 throughout the experiment. Fresh gas flow was 1.2 L/min. Inspiration rate was 50/min. The tidal volume was 4.5 ml using a 50% inspiration and a peak gas pressure of 22.3 kPa. Core temperature of the animals was maintained at 38 ± 0.5 °C using a rectal probe and thermostatically controlled heating pad. When necessary a heating lamp was used to quickly normalize the temperature. Recordings were interrupted when temperature deviated more than 0.5 °C. Heart rate was continuously monitored during anesthesia on an oscilloscope and was between 240 and 300 bpm. Heart rate tended to decrease from 300 to 360 bpm at 1-2% isoflurane to 240 to 300 bpm at 3% isoflurane. Normative physiological data of

the guinea pig are as follows: respiratory rate: 42-104/min, tidal volume: 2.3-5.3 ml, rectal temperature: 37.2-40 °C, heart rate: 280-380 bpm (Wolfensohn and Lloyd, 1994; Hauser et al., 2005). Different concentrations of isoflurane were tested, starting with the lowest. Isoflurane was supplied using a calibrated isoflurane vaporizer (Ohmeda Isotec 4, Boc Healthcare). After an equilibration period of 15 min, recordings were initiated. Isoflurane was then increased to a higher concentration and recordings were repeated after equilibration.

2.2.3. Stimulus generation and electrophysiology

Stimulus generation and response recording were performed using a Tucker-Davis Technologies laboratory interface (TDT2 and TDT3 system). Tonal stimuli consisted of 8 ms tone bursts with frequencies of 2, 4, 8, 8√2 (11.3) and 16 kHz and were presented with alternating polarity as described previously (Stengs et al., 1997). Clicks consisted of biphasic alternating acoustic pulses (100 ms/phase). Acoustic stimuli were presented using an interstimulus interval of 99 ms. Stimuli were fed to a speaker (Compression Driver, Skytronic) placed 15 cm from the right pinna in an open-field configuration. Tonal sound level was varied from approximately 100 dB SPL (frequency dependent) to below-threshold level in 10-dB steps. Click stimuli were presented from 85 dB above the average threshold of ABR peak IV (107 dB peak-to-peak equivalent SPL) down to threshold in 10-dB steps. Peak IV typically showed the lowest threshold. Sound levels were calibrated using a sound level meter (Brüel & Kjær type 2610) and a ¼'' condenser microphone (Brüel & Kjær type 4136).

CAP and ABR recordings were performed as described previously (Versnel et al., 2007). Briefly, signals were differentially amplified (EG&G instruments model 5113) with a gain of 5000, band-pass filtered at 1 Hz30 kHz (CAP and CM) or 100 Hz-10 kHz (ABR) and recorded at a sample rate of 50 or 100 kHz. Signals were averaged to a maximum of 250 sweeps (CAP) or 500 (ABR) per stimulus polarity. CM and CAP were mathematically separated as described previously (Stengs et al., 1997). Data acquisition and analysis software were custom made in a Delphi (Borland) and Matlab© (The Mathworks™) programming environment, respectively.

2.2.4. Data analysis and statistics

CAP waveforms were analyzed by determining the amplitude and latency of the first and second negative peak (N_1 and N_2). Amplitudes were expressed relative to the summating potential (SP) as shown in Fig. 2.1. CM amplitude was obtained by fast Fourier transformation (FFT) using a window from stimulus onset to 2 ms after offset. The amplitude of the frequency component corresponding to the tonal stimulus in the frequency spectrum was determined. ABR recordings were analyzed by determining the amplitude and latency of four different peaks (P_I,

P_{III}, P_{IV} and P_V) in the waveform. Peak II was absent in some animals or showed high thresholds and was therefore not analyzed. In some animals peaks II and III were difficult to discern. In these cases the highest peak of these two was analyzed as being peak III. ABR peak amplitudes were determined relative to the preceding or following negative peak as shown in Fig. 2.4. Threshold response levels were defined as the interpolated sound level at which the amplitude was 3 mV (CAP), 0.25 mV (CM), or 0.5 mV (ABR).

Effects of isoflurane on the different variables were statistically analyzed using repeated measures analysis of variance (RM ANOVA) with Dunnett's post hoc test, using GraphPad Prism 5.01 (GraphPad Software). Amplitude data were logarithmically transformed for statistical analysis. Isoflurane concentration was treated as within factor.

2.3. Results

2.3.1. Effects of isoflurane on CAP and CM in individual animals

CAP and CM data were obtained in five animals. Fig. 2.1 shows CAP recordings at 11.3 kHz in a representative animal in the awake condition and when artificially ventilated with 3% isoflurane. Isoflurane clearly decreased CAP amplitudes. Fig. 2.2 displays the effects of isoflurane on CAP and CM in individual animals to illustrate inter-animal variability. Cochlear responses were evoked with 11.3 kHz tones of 63 dB SPL and are shown as a function of isoflurane concentration. In four out of five animals a dose-dependent (monotonic) suppression of the CAP amplitude was observed. All animals showed CAP suppression at 2.5% and 3% isoflurane. CAP suppression was accompanied by an increased CAP latency. Effects on CM amplitude were more variable. At 2.5% and 3% CM amplitude was invariably decreased relative to the awake condition in all animals.

2.3.2. Averaged effects of isoflurane on CAP and CM amplitude

Fig. 2.3 shows the averaged CAP and CM growth functions with stimulus level at three stimulus frequencies in the awake condition and when 2% and 3% isoflurane was applied. At a low frequency of 2 kHz no effect on N_1 amplitude was seen, while the amplitudes of N_2 and CM were marginally decreased at the highest dose of isoflurane (3%). At high tonal frequencies, effects of isoflurane were more pronounced and at 11.3 and 16 kHz a dose-dependent decrease of the N_1, N_2 and CM amplitude was observed. A small tendency toward larger effects on N_1 amplitude at low sound levels was present, but effects on N_2 or CM were similar between sound levels. The observed effects thus appeared mainly dependent on the isoflurane concentration and stimulus frequency. We statistically tested this observation using RM ANOVA and Dunnett's post hoc test ($\alpha = 0.05$) at

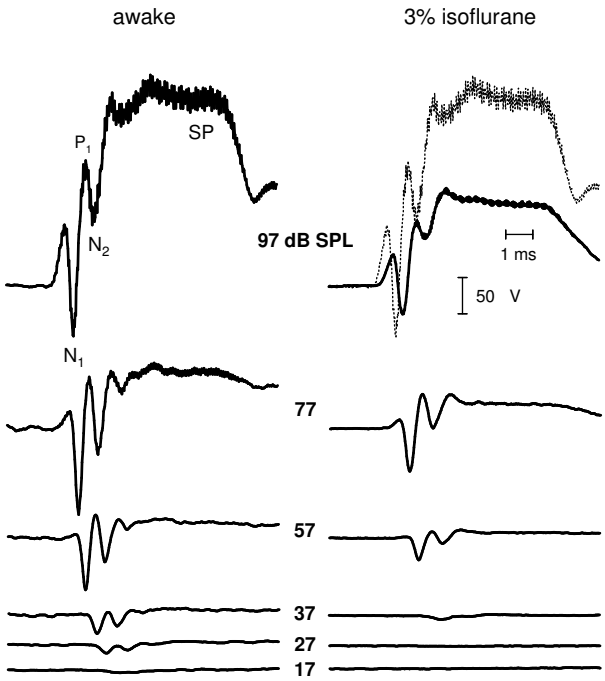

Fig. 2.1. Example compound action potential (CAP) recordings evoked at 11.3 kHz at different sound levels. The left panel shows CAPs evoked in the awake animal, the right panel shows recordings in the same animal when ventilated with 3% isoflurane. At 100 dB SPL the CAP in the awake condition is also shown by a dotted line aligned with the CAP under 3% isoflurane. The start of the traces corresponds to stimulus onset. CAP amplitude was defined as the difference between the first or second negative peak (N_1 or N_2) and summating potential (SP).

each individual stimulus frequency. Analysis was carried out per frequency at a moderate sound level of around 60 dB SPL (frequency dependent) with the concentration of isoflurane as within factor. A concentration of 1% isoflurane was tested in three out of five animals. The amplitude of N_1 was significantly affected at high stimulus frequencies of 11.3 kHz (at 2.5-3% isoflurane) and 16 kHz (at 3% isoflurane). The amplitude of N_2 was significantly reduced at 8 and 11.3 kHz (2-3% isoflurane) and at 16 kHz (2.5-3% isoflurane). Hence, the effects on N_2 were present at an extended range of stimulus frequencies and were statistically significant at lower isoflurane concentrations. The effects of isoflurane on the amplitude of N_2 were also larger compared to N_1 (Table 2.1). Effects on CM amplitude were comparable to effects on N_1. CM amplitude was significantly reduced at 8 and 16 kHz (2.5-3% isoflurane), but not at 11.3 kHz. The CAP and CM thresholds, corresponding to the sound level at an iso-response level of 3 and 0.25 mV, respectively (the horizontal axis intercepts in Fig. 2.3), were also statistically analyzed. Effects on CAP and CM thresholds were comparable to the

effects on amplitude at 60 dB SPL. N_1 threshold was significantly increased at 8 kHz (3% isoflurane) and 16 kHz (2.5-3% isoflurane). N_2 threshold was increased at 8-16 kHz (2.5-3% isoflurane) and the CM threshold was increased at 11.3 and 16 kHz (2.5-3% isoflurane). Table 2.1 shows a summary of the effects of isoflurane on amplitude and threshold of the CAP and CM at a stimulus frequency of 11.3 kHz. The table clearly shows that isoflurane affects the CAP in a dose-dependent manner. Effects on CM were more variable and were not significant in any of the conditions tested at 11.3 kHz.

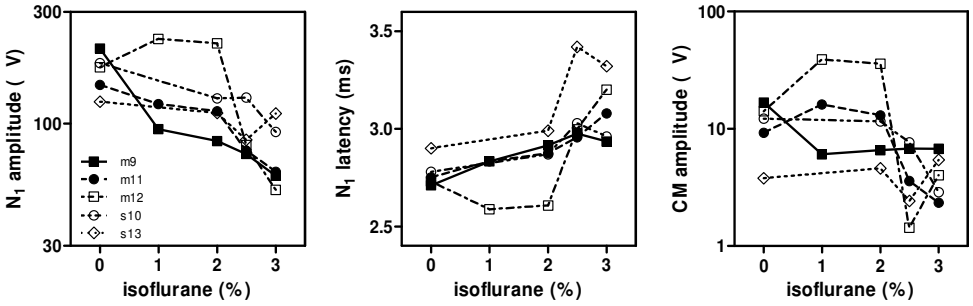

Fig. 2.2. Effects of different concentrations of isoflurane on the amplitude of the first negative (N_1) peak of the CAP (A), N_1 latency (B) and CM amplitude (C) in individual animals (n = 5). Isoflurane at 1% was tested in only three animals. Cochlear potentials were evoked with 11.3 kHz tones of 63 dB SPL. Increasing concentrations of isoflurane were recorded sequentially.

We have determined CAP amplitude relative to SP in order to isolate the CAP response from the hair cell response. In principle, these responses are superimposed. The CAP amplitude can also be reliably determined relative to the first positive peak (P_1) in the CAP signal (e.g. Charlet de Sauvage et al., 1996) or by removing the SP from the CAP response by more stringent high-pass filtering (e.g. Puel et al., 1991). We have additionally analyzed the N_1 and N_2 amplitude and threshold data relative to P_1 (Fig. 2.1) to compare our results with those using other analysis methods (results not shown). Absolute CAP amplitudes and thresholds were comparable and more importantly, the overall effect of isoflurane was very similar between the two methods. For example, effects of isoflurane on N_1 and N_2 amplitude increased with tone frequency, and effects on N_2 were typically larger and significant at lower isoflurane concentrations compared to N_1.

2.3.3. Averaged effects of isoflurane on CAP latency

Fig. 2.4 shows the effects of isoflurane concentration on CAP latency as a function of sound level at three stimulus frequencies. The effects of isoflurane on CAP latency resembled those on the amplitude. Latencies tended to increase with

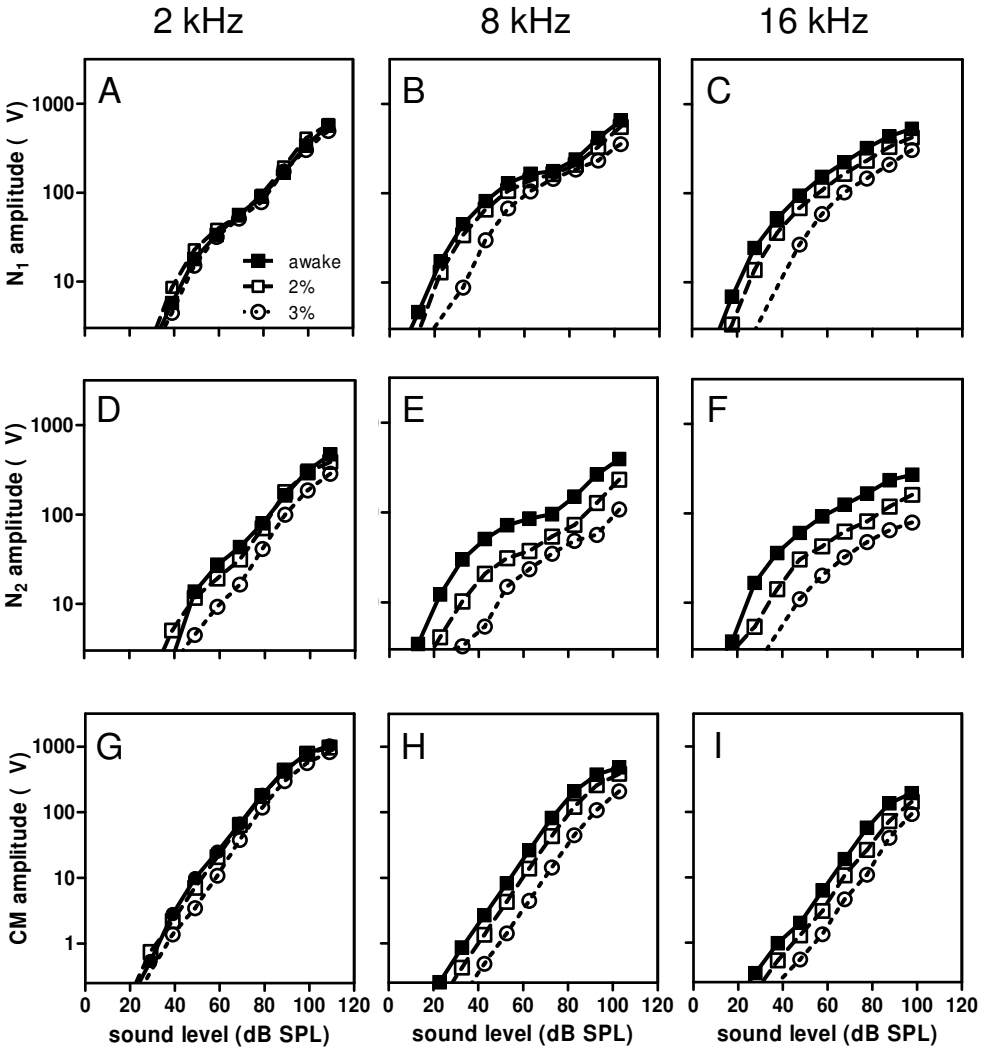

Fig. 2.3. Averaged (n = 5) amplitude as function of sound level of the first (AC) and second negative peak of the CAP (DF) and CM (GI). Responses were evoked with tones of 2 kHz, 8 kHz and 16 kHz tones in the awake animal, and when 2% and 3% isoflurane was applied.

isoflurane concentration and stimulus frequency. At 2 kHz the N_1 latency was unaffected, while the N_2 latency was increased only at 3% isoflurane. At 8 and 16 kHz, both the N_1 and N_2 latency increased dose-dependently. Statistical analysis was carried out for each frequency similarly to the analysis of the amplitude data (i.e. RM ANOVA per frequency at around 60 dB SPL). N_1 latency was significantly increased at 4, 8 and 16 kHz (2.5-3% isoflurane). Effects on N_2 latency were larger and present at all frequencies tested. Effects were significant at

2.5-3% isoflurane, except at 16 kHz where the effects were significant only at 3% isoflurane. Table 2.2 shows a summary of the effects of isoflurane on CAP latency at a stimulus frequency of 11.3 kHz.

Table 2.1

Effects of isoflurane on CAP and CM amplitude and threshold at a stimulus frequency of 11.3 kHz.

Isoflurane (%)	**N_1**				**N_2**				**CM**			
	Amp (%)	*P*	Thr (dB)	*P*	Amp (%)	*P*	Thr (dB)	*P*	Amp (%)	*P*	Thr (dB)	*P*
2	-19	ns	-0.2	ns	-47	**	+2	ns	+30	ns	+1	ns
2.5	-45	*	+10	ns	-65	***	+14	**	-57	ns	+10	ns
3	-52	**	+14	*	-68	***	+17	**	-48	ns	+9	ns

*Average percentage difference in absolute amplitude (amp) of the first and second negative peak (N_1 and N_2) of the compound action potential (CAP) and of the cochlear microphonic (CM) evoked with 11.3 kHz tones at 57 dB SPL. Threshold (Thr) was defined as the sound level that evoked a response of 3 mV (CAP) or 0.25 mV (CM). Significance levels (P) of the logarithmically transformed amplitude data and absolute thresholds were determined relative to the awake condition using repeated measures ANOVA and Dunnett's post hoc test (n = 5). *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant.*

2.3.4. Effects of isoflurane on ABR in individual animals

ABR data were obtained in seven animals, five of which were also used for CAP and CM recordings. Two additional animals were included in which the cochlear electrode was malfunctioning, but from which ABRs were recordable. Fig. 2.5 shows example recordings of click-evoked ABRs in a representative animal (the same animal as used for Fig. 2.1) in the awake condition and when ventilated with 3% isoflurane. The recordings clearly show decreased amplitudes and an altered morphology of the ABR when isoflurane was applied. Fig. 2.6 displays the effects in individual animals on the amplitude and latency of peak IV of the ABR evoked with 67 dB peSPL clicks, as a function of isoflurane concentration. One animal shown (m12) was discarded from statistical analysis (see next sections) due to a large drop in heart rate during ABR recordings at 3% isoflurane (marked with an asterisk). In all animals a suppression of the ABR amplitude was observed which was accompanied by a dose-dependent increase in latency. In four animals amplitude effects at 1%, 2% and 2.5% isoflurane appeared similar. Latency increases in these animals seemed however dose-dependent.

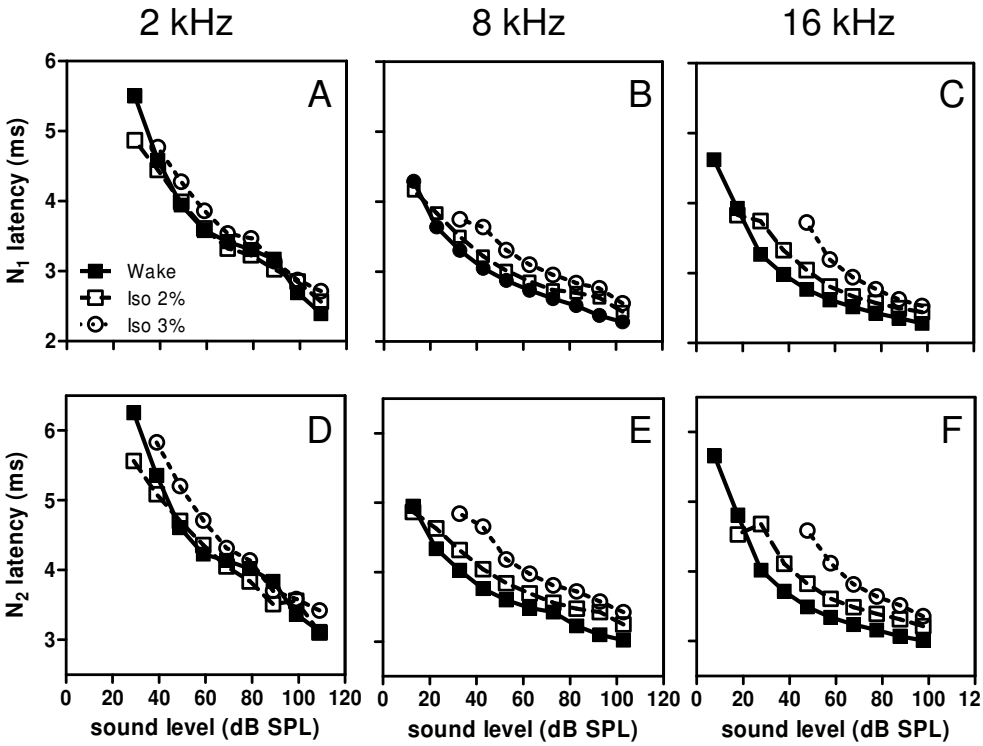

Fig. 2.4. Averaged (n = 5) CAP latency of N_1 (AC) and N_2 (DF) as function of sound level. CAPs were evoked with 2 kHz, 8 kHz and 16 kHz tones in the awake animal, and when 2% and 3% isoflurane was applied.

Table 2.2

Effects of isoflurane on CAP latency at a stimulus frequency of 11.3 kHz.
Isoflurane (%)

Isoflurane (%)	**N_1**		**N_2**	
	Latency (ms)	*P*	Latency (ms)	*P*
2	+0.1	ns	+0.1	ns
2.5	+0.3	**	+0.4	**
3	+0.3	**	+0.4	**

*Average latency difference of the first and second negative peak (N_1 and N_2) of the compound action potential (CAP) evoked with 11.3 kHz tones at 57 dB SPL. Significance levels (P) were determined relative to the awake condition with repeated measures ANOVA and Dunnett's post hoc test (n = 5). **P < 0.01; ns, not significant.*

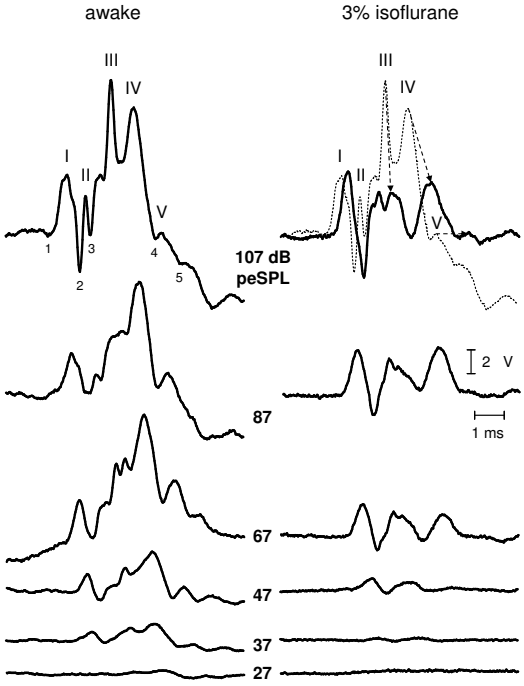

Fig. 2.5. Example auditory brainstem (ABR) recordings at different sound levels. The left panel shows waveforms in the awake animal, the right panel shows recordings in the same animal when ventilated with 3% isoflurane. At 107 dB SPL the ABR in the awake condition is also shown by a dotted line aligned with the ABR under 3% isoflurane. Arrows illustrate the latency shift of peaks III, IV and V. The start of the traces corresponds to stimulus onset. The four analyzed positive peaks are indicated (I, III, IV and V). The amplitude of peak I was determined relative to baseline (1) and the amplitude of later peaks were determined using an adjacent negative peak: 3 for peak III, 4 for peak IV and 5 for peak V.

2.3.5. Averaged effects of isoflurane on ABR amplitude

Fig. 2.7 shows the growth functions with stimulus level of the four analyzed peaks which were easily discernable in all animals (P_I and P_{III}-P_V). Growth functions are shown in the awake state, and when the animals were ventilated with 2% and 3% isoflurane. Visual inspection appears to show that effects of isoflurane on the first peak (P_I) are negligible, while the third peak (P_{III}) is decreased to a small extent. Amplitudes of the fourth (P_{IV}) and fifth peak (P_V) are clearly decreased in a dose-dependent manner, while effects of sound level appear small. Statistical analysis of the peak amplitudes was therefore carried out at one (moderate) stimulus level of 67 dB peak-to-peak equivalent SPL (67 dB peSPL). Analysis consisted of RM

ANOVA with the concentration of isoflurane as within factor followed by Dunnett's post hoc test ($\alpha = 0.05$). A concentration of 1% isoflurane was investigated in only four out of six animals. Each ABR peak was analyzed separately. Sound level thresholds, corresponding to the sound level at an iso-response level of 1 mV (the horizontal axis intercepts in Fig. 2.5), were also statistically analyzed. The amplitude of peaks I and III were not significantly affected by isoflurane, while those of P_{IV} and P_V were significantly decreased at 2-3% isoflurane. Thresholds of P_{III}-P_V were significantly increased. Later peaks showed significantly increased thresholds at lower isoflurane concentrations: P_{III} only at 3%, P_{IV} from 2.5% and P_V from 2%. Table 2.3 shows a summary of the effects of isoflurane on amplitude and threshold of P_{III}-P_V. The table reveals that effects of isoflurane increased with concentration and ABR peak number.

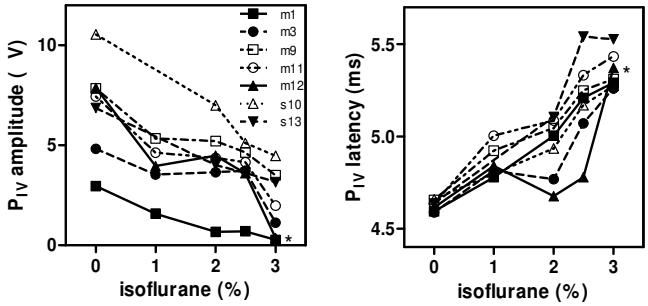

Fig. 2.6. Effects of different concentrations of isoflurane on the amplitude (A) and latency (B) of peak IV of the ABR in individual animals (n = 7). Isoflurane at 1% was tested in five animals. Cochlear potentials were evoked with acoustic clicks of 67 dB peSPL. Increasing concentrations of isoflurane were recorded sequentially. Animal m12 was discarded from the dataset due to bradycardia during the recording at 3%, which was accompanied by a sharp decline in amplitude and increase in latency of peak IV (asterisks).

The absence of effects on P_I was unexpected, since P_I represents the auditory nerve response (i.e. the CAP) and high-frequency-evoked CAPs showed significantly reduced amplitudes. To compare click-evoked CAP responses with P_I of click-evoked ABRs we additionally recorded click-evoked CAPs in three animals (results not shown). We compared the CAP amplitude in the awake condition with the amplitude when animals were ventilated with 3% isoflurane. We found an average isoflurane-induced reduction of 23% and 60% of the N_1 and N_2 amplitude at 67 dB peSPL, respectively. The reduction of the P_I ABR amplitude averaged across six animals under these conditions was 19%, but was not significant. The slightly smaller P_I reduction relative to N_1 was possibly caused by the fact that CAP amplitudes are larger and provide a more robust measure for the auditory nerve activity compared to P_I of the ABR. The finding that isoflurane had only moderate and insignificant effects on click-evoked P_I and N_1 amplitude (20%),

while high-frequency-evoked CAPs were significantly reduced (50% at 12 kHz, Table 2.1) can possibly be explained by the fact that clicks instead of tonal stimuli were used for ABR measurements. Clicks have a broad frequency content and effects of isoflurane on the CAP increased with stimulus frequency. Therefore the effects on click-evoked auditory nerve activity will have been moderate, being the average across large effects on high-frequency evoked responses and small effects on low-frequency evoked responses.

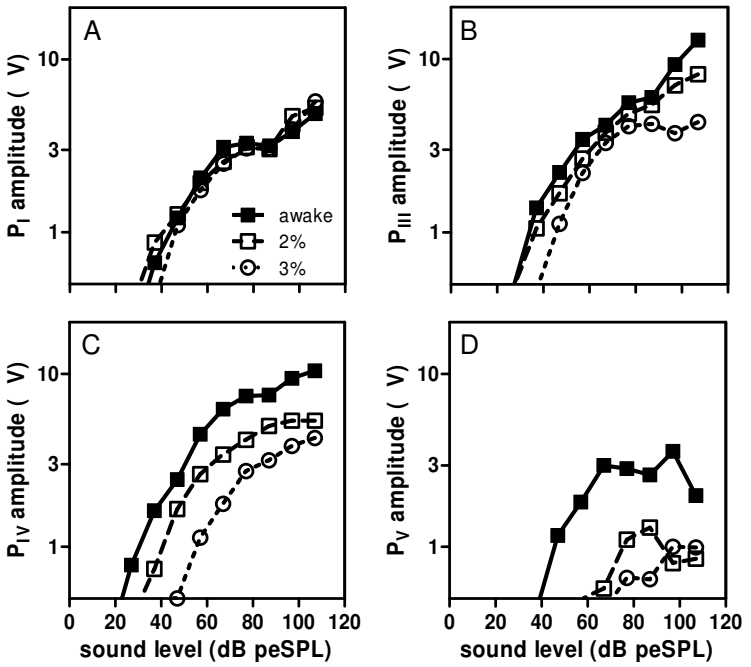

Fig. 2.7. Averaged (n = 6) amplitude of the four analyzed click-evoked ABR peaks P_I (A), P_{III} (B), P_{IV} (C) and P_V (D) as function of sound level. ABRs were recorded in the awake animal, and when 2% and 3% isoflurane was applied.

2.3.6. Averaged effects of isoflurane on ABR peak latency

Fig. 2.8 shows the effects of isoflurane concentration on the latency of the four analyzed ABR peaks as a function of sound level. Visual inspection shows that the effects of isoflurane on latency resemble those on the amplitude. Isoflurane's effects increased with ABR peak number and were small on P_I and most pronounced on P_V. Latency increases were dose-dependent. Statistical analysis was carried out similarly to the analysis of the amplitude data (i.e. RM ANOVA per frequency at 67 dB peSPL). The latency increase of P_I was small, but statistically

significant at all isoflurane concentrations. Effects on P_{III} latency were larger, but insignificant. Effects on P_{IV} were statistically significant at all isoflurane concentrations, while P_V showed a significant latency increase at 2.5% and 3%. Latency increases were larger at later peaks. Statistical analysis of interpeak latencies relative to P_I confirmed these findings. The interpeak latency of P_{III} to P_I (P_{III}-P_I) was not significantly affected, while P_{IV}-P_I and P_V-P_I increased significantly in a dose-dependent manner. P_{IV}-P_I increased with 0.5 ms and P_V-P_I with 1.2 ms at 3% isoflurane, respectively (results not shown). Table 2.4 shows a summary of the effects of isoflurane on the latency of ABR peaks III, IV and V. The table reveals a dose-dependent latency increase at peaks IV and V.

Table 2.3

Effects of isoflurane on the amplitude and threshold of ABR peaks P_{III}, P_{IV} and P_V.

Isoflurane (%)	**P_{III}**				**P_{IV}**				**P_V**			
	Amp (%)	*P*	Thr (dB)	*P*	Amp (%)	*P*	Thr (dB)	*P*	Amp (%)	*P*	Thr (dB)	*P*
2	-3	ns	+0.4	ns	-42	**	+9	ns	-76	***	+19	**
3.5	-5	ns	+4	ns	-47	***	+15	**	-85	***	+30	***
3	-12	ns	+11	***	-68	***	+22	***	-86	***	+32	***

*Average percentage difference in absolute amplitude (amp) of the third, fourth and fifth peak (P_{III}, P_{IV} and P_V) of the auditory brainstem response (ABR) evoked with 67 dB peSPL clicks. Threshold was defined as that sound level that evoked a peak amplitude of 1 mV. Significance levels (P) of the logarithmically transformed amplitude data and absolute thresholds were determined relative to the awake condition using repeated measures ANOVA and Dunnett's post hoc test (n = 5). **P < 0.01; ***P < 0.001; ns, not significant.*

2.4. Discussion

We have shown that isoflurane affects the auditory system from periphery (CAP, CM) to brainstem (ABR) in the guinea pig. On average, isoflurane dose-dependently suppressed the amplitude and increased the threshold of the CAP. Effects on CM amplitude were more variable, but at high concentrations CM was invariably suppressed. Isoflurane dose-dependently increased CAP latency. Effects were most pronounced at high frequencies (Figs. 2.3 and 2.4) and were typically significant at 2.5-3% isoflurane. Amplitude and latency of N_2 were affected to a greater extent compared to N_1, present at a wider frequency range and amplitudes were significantly decreased at lower isoflurane concentrations (2%). On average, isoflurane dose-dependently suppressed ABR amplitude, increased ABR threshold and increased the ABR latency especially of the later peaks (Figs. 2.7 and 2.8). Effects typically reached significance at 2% isoflurane.

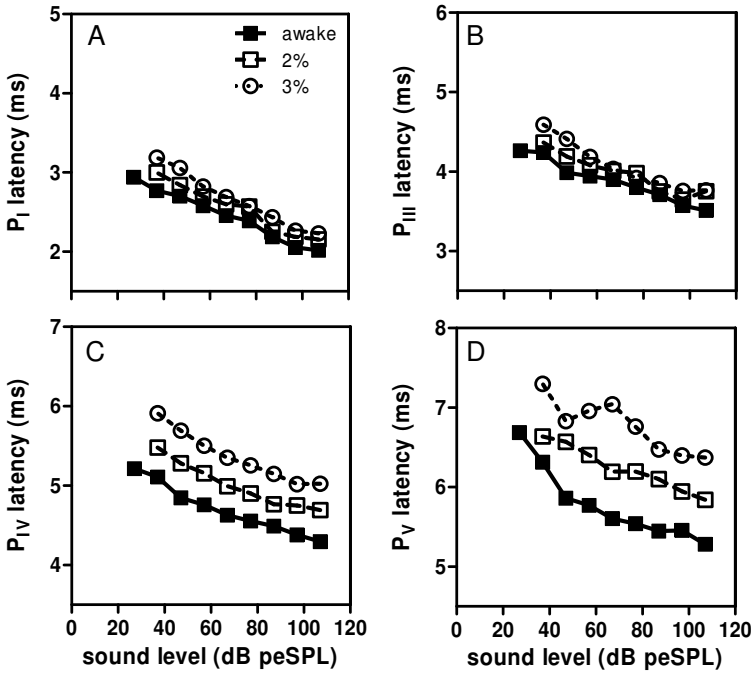

Fig. 2.8. Averaged (n = 6) latency of the four analyzed click-evoked ABR peaks P_I (A), P_{III} (B), P_{IV} (C) and P_V (D) as function of sound level. ABRs were recorded in the awake animal and when 2% and 3% isoflurane was applied.

The concentrations of isoflurane tested were 1-3%, corresponding to a minimum alveolar concentration (MAC) of 0.9-2.6 in the guinea pig (Seifen et al., 1989). Isoflurane concentrations were set with a calibrated vaporizer. End-tidal isoflurane concentrations were however not determined and actual isoflurane concentrations that the animal received could have deviated from the given concentration. Significance was typically reached at an isoflurane concentration of 2.5% for CAP parameters (MAC 2.2) and at 2% for the ABR parameters (MAC 1.7). Long-term experiments (8-20 h) in our lab requiring anesthesia are typically performed using isoflurane concentrations of 1-2% (MAC 0.9-1.7) in nitrous oxide and oxygen (N_2O:O_2= 2:1). Under these conditions effects on CAP and CM were typically insignificant. ABR amplitude of P_{IV} under 1-2% isoflurane anesthesia was invariably suppressed (up to 80% in one animal) and P_{IV} latency was increased up to 0.5 ms (Fig. 2.6).

Effects of isoflurane on the CAP and CM have not been reported before. Isoflurane has been shown to suppress the amplitude of evoked otoacoustic emissions (Ferber-Viart et al., 1998), which is in agreement with the suppressive effect on the CM found in this study. Isoflurane's effects on the ABR have been

reported previously and the present results are for the most part consistent with these reports, but there are some differences. Isoflurane increased latency of ABR peaks, but had no significant effects on ABR amplitude in humans (Manninen et al., 1985; Sebel et al., 1986) and rats (Santarelli et al., 2003) at concentrations up to 2%. We have shown a clear effect of isoflurane on amplitude, threshold and latency of ABR peaks IV and V at 1% and 2% isoflurane in guinea pigs.

Table 2.4

Effects of isoflurane on the latency of ABR peaks P_{III}, P_{IV} and P_V.

Isoflurane (%)	**P_{III}**		**P_{IV}**		**P_V**	
	Latency (ms)	*P*	Latency (ms)	*P*	Latency (ms)	*P*
2	0.1	ns	0.4	***	0.6	ns
2.5	0.1	ns	0.6	***	1.1	*
3	0.1	ns	0.7	***	1.4	**

*Average latency difference of the third, fourth and fifth peak (P_{III}, P_{IV} and P_V) of the auditory brainstem response (ABR) evoked with 67 dB peSPL clicks. Significance levels (P) were determined relative to the awake condition with repeated measures ANOVA and Dunnett's post hoc test (n = 6). *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant.*

Isoflurane has multiple mechanisms of action on the nervous system that can explain its broad spectrum of effects observed in this study. Isoflurane for example suppresses excitatory glutamatergic transmission via pre- and postsynaptic mechanisms (MacIver et al., 1996; Nishikawa and MacIver, 2000), enhances inhibitory GABAergic transmission (Verbny et al., 2005; Jia et al., 2008) and hyperpolarizes neurons by an increase in K^+ leak conductance (Berg-Johnsen and Langmoen, 1990; Ries and Puil, 1999). Isoflurane can hypothetically suppress the CAP by inhibiting glutamatergic synaptic transmission between hair cells and spiral ganglion cells, or by increasing efferent GABAergic input (Puel, 1995). Effects increased with frequency, indicating that isoflurane differentially affects signal transduction along the cochlea. Suppressive effects on N_2 were larger than on N_1. The N_1 represents the synchronized firing of auditory nerve fibers. The origin of N_2 is less well understood, but it can reflect secondary firing of auditory nerve fibers (Teas et al., 1962), or firing of neurons in the cochlear nucleus (Moller, 1983). Suppression of the N_2 could therefore be due to increased recovery times of nerve fibers, or suppressive effects on the cochlear nucleus. Effects on the CM could be mediated by isoflurane's hyperpolarizing effects. The observed frequency-specific effect of isoflurane on the CM is not readily explained, since the CM response is determined mostly by OHCs near the recording electrode (Tasaki and Fernandez, 1952; Dallos et al., 1972). Hence, isoflurane apparently differentially affected basal OHCs dependent on stimulus frequency. One possible explanation

could be that isoflurane affects the kinetics of transduction channels in OHCs. Suppressive effects on the ABR can be due to for example suppressed glutamate transmission and increased GABAergic neurotransmission, which represent the principal excitatory and inhibitory transmitter systems in the central nervous system, respectively.

A number of factors could have influenced our results. First, a possible time-dependent effect of isoflurane could have played a role, inherent to a repeated measures design. We recorded every animal starting with the lowest concentration and ending with the highest concentration. A typical experiment lasted approximately 3 h. Hence a cumulative effect of isoflurane independent of its concentration could have played a role. However, in experiments other than reported here in which we kept guinea pigs for prolonged periods (up to 20 h) under isoflurane anesthesia (1-2%), we found no evidence for cumulative depressant effects of isoflurane on the CAP (unpublished results). In humans it has been shown that ABRs remain constant during prolonged isoflurane anesthesia (Lloyd-Thomas et al., 1990).

Anesthesia decreases body and cochlear temperature. Rectal temperature of our animals was kept at 38 ± 0.5 °C, but cochlear temperature was not monitored separately. A body or cochlear temperature change of ±0.5 °C in guinea pigs affects CAP and CM amplitude by less than 10% and CAP latency by 0.03 ms in guinea pigs (Charlet de Sauvage et al., 1996). ABR latency is affected by no more than 3% after a change of ±0.5 °C in body temperature (Marsh et al., 1984), which corresponds to <0.2 ms in P_{IV} latency at 67 dB peSPL (Table 2.4). ABR amplitudes after changes in body temperature are variable (Williston and Jewett, 1982; Marsh et al., 1984). We found CAP (N_1) and CM amplitude changes up to 52% accompanied by a large N_1 latency increase of 0.3 ms (Tables 2.1 and 2.2). ABR latency was increased up to 0.7 ms (Table 2.4). Therefore we conclude that temperature differences in our animals cannot have played an important role in the observed effects of isoflurane on CAP, CM and ABR.

Anesthetics also affect heart rate. Heart rate was monitored and tended to decrease from 300-360 bpm at 1-2% isoflurane to 240-300 bpm under 3% isoflurane anesthesia. This in turn could have affected arterial pressure, blood oxygenation levels and partial CO_2 pressure in the blood. Furthermore, halogenated inhalants and nitrous oxide have been shown to diffuse into the middle ear (Perreault et al., 1982; Chinn et al., 1997; Doyle and Banks, 2003; Ozturk et al., 2006, 2007) which can have resulted in altered middle ear mechanics. We cannot exclude that an altered middle ear status and changes in physiological parameters affected our recordings. However, effects of isoflurane anesthesia on the CAP and CM were typically significant only at high frequencies, whereas modified middle ear characteristics and an altered physiological status of the animal would likely affect CAPs and CM evoked at both high and low frequencies. Hence, the effects reported here were likely caused by direct effects of isoflurane on hair cells and auditory nerve. Nevertheless, indirect effects on

cochlear and brain stem responses due to changes in physiology of the animal are probably inherent to isoflurane anesthesia and have to be taken into account when isoflurane is used as anesthetic during electrophysiological recordings on the auditory system.

Last, effects of the "background" anesthesia of Hypnorm® and nitrous oxide could have played a role. Hypnorm® consists of a neuroleptanalgesic combination of the short-acting opioid fentanyl and the dopamine antagonist fluanisone. Fentanyl has no effect on the CAP (Sahley et al., 1991) or ABR (Samra et al., 1984), but data on the effects of fentanyl on CM are not available. Also, data on the effects of fluanisone on CAP, CM or ABR are not available. Nitrous oxide does not affect the ABR (Manninen et al., 1985), but effects on CAP or CM were never investigated. Whatever the effect (of the combination) of these compounds has been, on average isoflurane dose-dependently affected the CAP, CM and ABR. This is a strong indication that the effects we found were related to isoflurane and not to the other compounds. However, possible additive or synergistic effects of background anesthesia on the observed effects of isoflurane cannot be ruled out.

The effects of isoflurane on the ABR are generally considered too small to be of use as an indicator for anesthetic depth (Sebel et al., 1986). The auditory middle latency response (MLR) has been proposed as an indicator of anesthetic depth (Thornton et al., 1989; Schwender et al., 1997; Sharpe et al., 1997). MLR peaks are however not as well defined compared to the ABR and myogenic potentials can interfere with the MLR limiting its usefulness (Sebel et al., 1986). Prediction of anesthetic depth based on the MLR recording indeed is challenging (Kochs et al., 1999) and computerized analyses seem necessary (Dutton et al., 1999; Kochs et al., 2001; Leistritz et al., 2002). We have shown that conventional ABR recordings yield significant, dose-dependent effects on latency and amplitude of the ABR at isoflurane concentrations of 1-3%. Guinea pigs however seem especially sensitive to isoflurane compared to other volatile anesthetics (Seifen et al., 1989). This could explain the large effects on ABR amplitude in this study. Other studies in humans and rats have not shown significant effects of isoflurane on ABR amplitude using isoflurane concentrations up to 2% (Manninen et al., 1985; Sebel et al., 1986; Lloyd-Thomas et al., 1990; Santarelli et al., 2003). Thus ABRs could be considered as an indicator of anesthetic depth for isoflurane in guinea pigs, but probably not for humans.

The findings that isoflurane suppressed the amplitude of the CAP, CM and ABR, and increased the latency of the CAP and ABR have to be taken into account when these potentials are recorded in isoflurane-anesthetized animals such as guinea pigs. In fundamental studies on the auditory pathway, effects of anesthesia are often not taken into account. Our results show that with modern anesthetics like isoflurane, this position requires reconsideration.

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Abbreviations

ABR:	Auditory brainstem response
bpm:	beats per minute (heart rate)
CAP:	Compound action potential
CM:	Cochlear microphonic
MAC:	Minimum alveolar concentration
N_x:	Negative peak (x= 1 or 2) of the CAP
peSPL:	Peak-to-peak equivalent sound pressure level
P_y:	Positive peak (y= I–V) of the ABR

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Suppression of the acoustically evoked auditory-nerve response by electrical stimulation in the cochlea of the guinea pig

3

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Abstract

There is increasing interest in the use of electro-acoustical stimulation in people with a cochlear implant that have residual low-frequency hearing in the implanted ear. This raises the issue of how electrical and acoustical stimulation interact in the cochlea. We have investigated the effect of electrical stimulation on the acoustically evoked compound action potential (CAP) in normal-hearing guinea pigs. CAPs were evoked by tone bursts, and electric stimuli were delivered at the base of the cochlea using extracochlear electrodes. CAPs could be suppressed by electrical stimulation under various conditions. The dependence of CAP suppression on several parameters was investigated, including frequency and level of the acoustic stimulus, current level of the electric stimulus and the interval between electric and acoustic stimulus (EAI). Most pronounced suppression was observed when CAPs were evoked with high-frequency tones of low level. Suppression increased with current level and at high currents low-frequency evoked CAPs could also be suppressed. Suppression was typically absent several milliseconds after the electric stimulus. Suppression mediated by direct neural responses and hair cell mediated (electrophonic) responses is discussed. We conclude that the high-frequency part of the cochlea can be stimulated electrically with little detrimental effects on CAPs evoked by low-frequency tones.

Keywords: Electro-acoustical stimulation; Electrocochleography; Compound action potential; Cochlear implant

3.1. Introduction

Cochlear implantation is currently the method of choice for treatment of severe to profound sensorineural hearing loss.Ongoing improvement of the performance of cochlear implants (CIs) has led to a relaxation of the clinical criteria for candidacy for implantation (Lorens et al., 2008). Nowadays, patients with considerable residual low-frequency hearing are implanted (Wilson et al., 2003; Gantz et al., 2005). Clinical studies have indicated that it is possible to take advantage of residual hearing after implantation. While the use of a hearing aid in the non-implanted ear can be advantageous for speech and melody recognition, and for sound localization (Dooley et al., 1993; Kong et al., 2005; Ching et al., 2006; Mok et al., 2006; Novak et al., 2007), most research has focussed on electro-acoustical stimulation (EAS) by means of hybrid implants. Hybrid implants combine a CI with a conventional hearing aid in the same ear. EAS can improve speech understanding in noise and can increase the aesthetic quality of sound (Von Ilberg et al., 1999; Fraysse et al., 2006; Gstoettner et al., 2008;

Turner et al., 2008).

To preserve residual hearing, surgical techniques have been adapted to minimize surgical trauma during implantation (Adunka et al., 2004; Gantz et al., 2005). In addition, short electrode arrays have been developed that do not penetrate the acoustically sensitive apical parts of the cochlea (Gantz and Turner, 2003; Gstoettner et al., 2004). Besides minimizing insertion trauma, short electrode arrays can decrease the interaction between electrical and acoustical stimulation by spatially segregating both stimulus modalities.

It seems a reasonable assumption that the beneficial effect of residual hearing in patients using EAS is optimal when electrical stimulation does not interfere with the acoustically evoked responses. Electro-acoustic interaction might be a factor contributing to the variability in performance of patients using EAS (Luetje et al., 2007; Turner et al., 2008) and may be a contributing factor to the occasionally observed detrimental effect of residual hearing on speech understanding (Gstoettner et al., 2008). Recent research on electro-acoustic interaction by Abbas and co-workers has focussed on the (suppressive) effects of acoustical stimulation on electrically evoked activity in the auditory nerve (Hu et al., 2003; Nourski et al., 2005, 2007; Miller et al., 2006). These studies have shown that remaining hair cell activity can suppress electrically evoked auditory-nerve activity. We have focussed on the effect of electrical stimulation on the acoustically evoked compound action potential (CAP) and cochlear microphonic (CM). Early reports demonstrated that direct current can either facilitate or suppress the acoustically evoked CAP and CM response in guinea pigs, dependent on the polarity of the current (Tasaki and Fernandez, 1951, 1952). Teas et al. (1970) subsequently showed that tone-evoked single-fiber discharges increased or decreased dependent on the phase of the applied 530 Hz sinusoidal current.Ball (1982) reported a potentiation of the CAP after 510 min of electrical stimulation at 100 Hz using biphasic pulses with 500 μs pulse width. Subsequent studies have shown that acoustic CAPs could be suppressed by electric stimulation, an effect attributed to electro-mechanical transduction mechanisms (McAnally et al., 1993, 1997a,b; Kirk and Yates, 1994; McAnally and Clark, 1994).

The major goal of this study was to investigate the extent to which tone-evoked CAPs are affected by electrical stimuli applied in present day cochlear implants. The CAP represents the synchronized activity of auditory-nerve fibers and is used as a measure of auditory-nerve activity (e.g. Goldstein and Kiang, 1958; Versnel et al., 1990). We used normal-hearing guinea pigs and tested the effects of a train of 10 biphasic current pulses (1 ms interpulse interval) on CAPs evoked by tone bursts presented up to 10 ms after the electric stimulus. Typically, the acoustic stimulus was presented after the electric stimulus in order to temporally separate the electrical artifact from the acoustic response. Cochlear trauma was minimized by using extracochlear stimulation electrodes on the round window and basal turn of the cochlea. Several parameters were investigated, including frequency and level of the acoustic stimulus, current level of the electric

stimulus, and interval between the electric and acoustic stimulus (EAI). We were especially interested in the frequency dependence of electroacoustic interactions, in order to verify whether the basal part of the cochlea could be stimulated electrically without interfering with responses evoked by low-frequency acoustic stimuli. Electro-acoustic interaction was also investigated as a function of current level. By determining the frequency range that was affected by electrical stimulation, we could estimate the spread of excitation through the cochlea at different current levels. EAI was varied to investigate the range of intervals at which electro-acoustic interaction occurs.

3.2. Materials and methods

3.2.1. Animal preparation

Healthy, normal-hearing adult female albino guinea pigs (n = 10, weight range: 350-600 g; strain: HSD POC; supplier: Duncan Hartley, Harlan) were used in acute experimental sessions. Average thresholds (in dB SPL ± SD), based on the CAP amplitude threshold criterion described in Section 2.4, were 39 ± 11 at 0.5 kHz, 44 ± 8 at 1 kHz, 46 ± 10 at 2 kHz, 38 ± 15 at 4 kHz, 18 ± 13 at 8 kHz and 29 ± 13 at 16 kHz. These values are comparable to the thresholds reported by Stengs et al. (1997) in normal-hearing albino guinea pigs. Anesthesia was initiated with 0.3 ml/kg Hypnorm® (Vetapharma; 0.315 mg/ml fentanyl + 10 mg/ml fluanisone) administered intramuscularly. Surgical anesthesia was induced with a gas mixture of N_2O (2 l/min), O_2 (1 l/min) and 2% isoflurane using a mouth cap. A single dose of atropine (0.05 mg/kg) was given subcutaneously to reduce bronchial secretion. The animal was subsequently tracheostomized and artificially ventilated (Amsterdam infant ventilator mk3, Hoekloos) with a gas mixture of N_2O, O_2 (2:1) and 1-2% isoflurane (50 cycles/min respiration rate, 2-2.3 kPa) throughout the experiment. The effectiveness of anesthesia was assessed regularly using a paw-pinch reflex. Heart rate (180-360/min) was monitored, and optionally atropine (0.05 mg/kg) was given subcutaneously. Rectal temperature was maintained at 38 ± 1 °C with a heating pad. Every 1-2 h, a volume of 1% body weight of warm glucose/saline solution was administered subcutaneously to prevent dehydration.

The animal's head was immobilized in a head holder and the right bulla was exposed by a ventrolateral approach. The cartilage of the right pinna was removed to facilitate coupling with a metal ear probe. An opening was made in the bulla with forceps to expose the cochlea. Cochlear potentials were recorded using a pair of silver ball electrodes with Teflon-insulated shanks. The recording electrode was placed extracochlearly on the apex of the cochlea (Van Deelen and Smoorenburg, 1986), the reference electrode was placed on the bulla wall. Stimulation electrodes were made of Teflon-insulated stainless steel wires with a

gold ball (diameter: 400-600 µm) attached. One electrode was placed in the bony notch near the round window, the other on or near the basal turn of the cochlea. Recording and stimulation were performed in a sound-attenuated booth.

3.2.2. Stimulus generation

Effects of electrical stimulation on the acoustically evoked CAP were tested by presentation of an electric stimulus followed by a tone burst (Fig. 3.1). Stimuli were generated by a pc (Dell) with custom-designed software in a Delphi 7© (Borland) programming environment, and were fed to a 24-bit DA converter (RP2.1, Tucker-Davis Technologies (TDT)) at a sampling rate of 49 kHz. Acoustic stimuli for CAP measurements were presented as 12 ms (0.5 kHz) or 8 ms (1-16 kHz) tone bursts, with $\cos^2$-shaped rise and fall times of 4 ms at 0.5 kHz, 2 ms at 1 kHz, 1.5 ms at 2 kHz and 1 ms at 4, 8 and 16 kHz. The acoustic signal was fed via a pair of attenuators (PA5, TDT) and a headphone amplifier (HB7, TDT) to a speaker (Beyer DT48) fixed on a metal intermeatal probe. Sound levels were determined with a sound level meter (2610, Brüel & Kjær (B&K) and a $^1/_4$'' condenser microphone (4136, B&K), calibrated with a 94 dB SPL 1 kHz reference source. Electric stimuli typically consisted of a train of 10 biphasic rectangular pulses (1000 pulses/s, 40 µs/phase) that were fed to a current source (Linear Stimulus Isolator A395, World Precision Instruments). On some occasions, a single biphasic pulse was also tested (40 µs/phase). The current level of the electric stimulus was typically set at a level at which a clear suppressive effect was observed on an 8 kHz-evoked acoustic response at 60 dB SPL. The inter stimulus interval was 111 ms plus the duration of the pulse train (9 ms) and EAI (2 to 10 ms). The inter stimulus interval was identical when the electric stimulus was not applied. The phase of the acoustic stimuli and the polarity of the electric stimuli alternated each cycle, such that one of the two acoustic polarities was always presented with one of the two electric polarities.

Various parameters were investigated (Fig. 3.1): (1) acoustic frequency (0.5-16 kHz); (2) acoustic level (from CAP threshold to 100 dB SPL); (3) current level of the electric stimulus (0-900 µA); (4) interval between electric and acoustic stimulus (2 to 10 ms); (5) single electric pulse versus 10-pulse train; (6) stimulating electrode position. The acoustic frequency and level were always co-varied with the parameter under investigation. Sound level was expressed in dB above CAP threshold. CAP thresholds were determined at the start of each experiment. Electrode position was tested by shifting the basal-turn electrode to a more basal or apical region on the cochlea, while keeping the round-window electrode in place.

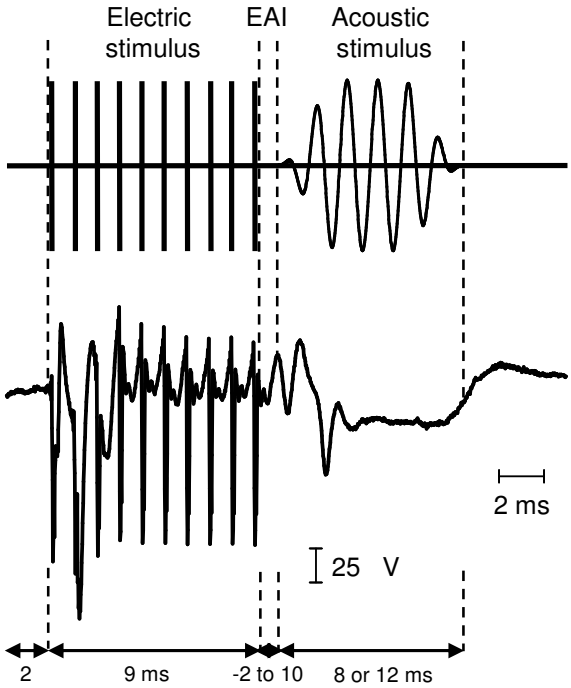

Fig. 3.1. Scheme of the stimulus paradigm (top) and an example CAP recording when an 8 kHz acoustic stimulus was applied at 60 dB SPL (50 dB above threshold) preceded by a current pulse train of 800 μA (bottom). Stimuli were separated by an electric-to-acoustic interval (EAI) of 1 ms. In this study, the varied parameters were acoustic frequency (0.5-16 kHz) and level (10100 dB SPL), current level (0900 μA) of the electric stimulus (1000 pulses/s), and interval between electric and acoustic stimulus (-2 up to 10 ms).

3.2.3. Recording technique

Cochlear potentials were differentially amplified (5000x), band-pass (1-30 kHz) filtered (preamplifier 5113, EG&G Instruments) and AD converted at 49 kHz (RP2.1, TDT). Responses to acoustic and electro-acoustic stimulation were recorded independently. Stimuli with opposite phases were separately averaged (to a maximum of 250 sweeps/polarity) and stored for off-line analysis. The sum of and difference between the two phases of the acoustic stimulus yielded the CAP and CM signal, respectively. In case of the acoustic CAP signal the electric stimulus artifact was reduced after addition of the two opposite polarities of the electric stimulus. In the CM signal the electrical artifact was increased due to subtraction of the polarities. In addition to recording the acoustically and electro-acoustically evoked responses, we recorded electrically evoked waveforms in the absence of an acoustically evoked response. These electrically evoked waveforms

were subtracted off-line from the electro-acoustically evoked recordings to further reduce the electric stimulus artifact, comparable to the method used by Charlet de Sauvage et al. (1983).

3.2.4. Data analysis

Data were analyzed using custom-written software in a Matlab® 6.5 (The Mathworks, Inc.) programming environment. The CAP amplitude was determined as a measure of auditory-nerve activity. CAP recordings at 0.5 and 1 kHz showed a multitude of negative peaks, due to frequency following of the CAP to the acoustic stimulus (Fig. 3.2A). The frequency of this response is twice that of the original tonal stimulus due to the subtraction procedure applied for the two phases of the tone burst (Section 2.3). At 0.5 and 1 kHz the CAP amplitude was defined as the difference between the CAP minimum and the summating potential (SP), while at high frequencies (2-16 kHz) it was defined as the difference between the 1st negative peak (N_1) and SP (Fig. 3.2). SP was defined as the mean response during the last 2 ms of the plateau of the tone burst. The CAP response criterion was defined as 4 times the standard deviation of 2 ms of baseline recording. Low-frequency (0.5 and 1 kHz) evoked CAPs were additionally analyzed with fast Fourier Transform (FFT) analysis using a window extending from acoustic stimulus onset to 2 ms after offset. In the spectrogram the peak amplitude was determined in a window at twice the tone frequency. CM data were also analyzed with FFT analysis in the same time window as the CAP. Peak CM amplitude was determined in the spectrogram in a window at the stimulus frequency.

CAP latency was defined by the latency of the CAP minimum (low frequencies), or by the N_1 latency (high frequencies). In addition, at low frequencies the latency of the 1st visually identifiable peak was examined, and at high frequencies the amplitude and latency of the second CAP peak (N_2) were also analyzed.

The effect of electrical stimulation on the acoustically evoked CAP (or CM) amplitude was expressed as a ratio R of the amplitude with electrical stimulation (A_{EAS}) and the amplitude without electrical stimulation (A_{AS}):

$$R = \frac{A_{AES}}{A_{S}}$$

When A_{AS}> response criterion and A_{EAS}< response criterion, R was set at 0 to reflect a complete suppression. The EAS effect criterion was set at 0.2. Hence, a ratio of <0.8 or >1.2 was defined as a relevant suppression and facilitation, respectively.

For averaging between animals, amplitude ratios were converted to a ratio scale between 1 and 1 defined by R'= (R - 1)/(R + 1). This ratio allows linear averaging. After determining the average, R' was converted back to the normal amplitude ratio by R = (1 + R')/(1 – R') for graphical presentation. This procedure

delivers an averaged ratio of 1 (i.e. "no effect"), when e.g. a CAP ratio of 0.5 (i.e. a factor 2 decrease) and 2 (i.e. a factor 2 increase) are averaged. Direct averaging in this example would have generated an erroneous ratio of 1.25 (i.e. a net facilitative effect).

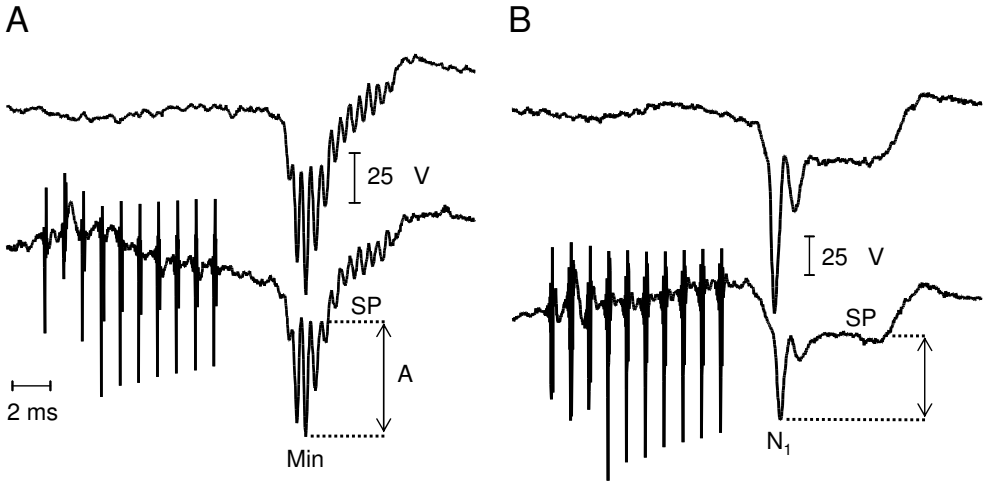

Fig. 3.2. Example compound action potential (CAP) recordings. The upper panel shows recordings without electrical stimulation, the lower panel shows recordings with electrical stimulation. The recordings with electrical stimulation are the resultant waveforms after subtraction of a recording with electrical stimulation without acoustic response. The 10 transients in the first part of the waveform are residual electric stimulus artifacts, followed by the CAP. CAP amplitude A was defined as the difference between CAP minimum (min) and summating potential (SP) at 0.5 and 1 kHz, or first negative peak (N_1) and SP at 216 kHz. SP was defined as the mean potential recorded during the last 2 ms of the plateau of the tone burst. (A) 1 kHzevoked CAP at 60 dB SPL (30 dB above CAP threshold). Note that the frequency of the so-called frequency following response (2 kHz) is twice that of the original stimulus (see Section 2.4). CAP latencies were determined on the 1st identifiable peak (N_1) and the CAP minimum. (B) 8 kHz-evoked CAP at 60 dB SPL (50 dB above CAP threshold). CAP latency was determined using N_1.

Statistical analyses consisted of standard linear regression analysis. Slopes and intercepts were tested for significance with *F* tests ($\alpha = 0.05$).

Surgical and experimental protocols were approved by the Animal Ethical Committee of the Academic Biomedical Center of the University Utrecht under number DEC 2007.I.02.025. Animals were housed according to the standards of the animal care facility of the University of Utrecht.

3.3. Results

We investigated the effects of current pulse trains on CAPs evoked by tone bursts in anesthetized guinea pigs. The main parameters that were varied were acoustic frequency and level, current level and interval between the pulse train and acoustic tones (EAI).

3.3.1. Dependence of CAP ratio on acoustic frequency and level

The effect of electrical stimulation on tone-evoked CAPs as a function of frequency and level was investigated in 8 animals. Current level of the pulse train was fixed per animal at a moderate to high level (600-900 μA) and EAI was set at a short interval (1 ms). The typical effect of electrical stimulation was a suppression of the CAP. Fig. 3.3 shows example waveforms of CAPs in response to 8 kHz tones with and without electrical stimulation, as a function of sound level. The amplitudes of these waveforms (Fig. 3.3B) were used to obtain the CAP ratio, which expressed the effect of electrical stimulation on the CAP (Fig. 3.3C). Suppressive effects in this example were pronounced and level dependent.

Suppression as a function of frequency and level is shown in Fig. 3.4, averaged across 4 animals which had comparable absolute thresholds at each frequency (within a range of 20 dB). The amount of suppression depended on acoustic frequency and level. At the lowest frequencies tested (0.5 and 1 kHz) there was a modest suppression of the CAP amplitude of ~25% (CAP ratios of ~0.75) that was virtually independent of sound level (Fig. 3.4A). At 8 and 16 kHz however, CAP ratios clearly depended on sound level and were lowest around CAP threshold (Fig. 3.4B). Most pronounced suppression was found at 8 kHz, with more than 90% suppression (CAP ratios <0.1) 10 dB above threshold.

The dependence of the CAP ratio on sound level was examined with linear regression analysis (Fig. 3.4C). CAP ratios at 8 and 16 kHz showed a significant dependence on sound level (F test, $r = 0.9$, $P < 0.05$), and at 4 kHz an almost significant level dependence was found (F test, $r = 0.8$, $P = 0.05$). Since maximal suppression was observed around CAP threshold, the intercepts of the regression curves at 0 dB sound level (Fig. 3.4A and B) were determined (Fig. 3.4D). At 0.5 and 1 kHz a horizontal line was used to calculate the intercept (i.e. the mean CAP ratio of the different sound levels tested). Except at 2 kHz, intercepts at all frequencies tested significantly differed from 1 (F test, $P < 0.05$). The CAP ratio at threshold was lowest at 8 kHz (~0.1), while at 0.5 and 1 kHz the highest ratios were found (~0.75). At the remaining frequencies intermediate values were found (~0.5).

The suppressive effect of electrical stimulation was further investigated by determining the CAP threshold shift at each acoustic frequency. At 8 kHz the CAP threshold was significantly increased by 14 dB (2-tailed 1-sample t test, $P < 0.01$, $n = 8$). For 16 kHz this effect was small and not significant. At lower frequencies

no effect on threshold was seen.

To summarize, at all frequencies but 2 kHz, significant suppression of tone-evoked CAPs by electrical stimulation was found at threshold sound level. Suppression was much more pronounced at high, than at low frequencies. Furthermore, CAP suppression was sound level dependent at high frequencies, but not at low frequencies. These results were obtained at relatively high current levels of 600-900 μA and a short EAI of 1 ms. The following sections will describe the dependence on current level and EAI.

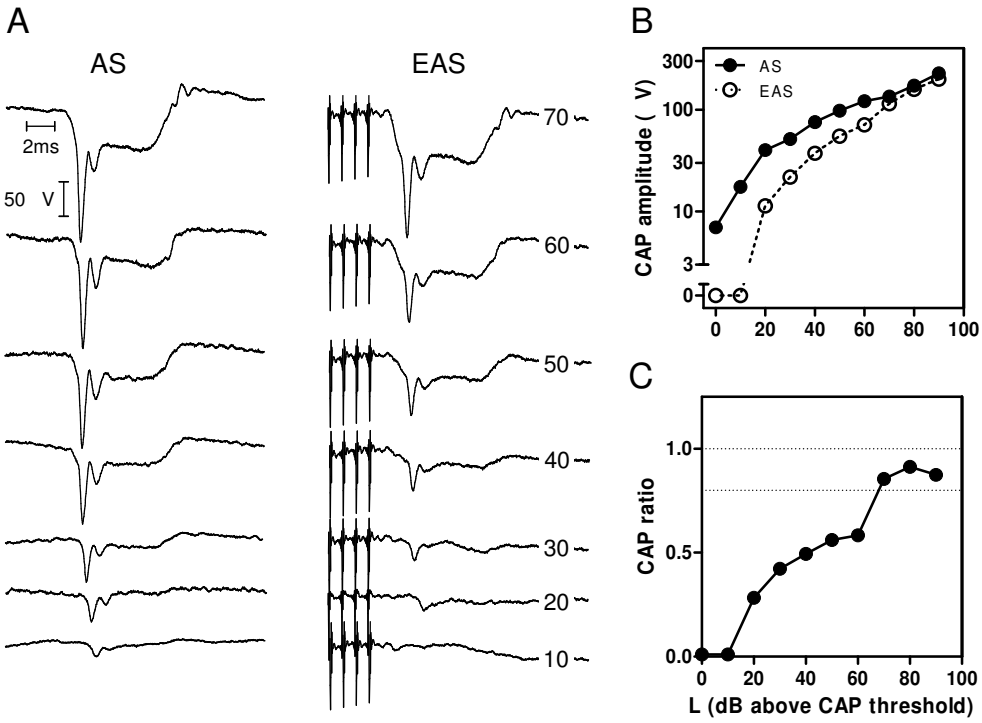

Fig. 3.3. Example CAP recordings at 8 kHz as a function of sound level expressed in dB above CAP threshold. Current level was 800 μA and the electric-to-acoustic stimulus interval was 1 ms. (A) CAP waveforms with acoustic stimulation (AS, left panel) and with electro-acoustic stimulation (EAS, right panel) as a function of sound level. Theelectrically evoked response, including the stimulus artifact, was subtracted from the EAS waveforms. (B) Amplitude of the CAPs with AS (filled circles) and EAS (open circles) as a function of sound level. (C) CAP ratio based on the data in (B) as a function of sound level. CAP ratios were determined by dividing the amplitude of the response to AS by that to EAS.

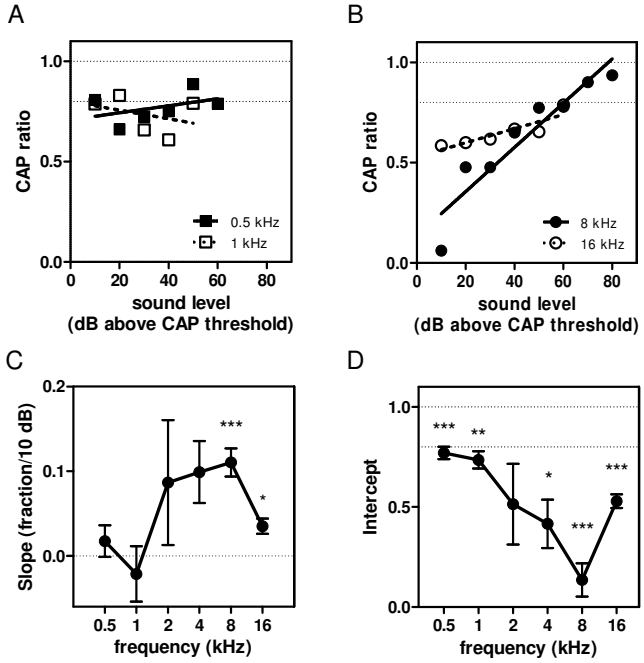

Fig. 3.4. Dependence of the CAP ratio on frequency and sound level averaged across 4 animals (Ela14, Ela17, Ela21 and Ela27). Sound level is expressed as dB above CAP threshold. The electric-to-acoustic stimulus interval (EAI) was fixed at 1 ms. The current level of the electric stimulus (10 pulses; 1000 pulses/s) was fixed per animal (mean: 750 μA; range 600900 μA). The dotted lines represent CAP ratios of 1 (i.e. no effect) and 0.8 (i.e. the suppression criterion used). (A) Level dependence of the CAP ratio at 0.5 and 1 kHz showing no dependence on sound level ($r < 0.4$). (B) Level dependence of the CAP ratio at 8 and 16 kHz showing a monotonic dependence of CAP ratio on sound level ($r = 0.9$). (C) Slopes of the regression lines of the CAP ratio as a function of sound level at all frequencies tested. (D) Intercepts of the regression lines. At 0.5 and 1 kHz, the intercept was based on a horizontal fit (i.e. the mean). Error bars represent the standard deviation. Asterisks indicate the significance level from the deviation of the slope from 0, or the deviation of the intercept from 1 according to F tests. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

3.3.2. Dependence of CAP ratio on current level

The dependence of CAP suppression on current level of the electric stimulus was tested in 4 animals. Different acoustic frequencies were tested at moderate and low sound levels, while the EAI was fixed at 1 ms. Fig. 3.5 shows the CAP ratio as a function of current level at 0.5 kHz (A) and at 8 kHz (B) averaged across 4 animals. Above a certain current threshold the CAP ratio decreased with current

level at both frequencies. In line with the results presented in Section 3.1, CAP suppression was most pronounced at 8 kHz, especially at low sound levels. At high frequencies (4-16 kHz), the dependence of CAP ratio on current level could be non-monotonic. At high current levels (>600 μA) CAP ratios were higher than at moderate levels in about half of the animals. At 4 and 16 kHz this was observed at both a high and low sound level (data not shown), and at 8 kHz non-monotonic dependence was seen at a low sound level (Fig. 3.5B).

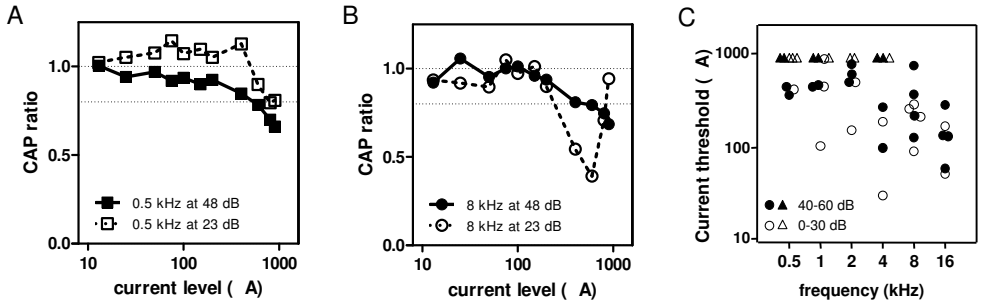

Fig. 3.5. Dependence of the CAP ratio on current level. For each frequency typically two sound levels were applied. These levels were expressed in dB above CAP threshold and grouped in a moderate sound level group (range: 4060 dB; filled symbols) and a low sound level group (range: 030 dB; open symbols). EAI was fixed at 1 ms. (A) Averaged CAP ratio (0.5 kHz stimulation) of 4 animals (Ela21, Ela22, Ela25 and Ela27) as a function of current level at moderate and low sound levels. (B) Averaged CAP ratio as in (A), but for 8 kHz stimuli. (C) Individual current thresholds (the interpolated current level at which the CAP ratio was 0.8) as a function of frequency at moderate and low sound levels. When CAP ratios were >0.8 in the current range tested (0900 μA) threshold current level was represented by a triangle at 900 μA (the maximal current tested).

Current threshold was defined as the (interpolated) current level at which the CAP ratio was 0.8 (i.e. the current level at 20% suppression). In Fig. 3.5C individual current thresholds are shown as a function of frequency. Current thresholds tended to decrease with frequency. At low and intermediate frequencies (0.5-4 kHz) threshold currents could not be determined in some animals because CAP ratios were >0.8 at the highest current level tested (900 μA), indicated with triangles. At 8 and 16 kHz thresholds were <900 μA in each animal with an average threshold at moderate sound level of 360 and 150 μA, respectively.

To summarize, CAP suppression increased with current level and was most pronounced at high acoustic frequencies. At high current levels and low acoustic frequencies above-threshold suppression occurred in some, but not all animals.

3.3.3. Dependence of CAP ratio on the electro-acoustic interval

The dependence of CAP suppression on the interval between the electric and acoustic stimulus (EAI) was tested in 5 animals. In some animals the EAI dependence was not obtained at all acoustic frequencies tested. The current level of the electric stimulus was fixed per animal (500-800 μA). Fig. 3.6A shows the CAP ratio as afunction of EAI at 1 and 8 kHz at a moderate sound level, averaged across 3 animals. At both frequencies CAP suppression decreased with EAI. In line with the results presented in Sections 3.1 and 3.2, suppression at 8 kHz was more pronounced than at 1 kHz at all EAIs tested. Note that we also tested negative EAIs. At the lowest EAI value of 2 ms the 8 kHz-evoked CAP occurred nearly simultaneously with the last pulse in the pulse train, since the CAP latency was ~2 ms.

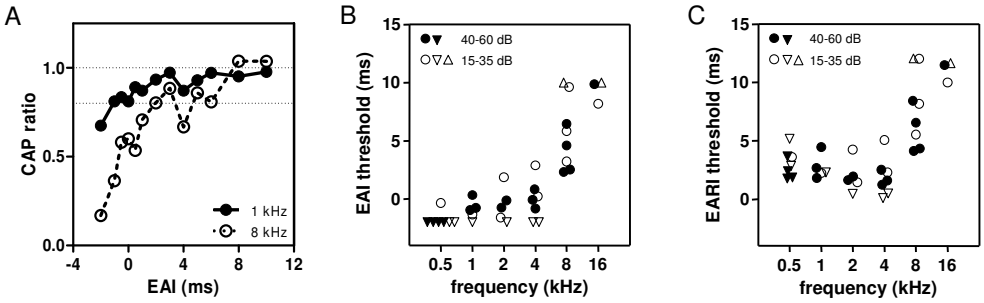

Fig. 3.6. Dependence of the CAP ratio on the electric-to-acoustic stimulus interval (EAI), and on the electric stimulus-to-acoustic response interval (EARI). Current levels were fixed per animal (range 500900 μA, mean: 760 μA). (A) Averaged CAP ratio of 3 animals (Ela22, Ela25 and Ela26) at 1 kHz (filled circles) and 8 kHz (open circles) as a function of EAI at moderate sound levels. (B) Individual interval thresholds (interpolated EAI at which the CAP ratio was 0.8) as a function of frequency at moderate (3060 dB; filled circles) and low sound levels (1040 dB; open circles) of 5 animals (Ela21, Ela22, Ela24, Ela25 and Ela26). When CAP ratios were >0.8 in the tested EAI range (2 to 10 ms) the interval threshold was represented by a downward pointing triangle at 2 ms (the shortest tested EAI). When CAP ratios were >0.8 in the tested EAI range interval threshold was represented by an upward pointing triangle at 10 ms (the longest EAI tested). In some animals data were not obtained at some acoustic frequencies. (C) Intervals thresholds of (B) converted to EARI thresholds by addition of the CAP latency to the EAI.

Interval threshold was defined as the EAI at which the CAP ratio was 0.8. Thus, below the interval threshold there is substantial suppression and above there is not. Fig. 3.6B shows individual interval thresholds as a function of frequency. The interval threshold increased with frequency, indicating longer lasting suppressive

effects of the electric stimulus at high frequencies. At low frequencies, particularly at 0.5 kHz, interval thresholds could often not be determined because CAP ratios were >0.8 even at the shortest EAI tested (indicated with downward pointing triangles). At 8 kHz interval thresholds ranged from 2 to 10 ms.

Low-frequency evoked CAPs at 0.5 and 1 kHz showed CAP minima occurring up to several milliseconds later compared to the N_1 peak in the CAP at high frequencies. This was caused by the longer rise times of the tone burst applied at low frequencies (see Section 2.2) and by a longer travelling time in the cochlea at low frequencies. To account for these latency differences, EAI was converted to the interval between electric stimulus offset and CAP latency. This "electro-acoustic response interval" (EARI) was calculated by adding the CAP latency to the EAI. Fig. 3.6C shows the interval thresholds expressed in EARI as a function of frequency. The overall picture remained the same in that high frequencies showed longer interval thresholds. However, the interval threshold increase from 0.5 to 4 kHz observed in the EAI data (Fig. 3.6B) disappeared in the EARI data (Fig. 3.6C). Therefore the dependence on EAI from 0.5 to 4 kHz could be ascribed to the frequency dependence of the CAP latency.

To summarize, CAP suppression decreased with the interval between the electric and acoustic stimulus, and suppression was observed at intervals up to 10 ms at high frequencies.

3.3.4. Dependence of CAP ratio using a single current pulse

Dependence of the CAP ratio on EAI was also investigated using a single pulse as electric stimulus and compared to suppression evoked by a pulse train. In this way we hoped to distinguish between refractoriness and fatigue (Killian et al., 1994). In two animals we obtained the EAI dependence with both the 10-pulse train and the single pulse. Fig. 3.7A shows the CAP ratios at 8 kHz averaged across these two animals. The current level used was 800 μA in both animals. At 8 kHz both the single pulse and pulse train clearly evoked CAP suppression. The magnitude of suppression was similar for both stimuli at short EAIs, but at EAIs of 46 ms suppression was larger using the pulse train.

Interval thresholds were determined as described in Section 3.3 for both the single pulse and pulse train stimulus (Fig. 3.7B). Rise and fall times of the tone bursts were 1 ms at all frequencies, which resulted in nearly uniform CAP latencies between frequencies. EARI dependence was therefore essentially the same as the EAI dependence. Interval thresholds were longer for a pulse train compared to a single-pulse stimulus in one animal, but not in the other.

3.3.5. Dependence of CAP ratio on electrode configuration

The influence of stimulation electrode configuration was investigated by displacing the basal-turn electrode either ~1 mm basally, or ~2 mm apically on the otic

capsule of the cochlea. In the latter situation the electrode was moved from the basal to the middle turn of the cochlea, which includes the characteristic frequencies of 2.7-5.1 kHz in the guinea pig (Greenwood, 1990). The round-window electrode was left in place. In 3 animals we obtained the acoustic frequency and level dependence in all three electrode configurations. The applied current range was 600-800 μA and EAI was fixed at 1 ms. No clear dependence of CAP suppression on electrode configuration was observed (data not shown). For example, no increased suppression was found at 16 kHz when the electrode was placed more basally on the cochlea, and no increased suppression at 2 or 4 kHz was observed when the electrode was placed on the middle turn.

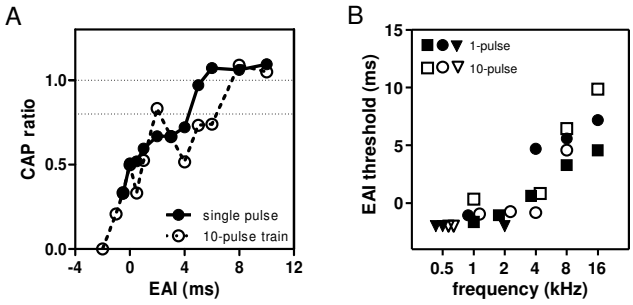

Fig. 3.7. Dependence of the CAP ratio on the electric-to-acoustic stimulus interval (EAI) using a single pulse (filled symbols) and a 10-pulse train as electric stimulus (open symbols) averaged across two animals (Ela25 and Ela26). The current level was fixed at 800 μA and the applied sound levels were of a moderate level (range: 4060 dB above CAP threshold). (A) Averaged CAP ratios at 8 kHz as a function of EAI when a single pulse and pulse train were applied as electric stimulus. (B) Interpolated interval thresholds (EAI interpolated at which the CAP ratio was 0.8) of animal Ela25 (squares) and Ela26 (circles) as a function of frequency when a single pulse was applied or a pulse train as electric stimulus. When CAP ratios were >0.8 in the entire EAI range tested this was represented by a downward pointing triangle at 2 ms (the shortest EAI tested) for both animals.

3.3.6. CAP latency

To address the question whether suppression concomitantly affected CAP latency, we examined CAP latency shifts at 8 and 16 kHz. Latency shifts were plotted as a function of CAP ratio using the datasets presented above with the pulse train as electric stimulus (Fig. 3.8). The CAP latency increased with suppression. Regression analysis showed a monotonic relation between latency shift and CAP ratio (F test, $r > 0.8$, $P < 0.0001$).

Latency shifts of the CAP minimum at 0.5 and 1 kHz were difficult to analyze due to the fact that slight baseline changes after electrical stimulation

could cause shifts of the CAP minimum to preceding or following peaks. We did not observe clear latency shifts at 0.5 and 1 kHz such as those observed at 8 kHz (for examples see Fig. 3.2). CAP latency at low frequencies was also analyzed using the first identifiable CAP in the frequency following response. With this method also no effects of electrical stimulation on CAP latency were found. Latency shifts at intermediate frequencies (2 and 4 kHz) were either small or absent.

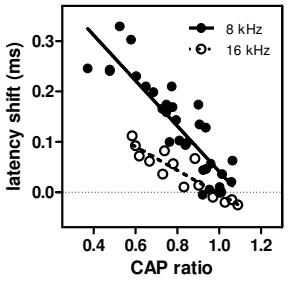

Fig. 3.8. CAP latency shift as a function of CAP ratio at 8 kHz (filled circles) and 16 kHz (open circles) at moderate sound levels. The CAP ratio data correspond to the averaged data presented in Figs. 3.3-3.5. Linear regression analysis showed a significant relationship at both frequencies (F test, $r > 0.8$, $P < 0.0001$).

3.3.7. Miscellaneous variables

In addition to the 1st negative CAP peak (N_1), the 2nd negative peak (N_2) was investigated for 2-16 kHz. When the amplitude and latency of N_1 were affected by electrical stimulation, amplitude and latency of N_2 were typically affected in a similar way. When effects were present, effects on N_2 were generally larger compared to N_1 (results not shown).

Suppression of low-frequency evoked CAPs was determined by analyzing the CAP amplitude defined by the difference between CAP minimum and summating potential (SP). In addition, suppressive effects on the CAP amplitude at low frequencies (0.5 and 1 kHz) were determined by FFT analysis (see Section 2.4). CAP ratios determined with FFT analysis were typically similar compared to the ratio of the CAP amplitude determined by the minimum-to-SP method.

The cochlear microphonic (CM) was investigated using FFT analysis. Effects of electrical stimulation on CM were negligible at all frequencies tested, and CM ratios were typically between 0.8 and 1.2 (i.e. below-threshold effects).

3.3.8. Electrically evoked responses

Apart from the expected electrically evoked CAPs, which were typically not observed because the short latency (<0.5 ms) of these responses placed them

within the stimulus artifact, a late electrical response was consistently observed with a latency of 1.2 ms at high current levels. In Fig. 3.1 these responses can be seen as adapting potentials during the pulse train. Fig. 3.9 shows these late electrical responses when evoked with a single pulse. The late electrical response and acoustically evoked CAPs were very similar in appearance (Fig. 3.9A), indicative of a possible electrophonic origin of this response. Further evidence for an electrophonic origin came from the observation that the amplitude and latency of the late electric responses were comparable with those of 8 kHz-evoked acoustically evoked CAPs (Fig. 3.9B). Typical minor differences were also observed, including a steeper inputoutput relation and latencies (1.2-1.5 ms) were less affected by different stimulus levels when compared with acoustically evoked CAPs of comparable amplitude. These characteristics closely agree with the characteristics of the electrophonic response described previously (Prijs, 1980). Therefore, from here on we refer to this late response as electrophonic response. The location in the cochlea that contributed most to the electrophonic response could be related to the spectral content of the input stimulus (Fig 3.9C) when cochlear filtering would apply to the input stimulus. We tested this hypothesis by investigating suppression of the electrophonic response (Fig. 3.9A) when acoustical tones of 80 and 60 dB SPL were simultaneously presented during pulse presentation (Fig. 3.9D). Fig. 3.9D shows that the electrophonic response was suppressed by acoustic stimuli of 80 and 60 dB SPL. Suppression at 60 dB clearly peaked at 8 kHz, showing a suppression of the electrophonic response by more than 50%. The spectral content of the applied pulsatile stimulus peaks at 10 kHz (Fig. 3.9C) and the observed tuning to 8 kHz could be a reflection of this peak.

3.4. Discussion

3.4.1. Summary of the results

The results presented in this study show that electrical stimulation can reduce the amplitude of acoustically evoked CAPs. CAP suppression was most pronounced at high acoustic frequencies (8 and 16 kHz) and low acoustic levels. Suppression increased with current, and at high current levels low-frequency evoked CAPs were also suppressed. Finally, suppression decreased with electric-to-acoustic interval (EAI).

The interdependence of the parameters tested on CAP suppression proved complex. First, CAP suppression at high tone frequencies was sound level dependent, while at low frequencies it was not. Second, CAP suppression at 0.5-2 kHz increased monotonically with current level, while suppression at 4-16 kHz revealed a non-monotonic dependence on current level at some sound levels.

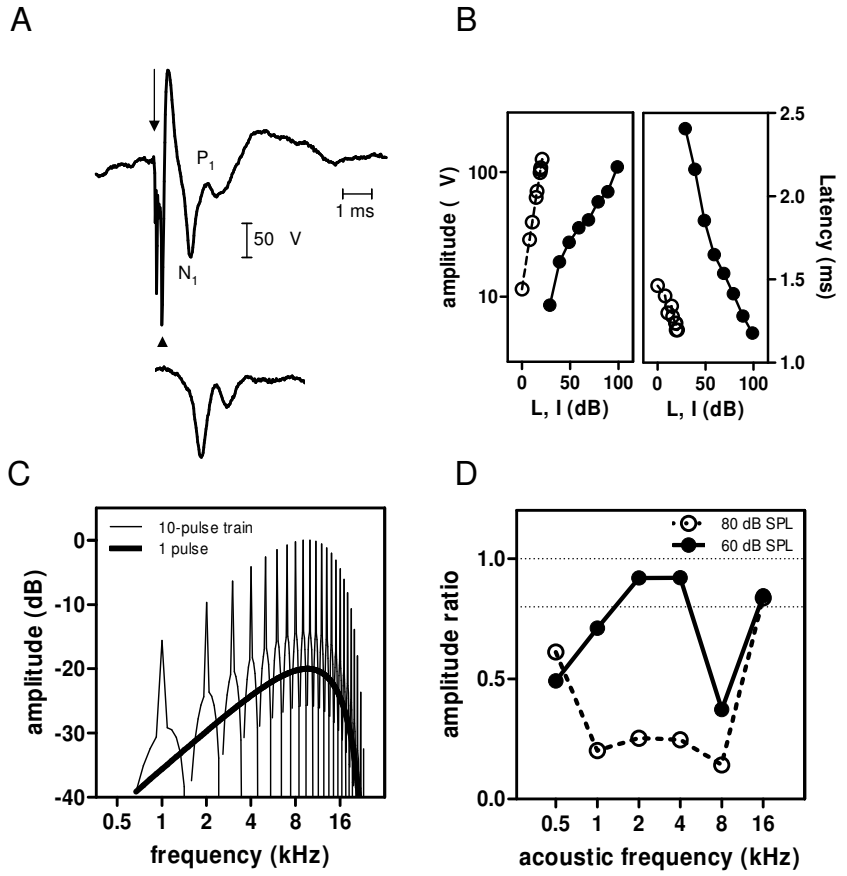

Fig. 3.9. Electrophonic response using a single-pulse electric stimulus. (A) Example recording showing the electrophonic response (top graph) after 1.2 ms following the pulse stimulus (arrow). Amplitude was defined as the difference between the first negative peak (N1) and the positive peak (P1). The electrically evoked CAP is visible after 0.3 ms (arrowhead). For comparison an acoustically evoked CAP (8 kHz, 60 dB SPL) in this animal is shown (bottom graph). Acoustic stimulus onset was corrected for approximate travelling time of the acoustic stimulus from speaker to stapes and is aligned with the electrical pulse in the upper graph. (B) Amplitude and latency of the electrophonic response (open circles) and acoustically evoked CAP at 8 kHz (filled circles) as a function of stimulus intensity (sound level in dB SPL, current level in dB re: 75 μA) in the same animal. (C) Spectrum of the single-pulse stimulus (thick line) and of the 10-pulse train (thin line). (D) Acoustical suppression of the electrophonic response (stimulus current level: 600800 μA, mean: 733 μA) averaged across 3 animals (Ela25, Ela26 and Ela27). Tone onsets preceded the electric stimulus with 2 ms and were presented at 60 dB SPL (filled circles) or 80 dB SPL (open circles). The acoustically evoked CAP response was subtracted from the electrophonic response evoked with electro-acoustical stimulation, delivering the electrophonic response.

3.4.2. Mechanisms of CAP suppression

The underlying mechanisms of the observed electrical suppression of acoustically evoked CAPs could have included (a) auditory-nerve activation via direct electrically evoked neural responses (refractoriness), (b) auditory-nerve activity via hair cell-mediated mechanisms (electrophonics), (c) stimulation of efferents and (d) contraction of the middle-ear muscles.

3.4.2.1. Refractoriness

Direct electrically evoked neural responses were occasionally observed as electrically evoked compound action potentials (eCAPs), though the short latency (<0.5 ms) of these responses (Fig. 3.9A) typically placed the eCAPs within the stimulus artifact. Direct electrically evoked neural responses and subsequent refractoriness of the auditory nerve can have suppressed acoustically evoked CAPs. Brown (1994) reported for double-pulse experiments in guinea pigs an absolute and relative refractory period of the auditory nerve of ~0.5 and ~5 ms, respectively. At ~1.5 ms interstimulus interval the eCAP amplitude had recovered to 80% (Fig. 5 in Brown, 1994). The recovery function of spontaneous firing of single fibers in guinea pigs reported by Prijs et al. (1993) delivers an 80% recovery after 2.6 ms. In the present experiments CAP suppression typically recovered to 80% within 2-10 ms (Figs. 3.6 and 3.7), which is somewhat longer compared to the reported refractory characteristics of the auditory nerve. This suggests that refractory mechanisms alone cannot completely explain the observed suppression.

Based on the position of the stimulating electrodes we expected current density to be larger in the vicinity of the round-window electrode than at the more apical electrode, because of the insulating effect of the otic capsule (Van den Honert and Stypulkowski, 1987). We found no evidence that the position of the more apical electrode affected the frequency dependence of suppression, which supports this expectation. A larger current density at the round window would mean that CAP suppression would increase with acoustic frequency. Our data largely agree with this expected pattern. Absolute suppression was however larger at 8 kHz than at 16 kHz (Fig. 3.4), which cannot easily be explained by a direct stimulation of the auditory nerve.

3.4.2.2. Electrophonics

Electrically evoked activity mediated by hair cells (i.e. electrophonic responses) can also have been responsible for the observed suppression of acoustically evoked CAPs. We observed electrical responses with a latency of 1.2-1.5 ms (Fig. 3.9A and B) that resembled the electrophonic response described by Prijs (1980) She found that morphology, latency (1.1-1.5 ms) and absolute amplitudes of

the electrophonic response were comparable to acoustically evoked CAPs, very similar to the present findings (Fig. 3.9A). The electrophonic response in our experiments could be suppressed by tonal stimuli. Suppression at moderate sound levels peaked at 8 kHz (Fig. 3.9D), which could reflect the peak in the frequency spectrum of the current stimuli used (Fig. 3.9C).

Indirect evidence for electrophonic responses interacting with acoustically evoked CAPs were reported previously. McAnally et al. (1997b) showed that electrical suppression of acoustically evoked CAPs was tuned to the spectral content of the applied pulsatile stimuli. They explained this as an electrically evoked basilar membrane response (Nuttall and Ren, 1995; Xue et al.,1995) generating a travelling wave (Kirk and Yates, 1994) that activated cochlear sites corresponding to the frequency content of the electric stimuli. Since the frequency content of our electrical stimulus peaked at around 8 kHz (Fig. 3.9C), this mechanism would explain that electrical suppression of acoustically evoked CAPs yielded lowest CAP ratios at an acoustic frequency of 8 kHz (Fig. 3.4).

As already mentioned above, the recovery of suppression in our experiments was somewhat longer than expected on the basis of refractoriness alone. This is an extra argument for the contribution of additional mechanisms, such as electrophonically mediated suppression. Electrophonic responses are associated with travelling waves and excitation of hair cells much like acoustical responses. Suppression due to hair cell excitation typically lasts longer than suppression due to neural excitation alone. For example, acoustically evoked CAPs recover to 80% after 10-20 ms when a suppressor tone burst 10 ms in duration (comparable to the pulse train duration typically used in our experiments) is applied (Fig. 4 in Abbas and Gorga, 1981 and Fig. 3 in Gorga and Abbas, 1981).

Summarizing, there are several arguments to suggest that elecrophonic responses, associated with electrical stimuli, played a role in the observed suppression of acoustic potentials.

3.4.2.3. Stimulation of efferents

Electrical stimulation at the round window has also been shown to activate efferent nerve fibers. Rajan and Johnstone (1983) report efferent suppression of click-evoked CAPs that was maximal at low acoustic levels in combination with a high current level and short EAI, very similar to the data presented in this paper. However, optimal efferent stimulation occurs at low electrical pulse rates (5-400 Hz) and long (e.g. 150 μs) pulse widths (Desmedt, 1962; Rajan and Johnstone, 1983). In the present study both electrical stimulation frequency (1 kHz) and pulsewidth (40 μs/phase) were suboptimal. Furthermore, single pulses do not evoke efferent-mediated CAP suppression (Rajan and Johnstone, 1983), while in our experiments single pulses effectively suppressed high-frequency evoked CAPs. In addition to suboptimal stimulus parameters, the recovery from CAP suppression seen in the present data deviates from efferent-mediated

suppression. While efferent-mediated suppression persists up to 80 ms, with a recovery time constant of 90-180 ms (Wiederhold and Kiang, 1970), recovery of suppression in the present experiments was considerably faster (generally <10 ms). Taken together, we conclude that the contribution of efferent inhibition on CAP suppression was small or non-existent.

3.4.2.4. Contraction of the middle-ear muscles

Finally, contraction of the middle-ear muscles could have been a factor contributing to the observed CAP suppression, since electrical stimulation at the round window can stimulate the stapedius muscle (Pang and Guinan, 1997). Attenuations of the CM due to the middle-ear muscle reflex are reported to be up to 10 dB (Van den Berge et al., 1990), corresponding to a CM ratio of 0.3. Effects on CM ratio in our experiments were however sporadically encountered and generally below threshold (CM ratio >0.8). Furthermore, in guinea pigs the largest attenuation by contraction of the middle-ear muscles is generated at frequencies below 0.3 kHz (Nuttall, 1974), whereas in the present experiments CAP suppression was most pronounced at CAPs evoked by 8 and 16 kHz tones. We therefore conclude that in our experiments middle-ear muscle contraction would not have had a significant contribution to the observed CAP suppression.

3.4.2.5. General conclusion on the mechanism of suppression in EAS

Since stimulation of efferents and middle-ear muscle contraction would not have played an important role, we conclude that direct neural stimulation and electrophonic stimulation probably both have contributed to the suppression of acoustically evoked CAPs in our experiments. The relative contribution of both cannot be determined using the present data.

3.4.3. Remaining observations

Suppression at moderate current levels was limited to high frequencies (Fig. 3.5C). At high current levels CAPs evoked at low frequencies were also suppressed in some animals. These findings are in line with earlier observations that current spread is limited at moderate current level, but that nerve fibers are excited and thus become refractory throughout the cochlea at high current levels (Van den Honert and Stypulkowski, 1987). These findings can also be attributed to electrophonic activation since the spectrum of the pulsatile stimuli used was broad (Fig. 3.9C). Electrophonic excitation spreads along the cochlea with increasing current levels because of broadening of neural frequency tuning with current level (Fig. 3.9D and McAnally et al., 1997b).

The longer CAP latencies at low frequencies could have contributed to the frequency dependence of suppression, since suppression decreased with EAI

(Fig. 3.6A) and low-frequency evoked CAPs have longer latencies (Fig. 3.6B and C). This contribution proved to be small, since interval thresholds corrected for CAP latency remained clearly higher at high frequencies (Fig. 3.6C).

CAP latencies increased with suppression at high frequencies (Fig. 3.8). This phenomenon could have resulted from a relatively decreased contribution to the CAP of nerve fibers with high characteristic frequencies and short latencies (Prijs and Eggermont, 1981, Fig. 3.10).

Suppressive effects tended to be higher at EAIs of 4-6 ms when 10 pulses were applied compared to a single pulse (Fig. 3.7). This could be caused by a cumulative refractory effect in the auditory nerve, sometimes called fatigue (Killian et al., 1994; Matsuoka et al., 2000).

3.4.4. Clinical implications

We showed that extracochlear electrical stimulation in the basal region of the cochlea suppressed high-frequency evoked CAPs (especially 8 and 16 kHz), while low-frequency evoked CAPs were less affected. When similar electro-acoustic interactions occur in cochlear implant users with residual low-frequency hearing, the high-frequency region of the cochlea can be stimulated electrically with little detrimental effects on low-frequency acoustic responses.

In the present study electric stimuli were presented as biphasic pulses of alternating polarity. In cochlear implants stimulus polarity is typically not alternated (Wilson et al., 1991). Since positive and negative starting currents evoke eCAPs differing in amplitude and latency (Miller et al., 1998), the opposite polarities of the biphasic currents used in the present study may have had slightly different effects on subsequent acoustically evoked CAPs.

The implied dual mechanism of suppression (refractoriness and electrophonics) suggests two strategies to minimize electro-acoustic interaction in implant users with residual low-frequency hearing. With respect to refractoriness, the use of short electrode arrays to segregate electrical and acoustical stimulation within the cochlea to minimize interaction is recommended. With respect to electrophonics, the pulse width of the pulses would be important and shorter pulse widths are advisable to shift the spectral content of the pulse stimuli toward the high frequencies.

High current levels increased suppression at low frequencies in our experiments. This indicates that current levels should be kept relatively low.
The finding that suppression decreased rapidly in the first few milliseconds after the electric stimulus can possibly be adopted in hybrid implant processor strategies.

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Abbreviations

ABR:	auditory brainstem response
CAP:	acoustically evoked compound action potential
CI:	cochlear implant
CM:	cochlear microphonics
eABR:	electrically evoked ABR
eCAP:	electrically evoked compound action potential
EAI:	electric-to-acoustic stimulus interval
EARI:	electric-to-acoustic response interval;
EAS:	electro-acoustical stimulation
FFT:	fast Fourier transform
N_1:	1st negative peak
N_2:	2nd negative peak
SP:	summating potential
SPL:	sound pressure level

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Effects of electrical stimulation on the acoustically evoked auditory-nerve response in guinea pigs with a high-frequency hearing loss

4

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Abstract

Criteria for cochlear implantation keep expanding and people with substantial residual low-frequency hearing are considered candidates for implantation nowadays. Therefore, electrical and acoustical stimulation in the same ear is receiving increasing interest. We have investigated the effects of intracochlear electrical stimulation on acoustically evoked auditory-nerve activity, using a forward masking paradigm. The stimulation electrode was placed in the basal turn of the cochlea. Compound action potential (CAP) recordings were performed in guinea pigs with severe high-frequency hearing loss and in normal-hearing control animals. In normal-hearing animals, electrical stimulation generally suppressed CAPs. This suppression was most pronounced when CAPs were evoked at high acoustic frequencies and low sound levels. At low frequencies, suppression was observed only at high sound levels. In animals with a high-frequency hearing loss, suppression of CAPs at low frequencies was substantially less compared to control animals. Even under conditions of high current level and temporal overlap of the acoustic and electric stimulus, suppression was absent in most animals with a high-frequency hearing loss. We conclude that in case of high-frequency loss, the basal part of the cochlea can be stimulated electrically with little effect on responses to low-frequency acoustic stimuli.

Key words: electro-acoustical stimulation; residual hearing; cochlear implant; electrocochleography; hair cell; spiral ganglion cell

4.1. Introduction

The method of choice for treatment of profound sensorineural hearing loss is cochlear implantation. In the last decades there has been considerable progress in improving speech processor strategies and stimulation paradigms that have led to excellent results regarding speech understanding by means of a cochlear implant (CI) (Wilson et al., 1991; Wilson and Dorman, 2008). Consequently, the focus of improvement of these devices has shifted from improving speech understanding in quiet to speech understanding in noise and improving the esthetical quality of complex sounds such as music. In this regard there is increasing interest in the use of combined electrical and acoustical stimulation to utilize residual low-frequency hearing that is present in a subpopulation of CI candidates. Aetiologies in this population range from presbycusis (Gstoettner et al., 2008) to acoustic trauma such as blast injuries (Turner et al., 2008a). Especially electro-acoustical stimulation in the same ear (EAS) via hybrid implants, as opposed to bimodal stimulation (Olson and Shinn, 2008), has received much attention. Hybrid implants combine a CI with a conventional hearing aid (Talbot and Hartley, 2008;

Turner et al., 2008b). EAS has been shown to increase speech understanding in noise and improve the appreciation of music (Fraysse et al., 2006; Gantz et al., 2006). Selection criteria for cochlear implantation continue to expand and consequently, the population of cochlear implant candidates with residual low-frequency hearing increases (Cohen, 2004). Cochlear implantation and chronic electrical stimulation may leave residual hair cells unaffected and can even be beneficial for spiral ganglion cell (SGC) survival (Coco et al., 2007).

We may assume that an interaction between electric and acoustic stimuli will reduce the beneficial effects of residual hearing in EAS strategies. For example, recruitment of auditory nerve fibers by electric stimuli might leave those fibers unavailable for processing of acoustic stimuli.

In the present paper we study the effect of electrical stimulation on acoustically evoked compound action potentials (CAPs). CAPs are widely used as a measure of synchronized auditory nerve activity (Goldstein and Kiang, 1958; Eggermont, 1976). CAP recordings by means of electrocochleography enabled us to examine electro-acoustical interactions in that neural stage along the auditory pathway in which the interactions first take place. In a previous study we have investigated this topic by testing the effects of electrical pulse trains on CAPs in normal-hearing animals (Stronks et al., 2010b). We found that CAPs were suppressed by preceding electrical stimuli (i.e. when using a forward masking paradigm). Suppression was most pronounced when CAPs were evoked with tones of high acoustic frequency presented at a low sound level. Suppression at low acoustic frequencies was substantially less. In that study electrical stimuli were delivered via extracochlear electrodes.

In the present study we examined electro-acoustical interaction in settings that better approach the circumstances in EAS candidates. First, we used an animal model for severe high-frequency hearing loss to mimic the type of hearing loss encountered in EAS candidates. Animals received an ototoxic treatment and CAP recordings were performed either 2 or 10 weeks thereafter. After 2 weeks cochlear hair cells are damaged without significant damage to the auditory nerve. After prolonged deafness (e.g. 10 weeks) hair cell loss is complemented with auditory nerve degeneration (Versnel et al., 2007). We used this model to mimic short-term and long-term sensorineural high-frequency hearing loss. Second, the present data were obtained using an intracochlear platinum wire electrode in the basal turn of the cochlea to mimic a cochlear implant electrode in the high-frequency region of the cochlea. Electric stimuli approached those used in contemporary implants, consisting of brief electric pulse trains.

Of particular interest was the question whether acoustically evoked responses at low frequencies (~1 kHz) were affected by electrical stimulation in the base of the cochlea under conditions of a high-frequency hearing loss. This question was addressed using various stimulus conditions; acoustic sound level, electric current level, and interval between electric and acoustic stimulus were systematically varied. Most experiments were conducted by presenting acoustic

tonal stimuli 1 ms after the electric stimulus, which in essence represents a "forward masking" paradigm. Experiments testing the effects of the interval between both stimuli also included "simultaneous masking", in which tone bursts partly overlapped with the electric pulse train.

4.2. Methods

4.2.1 Animal preparation

Experiments were performed on 22 healthy, female albino guinea pigs with a weight range of 350 – 910 g at the time of recording. This group was divided into a normal-hearing group (n= 12) and two groups of guinea pigs with a high frequency hearing loss recorded 2 weeks (n= 7), or 10 weeks after treatment (n= 3).

The procedure to induce a severe high-frequency hearing loss was performed using kanamycin and furosemide co-treatment as described previously (Versnel et al., 2007). Lower kanamycin doses were used than in that report to partially preserve low-frequency hearing (Brummett et al., 1979). Briefly, animals were anesthetized with intramuscular injections of 40 mg/kg ketamine (Ketanest-S®, Pfizer BV) and 0.5 mg/kg medetomidine (Domitor®, Pfizer BV). Freshly prepared kanamycin sulphate (Sigma) in saline was injected subcutaneously. Kanamycin doses were 200, 250 or 300 mg/kg. The jugular vein was then exposed and 100 mg/kg furosemide (Centrafarm®) was infused intravenously. After surgery, 0.01 ml atipamezole 5 mg/ml (Antisedan®, Pfizer BV) was given intramuscularly for a fast recovery from anesthesia. Dosages of 250 and 300 mg/kg could result in severe hearing loss extending to low frequencies and some (pilot) animals were excluded because of insufficiently large responses to low frequencies.

Electrophysiological recordings were performed in acute experiments as described previously (Stronks et al., 2010b). Anesthesia was initiated with an intramuscular injection of 0.1 ml/kg Hypnorm® (Vetapharma; 0.315 mg/ml fentanyl + 10 mg/ml fluanisone) followed by induction with 2% isoflurane (Nicholas Piramal Limited) evaporated in a gas mixture consisting of 67% N_20 and 33% O_2, using a mouth cap. A single subcutaneous injection of 0.05 mg/kg atropine (Pharmachemie BV) was given to reduce bronchial secretion. The animal was tracheotomized, intubated and artificially ventilated (Amsterdam infant ventilator mk3, Hoekloos) throughout the experiment with 1 – 2% isoflurane in 67% N_2O and 33% O_2. This anesthetic regime reduces CAP amplitudes only to some extent (Stronks et al., 2010a). Anesthetic depth was assessed regularly by testing the pedal-withdrawal reflex (front paw). Heart rate was monitored (180 – 360 bpm) and optionally 0.05 mg/kg atropine was administered subcutaneously. Rectal temperature was maintained at 38 ± 0.5°C using a thermostatically controlled heating pad. Every 1 – 2 hours a volume of 1% body weight of warmed glucose

in saline was administered subcutaneously for rehydration.

The right bulla was ventrally exposed and opened with a scalpel and forceps. The cartilage of the right pinna was removed and a metal ear probe inserted in the auditory meatus. Cochlear potentials were recorded using silver ball electrodes with Teflon-insulated shanks. The recording electrode was placed on the apex of the cochlea (Van Deelen and Smoorenburg, 1986), the reference electrode on the bulla wall. For electrical stimulation a Teflon-insulated platinum wire was partly stripped of its insulation (wire diameter 125 mm) and advanced 1 mm in the scala tympani of the basal turn through a cochleostomy 0.2 mm in diameter. After electrode insertion, the cochleostomy was sealed with silicone rubber (Dow Corning®). The return electrode, consisting of an insulated stainless steel wire with a gold ball 400 mm in diameter attached to it, was placed extracochlearly on the basal turn of the cochlea. Recording was performed in a sound attenuated booth.

CAP thresholds at 0.5 – 16 kHz were determined before and after the cochleostomy. Averaged thresholds (defined as 10 μV iso-response levels) of normal hearing animals (in dB SPL ± SD) before cochleostomy were: 0.5 kHz: 34 ± 11; 1 kHz: 49 ± 9; 2 kHz: 55 ± 6; 4 kHz: 50 ± 6; 8 kHz: 18 ± 12; 16 kHz: 33 ± 9. These values are comparable to those reported earlier at our laboratory for normal-hearing guinea pigs (Stengs et al., 1997). In the normal-hearing group and in the groups with a high-frequency hearing loss, averaged threshold shifts induced by drilling the cochleostomy did not exceed 10 dB at any frequency. Individual animals, however, occasionally showed threshold shifts up to 30 dB at one or more frequencies. After introduction of the cochleostomy recordings lasted up to 16 hours. The introduction of the cochleostomy and prolonged electrical stimulation had no visible effect on the histology of the organ of Corti or Rosenthal's canal in normal-hearing animals (Fig. 4.1).

Surgical and experimental protocols were approved by the Animal Ethical Committee of the University Utrecht under DEC-UMC number 2007.I.02.025. Animals were housed according to the standards of the animal care facility of the University of Utrecht.

4.2.2. Stimulus generation

The effects of electrical stimulation on acoustically evoked CAPs were tested by presenting a current pulse train before the acoustic tone burst (electro-acoustic stimulation, EAS) and comparing this response to the acoustically evoked CAP (acoustic stimulation, AS) without electrical stimulation (Fig. 4.2). Stimuli were generated using custom designed software in a Delphi 7® (Borland) programming environment and sent to a 24 bit DA converter (RP2.1, Tucker Davis Technologies; TDT) operating at a sampling rate of 49 kHz. Acoustic stimuli consisted of 12 ms (0.5 kHz) or 8 ms (1-16 kHz) tone bursts with $\cos^2$ shaped ramps of 4 ms (0.5 kHz), 2 ms (1 kHz), 1.5 ms (2 kHz) or 1 ms (4 - 16 kHz). Acoustic stimuli were

attenuated (PA5, TDT), and sent via a headphone amplifier (HB7, TDT) to a speaker (Beyer DT48) mounted on the metal probe. Sound levels were calibrated with a sound level meter (type 2610, Brüel & Kjær) and a ¼'' condenser microphone (type 4136, Brüel & Kjær). Electric stimuli consisted of a train of 10 biphasic (40 ms/phase) rectangular pulses with a pulse rate of 1000 pulses/s that were fed to a current source (Linear Stimulus Isolator A395, World Precision Instruments). Interstimulus interval was 120 ms plus the electric-to-acoustic interval (EAI). Interstimulus interval was identical under AS and EAS conditions. The phase of the acoustic stimuli and polarity of the electric stimuli were alternated each cycle such that one phase of the acoustic stimulus was always accompanied by the same polarity of the electric stimulus.

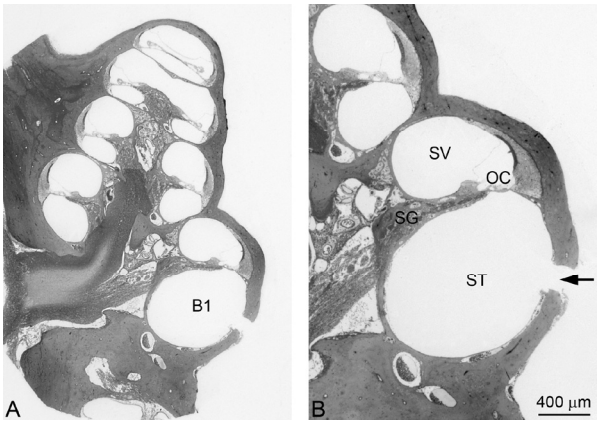

Fig. 4.1. (A) Low-magification overview of a cochlea sectioned along a plane just off the standard midmodiolar plane showing the location of the cochleostomy in the scala tympani of the lower basal turn (B1). (B) Light micrograph (detail from A) showing the cochleostomy (arrow) drilled through the bony capsule of the lower basal turn into the scala tympani (ST). There is no obvious damage to the basilar membrane, and organ of Corti (OC), or to the bony modiolar wall and the spiral ganglion (SG). SV: scala vestibuli.

Various parameters were investigated. Acoustic frequency (0.5 – 16 kHz) was always co-varied. Sound level was systematically varied from 100 dB SPL to threshold, current level was varied from 0 to 900 µA and EAI from -2 to 10 ms (Fig. 4.2). Due to technical limitations, current levels higher than 900 µA were not applied.

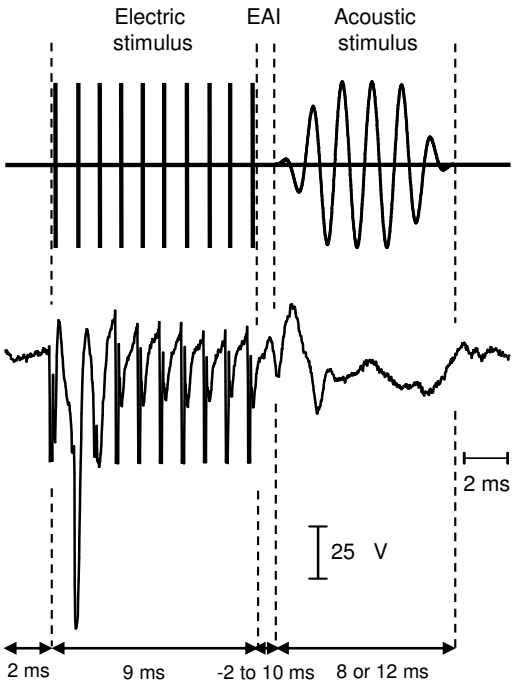

Fig. 4.2. Schematic of the stimulus paradigm. After 2 ms of baseline recording the electric stimulus was presented consisting of a train of 10 biphasic pulses (1000 pulses/s, 40 µs/phase). The tone burst was temporally separated from the pulse train by the electric-to-acoustic interval (EAI). The acoustically evoked compound action potential (CAP) was recorded and compared to the CAP without the electric stimulus present. Acoustic frequency and acoustic level, electric current level and EAI were systematically varied in this study.

4.2.3. Recording technique

Cochlear potentials were differentially amplified (2500 or 5000x) and band pass (1 Hz – 30 kHz) filtered (type 5113, EG & G Instruments) and subsequently digitized using a 16 bit AD converter at a sample rate of 49 kHz (RP 2.1, TDT). AS and EAS responses were recorded independently to a maximum of 250 sweeps per acoustic phase. Responses to acoustic stimuli of opposite phases were separately stored for off-line analysis and added and divided by two for CAP analysis, or subtracted and divided by two for CM analysis. In addition, responses to the electric stimuli (ES) were recorded and processed identically to the AS and EAS responses. ES waveforms were used for off-line EAS artefact reduction by subtracting them from the EAS waveforms, comparable to the method described by Charlet de Sauvage et al. (1983). ES waveform subtraction was especially useful to normalize the baseline and to remove electrophonics

from EAS waveforms. Electrophonic potentials often overlapped with acoustically evoked CAPs in the EAS waveforms. Electrophonic potentials had a latency of ~1.2 ms at high current levels and can be seen in Fig. 4.2 as adapting potentials during the pulse train. Electrophonic responses were previously described by us (Stronks et al., 2010b) and by McAnally et al. (1997).

4.2.4. Data analysis

Data were analyzed using custom written software in a Matlab® 6.5 (The Mathworks Inc.) programming environment. CAP amplitude at intermediate and high frequencies (2-16 kHz) was defined from the first negative peak (N_1) to the summating potential (SP) as depicted in Fig. 4.3A. At low acoustic stimulus frequencies (0.5 and 1 kHz) the CAP amplitude was defined from CAP minimum to SP (Fig. 4.3B). CAP minimum instead of N_1 was used because low-frequency evoked CAPs show a multitude of negative peaks, the so-called frequency following response. In our recordings the frequency of this frequency-following response was twice that of the stimulus frequency, due to the averaging procedure applied at the opposite phases described above. Because of the complex waveforms of the CAPs evoked at low frequencies, we additionally characterized the amplitude of these CAPs using the frequency-following characteristic by means of fast Fourier transform (FFT) analysis. The analysis window was set from tone onset to 2 ms after offset. The FFT amplitude was determined in a window centered on twice the frequency of the tonal frequency of the input stimulus. The CM was analyzed identically, but the center frequency was equal to the input acoustic frequency.

Effects of electrical stimulation on acoustically evoked CAPs were assessed by determining the ratio (R) of the amplitude of the EAS response (A_{EAS}) and the acoustical response amplitude (A_{AS}) (Fig. 4.6):

$$R = \frac{A_{AES}}{A_{S}}$$

To average these ratios linearly we converted ratios to a scale between -1 and 1 by means of R'= (R-1)/(R+1) after which the ratios R' were averaged. For graphical presentation these were re-converted to R by means of R= (1+R')/(1-R').

Statistical analysis of the effects of electrical stimulation (i.e., within animals) consisted of 1-sample t-tests against the no-effect level (i.e., CAP ratio= 1). To test for significance of effects of high-frequency hearing loss, data of ototoxically treated animals were compared to normal-hearing animals (i.e., between animals) using 2-sample t-tests, or ANOVA with ototoxic treatment as between factor.

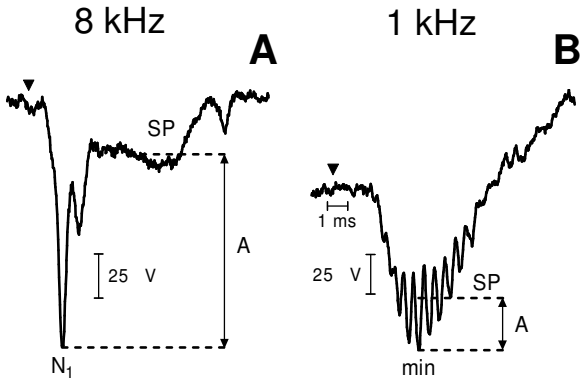

Fig. 4.3. Example CAPs evoked with 8 kHz (A) and 1 kHz tones (B) at 80 dB SPL. Triangles indicate tone onset. The amplitude of CAPs evoked with high-frequency tone bursts (2 – 16 kHz) was defined as the difference between the first negative peak (N_1) and SP. SP was defined as the mean potential of the last 2 ms of the plateau of the tone burst. The amplitude of low-frequency (0.5 and 1 kHz) evoked CAPs was defined as the difference between CAP minimum and summating potential (SP).

4.2.5. Histology

Immediately after finishing recordings, cochleas were fixed and processed for histological examination described in detail previously (De Groot et al., 1987). Cochleas were divided along a midmodiolar plane (Fig. 4.5B) and, after sectioning, the inner hair cells (IHCs) and outer hair cells (OHCs) in the organ of Corti were counted in each transection of the half turn as described previously. The number of spiral ganglion cells (SGCs) in Rosenthal's canal was quantitatively analyzed in each half turn by determining SGC packing densities (Van Ruijven et al., 2004). HC counts and SGC packing densities were analyzed only in the right cochlea, from which electrophysiological data were obtained. HC counts or SGC packing densities could occasionally not be obtained in apical cochlear locations. These data were complemented with HC counts or SGC counts from the left ear. Since ototoxic effects were minimal in these locations (see Results section), effects of this procedure on our results will have been minimal.

4.3. Results

We have investigated the effect of current pulse trains on CAPs evoked by tone bursts of variable frequency in normal-hearing guinea pigs and in animals with a high-frequency hearing loss either 2 weeks, or 10 weeks after ototoxic treatment. First we evaluate the animal model functionally (CAP thresholds) and histologically (hair cell and SGC loss). Next we show which current levels are

effective in altering acoustic responses evoked at variable acoustic frequency. Thereafter results are presented in which the sound level of these acoustic stimuli was varied. Last, the dependence on duration of the interval between electric and acoustic stimulus is shown.

4.3.1. Evaluation of the animal model

Effects of ototoxic treatment on the histology of the basal turn of the cochlea are illustrated in Fig. 4.4, showing representative sections of the organ of Corti and Rosenthal's canal in the lower basal turn of normal-hearing animals (Fig. 4.4A, D) and animals 2 weeks (Fig. 4.4B, E) and 10 weeks after ototoxic treatment (Fig. 4.4C, F). After ototoxic treatment, all OHCs were lost in the basal turn in these sections. IHCs were typically still present 2 weeks after ototoxic treatment, but not after 10 weeks. SGC packing densities were clearly decreased in the basal turn after 10 weeks, while after 2 weeks SGC loss was not visually identifiable.

Effects of ototoxic treatment on CAP thresholds, hair cell loss and SGC loss were quantified as shown in Fig. 4.5. CAP threshold shifts increased with frequency and were comparable 2 and 10 weeks after treatment (Fig. 4.5A). On average, CAP thresholds at 0.5 kHz and 1 kHz were increased by no more than 15 dB. At 2 and 4 kHz they were moderately increased by 15 – 35 dB, while at 8 and 16 kHz drastic increases of more than 50 dB were observed. Corresponding to the observed threshold shifts, OHC loss increased from apex to base from 20% to 100% (Fig. 4.5C). OHC counts were similar 2 weeks and 10 weeks after treatment. In contrast, IHC loss (Fig. 4.5D) was mild throughout the cochlea 2 and 10 weeks after treatment (<15%), and was only substantial in the lower basal turn 10 weeks after treatment. SGC loss was evident only after 10 weeks and reflected IHC loss, being severe (62%) only in the most basal location (Fig. 4.5E).

In conclusion, we obtained an appropriate model for severe high-frequency hearing loss with near-normal low-frequency hearing.

4.3.2. Dependence of CAP ratio on electric current level

We determined at which current levels effects of electrical stimulation became evident on CAPs evoked at variable tonal frequencies. Tonal stimuli were presented 1 ms after the pulse train (i.e., a forward masking paradigm). Example CAPs, evoked at 8 kHz in a normal-hearing animal, are shown as a function of current level in Fig. 4.6A. Under these conditions electrical stimulation decreased CAP amplitude. This suppression of the CAP increased with current level. In Fig. 4.6B this effect of electric stimulation on CAP amplitude is graphically presented by determining CAP ratio (A_{EAS}/A_{AS}) per current level. It can be seen in the figure that electric currents of 400 μA and higher resulted in CAP suppression in this example.

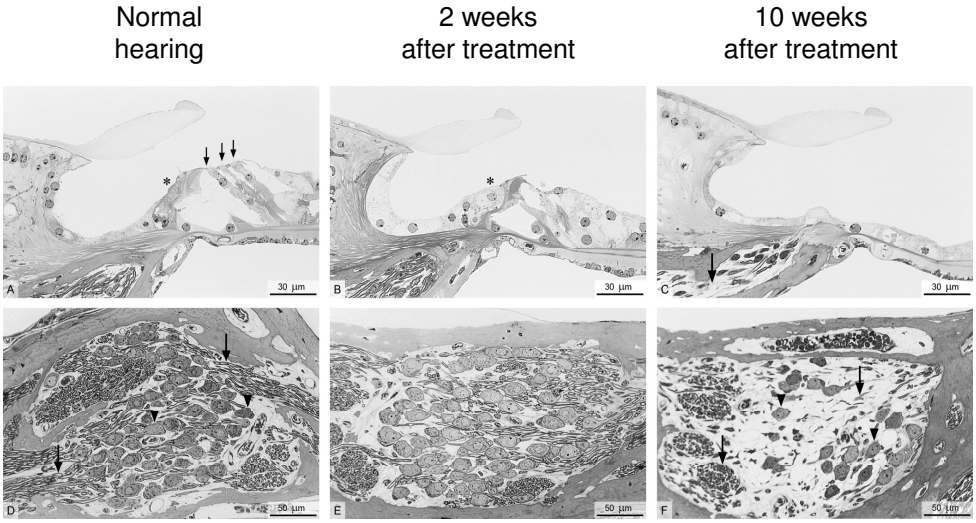

Fig. 4.4. Histological sections of the lower basal turn of representative cochleas. (A) Organ of Corti of a normal-hearing guinea pig. Arrows indicate the outer hair cells (OHCs) and the asterisk the inner hair cell (IHC). (B) Organ of Corti of an animal 2 weeks after ototoxic treatment. OHCs were completely lost, but IHCs were present (asterisk). (C) Organ of Corti of an animal 10 weeks after treatment showing a complete loss of OHCs and IHCs. Note the loss of nerve fibers in the spiral osseous lamina (arrow). (D) Rosenthal's canal of a normal-hearing animal showing a normal population of spiral ganglion cells (SGCs, arrowheads) and nerve fibers (arrows). (E) Rosenthal's canal of an animal 2 weeks after treatment showing SGC and nerve fiber populations comparable to normal-hearing animals. (F) Rosenthal's canal of an animal 10 weeks after treatment showing clear SGC and nerve fiber degeneration.

The effect of electric current level was tested at various frequencies (0.5 – 16 kHz) at 80 dB SPL. Averaged CAP ratios, determined at low frequencies (0.5 and 1 kHz) and a high frequency (8 kHz, as in Fig. 4.6) are shown in Fig. 4.7. At all these frequencies, CAPs were typically suppressed (i.e., CAP ratios < 1) in normal-hearing animals (Fig. 4.7A). At high current levels (600 - 900 µA), maximal suppression was 20% at low frequencies and up to 40% at 8 kHz.

In animals with a high-frequency hearing loss, suppression at 0.5 and 1 kHz was less compared to the normal-hearing controls after 2 weeks (Fig. 4.7B) or 10 weeks (Fig. 4.7C). Some CAP enhancement was observed that did not seem to depend on current level in a systematic way.

On the basis of these results, we applied a current level around 600 µA in normal-hearing animals in the experiments described in the following sections. In animals with a high-frequency loss, somewhat higher current levels of 800 or 900 µA were applied, because of the small effects in these animals.

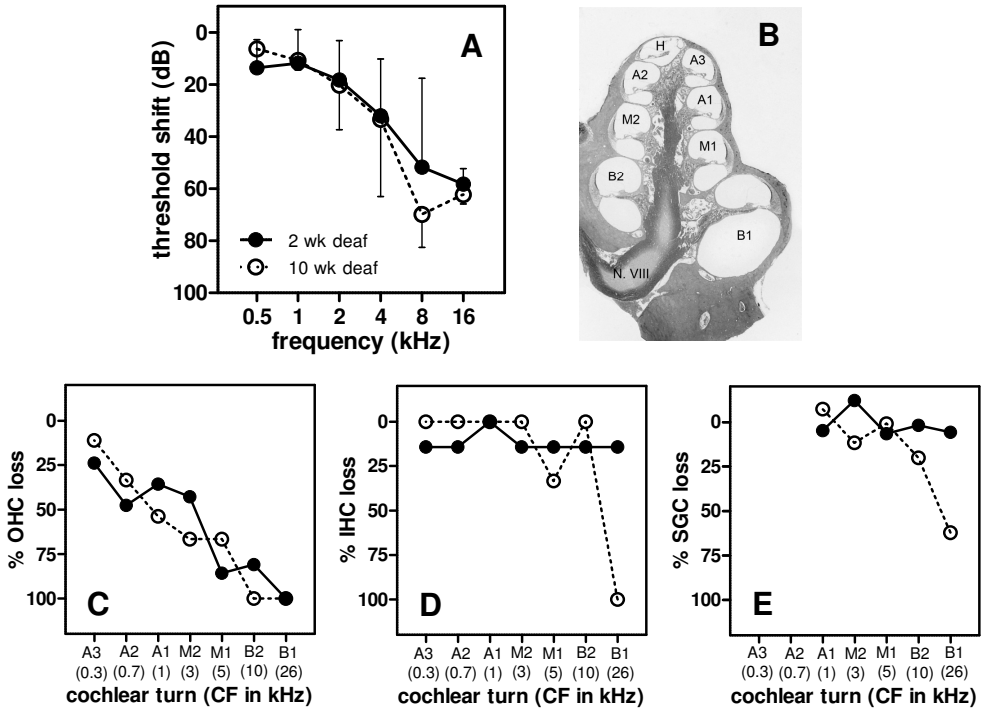

Fig. 4.5. Effects of ototoxic treatment after 2 weeks (n= 7) and 10 weeks (n= 3) relative to normal-hearing animals (n= 12). (A) Average threshold shifts of the treated groups relative to the normal-hearing group. Standard deviations are indicated. (B) Midmodiolar section of a cochlea showing the examined cochlear locations (A3 – B1). Helicotrema (H) and auditory nerve (N. VIII) are indicated. Cochleas of all ototoxically treated animals, and 9 out of 12 normal-hearing animals were histologically examined. (C) Outer hair cell (OHC) loss. (D) Inner hair cell (IHC) loss. (E) Spiral ganglion cell (SGC) loss expressed as packing density decrease. OHC, IHC and SGC loss are relative to the hearing group and plotted as function of cochlear location. Characteristic frequencies of these locations are given in parentheses in the graphs.

4.3.3. Dependence of CAP ratio on sound level

To determine the dependence of CAP ratio on sound level, we varied tonal stimulus levels in steps of 10 dB from 100 dB SPL down to threshold at various frequencies. Tone bursts were presented 1 ms after the pulse train (i.e. a forward masking paradigm). Figure 4.8A shows averaged CAP ratios determined at 0.5, 1 and 8 kHzaveraged across 8 normal-hearing animals. CAP ratios obtained at low frequencies (0.5 and 1 kHz) contrasted with ratios obtained at high acoustic

frequencies, in that they varied non-monotonically with sound level. Interestingly, some CAP enhancement at low frequencies was noted at 60 dB SPL, which was significant at 1 kHz (ratio= 1.3, 1-sample t-test against the no-effect level of 1, P< 0.05, Fig. 4.8E). With increasing sound level the effect of electrical stimulation turned from enhancement to suppression. At 80 dB SPL suppression was significant at 0.5 kHz (ratio= 0.9, P< 0.05, Fig. 4.8F) and 1 kHz (ratio= 0.8, P< 0.05, Fig. 4.8G). CAP enhancement around threshold and suppression at higher sound levels was also observed at 2 kHz, while at 4 and 16 kHz CAP ratios showed a monotonic dependence on sound level comparable to 8 kHz (results not shown).

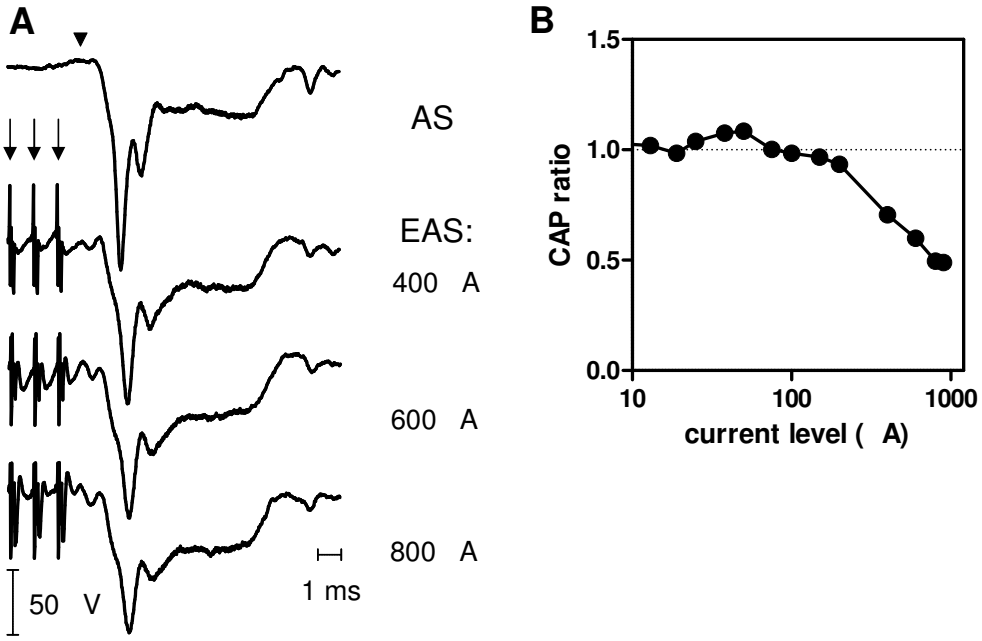

Fig. 4.6. Example CAPs evoked with 8-kHz tones at 80 dB SPL as a function of current level, obtained in a normal-hearing animal. Electric-to-acoustic stimulus interval was 1 ms. (A) CAP evoked with acoustical stimulation only (AS) and when an electric pulse train was presented (400 – 800 μA). The triangle in the upper trace indicates acoustic stimulus onset. The response to electrical stimulation (ES) was recorded separately and subtracted from the EAS recordings yielding the EAS waveforms shown. This subtraction procedure eliminated most, but not all electric artifact (arrows). (B) CAP amplitude ratios obtained by dividing the amplitude of the EAS response by that of the AS response.

Effects of electrical stimulation on CAPs evoked at low frequencies were further investigated in animals with a high-frequency hearing loss. In these animals, effects of electrical stimulation on CAPs were primarily investigated at

low-frequency tones (0.5 and 1 kHz). At these frequencies effects were generally mild in normal-hearing animals. In animals with a high-frequency hearing loss effects were similarly small, or even less. Two weeks after ototoxic treatment, CAP ratios showed a non-monotonic dependence on sound level, more or less comparable to normal-hearing controls (Fig. 4.8B). After 10 weeks, this trend with sound level had disappeared and effects of electrical stimulation were virtually absent at 0.5 and 1 kHz (Fig. 4.8C). Individual data (Fig. 4.8D – G) showed relatively large variability in animals with a high-frequency loss, compared to normal-hearing controls. CAP ratios did not depend significantly on ototoxic treatment at 0.5 or 1 kHz between the three groups of animals (1-way ANOVA with ototoxic treatment as between factor, $P > 0.1$). When CAP ratios 2 and 10 weeks after treatment were tested relative to the no-effect level, ratios did not differ significantly from 1. This was true when separate data were used (2 or 10 weeks), or when data from the 2 groups were combined (1-sample t-tests per frequency, at 60 or 80 dB SPL, $P > 0.05$).

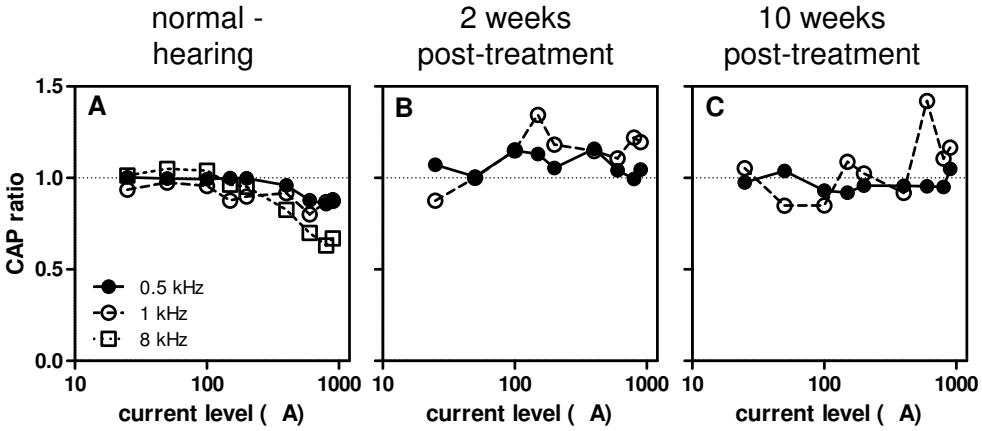

Fig. 4.7. Dependence of CAP ratio on current level at various acoustic frequencies. Animals were normal-hearing (A; n= 5), or had a high-frequency hearing loss and were recorded either 2 weeks (B; n= 4), or 10 weeks after ototoxic treatment (C; n= 3). Acoustic stimuli were presented 1 ms after the end of the electric pulse train (10 pulses, 1000 pps). Averaged CAP ratios are shown at 80 dB SPL in normal-hearing animals (A) at 0.5, 1 and 8 kHz, and at 0.5 and 1 kHz in animals 2 weeks (B), or 10 weeks after treatment (C).

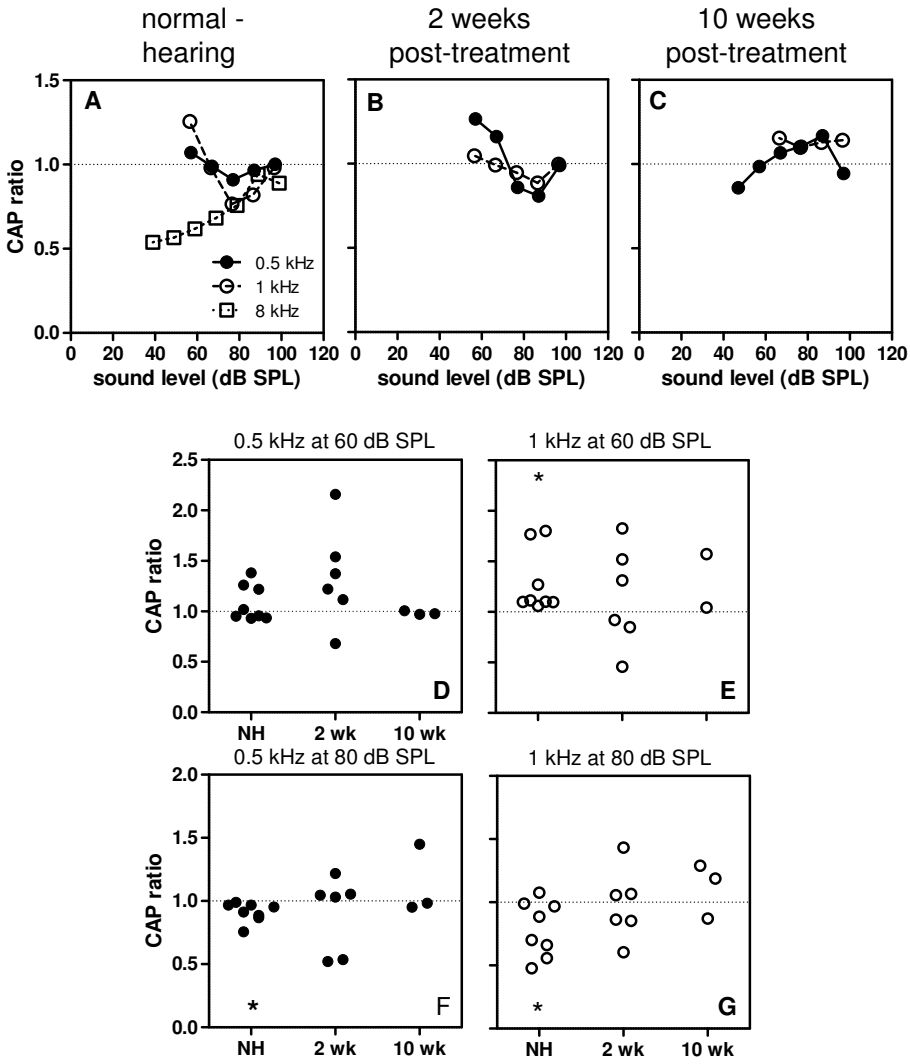

Fig. 4.8. Dependence of CAP ratio on sound level at various acoustic frequencies. Animals were normal-hearing (A: n= 8, current level 200 – 800 μA, mean: 650 μA), or had a high-frequency loss and were recorded either 2 weeks after ototoxic treatment (B: n= 6, current level: 800 – 1000 μA, mean: 833 μA) or 10 weeks after treatment (C: n= 3, current level: 800 – 1000 μA, mean: 867 μA). Tonal stimuli were presented 1 ms after the end of the electrical pulse train. Averaged CAP ratios are shown at 0.5 kHz, 1 kHz and 8 kHz in normal-hearing animals (A), and at 0.5 and 1 kHz in animals 2 weeks (B), or 10 weeks after treatment (C). Individual data in the three groups of animals are shown at 0.5 kHz at 60 SPL (D) and 80 dB SPL (E), and at 1 kHz at 60 dB SPL (F) and 80 dB SPL (G). Asterisks indicate a significant difference (P < 0.05) relative to the no-effect level (1-sample t-test relative to a CAP ratio of 1).

4.3.4. Dependence of CAP ratio on electric-to-acoustic interval

Effects of electrical stimulation on CAPs were tested in the previous sections using an electric-to-acoustic interval (EAI) of 1 ms to temporally segregate electrical stimulus artifacts from the subsequent acoustically evoked CAP waveform. Technically, such a procedure is a forward-masking paradigm. The difference between tonal stimulus and CM onset was about 0.3 ms. Hence, at EAIs of -0.3 and less, tone bursts (8 or 12 ms) temporally overlapped with the pulse train and represented simultaneous presentation of electric and acoustic stimuli within the cochlea. To investigate effects of simultaneous EAS, we applied EAIs of -2, -1 and -0.5 ms. To compare these effects with those under various conditions of forward masking, we additionally tested various EAIs from 0 to 10 ms.

Figure 4.9A shows the dependence of CAP ratio on EAI in normal-hearing animals at various acoustic frequencies at 80 dB SPL. On average, CAP suppression decreased with EAI. An EAI of -2 ms resulted in the largest suppression. This suppression was 10% at 0.5 kHz (CAP ratio = 0.9), 50% at 1 kHz (CAP ratio = 0.5), and 60% at 8 kHz (CAP ratio = 0.4). Differences between CAP ratios obtained at high frequencies and 1 kHz were small at an EAI of -2 ms, but increased rapidly at EAIs positive to -2 ms. CAP amplitudes generally recovered to near-normal values within several ms after pulse train offset. At lower sound levels (60 instead of 80 dB SPL) effects were similar at 0.5 and 1 kHz. At high frequencies suppression was more pronounced at low sound level and recovery could take considerably longer. For example, at 8 kHz averaged CAP suppression was 90% at the shortest EAI and recovery was not complete (ratio of 0.8) after 10 ms (not shown).

On average, no clear EAI dependence was observed in animals with a high-frequency hearing loss either 2 weeks (Fig. 4.9B) or 10 weeks (Fig. 4.9C) after treatment. In these animals, effects of electrical stimulation on CAPs evoked at low frequencies were insignificant, even at the shortest EAI tested (-2 ms, Fig. 4.9D, E). CAP ratios at both 0.5 and 1 kHz did not differ from 1 when the groups were tested separately, or when pooled data were used (1-sample t-tests, $P > 0.2$). In contrast, in normal-hearing animals a significant suppression at 1 kHz was observed at the shortest EAI tested (1-sample t-test, $P < 0.05$). When data of the animals with a high-frequency hearing loss were combined, CAP ratios differed significantly from normal-hearing animals (2-sample t-test, $P < 0.05$). Hence, there was significantly less CAP suppression at low frequencies (and high sound level) in animals with a high-frequency hearing loss than in normal-hearing animals.

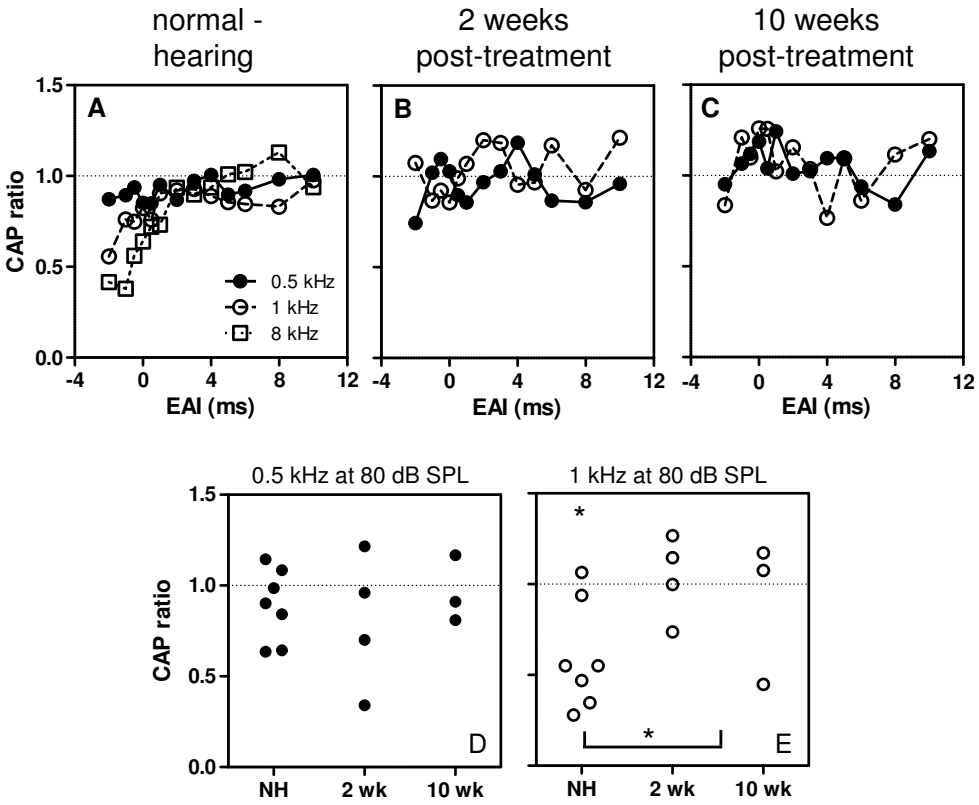

Fig. 4.9. Dependence of CAP ratio on the interval between electric and acoustic stimulus at various acoustic frequencies. Animals were normal-hearing (n= 7, current level 600 – 800 µA, mean: 743 µA), or had a high-frequency hearing loss and were recorded either 2 weeks after ototoxic treatment (n= 4, current level: 800 – 1000 µA, mean: 850 µA), or 10 weeks after treatment (n= 3, current level: 800 – 1000 µA, mean: 867 µA). EAI dependence of the averaged CAP ratio is shown at 80 dB SPL in normal-hearing animals (A) at 0.5, 1 and 8 kHz, and at 0.5 and 1 kHz in animals 2 weeks (B), or 10 weeks after treatment (C). Intervals of -2 to -0.5 represent simultaneous presentation of acoustic stimuli and electric stimuli. Individual data in the three groups of animals are shown when electric and acoustic stimuli were presented simultaneously (interval of -2 ms). Data are shown at a tonal frequency of 0.5 kHz (D) and 1 kHz (E), at a sound level of 80 dB SPL. At 0.5 kHz no significant effects were found on CAP ratios. At 1 kHz, normal-hearing animals had CAP ratios that were significantly below the no-effect level (asterisk, P< 0.05). Combined data of animals 2 and 10 weeks after treatment differed significantly from the normal-hearing group (P < 0.05).

4.3.5. Frequency following responses and cochlear microphonics

CAPs evoked at 0.5 and 1 kHz were additionally analyzed with FFT as a measure for the amplitude of the frequency following response. Results obtained with FFT analysis corresponded fairly well with the results described above for the CAP analysis based on the minimum-to-SP method (results not shown). For example, in normal-hearing animals CAPs at low frequencies were suppressed to some extent at high sound levels, and somewhat enhanced at low sound level. Cochlear microphonics (CM) analyzed with FFT analysis showed no effects under any condition (not shown).

4.4. Discussion

Effects of intracochlear electrical stimulation on acoustically evoked CAPs were investigated in guinea pigs with a high-frequency hearing loss and compared to normal-hearing animals. In normal-hearing animals, suppression of CAPs was most pronounced at high current levels (Fig. 4.7), high frequencies and low sound levels (Fig. 4.8), and short EAIs (Fig. 4.9). These results agree with our earlier study where we used minimally invasive techniques by using extracochlear stimulation electrodes on the round window and basal turn of the cochlea in normal-hearing animals (Stronks et al., 2010b). The major finding of the present study is that CAP suppression under conditions of low frequency and high sound level was virtually absent in animals with a high-frequency loss, while suppression under these conditions was significant in normal-hearing animals (though still not as large as suppression at high frequencies). In addition, the present data showed a significant CAP enhancement at 1 kHz at low sound levels in normal-hearing animals that was not observed in our earlier study using extracochlear stimulation. CAP enhancement was insignificant in animals with a high-frequency loss.

4.4.1. Effects of ototoxic treatment and high-frequency hearing loss

We used a guinea pig model for high-frequency hearing loss by co-administration of kanamycin and furosemide (West et al., 1973; Versnel et al., 2007). By using a moderate dose of kanamycin (Brummett et al., 1979) high-frequency hearing was severely impaired, while low-frequency hearing was spared (Fig. 4.5A). This pattern of hearing loss roughly mimicked the type of hearing loss associated with hybrid implant users. We note that hearing loss in our guinea pig model was mostly confined to high frequencies (8 – 32 kHz), while in EAS candidates hearing loss includes the moderate frequencies as well (2 – 20 kHz) (Gantz et al., 2005). It might be argued, however, that hearing sensitivity in both species is

shifted in the same direction, since normal-hearing guinea pigs are most sensitive to frequencies between 8 – 12 kHz, while humans hear best between 1 – 4 kHz. Hence, both hearing sensitivity and hearing loss seem to be shifted in our guinea pig model compared to EAS candidates. In all we conclude that, with regard to hearing function, we obtained a guinea pig model approaching the situation in EAS candidates.

Regarding histology, OHC loss was complete in the basal turn and gradually decreased towards apical regions, regardless of time after treatment (Fig. 4.5B). IHCs were lost in the basal turn after 10 weeks, but not after 2 weeks (Fig. 4.5C). Since CAP thresholds were very similar 2 and 10 weeks after treatment, we assume that after 2 weeks IHCs in the basal turn were in the process of degeneration and dysfunctional. SGC degeneration was extensive in the lower basal turn after 10 weeks. The concurrence of IHC and SGC loss in the lower basal turn agrees with the notion that long-term loss of IHCs is associated with a secondary loss of associated SGCs (Xu et al., 1993; Dodson, 1997; Versnel et al., 2007). We conclude that this model is an appropriate model for high-frequency hearing loss.

4.4.2. Effects of high-frequency hearing loss on electro-acoustic interaction

CAPs evoked by low-frequency tones at high sound levels were suppressed in normal-hearing animals, while at low sound levels CAPs at low frequencies were enhanced (Fig. 4.8). CAP suppression at 1 kHz was especially pronounced (50%) at very short EAIs when the acoustic stimulus was presented simultaneous with the electrical stimulus (Fig. 4.9). Suppression under these conditions was absent in animals with a high-frequency hearing loss (Fig. 4.9E). Suppression of low-frequency evoked CAPs in normal-hearing animals at high sound levels can be explained by the fact that auditory nerve fibers with a high characteristic frequency (CF) respond to low stimulus frequencies above sound levels of approximately 70 dB SPL (Kiang et al., 1967; Evans, 1972; Javel, 1994). The contribution of these high-CF fibers to CAPs evoked at 1 kHz will have been suppressed by electrical stimulation. This assumption can also explain the observation that animals with a high-frequency hearing loss showed no suppression at low acoustic frequencies even at very short EAIs, since high-CF nerve fibers cannot respond anymore.

The enhancement of CAPs at low frequencies and low sound level in normal-hearing animals might be related to the CAP-amplitude increase observed after intraneural stimulation reported earlier (Ball, 1982). The author explained CAP enhancing effects by efferent mechanisms involving the central nervous system, because CAPs were also enhanced on the contralateral side. Possibly OHCs were affected by electrical stimulation of efferents in that study. In the present report, CAP enhancement was absent in animals lacking functional OHCs in the basal regions. Therefore, enhancement of CAPs evoked at low frequencies

in normal-hearing animals could have been dependent on functional OHCs in the electrically stimulated basal regions of the cochlea in our experiments.

Both CAP suppression and enhancement at low frequencies were decreased or absent in animals with a high-frequency hearing loss, irrespective of duration after treatment. Hence, the differential effects observed in animals with a high-frequency hearing loss were likely mostly dependent on outer hair cells in the basal regions of the cochlea. IHC and SGC loss, which increased with duration after treatment, were probably of minor importance.

We conclude that effects of electrical stimulation on auditory nerve responses evoked at low frequency are predominantly caused by high-frequency regions in the cochlea. Hence, high-frequency regions of the cochlea can be stimulated electrically with little effect on low-frequency responses in cochleas with a basal loss of hair cells.

4.4.3. Intracochlear electrical stimulation in normal-hearing guinea pigs

In our earlier study we focussed on interactions of electrical stimulation with CAPs evoked at high acoustic frequencies in normal-hearing animals (Stronks et al., 2010b). In that study we used extracochlear stimulation electrodes. We expected to find more pronounced suppression in the present study using intracochlear stimulation, since intracochlear electrodes are likely more effective for current delivery in the cochlea. Suppression was however very similar in magnitude. For example, at 8 kHz averaged suppression around 40 dB SPL (corresponding to about 20 dB above CAP threshold in our previous article) was ~50% in both studies (Fig. 4.8A). Furthermore, these suppressive effects were obtained using very similar current levels in both studies (~600 μA).

In our earlier study we explained the observed suppression by a combination of direct neural effects and hair-cell mediated effects, i.e. electrophonics. Electrophonics refer to mechanical events in the cochlea evoked by electrical stimulation. In that study we showed that the frequency-dependence of acoustic suppression of the electrophonic response was related to the spectrum of the electric stimulus (Stronks et al., 2010b). The pulsatile stimulus in the present study was the same and had its spectral peak near 8 kHz. The observed maximal suppression at 8 kHz could be a reflection of that spectral peak, and electrophonics were probably an important factor underlying suppression in the present study as well.

Besides direct electrical neural activation and electrophonics, electrical activation of inhibitory efferents and electrical contraction of middle ear muscles could have played a role in the observed CAP suppression. These effects were probably small or absent, as was discussed previously (Stronks et al., 2010b).

At low frequencies we found some CAP suppression at a high sound level, and some enhancement at a moderate sound level, especially at 1 kHz

(Fig. 4.8D). At high frequencies suppression decreased with sound level and enhancement did not occur. These results correspond to our earlier study where we used extracochlear stimulation electrodes (Stronks et al., 2010b).

4.4.4. Clinical implications

We have shown that electrical stimulation had little effect on the amplitude of CAPs evoked at low frequencies in guinea pigs with a high frequency hearing loss. Electrical stimuli were delivered intracochlearly and resembled the biphasic pulsatile stimuli used in modern cochlear implants. When these results are extrapolated to hybrid-implant users, high-frequency regions of the cochlea can be stimulated electrically without large effects on the magnitude of low-frequency acoustic responses.

There are several considerations concerning the extrapolation of our findings in a guinea pig model to the situation in humans using hybrid implants. First of all, we used CAP amplitude as a measure of auditory nerve activity. CAPs represent the synchronized gross activity of multiple auditory nerve fibers. Although we based our results at low acoustic frequencies on peak-amplitude and FFT–derived amplitude (covering multiple peaks), other mechanisms not affecting gross potential amplitude may have played a role. For example, temporal aspects of auditory nerve firing might have been affected that were not reflected in CAP peak amplitudes.

We did not test current levels higher than 900 μA. Although this current level is probably a reasonable approximation of actual current levels used in CIs, higher current levels likely would have revealed additional effects. Furthermore, we deployed a short (1 mm) electrode, while hybrid implant arrays may extend up to 22 mm in the cochlea (Adunka et al., 2004) and may have larger effects on low-frequency evoked responses. Contemporary arrays are also thicker than our electrode and may therefore affect cochlear mechanics to a larger extent than the platinum wire used in our experiments.

Last, biphasic current pulses were used that alternated in polarity, whereas in cochlear implants trains of biphasic current pulses are used that do not alternate (Wilson et al., 1991). It has been shown in cats and guinea pigs that monophasic pulses of different polarity yield electrically evoked compound action potentials (eCAPs) that differ in amplitude, latency and threshold (Miller et al., 1998). Likewise, in cochlear implant users, current stimuli of opposite polarities were reported to yield eCAPs with different amplitudes, as well as different auditory sensations (Macherey et al., 2008). We have averaged responses to both polarities and the effects observed in the present study may therefore not completely apply to cochlear implant users.

Despite these limitations, important conclusions can be drawn from the present data. We used a single stimulating electrode in the basal turn of the cochlea, which will likely have spatially restricted the area of stimulation. If

the lack of effects on low-frequency acoustic responses was due to this spatial segregation, these findings would plead for the use of short electrodes in EAS strategies (Gantz and Turner, 2003; Gantz et al., 2006, 2009). Furthermore, suppression of CAPs increased with current level. Hence, ideally current levels should be kept low to minimize interactions of electrical stimuli with acoustically evoked auditory nerve activity. Last, when CAPs were suppressed by electrical stimuli, amplitudes recovered rapidly when the interval between electric pulse train and acoustic tone was increased. After approximately 5 ms, amplitudes had returned to near-normal values. These findings can possibly be adopted in hybrid implant stimulation strategies.

Acknowledgements

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Abbreviations

AS	acoustical stimulation
CAP	compound action potential
CI	cochlear implant
CF	characteristic frequency
EAI	electric-to-acoustic stimulus interval
EAS	electro-acoustical stimulation
ES	electrical stimulation
FFT	fast Fourier transform
IHC	inner hair cell
OHC	outer hair cell
SGC	spiral ganglion cell

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Effects of electrically evoked hair-cell activity on acoustically evoked auditory-nerve responses in the guinea pig

5

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In preparation

Abstract

There is increasing interest in electro-acoustical stimulation in cochlear implant candidates with residual hearing. This raises the issue of how responses to electric and acoustic stimuli interact. Previously we found that electric pulse trains could suppress acoustically evoked compound action potentials (CAPs). We suggested that suppression was mediated by direct neural activation and electrophonics. In the present study we investigated interaction of electrophonics and CAPs in the guinea pig cochlea. Electrophonics can excite cochlear regions corresponding to the frequency spectrum of the electrical stimulus. In case of pulsatile stimuli, the spectrum mainly depends on pulse width. We found that the frequency-dependence of CAP suppression could be related to the spectrum of the electric pulses. For instance, the spectrum of a biphasic pulse 80 μs in width peaks at 10 kHz, while at 200 μs there is a spectral notch at 10 kHz. A train of pulses of 80 μs in width suppressed CAPs evoked at acoustic frequencies around 10 kHz to a large extent (~40%), whereas suppression was minimal (~10%) using pulses of 200 μs. We conclude that electrophonics contribute to CAP suppression. Short pulse widths are therefore advisable in EAS strategies to minimize this interaction at low acoustic frequencies.

Key words: electro-acoustical stimulation; residual hearing; cochlear implant; compound action potential; electrophonics; hair cell; spiral ganglion cell

5.1. Introduction

Cochlear implantation is at present the method of choice for treatment of profound sensorineural hearing loss. Since the development of the cochlear implant (CI), speech understanding of CI users has improved considerably (Wilson et al., 1991; Wilson and Dorman, 2008). Therefore, selection criteria for implantation continue to expand and people with considerable residual low-frequency hearing are now considered candidates for cochlear implantation (Cohen, 2004). Recently, hybrid implants have been developed that combine a CI with a conventional hearing aid, delivering electro-acoustical stimulation in the same ear (Talbot and Hartley, 2008; Turner et al., 2008). Residual low-frequency hearing in CI users has been shown to increase speech understanding in noise and improve the esthetical quality of complex sounds such as music (Fraysse et al., 2006; Gantz et al., 2006).

We assume that an interaction between electric and acoustic stimuli will reduce the beneficial effects of residual hearing in EAS strategies. In previous studies we found that auditory-nerve responses evoked at high acoustic frequencies were suppressed by electrical stimulation of the basal part of the cochlea (Stronks et

al., 2010b). We suggested that this suppression was mediated by direct electric neural stimulation as well as electrophonic activity. Electrophonic responses are evoked by electrically evoked auditory nerve activity not mediated by direct neural activation (Stevens, 1937), but by activation of hair cells (Lusted and Simmons, 1988). McAnally et al. (1997) investigated the interaction between acoustically evoked CAPs and electrophonic responses evoked by pulse trains. The cochlear excitation pattern, derived from the frequency-dependence of suppression of acoustically evoked CAPs by pulse trains, was related to the frequency spectrum of the pulse trains. Hence, electrical stimuli seemed to excite cochlear regions corresponding to the frequency spectrum of the electric stimuli, as if they were acoustic stimuli. These results support the notion that electric stimuli can generate electro-mechanical responses on the basilar membrane (McAnally et al., 1993; Kirk and Yates, 1994). Electro-mechanical transduction is thought to rely on the electromotility of outer hair cells (e.g. Brownell et al., 1985; Ashmore, 1987) by generating intracochlear pressure differences and travelling waves on the basilar membrane (Nuttall and Ren, 1995). However, some findings indicate that electrophonics may be independent on OHCs. First, acoustically evoked CAPs were suppressed by electrophonic mechanisms after damage of basal OHCs close to the stimulating electrode (McAnally et al., 1993; McAnally and Clark, 1994). Second, changes in cochlear pressure by basal electrical stimulation occurred in the absence of OHCs and IHCs in the basal and middle turn, and even in cochleas tested *post mortem* (Moxon, 1971).

In the present study we investigated the role of electrophonics in suppression of acoustically evoked responses in normal-hearing guinea pigs by varying the frequency spectrum of the electric stimulus using variable pulse rates and pulse widths. While McAnally et al. applied long electric-to-acoustic intervals of 15 ms (McAnally, 1997), we aimed to mimic the situation in EAS users. Therefore, electric and acoustic stimuli were presented nearly simultaneously (simultaneous presentation was, however, not feasible due to electric stimulus artifacts). As a consequence, suppression of acoustical responses could be mediated by direct neural activation and subsequent refractory effects, by electrophonic mechanisms, or a combination of both. By varying the electric frequency spectrum we could estimate the role of electrophonics in CAP suppression under conditions more or less representative for EAS users.

Two additional experiments were conducted addressing the interaction between electrophonics and acoustically evoked responses. First, we recorded electrophonically evoked compound action potentials (epCAPs) directly and measured epCAP suppression by tonal stimuli as a function of acoustic frequency. If electrophonics depend on the frequency spectrum of the electric stimulus, epCAP suppression as a function of acoustic frequency would correspond to this spectrum. Second, the role of outer and inner hair cells in electrophonics was examined by recording epCAPs in animals with hair cell damage.

5.2. Materials and methods

5.2.1. Animal preparation and experimental design

Experiments were performed on 24 healthy, female albino guinea pigs with a weight range of 350 – 880 g at the time of recording. Experiments on CAP suppression by electric stimuli and epCAP suppression by acoustic stimuli were conducted on normal-hearing animals (n= 11). Two groups of partially deafened guinea pigs were used that were recorded 2 weeks (n= 7), or 10 weeks after ototoxic treatment (n= 6) to study dependence of epCAP amplitude on cochlear status. Acoustic sensitivity in these animals was characterized by determining CAP threshold (iso-response level interpolated at 10 μV) as a function of acoustic frequency. Averaged thresholds (±SD) of the normal-hearing animals, before cochleostomy, were: 0.5 kHz: 34 ± 11; 1 kHz: 49 ± 9; 2 kHz: 55 ± 6; 4 kHz: 50 ± 6; 8 kHz: 18 ± 12; 16 kHz: 33 ± 9. These values are comparable to those reported earlier for normal-hearing guinea pigs (Stengs et al., 1997). Effects of ototoxic treatment are presented in the Results section (Fig. 5.7).

Surgery and electrophysiological recordings were described in detail previously (Stronks et al., 2010b). Animals underwent surgery under general anesthesia using 1 – 2.5% isoflurane (Nicholas Piramal Limited) evaporated in a mixture of nitrous oxide and oxygen (2:1) delivered by active ventilation (Stronks et al., 2010a). Heart rate was monitored (180 – 360 bpm) and rectal temperature maintained at 38 ± 0.5°C using a heating pad. Every 1 – 2 hours a volume of 1% body weight of physiologic saline with glucose (37°C) was administered subcutaneously for rehydration. The right cochlea was exposed and a metal ear probe was inserted in the ear canal. Cochlear potentials were recorded using silver ball electrodes. The recording electrode was placed on the apex of the cochlea (Van Deelen and Smoorenburg, 1986), the reference on the bulla wall. Electrical stimuli were delivered by a platinum wire (diameter 125 mm) advanced 1 mm through a cochleostomy in the basal turn of the cochlea about 1 mm from the round window. The cochleostomy (~200 mm in diameter) was sealed with silicone rubber (Dow Corning®). A gold ball (~400 mm in diameter) placed extracochlearly on the basal turn of the cochlea was used as return electrode. Recordings were performed in a sound attenuated booth.

5.2.2 Ototoxic treatment

High-frequency hearing loss was induced using kanamycin and furosemide co-treatment as described in detail previously (Versnel et al., 2007). Kanamycin doses were lowered (200 – 300 mg/kg instead of 400 mg/kg) to (partly) preserve low-frequency hearing. Animals were anesthetized with intramuscular injections of 40 mg/kg ketamine (Ketanest-S®, Pfizer BV) and 0.5 mg/kg medetomidine (Domitor®, Pfizer BV). Freshly prepared kanamycin sulphate (Sigma) in saline

was injected subcutaneously. Kanamycin doses were 200, 250 or 300 mg/kg. The jugular vein was then exposed and 100 mg/kg furosemide (Centrafarm®) was infused intravenously. After closure of the incision 0.01 ml atipamezole 5 mg/ml (Antisedan®, Pfizer BV) was given intramuscularly for recovery from anesthesia.

Surgical and experimental protocols were approved by the Animal Ethical Committee of the University Utrecht under DEC-UMC number 2007.I.02.025. Animals were housed according to the standards of the animal care facility of the University of Utrecht.

5.2.3. Stimulus generation

Stimuli were generated as described previously (Stronks et al., 2010b) using Tucker Davis Technologies hardware and custom designed software in a Delphi 7® (Borland) programming environment. Tonal stimuli were delivered via a speaker (Beyer DT48). Electric stimuli consisted of a single biphasic pulse, or trains of these pulses. Current stimuli were delivered by a current source (Linear Stimulus Isolator A395, World Precision Instruments). Interstimulus interval was 111 ms plus the pulse train duration (9 or 10 ms) and the electric-to-acoustic interval (typically 1 ms). An example paradigm with a pulse rate of 500 pps is given in Fig. 5.1. Pulse train stimuli consisted of a number of pulses that depended on pulse rate (single pulse, 6 pulses at 500 pps, 11 at 1000 pps etc.). Interstimulus interval was identical under acoustical or electrical stimulation alone (AS or ES), and under electro-acoustical stimulation (EAS) conditions. The phase of the acoustic stimuli and polarity of the electric stimuli were alternated each cycle such that one phase of the acoustical stimulus was always accompanied by the same polarity of the electric stimulus.

Various parameters were varied, including pulse width (80 – 400 μs), pulse rate (500 - 4000 pulses/s (pps)), acoustic frequency (0.5 – 16 kHz), sound level (60 and 80 dB SPL), and interval between electric and acoustic stimulus (EAI; -2 to 10 ms).

Effects of electrical stimulation on CAP and CM were recorded by presenting acoustic tone bursts 1 ms after the last pulse in the pulse train. This forward masking paradigm was deployed to separate electric stimulus artefacts from the acoustically evoked CAP waveform. Effects of acoustic tones on epCAPs were performed by presenting a current pulse 2 or 5 ms after tone onset (simultaneous stimulus presentation).

5.2.4. Recording technique

Evoked potentials were differentially amplified (2500 or 5000x) using filter settings of 1 Hz - 30 kHz (type 5113, EG & G Instruments). Signals were AD converted at 49 kHz (Tucker Davis Technologies) and averaged to a maximum of 500 sweeps using custom-written software (Delphi 7®, Borland). Responses to

stimuli of opposite phases were separately stored for off-line analysis. CAP and epCAP waveforms were obtained by summating responses to opposite stimuli, and division by 2. CM was obtained by subtraction and division by 2. Responses to EAS (Fig. 5.1) were compared to the response to the probe-alone condition (AS for CAP and CM, or ES for epCAP). For artifact reduction, ES-only responses (when probe was AS), or AS-only responses (when probe was ES) were also recorded and processed identically to the probe and EAS responses. These waveforms were used for off-line artefact reduction by subtracting them from the EAS waveforms, comparable to the method described by Charlet de Sauvage et al. (1983).

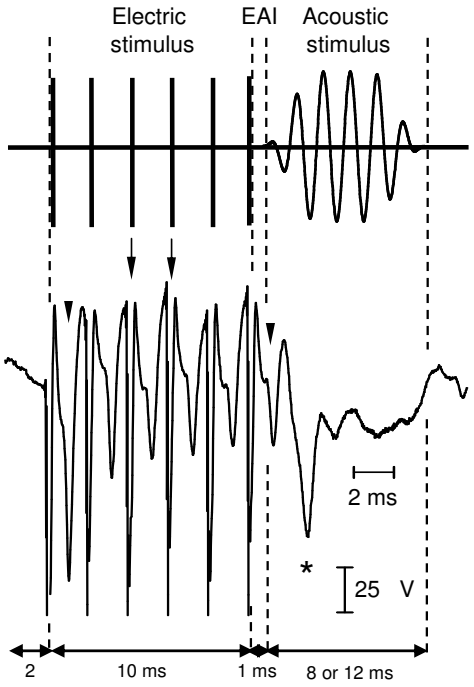

Fig. 5.1. Example of a stimulus paradigm and the corresponding recording. After 2 ms of baseline recording the electric stimulus was presented consisting of a train of biphasic pulses (here: 6 pulses at 500 pps, 80 µs pulse width). The tone burst followed after an electric-to-acoustic interval (EAI), which is here 1 ms. Pulse rate and pulse width, acoustic frequency, sound level, and EAI were systematically varied. The bottom plot shows the actual response using an 8 kHz stimulus of 80 dB SPL and a current level of 800 µA. Electrical stimulus artifact (arrows), electrophonic responses (arrow heads), as well as the acoustically evoked CAP (asterisk) are visible.

5.2.5. Data analysis

Data were analyzed with custom written software using Matlab® 6.5 (The Mathworks Inc.) as described previously for CAP and CM (Stronks et al., 2010b). Tone-evoked CAPs were accompanied by a tonic SP response, and CAP amplitude was determined relative to this SP as depicted in Fig. 5.2A, B. Pulse-evoked epCAP waveforms did not contain a discernable SP and amplitude was determined relative to the first positive peak (Fig. 5.2C). CM amplitude was determined using fast Fourier transformation (FFT) analysis (Stronks et al., 2010b).
Effects of EAS on evoked potentials by AS or ES were assessed by determining the ratio (R) of the amplitude of the EAS response (A_{EAS}) and the probe response amplitude, i.e. the amplitude of the response to AS or ES stimulation only (A_{AS} or A_{ES}):

$$R = \frac{A_{EAS}}{A_{AS}} \quad \text{or} \quad R = \frac{A_{EAS}}{A_{ES}}$$

Ratios were linearly averaged across animals by converting ratios to a scale between -1 and 1 by means of R'= (R-1)/(R+1) after which the ratios R' were averaged. For graphical presentation these were re-converted to R by means of R= (1+R')/(1-R').

Statistical analyses consisted of 1- or 2-way analysis of variance (ANOVA) or repeated measures ANOVA (RM ANOVA), followed by *post hoc* tests (Bonferroni corrected *t*-test, or Dunnett's *post hoc* test). For analyses of ratios (R) relying on mean values such as ANOVA, converted ratios (R') were used.

5.2.6. Histology

Immediately after electrophysiological recording, cochleas were fixed and processed for histology as described previously (De Groot et al., 1987). Right cochleas were divided along a midmodiolar plane and after sectioning the inner hair cells (IHCs) and outer hair cells (OHCs) in the organ of Corti were counted in each transection of the half turn. The degeneration pattern of the spiral ganglion cells (SGCs) in Rosenthal's canal was quantatively analyzed in each half turn by determining the SGC packing densities (Van Ruijven et al., 2004). In some cochleas, hair cell counts or SGC packing densities could not be obtained in apical regions, e.g. due to imperfect midmodiolar transections. In these cases, missing data were supplemented with that from the left ear. Since apical locations were least affected in ototoxically treated animals, this procedure will not have affected our data to a large extent.

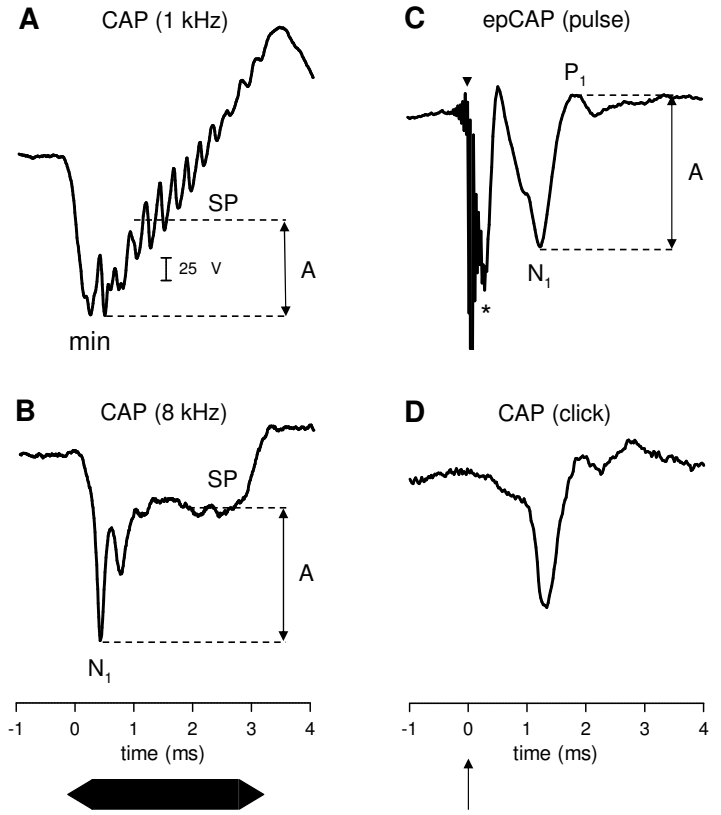

Fig. 5.2. Examples of CAP and epCAP waveforms. Tone-evoked CAPs were obtained at 1 kHz (A) and 8 kHz (B), at 80 dB SPL. CAP amplitude (indicated with A) at low frequencies (0.5 and 1 kHz) was defined from CAP minimum (min) to summating potential (SP). SP was defined as the mean potential of the last 2 ms of the plateau of the tone burst. The frequency-following response had a frequency twice that of the original stimulus due to averaging of responses to opposite phases. CAP amplitudes at high-frequency tones (2 – 16 kHz) were defined from the first negative peak (N_1) to SP. Rise and fall times of acoustic tones were 2 ms for 1 kHz bursts, and 1 ms for 8 kHz bursts (as drawn). The epCAP (C) was evoked by a biphasic pulse (80 µs pulse width, 800 µA) and had a latency of 1.2 ms. The electric stimulus artifact is visible (arrowhead). The short-latency response 0.3 ms after pulse onset represents the electrically evoked CAP (asterisk). For comparison, an acoustic click-evoked CAP (D) of approximately equal amplitude is shown below the epCAP (monophasic click, 40 µs click width, 67 dB peSPL). Amplitude of epCAPs were defined from N_1 to the first positive peak (P_1). Stimulus timing is shown below the time bar. Pulse and click stimulus timing are indicated by an arrow. CAP responses (A, B, D) and tone burst placement were corrected for CM onset (CM onset corresponds to t= 0). CM onset after acoustic stimulus onset was 0.3 ms, corresponding to a travelling distance of ~10 mm from speaker to tympanic membrane.

5.3. Results

We have investigated the effects of combined electrical and acoustical stimulation by recording acoustically evoked compound action potentials (CAPs) and electrophonically evoked compound action potentials (epCAPs). First, effects of electrical stimulation on tone-evoked CAPs will be presented, starting with the dependence on pulse rate (section 3.1), and followed by the dependence on pulse width (section 3.2). Next, the effects of acoustic stimulation on epCAPs will be shown, including the dependence on acoustic frequency (section 3.3) and electric pulse width (section 3.4). The dependence of the epCAP on cochlear condition is presented thereafter, for which animals were used that were treated with ototoxins. These animals had variable degrees of hearing losses mainly restricted to high frequencies (section 3.5).

5.3.1. Dependence of CAP amplitude on electric pulse rate

To test for tuning of CAP suppression to electric pulse rate we varied pulse rate from 500 to 4000 pps, and determined CAP ratios at corresponding acoustic frequencies of 0.5 to 4 kHz. Tonal stimuli were presented at sound levels of 60 or 80 dB SPL. All electric stimuli had a spectral maximum at a high frequency (~10 kHz) due to the short pulse width (80 μs). The number of harmonics differed between stimuli and was dependent on pulse rate (Fig. 5.3A – C).

CAP suppression did not depend on pulse rate (Fig. 5.3D, E) at any acoustic frequency at either 60 or 80 dB SPL (RM ANOVA performed per acoustic frequency with pulse rate as within factor, $P > 0.05$). These CAP ratio data were re-plotted as a function of acoustic frequency, additionally including acoustic frequencies of 8 and 16 kHz (Fig. 5.3F, G). On average, effects were frequency and level dependent. Irrespective of pulse rate, CAPs at acoustic frequencies of 1 and 2 kHz at a sound level of 60 dB SPL were somewhat enhanced, while at 80 dB SPL CAPs were somewhat suppressed. At 8 and 16 kHz suppression was observed irrespective of sound level. Statistically, CAP ratios at 60 dB SPL depended significantly on acoustic frequency at all pulse rates tested (RM ANOVA per pulse rate, frequency as within factor, $P < 0.05$), but not when a single pulse was applied ($P > 0.3$). At 80 dB SPL, no significant effect of acoustic frequency was found (RM ANOVA per pulse rate, frequency as within factor, $P > 0.05$).

5.3.2. Dependence of CAP amplitude on electric pulse width

We investigated whether CAP suppression depended on the shape of the frequency spectrum of the electric stimulus by varying the frequency spectrum of the electric stimulus and determining CAP suppression at variable tonal frequencies. The spectrum of pulsatile stimuli mainly depends on pulse width, and we varied this parameter from 80 μs (as used in the previous section) to 200 and 400 μs.

Increasing the pulse width shifted the maximum in the frequency spectrum to lower frequencies and introduced null harmonics at 5 and 10 kHz (Fig. 5.4A - C). Compared to Fig. 5.3, additional tonal frequencies were introduced around these null harmonics (5, 9, 10 and 11 kHz) and a linear frequency-axis was deployed to highlight effects at these frequencies. Statistics were carried out on CAP ratios at 5 and 10 kHz using 1-sample t-tests relative to the no-effect level (CAP ratio of 1).

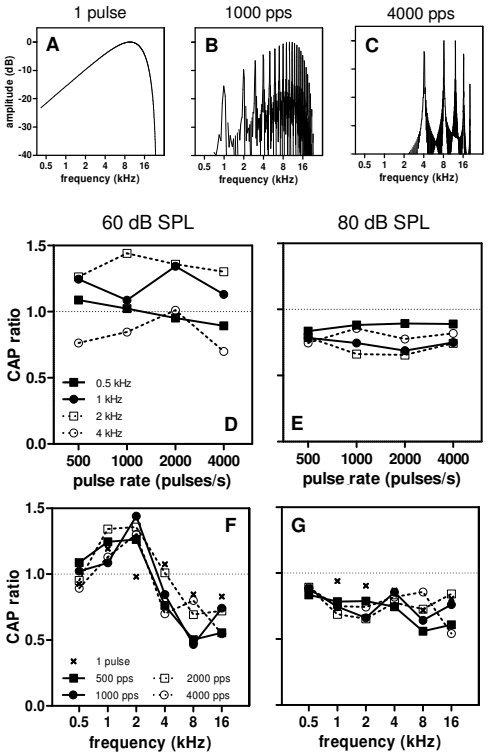

Fig. 5.3. Dependence of CAP ratio on electric pulse rate. (A – C) Power spectra for a single pulse (A) and 10 ms pulse trains at 1000 pps (B) and 4000 pps (C), scaled relative to 0 dB for every pulse rate. (D, E) Averaged CAP ratios (n= 5) plotted per acoustic frequency as a function of pulse rate at 60 dB SPL and 80 dB SPL. (F, G) Same data re-plotted as a function of acoustic frequency at 60 dB SPL and 80 dB SPL, including additional data of a single pulse and additional acoustic frequencies (8 and 16 kHz). Pulses consisted of biphasic pulses of 800 μ*A, 80* μ*s in width. Number of pulses depended on the pulse rate (6 pulses at 500 pps, 11 at 1000 pps etc.).*

At a short pulse width of 80 μs (Fig. 5.4D), CAP suppression was maximal around 10 kHz, corresponding to the maximum in the frequency spectrum of the electric

stimulus, and similar to the data presented in Fig. 5.3F. CAP ratios at 10 kHz were significantly lower than 1 at both sound levels tested (Fig. 5.5A and B, separate 1-sample t-tests, $P< 0.05$). At 5 kHz CAP ratios were significantly lower than 1 at 60 dB SPL ($P< 0.05$), but not at 80 dB SPL ($P > 0.1$).

Introduction of a null harmonic at 10 kHz by increasing pulse width to 200 μs (Fig. 5.4E) resulted in CAP ratios around 10 kHz that were clearly increased when compared to CAP ratios in that frequency range at a pulse width of 80 μs. Statistically CAP ratios were not different from 1 at 10 kHz ($P> 0.2$), despite a 250% increased charge injection with respect to a pulse width of 80 μs (Fig. 5.5A, B). At 5 kHz CAP ratios were significantly reduced at 80 dB SPL ($P< 0.05$). CAP ratios at frequencies below 5 kHz were lowered compared to those at a pulse width of 80 μs, especially so at 60 dB SPL which could be related to the shift of the spectral maximum to lower frequencies.

Introduction of null harmonics at 5 and 10 kHz by further increase of the pulse width to 400 μs (Fig. 5.4F) led to CAP ratios showing maxima at 5 and 10 kHz. At 5 kHz CAP ratios were not significantly different from 1 at either sound level ($P> 0.05$) and at 10 kHz only at 60 dB SPL (Fig. 5.5A and B, $P< 0.05$). CAP ratios at frequencies below and above 5 and 10 kHz were clearly lower, giving rise to local maxima at these frequencies. Absolute suppression at 10 kHz and 60 dB SPL was larger than at 200 μs pulse width.

At a pulse width of 400 μs, a marked suppression at low acoustic frequencies (0.5 and 1 kHz) was observed. CAP ratios under these conditions could not be determined, because "positive-first" pulse trains (i.e., trains of pulses with the positive phase of the biphasic pulse presented first) suppressed the CM signal to a greater extent than trains of opposite ("negative-first") current polarity (not shown). This unbalanced CM suppression prevented extraction of the CAP by the methods described in section 2.4. Therefore CAP data under these conditions were omitted in our analysis (Fig. 5.4E, F). CM amplitude could however be determined (Fig. 5.4G – I) and was decreased to a large extent at 0.5 and 1 kHz at both sound levels. CM suppression was especially pronounced at 0.5 kHz and a pulse width of 400 μs (more than 50% reduction). CM evoked at high frequencies was unaffected by electrical stimulation.

5.3.3. Effects of acoustic stimulation on electrophonic responses

When epCAPs evoked by an electric pulse (Fig. 5.2C) and click-evoked CAPs (Fig. 5.2D) of approximately equal amplitude were compared, a striking resemblance was observed with regard to waveform morphology. Peak latencies were nearly identical and were 1.29 ms for the epCAP and 1.32 ms for the click-evoked CAP, in the example given.

We tested the effects of acoustic stimulation on epCAPs by presenting simultaneous acoustic tonal stimuli of variable frequency and level (Fig. 5.6A). Electric pulses were presented 2 ms after tone onset. At 60 dB SPL, suppression

was sharply tuned to 8 kHz, which could be related to the frequency spectrum of the electric stimulus (Fig. 5.6B). Additional data obtained at 40 dB SPL indicated the same (not shown). At 80 dB SPL, epCAPs were suppressed by a range of tonal frequencies, without any clear tuning.

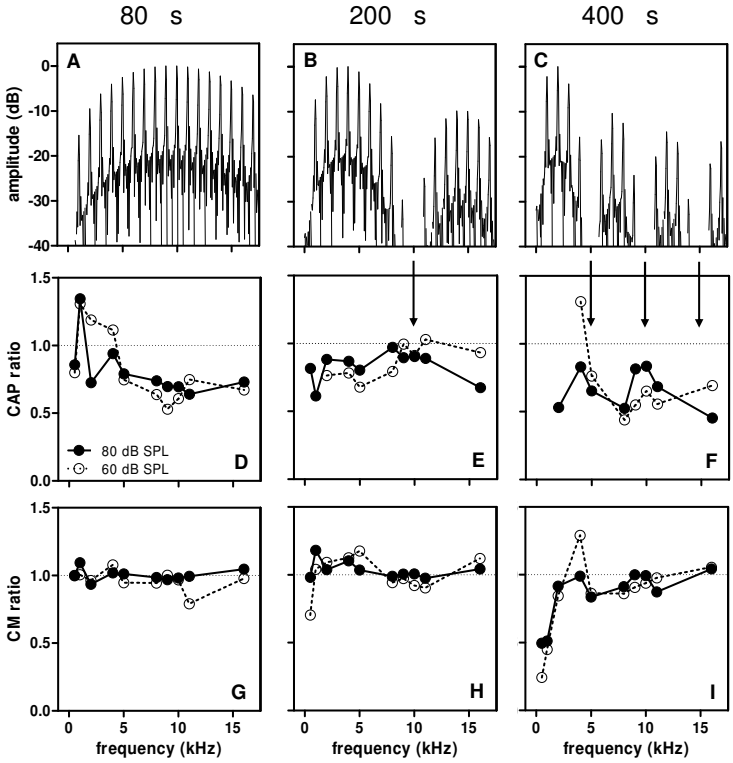

Fig. 5.4. Dependence of CAP ratio on electric pulse width. (A – C) Power spectra of electric pulse trains (10 biphasic pulses, 1000 pps, 800 mA) using different pulse widths of 80 ms (A), 200 ms (B), and (400 ms). Null harmonics in the spectra are indicated (arrows). (D – F) Dependence of CAP ratio as a function of acoustic frequency at a pulse width of 80 ms (D), 200 ms (E), and 400 ms (C). (G – I) Dependence of CM ratio as a function of acoustic frequency at a pulse width of 80 ms (G), 200 ms (H), and 400 ms (I). Sound level of tone bursts was 60 or 80 dB SPL. Data are averaged across 5 animals. Note the linear frequency scales. CAP ratios at low acoustic frequencies and long pulse widths are not shown, since effects strongly depended on stimulus polarity, which prevented extraction of the CAP from the raw signal.

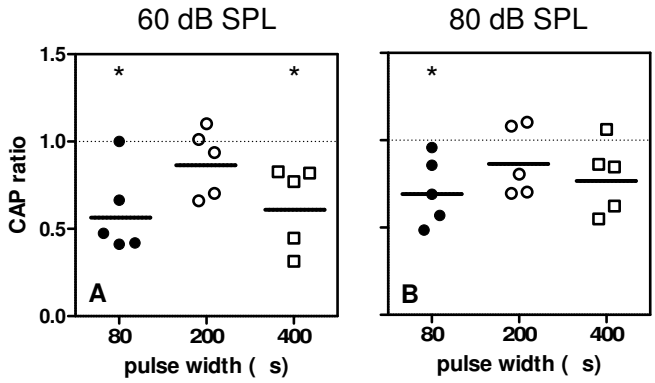

Fig. 5.5. Individual CAP ratios determined at 10 kHz using electric pulse trains with variable pulse widths (80, 200 and 400 μs). CAPs were evoked at 60 dB SPL (A) or 80 dB SPL (B). Horizontal bars indicate average values, corresponding to the 10-kHz data in Fig. 5.4D - F. Asterisks indicate significant CAP suppression (P< 0.05) relative to the no-effect level (i.e., CAP ratio= 1) according to 1-sample t-tests.

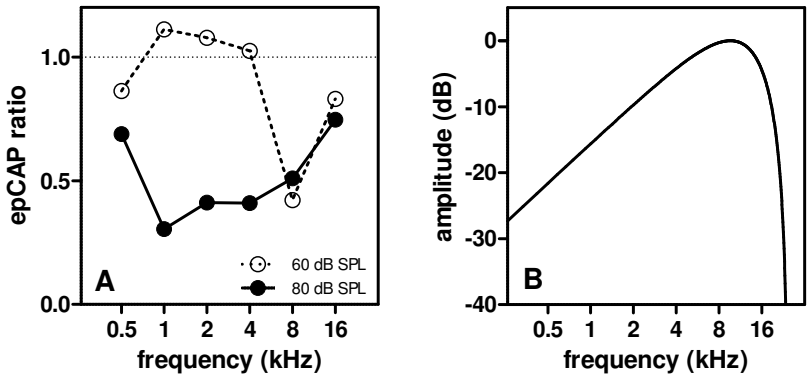

Fig. 5.6. Effect of acoustic tonal stimulation on electrophonic responses. (A) epCAP ratio as a function of acoustic frequency. Electric pulses (80 μs pulse width, 600 – 800 μA, mean: 750 μA) were presented simultaneously with acoustic tone bursts of 10 or 12 ms. Pulses were presented 2 ms after tone onset. Tones were presented at 60 or 80 dB SPL. Data were averaged across 4 animals. (B) Frequency spectrum of the biphasic pulse stimulus.

5.3.4. Dependence of epCAP amplitude on pulse width

In two animals, in which the acoustic frequency-dependence of electric of CAPs suppression as a function of pulse width was obtained (data from Fig. 5.4), we also tested the acoustic frequency-dependence of electric suppression of CAPs as a function of pulse width (Fig. 5.7). Effects of acoustic stimuli on epCAPs were

tested by presenting single pulses of 800 µA with a width of 80, 200 or 400 µs simultaneously with acoustic tones of 60 dB SPL, 5 ms after tone onset (similarly to the method used in section 3.3). CAP suppression was tested using the method described in section 3.2.

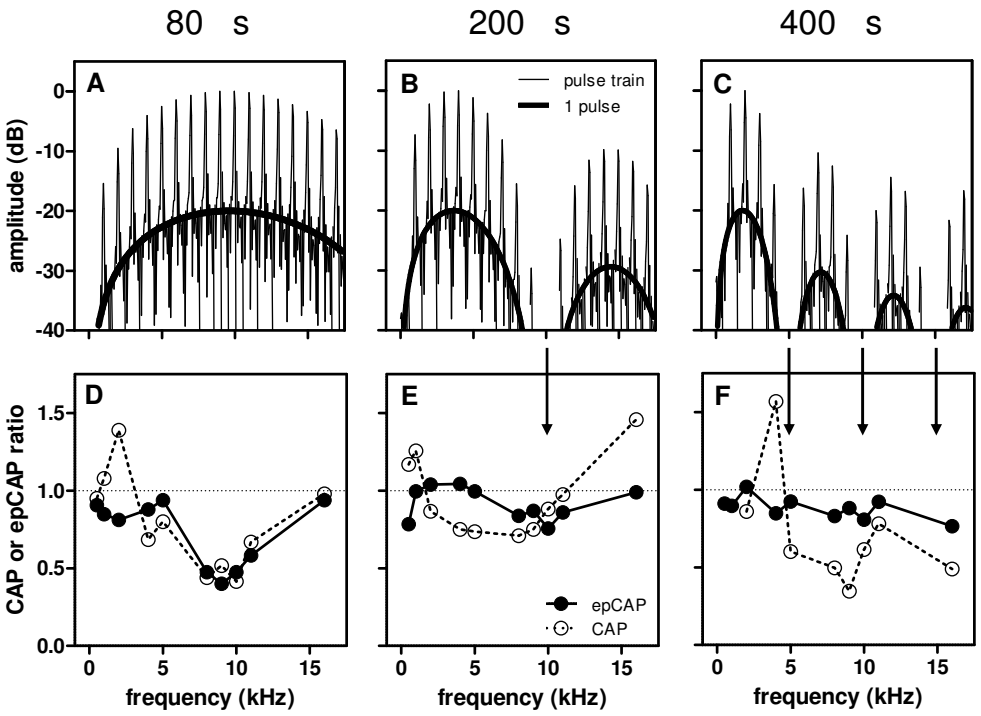

Fig. 5.7. Effect of acoustic tonal stimulation on epCAPs compared to the effects of electrical stimulation on tone-evoked CAPs, as function of acoustic frequency. The former is expressed as epCAP ratio, the latter as CAP ratio. (A – C) Spectra of single pulses (thick lines) and electric pulse trains (thin lines) at pulse widths of 80 µs *(A), 200* µs *(B), and 400* µs *(C). Current pulses were biphasic and presented at 800* µ*A. Trains consisted of 10 pulses (1000 pps, 800* µ*A). (D – F) Effects of acoustical stimulation on epCAPs evoked with a single biphasic pulse of 800* µ*A presented during the tonal stimulus, 5 ms after onset. Tone bursts were 10 ms (1 – 16 kHz) or 12 ms (0.5 kHz) presented at 60 dB SPL. Effect of electric pulse trains on CAPs (solid circles) evoked with acoustic tones (0.5 – 16 kHz) presented at 60 dB SPL are shown in the same figures. Electric-to-acoustic interval was 1 ms. Applied pulse widths for pulse trains and single pulses were 80* µs *(D), 200* µs *(E), and 400* µs *(F). Data are averaged across 2 animals.*

In Figs. 5.7A – C the spectra of the pulse trains and single pulses used are shown. Acoustic suppression of epCAPs and electric suppression of CAPs showed

a strikingly similar dependence on acoustic frequency at a short pulse width of 80 µs (Fig. 5.7D). Suppression of both epCAP and CAP was maximal at acoustic frequencies of 8 – 10 kHz, showing ratios of ~0.4. At a pulse width of 200 µs (Fig. 5.7E) both epCAP and CAP ratios were clearly higher (0.7 – 0.8) than with a pulse width of 80 µs. At 400 µs pulse width (Fig. 5.7F) epCAP and CAP suppression greatly differed. Suppression of epCAPs was absent, whereas a substantial CAP suppression was observed at high frequencies (ratios of ~0.4). A relatively high suppression of 16 kHz-evoked CAPs by electric stimuli was found at this pulse width. At a pulse width of 400 µs, epCAPs were also unaffected by acoustical stimuli of 80 dB SPL (results not shown).

5.3.5. Dependence of the epCAP on cochlear status

The dependence of electrophonics on cochlear status was examined by recording epCAPs in ototoxically treated and normal-hearing animals. Treated animals were recorded 2 or 10 weeks after ototoxic treatment. CAP threshold shifts were similar 2 and 10 weeks after treatment (Fig. 5.8A, B) and were generally small at low frequencies (< 25 dB) and increased with frequency (up to 75 dB at high frequencies). OHC loss (Fig. 5.8D) gradually increased from apex (25%) to base (100%). IHC loss (Fig. 5.8E) was mild throughout the cochlea 2 weeks after treatment (~25%), but more pronounced after 10 weeks (~50%). IHCs were completely lost in the lower basal turn in animals after 10 weeks. SGC loss was noted after 10 weeks and corresponded to the pattern of IHC loss (Fig. 5.8F).

Amplitude of epCAPs were determined at relatively high current levels of around 800 µA in ototoxically treated animals and compared to normal-hearing animals (Fig. 5.9). Averaged epCAP amplitude in normal-hearing animals was ~160 µV, which approximately corresponded to the amplitude of 8-kHz-evoked CAPs at a sound level of 80 dB SPL (Fig. 5.2B). The amplitude of epCAPs decreased after ototoxic treatment (ANOVA, $P< 0.01$) and was significantly reduced 10 weeks after treatment to 60 µV (Tukey's *post hoc* test, $P< 0.01$). Amplitudes did not differ from control after 2 weeks (120 µV, $P> 0.05$), or between the two treated groups ($P> 0.05$).

In 4 animals ototoxic treatment resulted in CAP thresholds of >75 dB SPL at all acoustic frequencies tested (indicated with open circles in Fig. 5.9). Note that one of these profoundly deaf animals had an epCAP amplitude comparable to that of normal-hearing animals.

Absolute epCAP amplitudes were analyzed as function of OHC, IHC and SGC loss. Cell losses were taken as the mean in the basal part of the cochlea (lower basal turn to upper middle turn, i.e., B1, B2, M1, M2 in Fig. 5.8C), or in the apical part of the cochlea (i.e., A1, A2 and A3 in Fig. 5.8C), and expressed as percentage cell loss relative to the normal-hearing group. In the basal part (Fig. 5.10A – C), no significant linear relation was found between epCAP amplitude and OHC loss (linear regression and F test, $P> 0.5$, $r^2= 0.04$), while a significant

correlation was found for IHC loss ($P < 0.05$, $r^2 = 0.4$) and SGC loss ($P < 0.01$, $r^2 = 0.6$). In the apical part (Fig. 5.10D, E), no significant relation was found on either OHC or IHC loss ($P > 0.1$, $r^2 < 0.1$). SGC packing densities could not reliably be obtained in the apical regions.

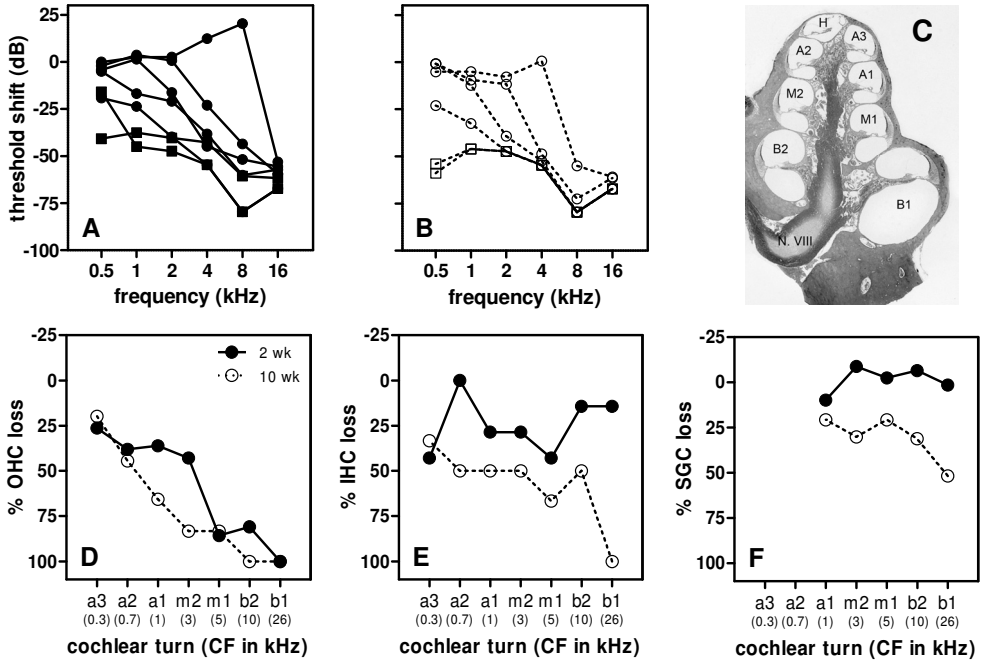

Fig. 5.8. Effects of ototoxic treatment on CAP thresholds and cochlear histology. (A – B) CAP threshold shift as a function of acoustic frequency averaged across 7 animals 2 weeks after treatment (A) and 6 animals 10 weeks after treatment (B) relative to a group of 11 normal-hearing animals. Four profoundly deaf animals had large threshold shifts over the entire frequency range (squares), 3 of these received a dose of 300 mg kanamycin/kg, the other 250 mg/kg. Most animals received 200 or 250 mg/kg. (C) Midmodiolar section of the cochlea showing the locations examined. Acoustic nerve (N. VIII) and helicotrema are indicated. Data are from the right (recorded) cochleas. SGCs could often not be counted in the apical turn. (D – F) Averaged percentage OHC loss (D), IHC loss (E) and SGC loss (F) relative to the control group are shown. Typical normative data: 3 OHCs, 1 IHC and ~1500 SGCs/mm² per location. Characteristic frequencies corresponding to the cochlear locations are shown below the x-axis in parentheses.

5.4. Discussion

We studied the role of electrophonics in electro-acoustic interaction with short intervals between electric and acoustic stimuli. Variation in the frequency spectrum of the electric stimulus by use of different pulse widths revealed a relation between the spectral characteristics of the electric stimulus and the effects of electric stimuli on acoustically evoked compound action potentials (CAPs). Moreover, effects of acoustic stimuli on electrophonically evoked compound action potentials (epCAPs) showed comparable dependence on the electric stimulus spectrum. Hence, electrophonics played a prominent role in suppression of acoustically evoked responses by electric pulses. Electrophonic responses decreased after severe loss of IHCs and SGCs in the basal part of the cochlea, while OHC loss appeared to have little effect on electrophonic response amplitudes.

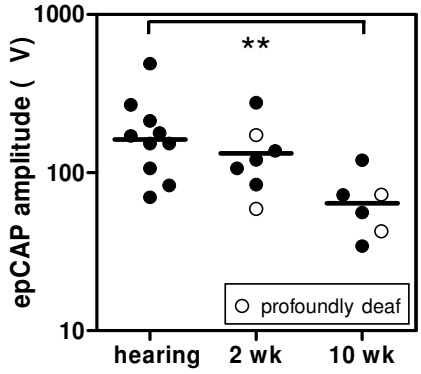

Fig. 5.9. Effect of ototoxic treatment on epCAP amplitude. Absolute epCAP amplitude is shown in 10 normal hearing animals, 7 animals 2 weeks after ototoxic treatment and 6 animals 10 weeks after treatment. Mean current level in the normal-hearing group was 780 µ*A (range: 600 – 800* µ*A), 860* µ*A in the group 2 weeks after treatment (range: 800 – 1000* µ*A), and: 850* µ*A in the group 10 weeks after treatment (range: 600 – 1000* µ*A). In the ototoxically treated groups, 4 animals were profoundly deaf (absolute thresholds >75 dB SPL) at all frequencies tested (open circles). The epCAP amplitudes depended significantly on ototoxic treatment (ANOVA, P< 0.01) and amplitudes differed significantly between the normal-hearing group and 10 weeks after treatment (Tukey's post hoc test, P< 0.01).*

5.4.1. Evidence for electrophonic mechanisms in electric suppression of acoustically evoked responses

Electrophonic responses are thought to depend on the frequency spectrum of the electric stimulus (McAnally et al., 1993; Kirk and Yates, 1994). Electric stimuli are thought to excite cochlear regions that tonotopically correspond to the frequency

spectrum of the electric stimulus (McAnally et al., 1997). With regard to pulse trains, the spectrum is mainly determined by pulse width. By varying pulse width, we found a relation between maxima and minima in the frequency spectrum of the electric stimulus, and the suppression of acoustically evoked CAPs (Fig. 5.4). Hence, we have demonstrated a substantial contribution of electrophonics to CAP suppression, which agrees with a study in cats from McAnally et al. (1997) and our previous study (Stronks et al., 2010b).

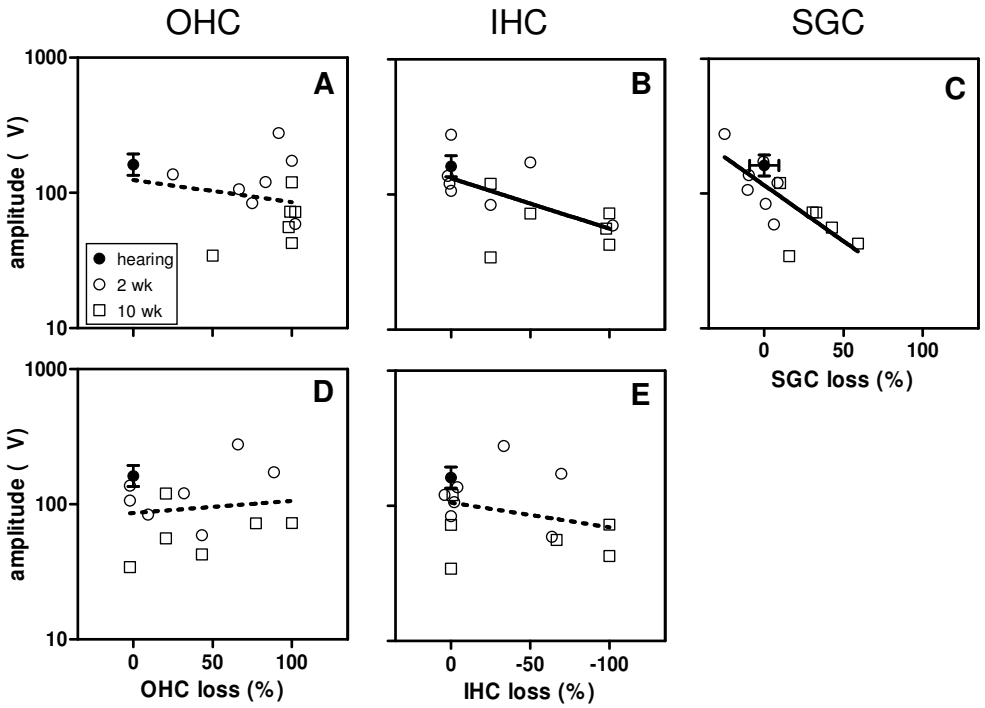

Fig. 5.10. Relation between epCAP amplitude and cochlear status. (A – C) epCAP amplitude as function of loss of OHCs (A), IHCs (B) and SGCs (C) determined in the basal part of the cochlea (mean of the cochlear locations B1, B2, M1 and M2). Cell counts were defined as the mean from the basal to middle turn of the cochlea (lower and upper basal turn, and lower and upper middle turn). (D – E) epCAP amplitude as function of OHC loss (D) and IHC loss (E) determined in the apical part of the cochlea (mean of the cochlear locations A1, A2 and A3). Cell loss was determined relative to the average of the normal-hearing group (filled symbol with SD). Linear regression was performed on the individual data of the treated groups (open symbols) and the single average of the normal-hearing group. There was a significant correlation with epCAP amplitude and IHC loss (r^2= 0.4) and SGC loss(r^2= 0.6) in the basal region of the cochlea (F-test, P< 0.05, solid lines). Insignificant (F-test, P> 0.3) linear relations are also shown (dotted lines). For clarity some data points are plotted in a slightly staggered fashion (e.g. at 100 % OHC loss).

Further evidence for the involvement of electrophonics came from suppression of low-frequency evoked CM that increased with pulse width (Fig. 5.4G - I). Suppression at low acoustic frequencies could have been a reflection of increased spectral power at the low frequencies of the electric stimuli compared to short pulse widths. Given the fact that CM responses are predominantly mediated by OHCs close to the recording electrode (Tasaki and Fernandez, 1952; Dallos and Cheatham, 1976), our CM recordings at low acoustic frequencies were assumed to be reliable since the recording electrode was situated on the apex of the cochlea. The placement of the recording electrode also provides an explanation for the lack of effect on CM amplitude at high frequencies.

At a pulse width of 400 μs, electric suppression of 10 kHz-evoked CAPs at 60 dB SPL was relatively pronounced (Fig. 5.4F), compared to a pulse width of 200 μs (Fig. 5.4E), despite the presence of a null harmonic at this frequency in the spectrum of the pulse train (Fig. 5.4B, C). This finding can be explained by the fact that these long pulse widths resulted in large charge injections. Consequently, nerve fibers with characteristic frequencies corresponding to null harmonics might have been excited by a spread of electrophonic activation from adjacent regions in the cochlea due to adjacent harmonics (see also McAnally et al., 1997). Conversely, at 400 μs pulse width epCAPs were unaffected by tonal acoustic suppression. This observation can also be explained by the large charges applied, resulting in pronounced electrophonic excitation. The acoustically evoked activity at a modest sound level of 60 dB SPL was probably not sufficient to suppress the strong electrophonic response.

5.4.2. Origin of epCAPs

The following findings indicated that epCAPs likely had a similar cochlear origin as CAPs (i.e., involving basilar membrane movement and stimulation of hair cells). First, latency and morphology of pulse-evoked epCAPs and click-evoked CAPs were very similar (Fig. 5.2C, D). Second, acoustic suppression of epCAPs showed a strikingly similar tuning to acoustic frequency compared to electric suppression of CAPs at short pulse widths (Fig. 5.7D). A similar tuning of epCAP suppression to acoustic frequency was also demonstrated using extracochlear stimulation and identical electric stimuli (Stronks et al., 2010b).

OHCs can show electromechanical movements up to at least 15 kHz (Reuter et al., 1992) and can therefore theoretically respond rate-dependently at pulse-rates used in present-day implants. However, pulse rates from 500 to 4000 pps did not alter the tuning of CAP suppression in normal-hearing animals (Fig. 5.3F). Furthermore, we found epCAPs in animals devoid of OHCs in the middle and basal turn of the cochlea (Fig. 5.10), while the stimulating electrode was located in the lower basal turn close to the round window. These results are in agreement with an earlier report that epCAP generation is independent

on OHCs close to the stimulating electrode (McAnally et al., 1993). We extend this observation with the notion that we found no significant dependence of epCAP amplitude on OHCs in the basal to middle turn (Fig. 5.10A), or apical turn (Fig. 5.10D).

These findings seem to contradict with the assumption that electrophonics depend on travelling waves on the basilar membrane generated by electromotile responses of OHCs (Nuttall and Ren, 1995). Even stronger evidence against this assumption was found by Moxon (1971), who reported electrically evoked pressure differences in *post mortem* cochleas. Possibly electrically evoked vibrations in accessory structures of the basilar membrane, such as Reissner's membrane, are alternative sources of electro-mechanical transduction (Clark Jones et al., 1940).

We used relatively large current levels (~800 μA) that evoked epCAPs with amplitudes roughly corresponding to CAPs evoked at 80 dB SPL (at 8 kHz). Therefore, we cannot exclude that at lower current levels OHCs would be involved in generating electrophonic responses by the generation and active amplification of basilar membrane movements.

In contrast to OHCs, a significant relation of epCAP amplitude with IHC loss was found in the basal and middle part (Fig. 5.10B, C). Furthermore, latencies of epCAPs and CAPs were very similar (Fig. 5.2C, D). These results confirm that epCAPs represented electrophonics (i.e., dependent on hair cells). IHC loss in the apical turn did not correlate with epCAP amplitude (Fig. 5.10E), confirming that epCAPs were generated in the high-frequency region of the cochlea when short pulse widths were used (i.e. electric stimuli with the spectral maximum at high frequencies). The dependence of epCAP amplitude on SGC loss was expected, since these cells are necessary for auditory nerve activity.

5.4.3. Other mechanisms behind CAP and epCAP suppression

Other studies examining interactions between acoustically and electrophonically evoked responses have used intervals between electric and acoustic stimuli of at least 10 ms. These long intervals were applied in order to isolate hair cell-mediated mechanisms from those evoked by direct electrical neural activation (e.g. Kirk and Yates, 1994; McAnally et al., 1997). We used shorter intervals (typically 1 ms) to approach the (near-) simultaneous electric and acoustic stimulation likely encountered in EAS users. As a consequence, direct electrical neural activation also could have contributed to suppression in our experiments (Stronks et al., 2010b, and present results). At the longest pulse width tested, the relatively large CAP suppression at 10 kHz (Fig. 5.4F) was likely caused by a spread of electrophonic activation from regions adjacent of the cochlear location with a characteristic frequency of 10 kHz, as explained earlier (section 4.1). Increased direct electrical activation of neural elements due to large charge injections may also have played a role in this. For instance, this mechanism might explain the pronounced suppression of 16-kHz evoked CAPs at the longest pulse

width tested (Fig. 5.7F). Since spectral energy at high frequencies at long pulse widths is low (Fig. 5.7C), electrophonics were probably of minor importance here. Therefore, direct electric activation of neural elements seems may have played a role, since the stimulation electrode was situated in the high-frequency region of the cochlea.

Previously we showed that 8-kHz-evoked CAPs recovered to 80% of their original amplitude within a few ms after electric stimulus offset. Electric stimuli were similar as we used in the present experiments and consisted of single pulses, or 10-pulse trains (Stronks et al., 2010b). We argued that this fast recovery might be indicative of refractory mechanisms underlying suppression. Though this might point towards suppression mediated by direct electric neural activation, hair cell stimulation followed by auditory-nerve activation might also have mediated refractoriness of the auditory-nerve. Hence, the temporal characteristics of electric CAP suppression support both assumptions of direct neural activation and electrophonic activation causing this suppression.

A number of factors besides the discussed acoustically and electrophonically evoked responses, and direct electrical neural activation can have influenced the observed electro-acoustic interaction. First, inhibitory efferents can be activated by acoustic and electric stimulation (Tasaki and Fernandez, 1952; Galambos, 1956) and reduce auditory nerve fiber activity around their CF (Wiederhold and Kiang, 1970), reduce CAP amplitude (Gifford and Guinan, 1987) and alter cochlear mechanics (Mountain, 1980; Russell and Murugasu, 1997). Electrical stimulation of efferents is optimal at low pulse rates (5 – 400 pps) and long (150 μs) pulse widths (Desmedt, 1962; Rajan and Johnstone, 1983). Most of the present experiments were performed at suboptimal conditions for efferent stimulation, using high pulse rates (1000 pps) and short pulse widths (80 μs). However, at long pulse widths of 200 and 400 μs, suppression of CAPs evoked at high frequencies was relatively high. Besides the above mentioned increased spread of electrophonic excitation and direct neural mechanisms, efferent activation might also have played a role here. The observed CM suppression at low acoustic frequencies under these conditions was probably unrelated to efferents, since efferents do not affect CM (Gifford and Guinan, 1987). With regard to acoustical suppression of epCAPs, acoustical stimulation of efferents mainly affects the contralateral ear. Since we tested the effects of acoustic stimuli on ipsilaterally evoked epCAPs, effects of efferents will likely have been small (Liberman and Brown, 1986).

Suppression of CAPs can also have been influenced by electrically induced contraction of middle ear muscles (Pang and Guinan, 1997). The effects of stapedius and tympanic muscle contraction are greatest at frequencies below 300 Hz in guinea pigs (Nuttall, 1974) and may therefore have contributed to the suppression of the CM at 0.5 and 1 kHz at long pulse widths.

5.4.4. Clinical relevance

We have shown that electrical stimulation can result in hair cell-mediated activation in cochlear locations distant from the stimulating electrode in the absence of outer hair cells in the vicinity of the stimulating electrode. Hence, spatial segregation of electrical and acoustical stimulation by using short electrode arrays as used in hybrid implant systems in patients with residual low-frequency hearing does not necessarily prevent interaction of electrophonic responses with acoustical responses. Interaction of electric and acoustic responses at low acoustic frequencies was most pronounced at long pulse widths of 200 and 400 μs, while at a short pulse width of 80 μs suppressive effects of biphasic pulse trains on acoustically evoked CAPs at low frequencies were small. Hence, the results obtained in this study indicate that short pulse widths are optimal for use in hybrid implants. Pulse rate did not affect tuning of suppression of CAPs. Nevertheless, since electrophonics contribute to suppression of acoustically evoked auditory nerve activity, it may be advisable to use high pulse rates to shift the frequency spectrum to high frequencies.

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Abbreviations

AS	acoustical stimulation
CAP	acoustically evoked compound action potential
CM	cochlear microphonics
EAI	electric-to-acoustic stimulus interval
EAS	electro-acoustical stimulation
epCAP	electrophonically evoked compound action potential
ES	electrical stimulation
FFT	fast Fourier transform
IHC	inner hair cell
OHC	outer hair cell
SGC	spiral ganglion cell

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Effects of acoustic noise on electrically evoked auditory-nerve activity in guinea pigs with a high-frequency hearing loss

6

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In preparation

Abstract

Criteria for cochlear implantation are broadened, and people with a severe high-frequency loss and residual low-frequency hearing are considered candidates for implantation. This raises the issue of how cochlear responses to electric and acoustic stimuli interact. We have investigated the effects of acoustic noise on electrically evoked compound action potentials (eCAPs) in normal-hearing guinea pigs and in animals with a high-frequency hearing loss. We used broadband noise and filtered variants of this noise, by applying high-pass, mid-frequency band-pass, and low-pass filters that had cut-off frequencies of 5.7 kHz, 1.4 – 5.7 kHz and 1.4 kHz, respectively. Electrical stimuli were delivered in the base of the cochlea. Broadband noise suppressed eCAP amplitudes, especially at high electric and acoustic stimulus levels. Effects of noise were largest just after noise onset and decreased with noise duration. After noise offset amplitudes were transiently increased. In normal-hearing animals, suppression could occur at noise levels as low as 40 dB SPL. Low-frequency noise was less effective and higher sound levels (~60 dB SPL) were needed. In animals with a high-frequency hearing loss, broadband and high-frequency noise were less effective compared to normal-hearing animals and higher sound levels were needed to suppress the eCAP. Mid-frequency and low-frequency noise were equally effective in both groups. We conclude that under conditions of high current and sound levels, low-frequency acoustic stimuli can suppress eCAPs evoked in the base of the cochlea in animals with a high-frequency hearing loss.

keywords: electro-acoustical stimulation; residual hearing; cochlear implant; electrically evoked compound action potential; hair cell; spiral ganglion cell

6.1. Introduction

Cochlear implantation is at present the method of choice for treatment of profound sensorineural hearing loss. The performance of cochlear implant (CI) users in terms of speech understanding has improved considerably (Wilson et al., 1991; Wilson and Dorman, 2008). As a consequence, selection criteria for implantation are expanded and people with considerable residual low-frequency hearing are considered for implantation (Cohen, 2004). Residual hearing can be stimulated using hybrid implants that combine a CI with a conventional hearing aid (Talbot and Hartley, 2008; Turner et al., 2008). Compared to electrical stimulation (ES), electro-acoustical stimulation (EAS) increases speech understanding in noise and improves the esthetical quality of complex sounds such as music (Fraysse et al., 2006; Gantz et al., 2006). There is some variability in these populations and post-operative speech understanding can even be reduced compared to pre-operative

scores. Besides proven predictors of post-operative performance, such as the age at implantation and duration of deafness (Gantz et al., 2009), interaction between electrically and acoustically evoked responses might play a role.

In earlier studies we found that electrical pulse trains can suppress acoustically-evoked auditory-nerve responses in the cochlea of guinea pigs (Stronks et al., 2010). Studies by Abbas and colleagues showed that acoustical stimulation can suppress electrically evoked responses in normal-hearing animals (Nourski et al., 2005, 2007). These effects were explained assuming a decreased synchrony of firing when electric stimuli were presented during acoustic stimulation. In the absence of acoustical stimulation, the presence of functional hair cells also decreases electrically evoked responses, as was shown by reversibly inactivating hair cells with furosemide (Hu et al., 2003). Auditory nerve fibers in acoustically sensitive ears also show different electrically evoked activity compared to deafened ears, such as electrophonic responses and burst firing (Miller et al., 2006). Electrophonics are mediated by electro-mechanical transduction processes, resulting in hair-cell mediated responses. We have found electrophonically evoked CAPs in earlier experiments in normal-hearing cochleas (Stronks et al., 2010). Responses to direct electrical neural activation, electrophonics, acoustically evoked responses and spontaneous hair cell-mediated activity may all interact in cochleas with functional hair cells when electro-acoustically stimulated. This interaction may be a factor determining the effectiveness of EAS strategies.

In the present study we characterized the effects of acoustic noise on electrically evoked compound action potentials (eCAPs) in the absence and presence of acoustic noise. This procedure was comparable to earlier work by Nourski et al. (2007), but with a few important differences. First, we used a model of short-term and long-term high-frequency hearing loss by using guinea pigs co-treated with kanamycin and furosemide. Second, we have used a forward masking paradigm for recording eCAPs, a technique generally applied in neural response telemetry (NRT) systems in CIs. Third, we used different types of filtered noise. Finally, we have complemented our eCAP data with electrically-evoked auditory brainstem responses (eABRs).

We will first present the evaluation of the animal model by relating eCAP amplitude to hair cell and spiral ganglion cell loss and by comparing these data to normal-hearing animals. Thereafter, the effects of acoustic noise on eCAP and eABR amplitude are presented. Finally, some deviating data is presented to illustrate problems that can be encountered when recording eCAPs from acoustically sensitive cochleas using the forward masking paradigm.

6.2. Materials and methods

6.2.1. Animal preparation

Nineteen healthy, female albino guinea pigs were used, weighing 400 – 800 g at the time of recording. Animals were normal-hearing (n= 6), or had a high frequency loss and were recorded 2 weeks (n= 7), or 12 weeks after ototoxic treatment (n= 6). The latter two groups were ototoxically treated prior to recording using a co-treatment of kanamycin and furosemide, as described by Versnel et al. (2007). We applied 100 mg/kg furosemide in combination with a lower dose of kanamycin (200 mg/kg) to spare low-freuency hearing. Assesment of acoustic hearing loss were performed as described previously (Stronks et al., 2010).

Electrophysiological recordings were performed in acute experiments. Surgical techniques were described previously (Stronks et al., 2010). A single pre-anesthetic dose of 0.05 ml/kg Hypnorm® (Vetapharma; 0.315 mg/ml fentanyl + 10 mg/ml fluanisone) was given i.m. Anesthesia was induced with 2% isoflurane (Nicholas Piramal Limited) evaporated in 67% N_20 and 33% O_2, delivered by a mouth cap. Atropine (Pharmachemie BV) was administered s.c. (0.05 mg/kg) to reduce bronchial secretion. Two transcranial screws served as auditory brainstem (ABR) electrodes. One was placed 1 cm caudal from bregma, a few mm right-lateral from midline. The other one was placed 2 cm rostrally from bregma on, or a few mm lateral from midline (Mitchell et al., 1997). After placement, screws were fixed with methyl-methacrylate resin (Technovit® 3040, Heraeus Kulzer). An s.c. hypodermic needle in the neck was used as ground. After intubation the animal was artificially ventilated (Amsterdam infant ventilator mk3, Hoekloos) with 1 – 2.5% isoflurane in 67% N_2O and 33% O_2. Anaesthetic depth was assessed regularly by means of the pedal withdrawal reflex (front paw). Heart rate was monitored (180 – 360 bpm) and optionally 0.05 mg/kg atropine was administered s.c. Temperature was maintained at 38 ± 0.5°C using a rectal probe thermostatically coupled to a heating pad. Every 1 – 2 hours a volume of 1% body weight of physiologic saline with glucose (37°C) was administered s.c. for rehydration.

The right bulla was exposed ventrally and opened with scalpel and forceps. After partial removal of the pinna, a metal probe was inserted in the auditory meatus. Cochlear potentials were recorded using a silver ball electrode with a Teflon-insulated shank on the apex of the cochlea (Van Deelen and Smoorenburg, 1986), the reference was a clamp on the caudal ABR screw. Electrically evoked ABRs (eABRs) were recorded with the caudal screw as active electrode and the rostral as reference. A cochleostomy with a diameter of 200 mm was made in the basal turn of the cochlea close to the round window. Care was taken to avoid damage to the basilar membrane and modiolus. A Teflon-insulated platinum wire, partly stripped of its insulation (wire diameter 125 mm), was advanced 1 mm through the cochleostomy. The cochleostomy was sealed with silicone

rubber (Dow Corning®). The return electrode was a s.c. hypodermic needle in the forepaw. Recording was performed in a sound attenuated booth.

Surgical and experimental protocols were approved by the Animal Ethical Committee of the University Utrecht under DEC-UMC number 2007.I.02.025. Animals were housed together according to the standards of the animal care facility of the University of Utrecht.

6.2.2. Stimulus generation

Electric stimuli consisted of biphasic rectangular pulses 80 ms in width and were generated by custom designed software (Delphi 7®, Borland). Stimuli were sent to a 24-bit DA converter (RP2.1, Tucker Davis Technologies; TDT) operating at a sampling rate of 49 kHz and, after attenuation (PA5, TDT), fed to a current source (Linear Stimulus Isolator A395, World Precision Instruments).

Acoustic stimuli consisted of bursts of Gaussian white noise that were semi-randomly sampled from a 10-minute template of "frozen noise" (LabVIEW 7, National Instruments). Randomization was established by assigning the starting point of the noise randomly within the first 200 ms of the template. Thereafter, the noise was running constantly on the background throughout the averaging process. This procedure ensured a near-infinite number of possible noise bursts. Noise bursts were generated via a pc sound card (EDIROL UA-1EX, Roland, operating at a sample rate of 96 kHz) and opening of a digital gate. This gate consisted of a custom-built voltage gated amplifier that introduced $\cos^2$-shaped on- and offset ramps of 1 ms. In a similar way filtered noise was generated from a template of digitally filtered Gaussian white noise (LabVIEW 7, National Instruments). Digital filters had cut-off frequencies of 1414 Hz (low-pass), 1414 – 5656 Hz (mid-frequency band-pass) and 5656 Hz (high-pass).

Noise stimuli were attenuated (PA5, TDT), and sent via a headphone amplifier (HB7, TDT) to a speaker (Beyer DT48). Sound levels were calibrated with a sound level meter (type 2610, Brüel & Kjær) and a ¼'' condenser microphone (type 4136, Brüel & Kjær) using a plastic, custom-made artificial guinea pig meatus mounted on the earprobe. The frequency-spectrum of the white noise and filtered noise was determined at a sound level of 80 dB SPL using a fast Fourier transform spectrum analyzer (model SR760, Stanford Research Systems). As shown in Fig. 6.1, moderate frequencies were over-represented in white noise due to the speaker transfer function. Therefore, we will refer to it as broadband noise. The filtered noise types will be referred to as high-frequency noise, mid-frequency noise and low-frequency noise.

6.2.3. Recording technique

Electrically evoked compound action potentials (eCAPs) were recorded using a forward masking paradigm (Fig. 6.2) described in detail by Miller et al. (2000).

The interval between masker pulse and probe pulse (MPI) was 0.7 ms. Miller et al. suggest an MPI of 0.2 - 0.5 ms, given the refractory characteristics of the auditory nerve. However, MPIs of <0.7 ms resulted in excessively large probe-stimulus artifacts, though the masker-pulse was almost completely cancelled. We ascribe this phenomenon to a polarization of the stimulating electrode by the masker-pulse. Polarization may have resulted in a deviation of the probe-artifact under the masker-probe condition from that of the probe-alone condition. The masker level was fixed at 900 μA.

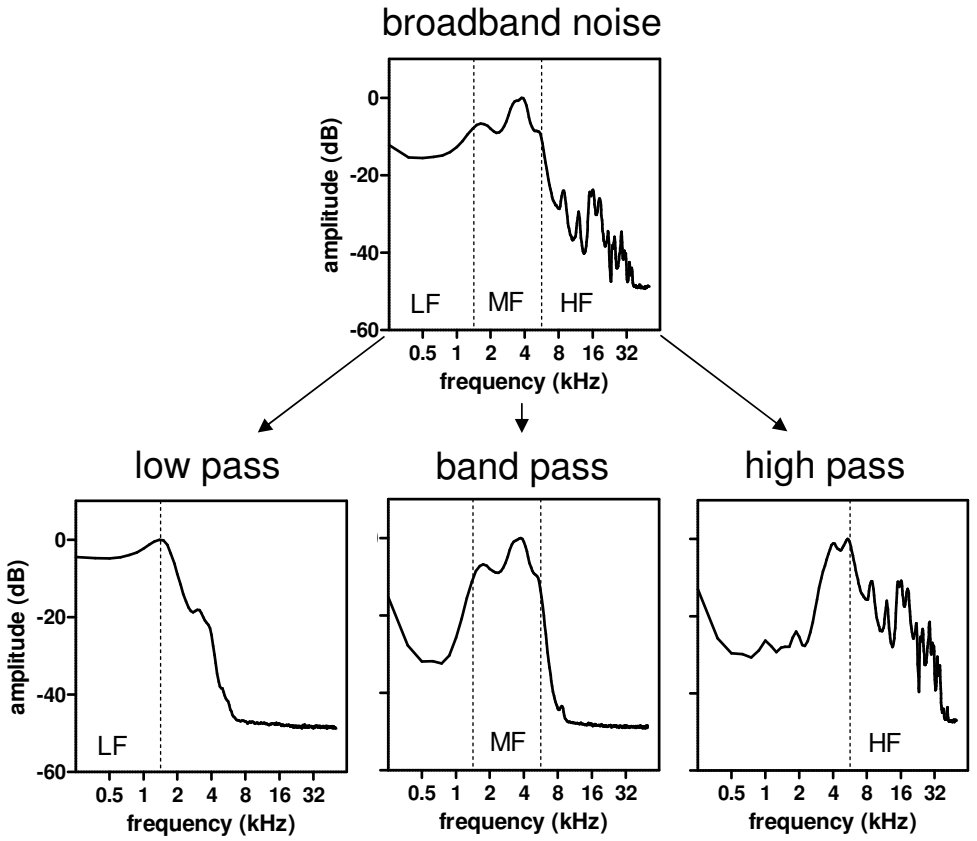

Fig. 6.1. Spectra of the applied broadband noise (upper graph) and broadband noise after digital filtering (lower graphs). Filters had cut-off frequencies of 1414 Hz (low-frequency noise, LF), 1414 – 5656 Hz (mid-frequency, MF) and 5656 Hz (high-frequency, MF). Broadband noise was digitally generated as Gaussian white noise. The acoustic frequency spectrum of the broadband noise was shaped according to the transfer function of the speaker. Each noise type was separately calibrated and presented at fixed sound pressure levels.

Electric stimuli alternated after each stimulus series, such that a single forward masking paradigm was completed only after 6 stimulus presentations (3 for each polarity). For eCAP recordings, responses were amplified (100 – 1000x) and filtered (1 Hz – 30 kHz, or occasionally 100 Hz – 10 kHz) using a differential amplifier (type 5113, EG & G Instruments). Signals were AD converted at 49 kHz (RP 2.1, TDT) and averaged to a maximum of 250 sweeps per stimulus polarity. Electrically evoked auditory brainstem responses (eABRs) were also recorded using a forward masking paradigm identical to that used for eCAP recording when testing the effects of noise. For the determination of eABR growth functions and visual thresholds, simple single-pulse stimuli were used. Both methods yielded similar amplitudes and thresholds of early and late peaks, as shown for one animal in Fig. 6.4B. For eABR recordings, signals were amplified (100 – 5000x), filtered (100 Hz – 10 kHz), AD converted at 49 kHz and averaged to a maximum of 500 sweeps per stimulus polarity.

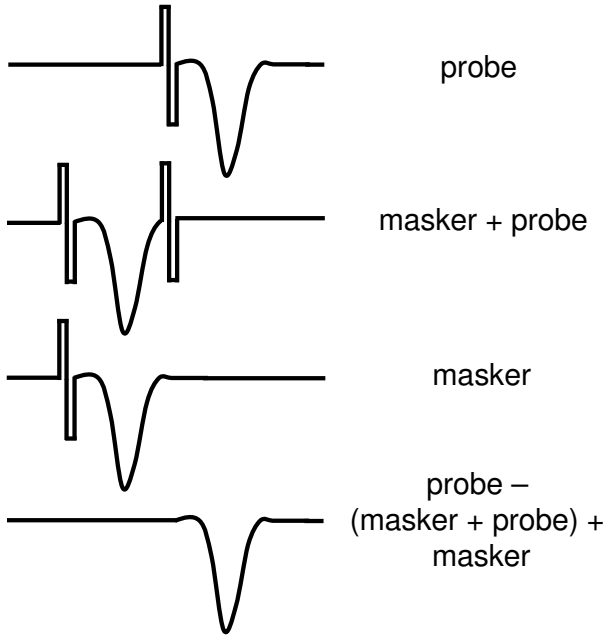

Fig. 6.2. Representation of the mathematical procedure of the "forward masking paradigm" used to eliminate the electric stimulus artifact. The probe-alone condition (probe) contains the eCAP with the electrical stimulus artifact. The artifact is eliminated by subtracting a masker-probe recording (masker + probe). The masker pulse puts the auditory nerve in a refractory state, preventing the generation of an eCAP after the probe pulse. The masker-pulse artifact is eliminated by adding a masker-only recording (masker). Time between masker and probe was 0.7 ms. Analyzed eCAPs were generated with "positive-first" pulses as shown (first phase of the pulse positive). Drawing after Miller et al. (2000).

For the occasional analysis of electrophonically evoked CAPs (epCAPs), separate recordings were performed using single alternating stimuli. A relatively high gain of 2500 – 5000x was applied, using the same filter settings and averaging procedure as described for the eCAP recordings.

Effects of acoustic noise on eCAPs and eABRs were tested by presenting electric probe stimuli simultaneously with noise bursts. Pulse stimuli were presented 5 ms after noise onset to prevent overlap of the eCAP and noise-evoked CAP. Effect of noise duration on eCAPs and subsequent recovery was tested by presenting probe pulses at variable time intervals after noise onset. Interstimulus interval was 99 ms, irrespective of the kind of recording (eCAP, eABR and epCAP) or type of variable under investigation.

Different parameters were varied, including current level of the electric stimulus (0 – 900 μA), sound level of the (filtered) acoustic noise (0 - 100 dB SPL), and timing of probe presentation (1 ms after noise onset, to 20 ms after offset).

6.2.4. Data analysis

Electrically evoked responses to stimuli of opposite polarity were separately stored for off-line analysis. Responses to opposite stimuli could be different in the same animal (Fig. 6.3). "Positive-first" stimuli (i.e., biphasic pulses starting with a rising flank) generally evoked the largest eCAPs (Fig. 6.3A) and evoked responses less contaminated by stimulus artifact (Fig. 6.3B, C). For these reasons analysis was performed on eCAPs (and eABRS) evoked with "positive-first" ("anodic-first") stimuli. For the occasional epCAP analyses, responses to both polarities were averaged to reduce stimulus artifact. In contrast to eCAP and eABR responses, epCAP amplitudes were independent on stimulus polarity (Fig. 6.3A, arrow). Responses to EAS were compared to responses to ES. To eliminate acoustically evoked CAPs, the response to AS alone was recorded and processed identically to the ES and EAS responses. These AS waveforms were used for off-line EAS artifact reduction by subtracting them from the EAS waveforms, similar to an artifact reduction technique described earlier (Charlet de Sauvage et al., 1983).

Repetitive electrical stimulation during recordings caused a steady decline of the eCAP amplitude over time in some animals, and notably so when performing multiple eCAP recordings at a constant current level (e.g. Fig. 6.8B). Therefore EAS and corresponding ES control data are always presented in the correct time frame. For example, noise level dependence was always tested from high sound level (100 dB SPL) to low level (0 dB SPL). During separate recordings of different EAS conditions, ES control data were periodically performed and graphically presented in the correct time frame (e.g. Fig. 6.8B).

Data were analyzed with custom written software in Matlab® 6.5 (The Mathworks Inc.). Amplitude of the eCAP was defined from the first negative peak (N_1) to the first positive peak (P_1) as shown in Fig. 6.3 and 6.6. Amplitude of eABR peaks

were determined for five peaks (P_I – P_V) relative to the following negative peak (Fig. 6.4). Analysis of epCAPs was identical to eCAPs (N_1 – P_1).

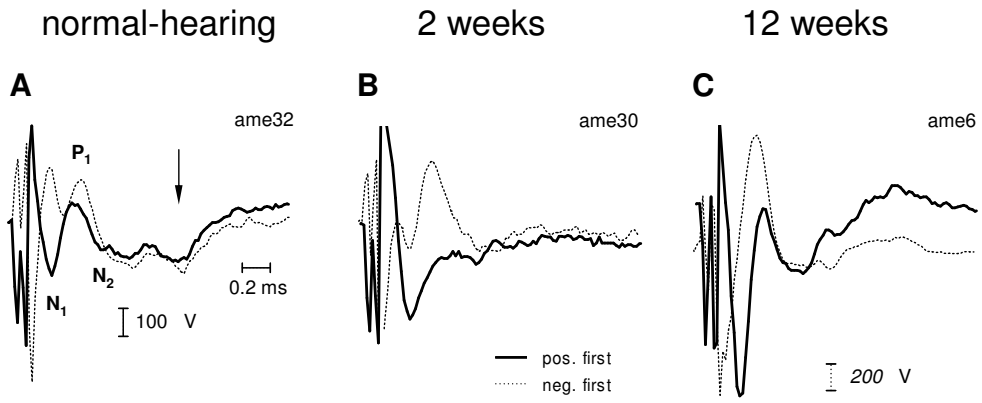

Fig. 6.3. Example eCAP recordings in a normal-hearing animal (A), and animals with a high-frequency hearing loss 2 weeks after treatment (B), and 12 weeks after treatment (C). The start of each trace corresponds to probe onset. Animal codes are provided in the upper right corner of each graph. Current pulses alternating in polarity were used to evoke eCAPs. Only eCAPs evoked with positive-first (anodic-first) stimuli (thick lines) were analyzed. For comparison, responses to negative-first (cathodic-first) stimuli are also shown (dotted lines). The positive-first stimuli generally evoked larger eCAPs (B), with less stimulus artifact (C). Note that scales are identical for all traces, except the negative-first trace in (C), emphasizing the large artifact in this recording. The first negative peak of the eCAP (N_1) had a latency of 0.3 ms. Amplitude of the eCAP was defined from the first negative peak (N_1) to the first positive peak (P_1). The second negative peak (N_2) is indicated in (A). Recordings in normal-hearing animals showed pronounced electrophonic responses (arrow) with a latency of 1.2 ms.

Effects of ototoxic treatment were assessed functionally by determining acoustic tone-evoked CAP thresholds (i.e., iso-response levels interpolated at 10 mV response level). CAP thresholds were determined before, and just after making the cochleostomy and placement of the stimulation electrode. Occasionally, CAP thresholds were obtained after completion of the experiment. Just after stimulation electrode placement, threshold shifts were generally not larger than 10 dB at any frequency (0.5 – 16 kHz). After recordings were finished, CAP thresholds were generally comparable, or even improved when compared to thresholds just after drilling the cochleostomy.

6.2.5. Histology

Immediately after recording, cochleas were fixed and processed for histology as described in detail previously (De Groot et al., 1987). Cochleas were divided along the midmodiolar plane and sectioned. Inner and outer hair cells were counted and spiral ganglion cell packing densities determined in each transection of a half turn (Van Ruijven et al., 2004). In some cochleas the most apical locations were damaged, or transection was not perfectly midmodiolar. In these cases hair cell counts and spiral ganglion cell (SGC) packing densities could not be reliably determined in these areas and data were supplemented with data from the left ear. This procedure will not have had large effects on our results, since effects of ototoxic treatment on apical regions were small.

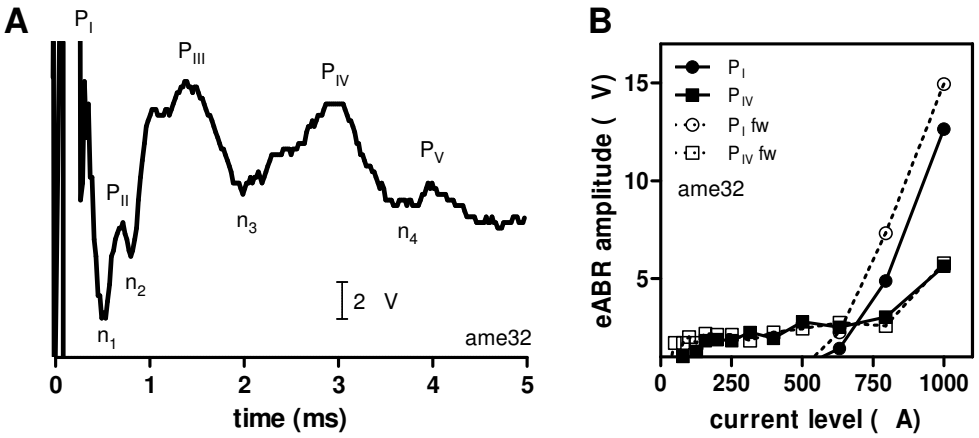

Fig. 6.4. Example recording of an electrically evoked auditory brainstem response (eABR) in a normal-hearing animal using a single, positive-first stimulus (A). Start of the sweep corresponds to probe onset. Peak amplitudes were determined from a positive peak (P_I – P_V) to the following adjacent negative peak (n_1-n_5). Thus, the amplitude of P_I was defined as P_I-n_1, of P_{II} as P_{II}-n_2 etc. When single stimuli were compared with a forward masking paradigm, amplitudes of early and late eABR peaks were comparable for both methods, as shown for P_I and P_{IV} (B).

6.2.6. Statistical analyses

Correlation between eCAP amplitudes and hair cell, or SGC loss were determined by linear regression. Significance of correlation was tested by performing post hoc F-tests on the slopes. Effects of various parameters (e.g. ototoxic treatment and acoustic noise presentation) on eCAP amplitude were tested for significance by using ANOVA, or repeated measures ANOVA, followed by Dunnett's or Tukey's post hoc test.

Effects of acoustic noise on eCAP amplitudes are mostly presented by means of individual examples, since averaging of absolute eCAP amplitudes between animals was inconvenient, because of the large variability in both eCAP amplitude (Fig. 6.55A), and effect of noise between animals (Fig. 6.9). Statistics were carried out on normalized eCAP amplitudes expressed as eCAP ratios, representing the eCAP amplitude in the EAS condition (A_{EAS}) divided by the amplitude in the ES condition (A_{ES}). Normalizing eCAP amplitudes was in some animals difficult due to the aforementioned adaptation of the eCAP due to repetitive electrical stimulation (Fig. 6.8). Statistical testing was carried out using ANOVA, or repeated measures ANOVA (RM ANOVA), followed by Tukey's or Dunnett's post hoc tests.

6.3. Results

6.3.1. Animal model

Ototoxic treatment increased thresholds of acoustically evoked compound action potentials (CAPs) relative to normal-hearing animals (n= 5). CAP threshold shifts increased with acoustic frequency up to 60 dB at 16 kHz and threshold shifts 2 weeks (n= 7) and 12 weeks after treatment (n= 6) were very similar (not shown). Histological examination showed that outer hair cell (OHC) loss increased gradually from apex (~10%) to base (100 %). Inner hair cell (IHC) loss was evident only in the lower basal turn after 2 (~40%) weeks and especially after 12 weeks (~70%). Spiral ganglion cell (SGC) loss was evident after 12 weeks in the lower basal turn (50%), more or less reflecting IHC loss (Fig. 6.5).

Figure 6.5A shows that electrically evoked CAP (eCAP) amplitudes were increased in animals with a high-frequency hearing loss (ANOVA, $P< 0.05$). This difference was significant after 12 weeks compared to normal-hearing animals (Tukey's post hoc test, $P< 0.05$). This gradual amplitude increase over time was also observed for eABR P_I (not shown). There were no effects of hearing loss on eCAP threshold (i.e., the interpolated current level to elicit an eCAP 25 μV in amplitude), or eCAP latency (not shown). We add that eCAP latency differences had a detection limit of 0.02 ms due to the sample rate used (eCAP latencies were 0.3 ms).

Effects on eCAP amplitude were further investigated by relating it to total hair cell (HC) count and spiral ganglion cell (SGC) packing densities in the basal turn. HC count was obtained by averaging the OHC and IHC count in the lower and upper basal turn. SGC packing densities were also determined by averaging the two basal half turns. Linear regression of the pooled data of all animals showed that eCAP amplitudes were negatively correlated with HC count ($P< 0.05$, $r^2= 0.2$, Fig. 6.5B). After 12 weeks, a positive correlation with SGC packing density was found ($P< 0.05$, $r^2= 0.7$, Fig. 6.5C). In normal-hearing animals and in animals 2 weeks after treatment, amplitude and SGC loss were not significantly correlated

(F-test, P> 0.2). In all, largest eCAP amplitudes were found in animals 12 weeks after treatment in which no apparent SGC loss was noted.

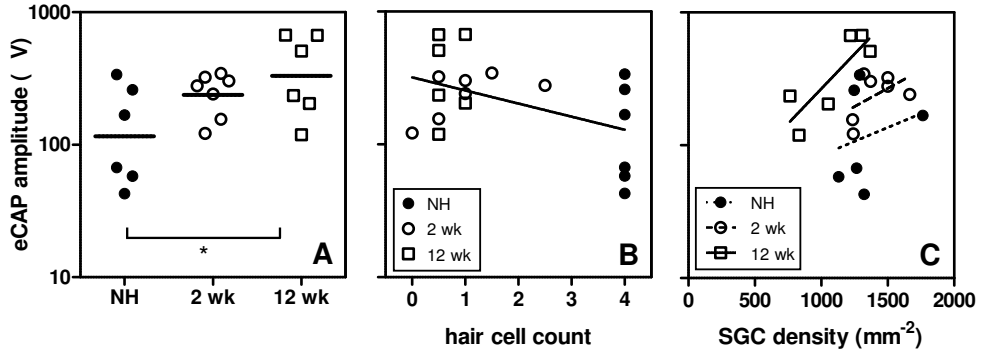

Fig. 6.5. Effects of short-term (2 weeks) and long-term (12 weeks) high-frequency hearing loss on eCAPs. Animals with a high-frequency hearing loss had larger eCAP amplitudes (A), this difference was significant in the group 12 weeks after treatment compared to normal-hearing animals (ANOVA and Tukey's post hoc test, P< 0.05). The eCAP amplitudes were re-plotted as function of outer hair cell (OHC) plus inner hair cell (IHC) count (B) and spiral ganglion cell (SGC) packing density (C) averaged across lower and upper basal turn. Linear regression showed a significant negative correlation of eCAP amplitude with OHC + IHC count (F-test, P< 0.05) when all the data were combined. A significant positive correlation of eCAP amplitude with SGC packing density was found only in the group of animals with a high-frequency hearing loss 12 weeks after treatment (F-test, P< 0.05).

6.3.2. Effects of acoustic noise on eCAP amplitude: dependence on current level

Effects of acoustic noise bursts on eCAPs were tested by presenting noise bursts at 80 dB SPL simultaneously with the electric stimuli. Recordings were performed at variable current level from 900 μA to eCAP threshold at 2 dB steps. Presentation of broadband noise suppressed eCAPs as shown by some example recordings in a normal-hearing animal (Fig. 6.6). Suppression of the first negative peak (N_1) was most evident at high current levels (600 and 800 μA in this example). Note the near complete suppression of the second negative peak (N_2) and of the later electrophonically evoked CAP (epCAP, arrow).

The growth functions of this animal with and without noise using various types of noise are presented in Fig. 6.7A. Clear suppression was observed using broadband noise, and the different types of filtered noise. Irrespective of the type of noise used, effects were always largest at high current levels. Animals with a high-frequency hearing loss also showed the most pronounced effects at high

current levels (Fig. 6.7B, C), though the magnitude of suppression depended on noise type in the animals shown (broadband and high-frequency noise were less effective).

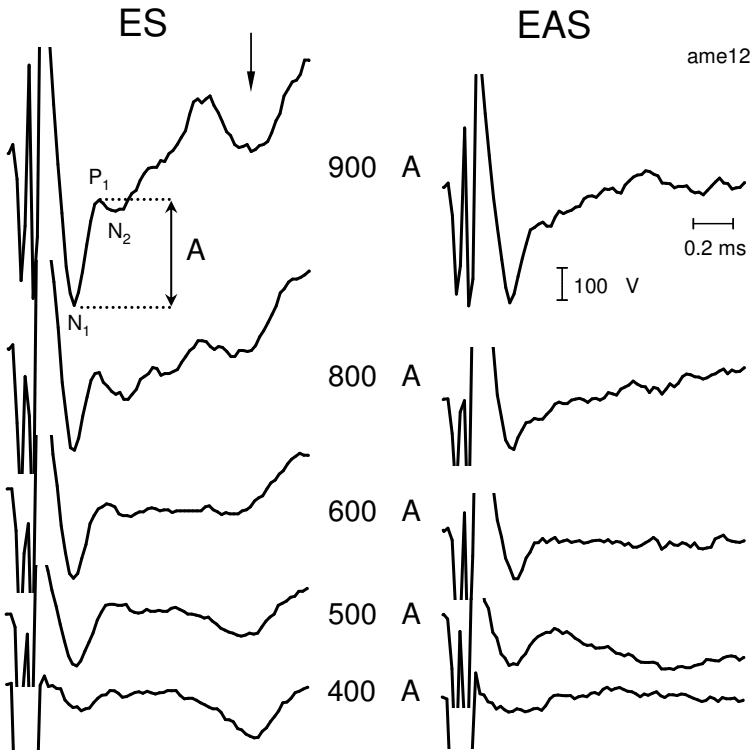

Fig. 6.6. Example eCAP recordings as function of probe current level in a normal-hearing animal (left) and when simultaneous broadband noise was presented at a sound level of 80 dB SPL (right). Amplitude was defined from the first negative peak (N_1) to the first positive peak (P_1). The second negative peak (N_2) and electrophonic CAP (arrow) are indicated. Start of the sweep corresponds to probe onset. Suppression of the eCAP is especially evident at 800 and 600 μA.

6.3.3. Effects of acoustic noise on eCAP amplitude: dependence on noise type and noise level

The effect of the different types of noise on eCAP amplitude were further investigated by applying various sound levels, as shown in Fig. 6.8. Current level was set at the highest level tested (900 μA). Suppression of eCAPs in normal-hearing animals increased with sound level, as shown for an example animal (Fig. 6.8A). Suppression was evident at relatively low sound levels of approximately

40 dB, except if low-frequency noise was applied. In the latter case higher levels were needed (~80 dB SPL).

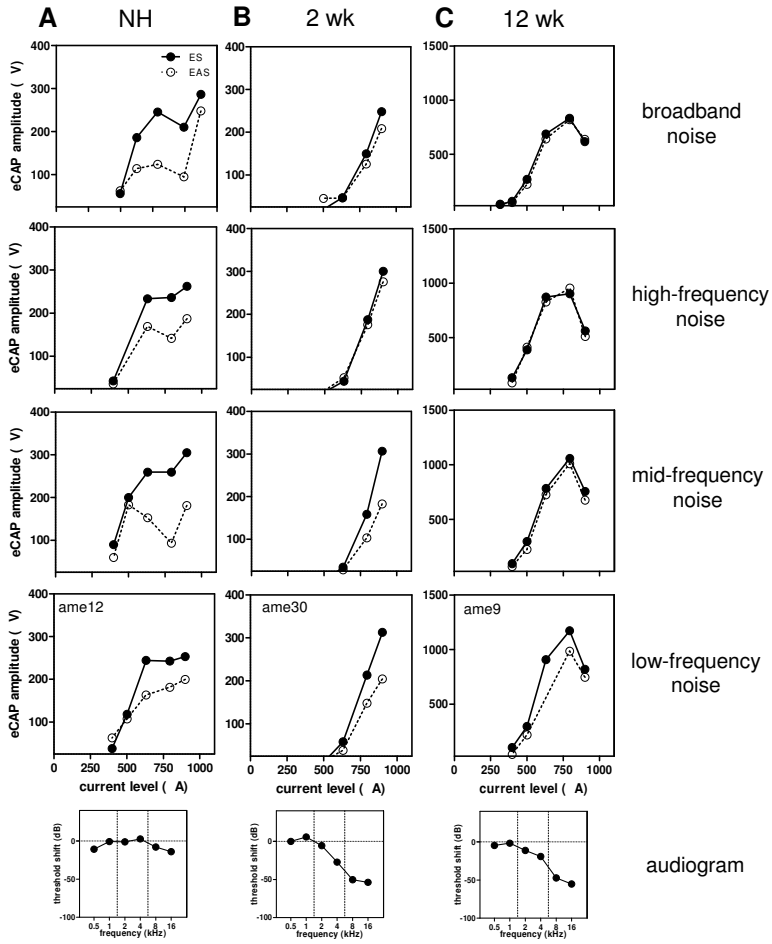

Fig. 6.7. Effects of noise on eCAP amplitude as function of probe level. Representative examples of a normal-hearing (NH) animals is shown (column A), and 2 animals with a high-frequency hearing loss 2 weeks (column C) and 12 weeks after ototoxic treatment (column D). Noise was broadband (first row), high-frequency noise), mid-frequency noise (third row), or low-frequency noise (fourth row). Electrical stimulation only (ES) is shown in filled circles, electro-acoustical stimulation (EAS) in open circles. For each animal acoustically evoked CAP thresholds are shown relative to the normal-hearing group before cochleostomy (bottom row). Dotted lines indicate cut-off frequencies of the noise filters (1.4 and 5.7 kHz).

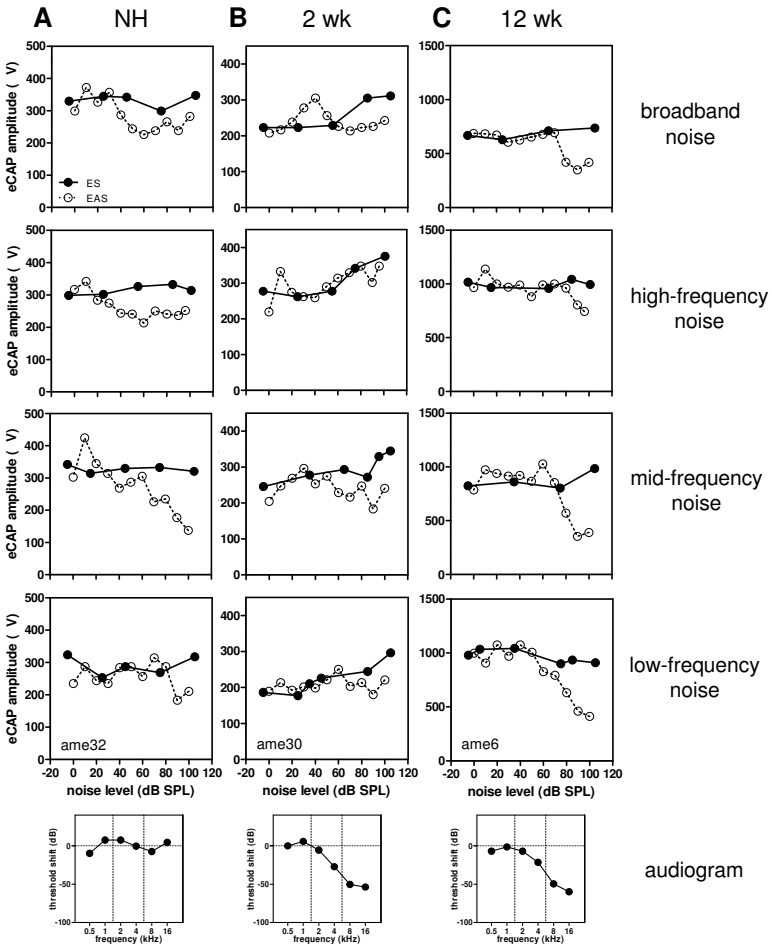

Fig. 6.8. Effects of noise on eCAP amplitude as function of noise level. Representative examples of a normal-hearing (NH) animal (column A) and 2 animals with a high-frequency hearing loss either 2 weeks (column B) or 12 weeks after ototoxic treatment (column C) are shown. Broadband noise was used (first row), or high-frequency noise (second row), mid-frequency noise (third row), or low-frequency noise (fourth row). Electrical stimulation only (ES) is shown in filled circles, electro-acoustical stimulation (EAS) in open circles. Note the different y-axis between animals. Recordings were performed sequentially from 100 dB to 0 dB SPL noise level. Hence, chronologically, graphs should be viewed from right to left. Control ES recordings are shown in the correct time frame. Note the eCAP adaptation in response to repetitive electrical stimulation (i.e. apparently irrespective of acoustic stimulation) in animal ame30. For each animal acoustically evoked CAP thresholds are shown relative to the normal-hearing group before cochleostomy (bottom row). Dotted lines indicate the cut-off frequencies of the noise filters (1.4 and 5.7 kHz).

In animals with a high-frequency hearing loss (Fig. 6.8B, C), eCAPs were suppressed only at high sound levels (~80 dB SPL) when broadband noise or high-frequency noise was applied. When presented at a sufficiently high sound level, effects of broadband noise could be as large as in normal-hearing animals, as shown for an animal 12 weeks after treatment (Fig. 6.8C). When mid-frequency band-pass or low-frequency noise was used, suppression was evident at lower sound levels (~60 dB SPL) and effects at high sound levels could be pronounced in these animals, as exemplified by the data shown of the animal 12 weeks after treatment using low-frequency noise (Fig. 6.8C). The threshold sound level at which eCAPs suppression became apparent could be related to the increased acoustic CAP thresholds (insets in Fig. 6.8).

Individual data of all animals tested are shown in Fig. 6.9, again using the highest current level tested (900 μA) where effects of noise were generally largest. Data are presented in the form of normalized eCAP amplitudes (eCAP ratios, see Materials and Methods). At a moderate noise level of 60 dB SPL, normal-hearing animals showed CAP ratios (i.e., <1) that significantly depended on noise type (Fig. 6.9A, RM ANOVA, noise type as within factor, $P< 0.05$). Broadband and high-frequency noise resulted in significantly lower eCAP ratios compared to low-frequency noise (Tukey's post hoc test, $P< 0.05$). Hence, low-frequency noise was less effective in suppressing the eCAP, compared to broadband and high-frequency noise. In contrast, animals 2 and 12 weeks after treatment showed no significant effects of noise type at 60 dB SPL (Fig. 6.9B, C, RM ANOVA, $P> 0.1$), and CAP ratios were more or less equally distributed around the no-effect level (i.e., CAP ratio= 1). At 80 dB SPL, no significant effect of noise type was found in any of the three groups (Fig. 6.9D - F, RM ANOVA, $P> 0.05$). At high sound levels, mid-frequency and low-frequency evoked a clear suppression in most animals that could be as high as in normal-hearing animals.

Effects of ototoxic treatment were tested on the same dataset. At both 60 and 80 dB SPL, ototoxic treatment significantly affected CAP ratios when broadband and high-frequency noise were used (Fig. 6.9, ANOVA, treatment as between factor, $P< 0.05$), but mid-frequency and low-frequency noise had similar effects on CAP ratios in all three groups ($P> 0.05$). CAP ratios tended to be lower in normal-hearing animals compared to animals 2 and 12 weeks after treatment for broadband and high-frequency noise (Fig. 6.9).

In some animals a clear facilitation of the eCAP was observed, most notably in some animals 2 weeks after ototoxic treatment. This effect was probably artificially introduced, as pointed out in section 3.7.

6.3.4. Effects of acoustic noise on eCAP amplitude: temporal aspects

Effects of noise duration on eCAP suppression were pronounced in normal-hearing animals (Fig. 6.10A, B). Initial suppressive effects of broadband noise

were largest after 2 – 3 ms (time between sound onset and hair cell activation was ~0.3 ms). At these noise durations, acoustically evoked CAP and eCAP more or less coincided and eCAPs could be suppressed by more than 80%. As noise duration increased, suppression quickly decreased to less than 20% after 10 ms. After noise offset, eCAP amplitudes were transiently increased up to as much as 40%.

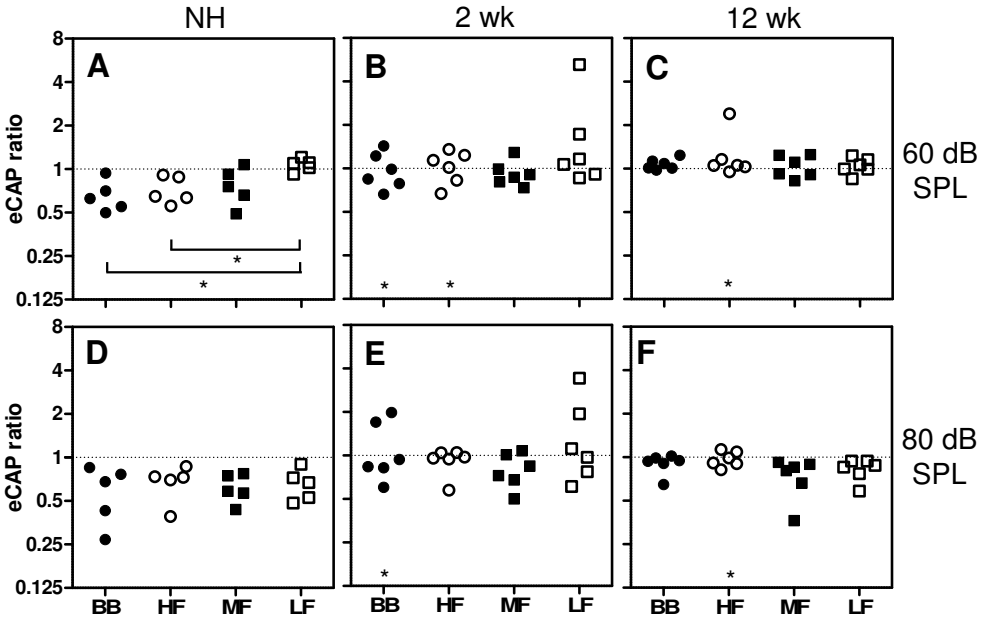

Fig. 6.9. Individual data of all animals tested for effects of noise on eCAP amplitude, including normal-hearing animals (left panels) and animals with a high-frequency hearing loss 2 weeks (middle panels) and 12 weeks after ototoxic treatment (right panels). Effects are represented as normalized amplitudes (A_{EAS}/A_{ES}). Different types of noise were applied: broadband noise (BB), high-frequency noise (HF), mid-frequency noise (MF) and low-frequency noise (LF) Noise was presented at 60 dB SPL (A-C) and 80 dB SPL (D-F). Asterisks indicate significant effects (P< 0.05) according to post hoc analyses, following (RM) ANOVA. Asterisks in (A) indicate significant differences between noise types. Asterisks in (B, C, E and F) indicate significant differences between eCAP ratios under the given condition in treated animals relative to normal-hearing animals.

Animals with a high-frequency hearing loss 2 and 12 weeks after treatment showed a temporal pattern of eCAP suppression and enhancement similar to normal-hearing animals (Fig. 6.10C – F). The most important difference was the magnitude of suppression and enhancement, which could be related to acoustic CAP threshold shifts. For example, one animal 2 weeks after treatment had normal thresholds at 0.5 – 8 kHz and a sharp threshold increase at 16 kHz.

This animal showed pronounced suppression and clear enhancement of the eCAP (Fig. 6.10C). In contrast, eCAPs in an animal 2 weeks after treatment with a large hearing loss extending to moderate and low frequencies were almost unaffected by broadband noise (Fig. 6.10D). Similarly, in animals 12 weeks after treatment, the magnitude of suppression and enhancement was larger in animals with small hearing loss (Fig. 6.10E) compared to those with hearing loss extending to moderate and low acoustic frequencies (Fig. 6.10F).

6.3.5. Characteristics of eABRs

Marked differences were observed in the growth functions of the different eABR peaks (Fig. 6.11A). While the early peaks P_I (and P_{II}, not shown) showed high thresholds (~500 μA) and growth functions with steep slopes, the later peaks P_{III} and P_{IV} were characterized by low thresholds (~100 μA) and growth functions with shallow slopes especially around threshold. P_{IV} showed a steeper growth function at high current levels well above threshold (~900 μA) that approximated that of early peaks. The characteristics of P_I closely matched those of eCAP N_1, while the characteristics of later peaks could be linked to the electrophonically evoked CAP (epCAP). Especially the threshold of the later eABR peaks and the epCAP were similar (Fig. 6.11B).

6.3.6. Effects of acoustic noise on eABRs

Effects of broadband noise at 80 dB SPL on eABRs were comparable to effects on eCAPs, in that eABRs were suppressed by noise especially at high current levels (Fig. 6.12). However, effects of noise differed between peaks and between normal-hearing animals and those with a high-frequency hearing loss. In normal hearing animals, broadband noise suppressed all peaks (P_I – P_V), but affected peak threshold only of later peaks (P_{III} – P_V). Suppression of P_I was generally mild (Fig. 6.12A), while P_{II} could be suppressed to a greater extent (not shown). Though absolute suppression of later peaks was relatively small, thresholds were increased substantially (up to ~500 μA for P_{III} in Fig. 6.12A). In the presence of noise, thresholds were very similar between early and late peaks (~500 μA).

Regarding suppression between normal-hearing animals and animals with a high frequency hearing loss, effects on early peaks were comparable, while suppression of later peaks in animals with a high-frequency hearing loss was reduced (fig. 6.12B, C). More importantly, no effect of noise on threshold was found on later peaks in animals with a high-frequency hearing loss, and thresholds of later peaks in animals with a hearing loss were similar as those of early peaks (~500 μA).

Dependence of eABR amplitude on sound level of broadband noise was similar to the dependence on the eCAP (not shown). In normal-hearing animals suppression of early and late peaks could be evident at low sound levels ~40 dB

SPL. Effects of noise were robust and suppression was also observed in animals with a high-frequency hearing loss. Though somewhat higher sound levels were needed, suppression could be pronounced on both early peaks and late peaks in animals with a high-frequency hearing loss.

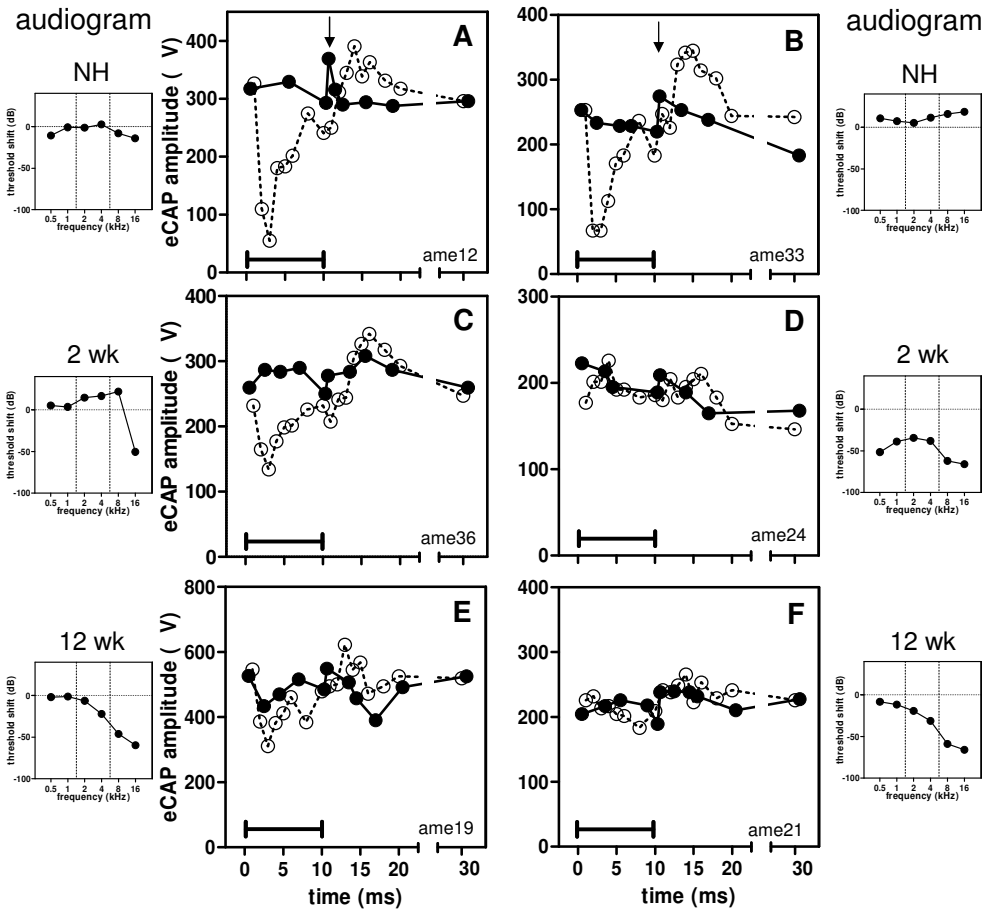

Fig. 6.10. Effects of noise duration during simultaneous presentation of broadband noise on eCAP amplitude and subsequent after-effects. 2 examples of each group are shown: normal-hearing (NH) animals (A, B), animals with a high-frequency hearing loss 2 weeks after ototoxic treatment (C, D) and 12 weeks after treatment (E, F). Noise was presented in bursts 10 ms in duration indicated by the black bar. Noise level was 80 dB SPL. Chronologically, the recordings were started at 0 ms and ended at 30 ms (i.e., from left to right). At the last recording with simultaneous noise presentation (at 10 ms), recordings were interrupted for a few minutes, resulting in a recovery from long-term adaptation (arrows). For each animal acoustically evoked CAP thresholds are shown relative to the normal-hearing group before cochleostomy (insets). Dotted lines indicate 1.4 and 5.7 kHz.

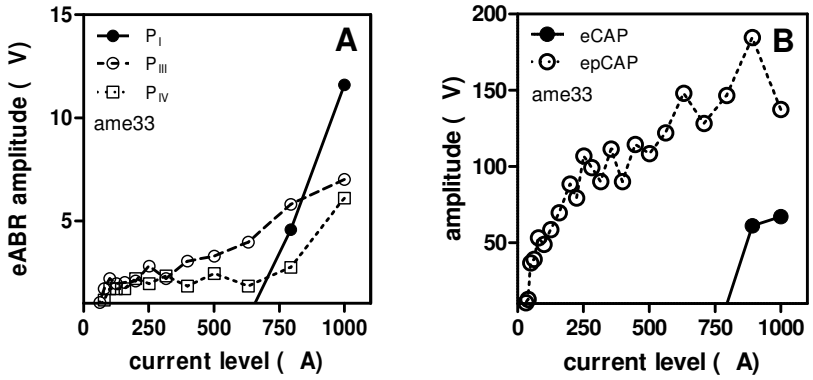

Fig. 6.11. Characteristics of early and late eABR peaks (A) compared to eCAP and electrophonically evoked CAPs (epCAPs) in normal-hearing animals (B). P_I of the eABR had high thresholds (~500 μA) and a steep growth function comparable to the eCAP. Later peaks (P_{III}, P_{IV}) of the eABR showed lower thresholds (~100 μA) and shallow slopes comparable to epCAPs. Stimuli for eABRs: positive-first, single biphasic pulses. Stimuli for epCAPs: single, alternating biphasic pulses. Stimuli for eCAPs: forward masking sequence using positive-first pulses.

6.3.7. Deviating observations on the effects of noise on eCAPs

In normal-hearing animals, noise had suppressive effects on eCAPs and eABRs. In some animals with a high-frequency hearing loss (2 out of 7 animals 2 weeks after treatment, and 1 of 6 animals 12 weeks after treatment) acoustic noise actually increased eCAP amplitude (Fig. 6.9). Example data are provided of an animal 2 weeks after treatment in which this phenomenon was particularly strong (Fig. 6.13). Acoustic noise led to somewhat more negative values of N_1 and a shorter latency. More importantly, the positive peak P_1 was increased, which was the most important factor in the amplitude increase. The second negative peak N_2 was less prominent when noise was presented (indicated with the arrowhead in the figure 6.13A), which was also noted in the normal-hearing animal shown in Fig. 6.6. Therefore, suppression of the N_2 might have increased P_1 due to its overlap with the N_1-P_1 peak complex, in turn increasing eCAP amplitude.

N_2 might have represnted a synchronous second firing of auditory nerve fibers, but its latency of ~0.5 ms might indicate that N_2 was related to the epCAP. The latency of the epCAP was approximately 1.2 ms (indicated with an arrow in Fig. 6.13A). Since the masker pulse was presented 0.7 ms prior to the probe pulse (t = 0), N_2 could theoretically be a residual epCAP signal left from the masker stimulus. Noise-induced suppression of this (residual) epCAP would then "unmask" the eCAP. Fig. 6.13C shows the dependence of eCAP amplitude on noise duration in the same animal and clearly reveals increased eCAP amplitudes.

The pattern of enhancement is actually an inverted pattern of eCAP suppression during noise presentation (Fig. 6.10). After-effects were similar, resulting in eCAP amplitudes of more than 300% of the original.

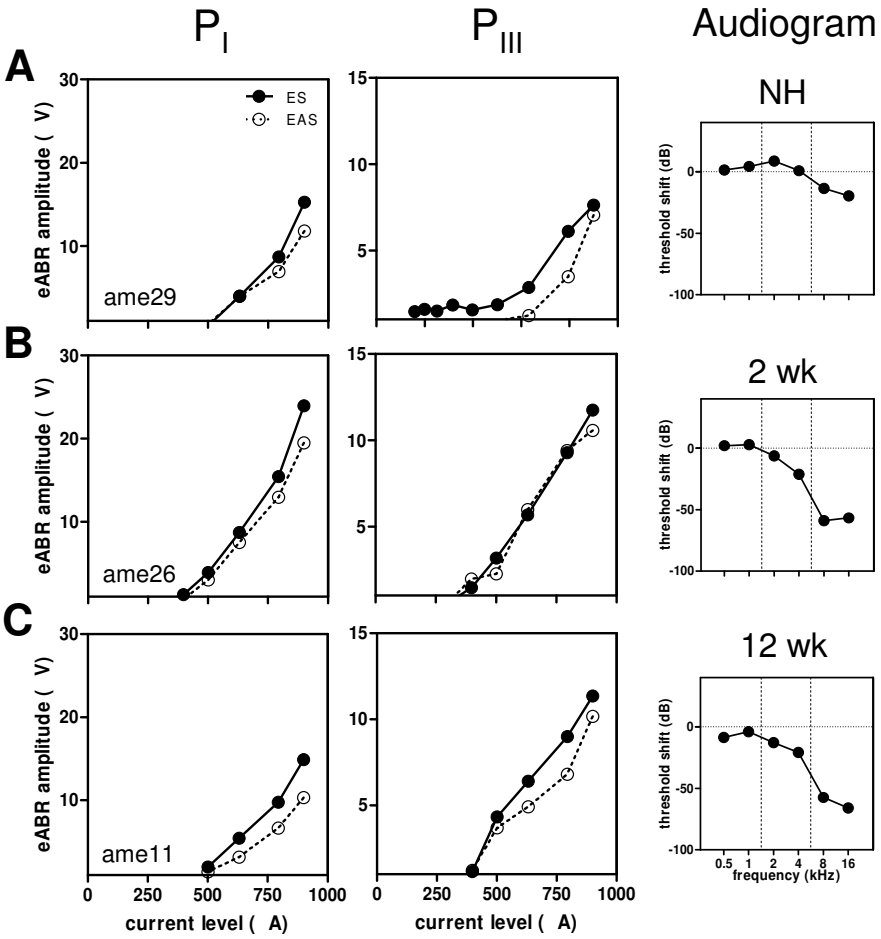

Fig. 6.12. Effects of broadband noise (80 dB SPL) on eABR amplitude as function of probe level. Representative examples of a normal-hearing (NH) animal (A), and animals with a high-frequency hearing loss recorded 2 weeks after ototoxic treatment (B) and 12 weeks after treatment (C) are shown. Effects are shown for P_I (left column), P_{II} (second column), P_{III} (third column), and P_{IV} (right column). Electrical stimulation only (ES) is shown in filled circles, electro-acoustical stimulation (EAS) in open circles. For each animal acoustically evoked CAP thresholds are shown relative to the normal-hearing group before cochleostomy (insets). Dotted lines indicate 1.4 and 5.7 kHz.

The data showing enhancement of eCAP amplitude contrasted with the effect of noise on eABR P_I in this animal (Fig. 6.13D). P_I was clearly suppressed, despite the fact that eCAPs were increased (Fig. 6.13B). Rather high noise levels were needed since the animal had a high-frequency hearing loss.

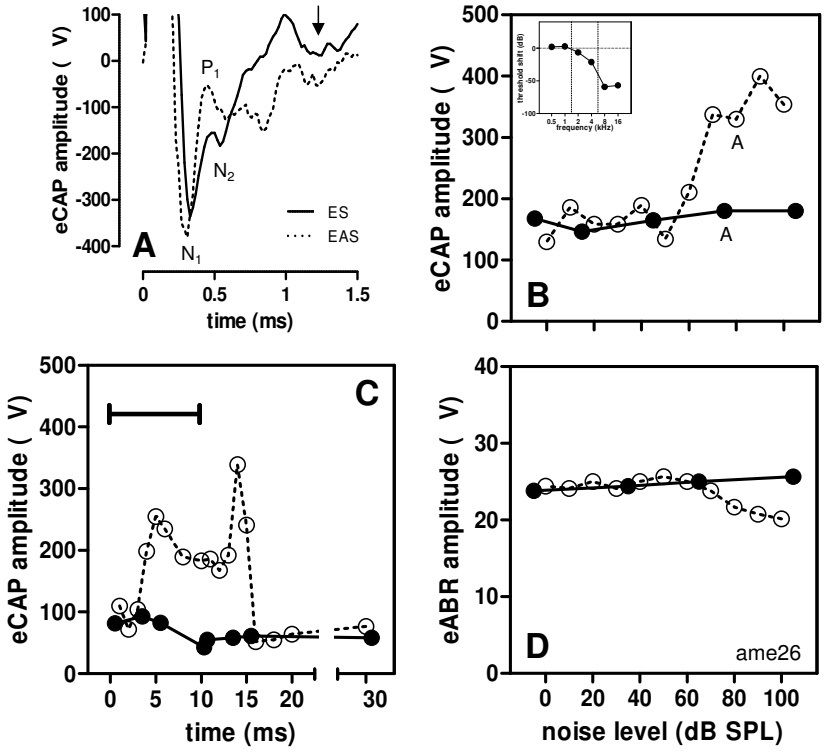

Fig. 6.13. Example of deviating effects of noise on the eCAP in an animal with high-frequency hearing loss 2 weeks after treatment. Presentation of broadband noise at 80 dB SPL resulted in a marked eCAP (900 µA stimulus pulse) amplitude increase in this animal (A). P_1 was shifted upward and the second negative peak (N_2) was reduced. Note the pronounced epCAP reduction (arrow). The eCAP amplitude increased with noise level (B). Recordings shown in (A) are indicated (arrows). The dependence of noise duration showed a reversal of the "normal" pattern, while after-effects were as expected (C). During noise presentation (indicated by the bar), a large initial enhancement can be seen that decreased over time. After the noise burst an enhancement "overshoot" can be seen. Contrasting effects of noise on eABR P_I (D), showing suppression in the same animal, under the same conditions as in (B). Acoustically evoked CAP thresholds are shown relative to the normal-hearing group before cochleostomy (inset). Dotted lines indicate 1.4 and 5.7 kHz.

6.4. Discussion

We have shown that acoustic noise can suppress eCAPs and eABRs. Suppression was largest at high current levels. Regarding dependence of suppression on type of noise, significantly more eCAP suppression was found using broadband and high-frequency noise in normal-hearing animals when moderate sound levels were applied (60 dB SPL). In contrast, at 80 dB SPL, no dependence on noise type was found. In animals with a high-frequency hearing loss, no significant effect of noise type was found at both sound levels. No differences of effects of noise were found between animals 2 and 12 weeks after treatment. High-frequency hearing loss significantly affected the effect of noise, and broadband and high-frequency noise tended to be more suppressive in normal-hearing animals compared to animals with a high-frequency hearing loss (Fig. 6.9).

Suppression was dependent on noise duration (Fig. 6.10) and after noise offset eCAP amplitudes could even be enhanced. Early eABR peaks resembled eCAPs in terms of growth-function characteristics and noise-induced suppression, while later peaks appeared to be related to electrophonic responses (Fig. 6.11 - 6.12).

6.4.1. Generation of eCAPs

Stimulus polarity affected eCAP morphology and biphasic pulses with an initial positive phase ("positive-first", or anodic-first stimuli) generally evoked larger responses (Fig. 6.3). An earlier study using monophasic stimuli showed that cathodic pulses yielded largest eCAP amplitudes in guinea pigs, while anodic pulses yielded lowest thresholds (Miller et al., 1998). For subsequent studies they used "cathodic-first" pulses for eCAP recording in guinea pigs for purposes similar to the present study (Nourski et al., 2005, 2007). On the basis of these data, the finding that "anodic-first" pulses typically yielded the largest eCAPs in our experiments was unexpected. However, differing experimental conditions might have been a factor, such as the fact that Nourski et al. used an electrode array, while we applied electrical stimuli via a thin platinum wire. Such differences in experimental setup might have affected the complex interaction between the two phases in the stimulus (Miller et al., 2001b). In addition, stimulus artifacts after the "anodic-first" stimuli were typically smaller in our experiments (Fig. 6.3C), which was another important factor for using these stimuli. Effects of polarity on threshold were not specifically addressed in this study, but no clear differences were observed.

6.4.2. Waveform of eCAPs

In normal-hearing animals, electrically evoked cochlear activity consisted of multiple peaks (Fig. 6.3A). The first negative peak (N_1) was associated with the

eCAP, given its short latency (~0.3 ms). A large broader peak with a long latency (~1.2 ms) was associated with electrophonics, as described earlier (Stronks et al., 2010). Amplitude of the eCAP was determined from N_1 to the first positive peak (P_1). The second negative peak (N_2) had a latency of 0.5 ms. Given the fact that the latency of N_2 corresponded to the latency of the epCAP (1.2 ms) when evoked by the masker pulse in the forward masking paradigm (0.7 ms before the probe pulse), N_2 might have been affected by epCAP responses that were artificially introduced by the applied subtraction procedure (Fig. 6.14A). This assumption was strengthened by the finding that potentials were visible following the masker-probe double-pulse with latencies corresponding to epCAPs evoked by the masker pulse (Fig. 6.14B). This suspected epCAP response evoked by the masker pulse has a latency of 0.5 ms relative to the probe pulse and might have affected the position of the probe-evoked P_1 of the eCAP. Cancellation of the masker-evoked epCAPs in the forward masking paradigm could have been imperfect, because masker-artifact and masker-response must be identical in the masker-only and masker-probe condition for perfect cancellation. It is conceivable that the epCAP evoked by the masker was altered by the probe pulse in the double-pulse stimulus, thereby disturbing cancellation of the masker response, as illustrated in Fig. 6.14.

The assumption of P_1 being affected by artificial residual epCAPs can possibly explain the finding of increased eCAP amplitudes in 2 out of 13 animals with a high-frequency hearing loss upon noise presentation (Fig. 6.9A). Addition of noise possibly reduced this interference and may have led to "unmasking" of the eCAP response.

The first peak of the eABR (P_I) is generally associated with the eCAP (Miller et al., 1993). The effects of noise on eABR P_I (Fig. 6.13D) contrasted with eCAPs, since P_I never showed any enhancement. This was surprising, since effects of noise on eABRs were tested using the same forward masking paradigm. Electrophonic responses, assumed to be responsible for the "unmasking" of the eCAP, were possibly represented to a lesser extent in the early eABR peaks due to the remote location of the eABR recording electrodes. This latter assumption is strengthened by the fact that early eABR peaks did not show any relation with epCAP responses as described earlier (Figs. 6.11, 6.12). However, we cannot explain why none of the normal-hearing animals showed "unmasking" of the eCAP, given the fact that these animals had the largest epCAPs.

6.4.3. Animal model

Ototoxic treatment with kanamycin and furosemide resulted in acoustic CAP threshold shifts that increased with acoustic frequency. Outer hair cell (OHC) loss gradually increased from apex to base. Inner hair cell (IHC) and spiral ganglion cell (SGC) loss were mild throughout the cochlea, except in the lower basal turn, especially after 12 weeks.

It was shown by others that a loss of functional hair cells increased eCAP amplitude (Hu et al., 2003), while a loss of SGCs decreased eABR amplitude (Hall, 1990; Agterberg et al., 2009) and the amplitude of optically evoked CAPs (Richter et al., 2008). Based on these findings we expected to find largest eCAP amplitudes 2 weeks after treatment (due to hair cell loss) and smallest eCAPs after 12 weeks of treatment (due to SGC loss). This assumption proved only partly true in our model. Compared to normal-hearing animals eCAPs were significantly larger after 12 weeks, while amplitudes were somewhat increased after 2 weeks, but not significantly (Fig. 6.5A). Largest amplitudes were observed in animals 12 weeks after treatment that had hair cell loss (Fig. 6.5B), but no apparent SGC loss (Fig. 6.5C). The balance between hair cell loss (increasing eCAP amplitude) and SGC loss (decreasing eCAP amplitude) will likely have determined eCAP amplitude.

6.4.4. Effects of acoustic noise on eCAP amplitude

Acoustic noise generally reduced eCAP amplitude, especially at high current levels which corresponds to earlier reports (Nourski et al., 2005, 2007). The authors explained eCAP suppression by assuming acoustic noise increased auditory-nerve fiber activity, thereby increasing the number of fibers in a (relative) refractory state and reducing the proportion of excitable fibers. Electrical stimulation was therefore assumed to result in excitation of less nerve fibers and hence in a decreased eCAP amplitude. Furthermore, fibers in a relative refractory state were expected to fire with less synchrony (Rubinstein et al., 1999) and respond with decreased spike amplitudes (Miller et al., 2001a). Hence, despite increased firing rates of fibers in response to electro-acoustical stimulation during noise presentation (Miller et al., 2009), eCAP amplitudes were expected to decrease.

We tested for effects of different types of noise on eCAP amplitude, including broadband noise, and the filtered variants after high-pass (5.7 kHz cut-off frequency), mid-frequency band-pass (1.4 – 5.7 kHz cut-off frequencies) and low-pass filtering (1.4 kHz cut-off frequency). We expected to find largest eCAP suppression in normal-hearing animals using high-pass filtered noise, since the stimulating electrode was placed in the basal part of the cochlea. However, we found similar suppressive effects of broadband and high-frequency noise on eCAP amplitude in normal –hearing animals (Fig. 6.9). Broadband and high-frequency noise both showed relatively high energy at middle-high frequencies of ~4 kHz (Fig. 6.1). This could be one of the reasons for the overall equal effectiveness of these noise types on eCAP amplitude.

In animals with a high-frequency hearing loss, broadband and high-frequency noise were less effective suppressors , while mid-frequency and low-frequency noise were still effective. These observations are readily explained by the loss of high-frequency hearing.

Regarding the dependence on noise level, suppression in normal-hearing animals could be evident at levels as low as 40 dB SPL (Fig. 6.8). At a moderate sound

level of 60 dB SPL, broadband and mid-frequency were significantly more effective in normal-hearing animals compared to low-frequency noise (Fig. 6.9). Low-frequency noise was likely less effective due spatial separation of electric and acoustic stimuli in the cochlea. At 80 dB SPL no effect of noise type was found, likely due to a spread of acoustic excitation throughout the cochlea.

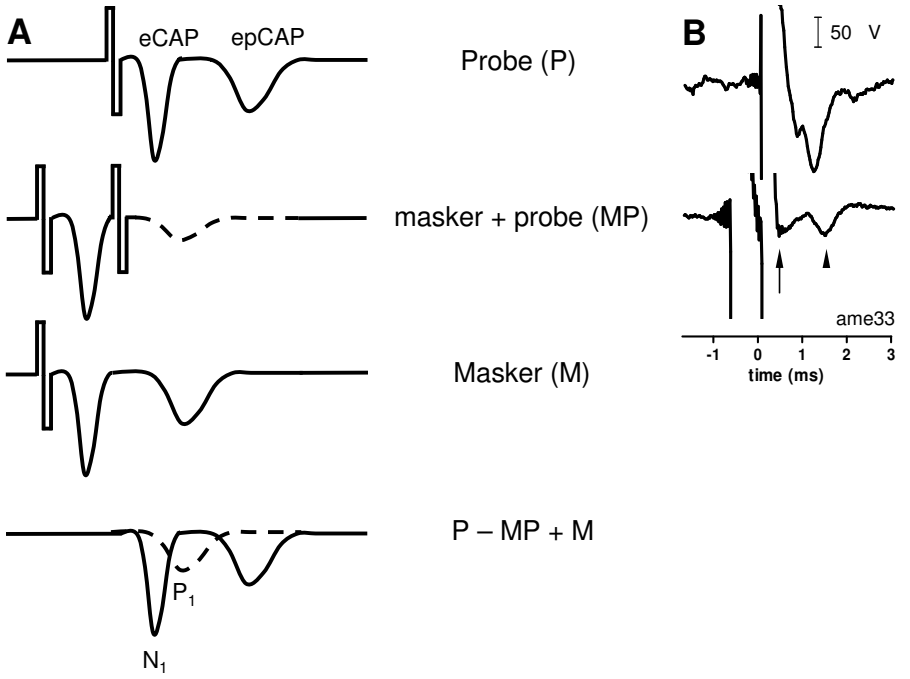

Fig. 6.14. Possible effects of epCAP responses on eCAP recording using a forward masking paradigm (A). The probe (P) elicits an eCAP and epCAP. In the masker+probe condition (MP), the epCAP in response to the masker pulse is altered in shape by the probe pulse (smaller epCAP). In the ideal situation shown, no responses to the probe pulse will be present. The masker (M) elicits the same response as the probe (P), but shifted in time. The resulting waveform (P – MP + M) shows a residual masker-pulse evoked epCAP at the place on N_2, thereby altering P_1 and the eCAP amplitude N_1-P_1. Example recordings of intermediate steps in the forward masking paradigm (B) show a clear epCAP 1.2 ms after the 900 μA probe pulse (t= 0, upper waveform). After the masker-probe pulse a response can be seen with a latency of 0.5 ms (arrow), corresponding to the latency of the epCAP relative to the masker pulse at -0.7 ms (bottom graph). A reduced epCAP evoked by the probe can also be seen (arrowhead). Note that eCAPs are obscured in these examples since they represent intermediate steps in the forward masking paradigm. Stimulus artifact was partly removed by averaging both stimulus polarities.

Regarding high-frequency hearing loss, ototoxically treated animals showed less suppression when broadband and high-frequency noise were applied and this effect could be related to the extent of hearing loss (e.g. Fig. 6.10). Nevertheless, the magnitude of suppression could be as pronounced as in normal-hearing animals when sound levels were high enough, possibly due to a spread of acoustical excitation due to high acoustic stimulus levels.

In all, eCAP suppression was most pronounced at high electric and acoustic levels. High current levels result in a spread of electrical excitation throughout the cochlea (Van den Honert and Stypulkowski, 1987). Likewise, at high sound levels, (low-frequency) acoustic excitation spreads to high-frequency regions of the cochlea (Kiang et al., 1967; Evans, 1972; Javel, 1994). Hence, these conditions will favour overlap between electrically and acoustically activated areas in the cochlea.

The temporal aspects of the effects of noise on eCAP amplitude in guinea pigs were investigated in detail by Nourski et al. (2005, 2007). Our data agree with these findings, in that suppression was most pronounced shortly after noise onset and decreased substantially within milliseconds during noise duration. After noise offset an "overshoot" was observed characterized by a temporary increase in eCAP amplitude. The decrease in suppression when noise duration increased and the temporary overshoot after noise offset was explained by Nourski et al. by assuming that during the presentation of noise the release of neurotransmitter decreases from acoustically stimulated hair cells due to peripheral adaptation. Consequently, noise-induced activation of auditory nerve fibers decreased and electrical stimuli recruited more fibers over time, thereby increasing eCAP amplitude during prolonged noise presentation (Nourski et al., 2007). We add the notion that noise onset will evoke a highly synchronous nerve response, after which a large proportion of nerve fibers will be in a refractory state, making them less responsive to electrical stimuli. During prolonged noise presentation, nerve fibers will be activated with decreased synchrony, thereby increasing responsiveness to electrical stimulation. Nourski et al. explained noise-offset responses by adaptation of hair cells, under the assumption that hair cells temporarily release below-normal amounts of neurotransmitter. This process will decrease spontaneous random activity of auditory nerve fibers and increased firing synchrony when the cochlea is electrically stimulated, thereby causing increased eCAP amplitudes to values above normal (Nourski et al., 2007). Temporal characteristics were very similar in animals with a high-frequency hearing loss, though suppression as well as enhancement was smaller in magnitude, likely due to a reduced number of hair cells.

6.4.5. Origin of eABR peaks and effects of noise

Comparable to the eCAP data, suppression of eABRs increased with current level (Fig. 6.12) and lower noise levels were sufficient to result in suppression in

normal-hearing animals compared to animals with a high frequency hearing loss. Furthermore, the magnitude of suppression was comparable between groups, though inter-animal variability was large.

The early peaks of the eABR (P_I and P_{II}) showed characteristics resembling those of the eCAP, including growth functions with steep slopes and high thresholds (Fig. 6.11). The resemblance of the eCAP and P_I was expected, since they are both thought to reflect the auditory nerve response (Stypulkowski and Van den Honert, 1984) and both had similar latencies in our recordings (0.3 ms). P_{II} had a somewhat longer latency of 0.7 ms (Fig. 6.4), which is somewhat long for an eCAP (typically <0.5 ms). It might represent N_2 of the eCAP (Fig. 6.3), which also had a latency of 0.7 ms. The early peaks P_I and P_{II} were suppressed by noise, while threshold was unaffected. Later peaks P_{III} and P_{IV} showed suppression and a substantial threshold increase, similar to the epCAP. Hence, P_I and P_{II} probably represented direct neural effects.

The later eABR peaks P_{III} and P_{IV} showed growth functions with shallow slopes and low thresholds in normal-hearing animals, features shared with the epCAP (Fig. 6.11). P_{III} had a latency of 1.3 ms (Fig. 6.4), similar to the epCAP (1.2 ms) and there might have be an epCAP component from the auditory nerve in this eABR peak. P_{IV} had a latency of ~5 ms, which excludes an epCAP component in this peak. The shallow slope of the eABR growth function around threshold in normal-hearing animals was ascribed previously to electrophonically evoked brainstem responses (Black et al., 1983). These authors reported that acoustic noise suppressed the electrophonic eABR component, resulting in steeper slopes of the eABR growth function due to a larger contribution of direct neural activation to the eABR response, similar to our data (Fig. 6.12). In addition, deafened animals were reported to have growth functions with steep slopes in the absence of noise, a characteristic also shared with our animals with a high-frequency hearing loss. Last, the authors report a steep slope of eABR growth functions at current levels well above threshold, probably due to increased contributions of activity due to direct neural activation. We observed this as well for P_{III} (Fig. 6.12). In all, though we cannot exclude a direct epCAP component in the eABR, it seems likely that low thresholds and shallow slopes of later eABR peaks were due to interference by electrophonically evoked eABR responses.

We add the notion that noise increased the threshold of later eABR peaks to values resembling those of early peaks, and thresholds of all eABR peaks fell in the same range as the threshold of the early peaks in animals with a high-frequency hearing loss. Hence, the early P_I (and P_{II}) represented the eCAP (i.e., auditory-nerve responses by direct electrical activation), while later peaks were generated by brainstem responses due to direct neural activation and by electrophonically evoked brainstem activity. The contribution of the latter was decreased in animals with a high-frequency hearing loss and by presentation of noise.

6.4.6. Clinical relevance

We have shown that in a model of high-frequency hearing loss eCAPs could be suppressed by mid-frequency and low-frequency noise at high sound levels (e.g. 80 dB SPL). Hence, electrically and acoustically excited areas overlapped, despite the theoretical spatial segregation of these two regions. In theory, similar mechanisms might also occur in hybrid implant users and acoustic stimuli might negatively affect electrical responses in hybrid implants.

It was shown earlier in CI users with a Nucleus implant that eCAP thresholds approximated the so-called maximum comfort level in patients (Brown et al., 1998). These eCAP thresholds were determined using neural response telemetry, based on the forward masking technique. Though eCAP threshold criteria might differ between implant systems, electrical stimuli delivered to the cochlea in CI users might be lower than those at which we found suppressive effects of noise. Hence, the large suppressive effects seen at high current levels in this study might not be reached in hybrid implant users. Furthermore, we applied alternating biphasic stimuli. Present cochlear implant stimulation strategies, such as the Continuous Interleaved Sampling method, apply non-alternating pulse trains to the electrodes. Though we analyzed the "positive-first" responses only, the use of alternating stimuli might have influenced our results. Therefore, direct extrapolation to EAS users must be done with caution.

In addition, suppressive effects on electrically evoked gross potentials might even reflect mechanisms beneficial to hearing. Hu et al. (2003) showed that the presence of functional hair cells alone (i.e. without acoustical stimulation) had a number of effects on the eCAPs. Observed effects included decreased maximum eCAP amplitude, shallower slope of the growth function and less suppression of the eCAP amplitude by electrically evoked refractoriness. These observations were explained by a decreased synchrony of the electrically activated population of auditory nerve fibers. Desynchronization of auditory nerve fibers in the absence of stimulation is caused by spontaneous release of neurotransmitter by hair cells, which leads to spontaneous random activity of auditory nerve fibers. Due to the loss of functional hair cells auditory nerve fibers in deafened cochleas show less spontaneous activity (Liberman and Dodds, 1984). As a consequence, a relatively low fraction of fibers is in a refractory state and electrical stimulation can excite a high number of fibers in synchronous fashion. All these fibers will subsequently be refractory and unresponsive to stimulation. This all-or-nothing mode of stimulation is unlike the normal-hearing cochlea, where random activity prevents mass synchrony since a proportion of fibers will be unresponsive to stimulation at any given time. Asynchronic auditory nerve fiber activity is believed to limit loudness growth and lower acoustic hearing thresholds (Hong and Rubinstein, 2006). Hence, while it is generally accepted that moderate-to-loud acoustic noise is disruptive to hearing function, low-level noise can actually improve hearing ability by increasing the dynamic range of sound perception. In CI users it has

been demonstrated that acoustic white noise lowers sound detection thresholds and increases frequency discrimination. In addition, "electric noise" in the form of random amplitude modulation of the carrier pulse train improves envelope modulation detection (Chatterjee and Robert, 2001; Zeng, 2002). Hence, the observed eCAP suppression in our experiments could actually reflect beneficial processes.

The finding that in some animals eCAP waveforms were probably contaminated by epCAP responses might be of relevance, since similar forward masking techniques are also applied in cochlear implant systems. Addition of acoustic noise eliminated epCAP responses, and eABRs did not show epCAP contamination of the early peaks either. These methods could thus be used to verify eCAP recordings in acoustically sensitive implanted cochleas in EAS users.

We found that eCAP recordings and eABR waveforms consisted of compound responses of direct neural activation and electrophonic responses. Furthermore, acoustic noise could suppress electrophonics, while preserving direct neural responses. These results indicate that EAS in cochleas with residual acoustic sensitivity results in complex responses consisting of direct electrically evoked neural activity, electrophonics and acoustically evoked responses, at least up to the level of the brainstem.

The finding that effects of acoustic noise drastically decreased after several milliseconds during noise presentation might be useful for the design of EAS strategies. Similarly, the transiently increased eCAP amplitude after noise offset might be relevant for EAS stimulation strategies.

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Abbreviations

AS	acoustical stimulation
CAP	acoustically evoked compound action potential
EAS	electro-acoustical stimulation
eCAP	electrically evoked compound action potential
epCAP	electrophonically evoked compound action potential
ES	electrical stimulation
IHC	inner hair cell
MPI	masker-to-probe interval
OHC	outer hair cell
SGC	spiral ganglion cell

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General discussion 7

Continuous improvement of cochlear implant (CI) systems has led to remarkably good speech understanding of CI users, resulting in a gradual broadening of the audiometric inclusion criteria for implantation. As a consequence, more and more people with a CI have considerable residual low-frequency hearing. These individuals receive electrical stimulation from their CI and acoustical stimulation from the remaining acoustically sensitive parts of the cochlea. This thesis aimed to characterize electrophysiological responses to combined electro-acoustical stimulation (EAS) evoked in the auditory system, with emphasis on cochlear potentials of the auditory nerve. As a measure for auditory nerve activity we used the amplitude of the acoustically evoked compound action potential (CAP) and electrically evoked compound action potential (eCAP). CAP and eCAP amplitude are widely used and represent synchronous auditory nerve activity. Parametric studies were conducted in guineas pigs in which evoked potentials by EAS were compared to responses evoked by acoustical stimulation alone (AS), or electrical stimulation alone (ES). The most important results will be discussed and concluding clinical implications will be presented.

7.1. Summary

First we investigated the guinea pig model with regard to anesthetic regime (chapter 2). Anesthesia was essential in our experiments due to the invasive nature of the required surgery. During all of our experiments guinea pigs were anesthetized using the volatile general anesthetic isoflurane evaporated in a mixture of nitrous oxide and oxygen. The effect of isoflurane and nitrous oxide on auditory nerve activity has not been investigated so far. Isoflurane (in nitrous oxide and oxygen) dose-dependently suppressed the amplitude of the CAP and increased its threshold. Effects increased with acoustic frequency and typically reached statistical significance at isoflurane concentrations of 2.5%.

Effects of electrical stimulation on CAPs were tested in normal-hearing guinea pigs and in animals with a high-frequency hearing loss. First the effects were tested in normal-hearing guinea pigs using extracochlear stimulation electrodes to minimize cochlear damage (chapter 3). Electric stimuli mimicked those used in present-day implants and were delivered via an electrode on the round window and a return electrode on the basal turn. CAPs were suppressed by electrical stimuli. Suppression was almost absent at low frequencies (0.5 and 1 kHz). CAP suppression increased with tonal frequency and current level, and decreased with sound level and interval between electric and acoustic stimulus (electric-to-acoustic interval, or EAI). At high current levels low-frequency evoked CAPs could also be suppressed. We concluded that the high-frequency part of the cochlea can be stimulated electrically with little detrimental effects on CAPs evoked by low-frequency tones.

Interestingly, we found evidence for dual responses to electric stimuli.

Fast responses were observed with a latency of less than 0.5 ms which were most likely related to direct electrical activation of neural elements (i.e., eCAPs). In addition, prominent responses were recorded with a latency of 1.2 ms, which were identified as electrophonic responses. Both electrical responses could be responsible for CAP suppression. The contribution of direct electrically evoked responses and electrophonics in CAP suppression was further investigated in chapter 5.

Effects of electrical stimulation on acoustically evoked CAPs were further investigated in a guinea pig model for high-frequency hearing loss to better mimic the situation in EAS users (chapter 4). To induce a high-frequency hearing loss, guinea pigs were treated with an ototoxic combination of furosemide and a relatively low dose of kanamycin. A low dose of kanamycin resulted in increased CAP thresholds at high frequencies, while hearing loss at low frequencies was minimal. Histologically, hair cells were damaged mainly in the high-frequency region of the cochlea, while those in low-frequency regions were mostly spared. Recordings were done after 2 or 10 weeks to mimic short-term (no degeneration of the auditory nerve) and long-term high-frequency hearing loss (additional nerve degeneration), respectively. Degeneration of the auditory nerve was characterized by determining the loss of auditory nerve cell bodies, i.e., the spiral ganglion cells (SGCs) in the cochlea. Current stimuli were presented via an intracochlear electrode to simulate a cochlear implant electrode.

First we re-tested effects of electrical stimulation in normal-hearing animals using intracochlear stimulation. Results were essentially similar to those obtained using extracochlear stimulation, in that effects on CAPs evoked at low frequencies were small, while CAPs at high acoustic frequencies were suppressed to a great extent. Nevertheless, a few differences were observed. Most notably, a more pronounced dependence on sound level at low acoustic frequencies was found using intracochlear stimulation. CAPs evoked at low acoustic frequencies were suppressed at high sound levels, but somewhat enhanced at low sound levels. CAP suppression at low frequencies and high sound levels was explained by the basal-ward spread of acoustical excitation in the cochlea at high sound levels. This phenomenon results in a stimulation of increasing numbers of fibers with a charachteristic frequency (CF) above the stimulus frequency when sound level increases. Since CAP suppression was most pronounced at high frequencies, suppression will therefore have increased with sound level at low frequencies. The small enhancement of CAPs at low frequencies and low sound levels was not seen when using extracochlear stimulation. CAP enhancement was observed by Ball after intraneural electrical stimulation (Ball, 1982). The author explained it by assuming an involvement of the central nervous system and efferents. Mechanistic explanations of CAP suppression are given below (section 7.3).

In animals with a high-frequency hearing loss virtually no CAP suppression was observed at low acoustic frequencies. This absence of suppression was explained by a reduction of the basal (high-frequency) contribution of fibers

under conditions of low-frequency stimulation at high sound levels.

Next we tested the dependence of CAP suppression on the pulse rate of the current pulse train and on the width of the pulses in the pulse trains in normal-hearing animals (chapter 5). Pulse rate had little effect, while pulse width had large effects on the acoustic frequency dependence of CAP suppression. This dependence on tone stimulus frequency could be related to the frequency spectrum of the electric stimulus and provided information about the underlying mechanisms of CAP suppression (section 7.3).

Last, we investigated effects of hair cells on electrically evoked CAPs, using our animal model for short-term and long-term high-frequency hearing loss (chapter 6). We first tested whether eCAP amplitude depended on cochlear status by measuring eCAP amplitudes in normal-hearing animals, and in animals with a high-frequency hearing loss 2 or 12 weeks after treatment in the absence of acoustic stimuli. We found that eCAP amplitudes were largest in cochleas with hair cell loss and a normal complement of SGCs. Hair cell activation by acoustic noise suppressed eCAP amplitude, especially at high electric and high acoustic stimulus levels.

Regarding effects of noise, eCAPs were suppressed in normal-hearing animals, especially at high current levels and high sound levels. At moderate sound levels, suppression was more pronounced when broadband noise and high-frequency noise were used and low-frequency noise was less effective. At high sound levels, suppression was similar irrespective of noise type. In animals with a high-frequency hearing loss, no dependence on noise type was found. Higher sound levels were needed for broadband noise and high-frequency noise to suppress the eCAP to a similar extent as in normal-hearing animals. Mid-frequency and low-frequency noise was equally effective in normal-hearing animals and those with a high-frequency hearing loss. Effects of noise were largest just after noise onset and decreased when noise duration increased. Mechanisms of eCAP suppression of are given below (section 7.3).

7.2. Animal model

Regarding our animal model for high-frequency hearing loss we concluded it to be an appropriate model, since CAP thresholds (defined as the 10 mV iso-response level) determined at high acoustic frequencies were increased by ~60 dB, while thresholds at low frequencies were nearly normal. Furthermore, the frequency-dependence of hearing loss more or less resembled the "steeply-sloping" audiograms of human EAS candidates. For example, compare the human audiometric criteria (Fig. 1.6) with the iso-response curves of our guinea pigs (Fig. 4.5). The gradual decrease of acoustic sensitivity reflected a sensorineural hearing loss from low to high acoustic frequencies, caused by an increasing loss of hair cells from apex to base. Long-term hearing loss (10 or 12 weeks) resulted

in additional SGC degeneration, mimicking the condition of degenerating nerve fibers in the human cochlea after prolonged sensorineural hearing loss.

During recording, animals were deeply anesthetized. The anesthetic regime during electrophysiological recordings typically consisted of 1 to 2% isoflurane and 67% nitrous oxide. Regarding CAP amplitude, one of the most important response parameter in this thesis, effects of isoflurane up to a concentration of 2% were insignificant. Hence, the anesthetic regime will not have had a large influence on our results regarding the effects of electrical stimulation on CAPs described in later chapters. In addition, recordings with and without electrical stimulation were performed within a short time span of a few minutes. Hence, despite the fact that isoflurane concentrations were animal-dependent and subject to change during experiments, the within-animal comparison design and short time span in which a given CAP ratio was determined will have excluded large time-dependent effects of anesthesia. Other auditory evoked potentials, such as cochlear microphonics (CM) and electrically evoked CAPs (eCAPs) were recorded and analyzed in much the same way. Hence, anesthesia will probably not have interfered with these recordings either. However, isoflurane might have affected the interaction between electrically and acoustically evoked responses. In addition, decreased response amplitudes might have negatively affected the quality of our recordings by reducing signal-to-noise ratios.

7.3. Mechanism behind suppression of combined electro-acoustic responses

CAPs evoked at high acoustic frequencies were suppressed to a great extent by electrical stimulation. CAPs typically recovered from suppression within several milliseconds after the electric stimulus. This fast recovery could have been mediated by refractoriness following direct neural activation by electrical stimulation. However, largest CAP suppression was found at 8 kHz and not at 16 kHz (the highest acoustic frequency tested). Largest CAP suppression was expected at 16 kHz when direct neural mechanisms were responsible for CAP suppression, since the stimulating electrode was placed on the round window and current densities will have decreased from places with a high CF to regions with low CF. A possible explanation for the deviating frequency-dependence of suppression was involvement of electrophonics. Electrophonic responses are electrically evoked responses generated by mechanical events in the cochlea. In contrast to electrically evoked responses evoked by direct neural activation, electrophonics depend on functional hair cells.

Electrophonic responses are thought to excite cochlear regions that tonotopically correspond to the frequency spectrum of the electrical stimulus. With regard to pulse trains, the spectrum is mainly determined by pulse width. The contribution of electrophonics to CAP suppression was therefore determined

by varying the pulse width of the electric stimuli. CAPs were maximally suppressed when the acoustic frequency of the tonal stimulus corresponded to a maximum in the frequency spectrum of the electric stimulus. Conversely, spectral minima resulted in small CAP suppression at the corresponding tonal frequency. These results strongly indicate that electrophonics played an important role in CAP suppression and are in agreement with an earlier study from McAnally et al. (1997). In addition, tuning of suppression of CAPs by electrical stimuli and suppression of electrophonically evoked CAPs (epCAPs) to tonal frequency were very similar. This finding confirms that electrophonic responses were generated mainly at cochlear locations with a CF corresponding to maxima in the frequency spectrum of the electric stimulus. These results agree with the notion that CAPs and eCAPs might have a common cochlear origin, involving cochlear filtering and the generation of a travelling wave (McAnally et al., 1993).

We applied mostly an interval of 1 ms between electric and acoustic stimulus, to mimic the near-simultaneous electrical and acoustical stimulation of the cochlea likely applicable to EAS users. As a consequence, CAP suppression in our experiments could have involved both direct neural and electrophonic mechanisms. In line with these assumptions, not all our results could be explained by electrophonic effects. At long pulse widths a relatively large suppression at high acoustic frequencies was noted that was not readily explained by the frequency spectrum of the electric stimulus. This observation was likely due to a direct neural activation at the base of the cochlea due to increased charge injections at long pulse widths. Nevertheless, a spread of electrophonic excitation could also have contributed to CAP suppression at frequencies not expected on the basis of the electric frequency spectrum. We conclude that electrophonic mechanisms played an important role in CAP suppression when short to moderate pulse widths were applied. Direct neural activation probably became increasingly important at high acoustic frequencies when long pulse widths were applied (i.e. when large charges were injected).

The amplitude of epCAPs depended on the presence of inner hair cells and spiral ganglion cells, but was independent on outer hair cells. These results agree with the notion that electrophonic responses can occur in cochleas devoid of outer hair cells (Moxon, 1971). Possibly accessory structures in the cochlea can act as electro-mechanical transducers (Clark Jones and Stevens, 1940). It seems likely, however, that at lower current levels than those used by us (e.g. around epCAP threshold) OHCs would have played a role in electrophonics by amplifying basilar membrane movements.

Last we investigated effects of hair cells on cochlear potentials evoked by direct stimulation of neural elements (eCAPs). In the absence of acoustic stimuli, largest eCAP amplitudes were observed in animals with hair cell loss and no SGC loss. Increased eCAP amplitudes after hair cell loss can be explained by the desynchronizing effect of hair cells on auditory nerve fiber firing due to the generation of spontaneous activity by functional hair cells (Liberman and

Dodds, 1984; Hu et al., 2003). Decreased eCAP amplitudes after SGC loss can be explained by the dependence of auditory nerve activity on SGCs (Hall, 1990).

Acoustical stimulation suppressed electrically evoked auditory-nerve activity. Suppression of eCAPs was explained by others by assuming that acoustic noise has a desynchronizing effect on hair cells and auditory nerve fibers, since acoustic noise increases hair cell activity in a random manner (Nourski et al., 2007). The observed pronounced eCAP suppression following noise onset can be explained by the fact that hair cells and nerve fibers are activated highly synchronously at the onset of acoustic stimulation. As a consequence, electrical stimulation can activate only a small proportion of auditory nerve fibers due to refractory mechanisms. The following rapid decrease of suppression during noise presentation can be caused by the fact that nerve fibers fire with less synchrony during prolonged acoustic stimulation, leaving more fibers in an excitable state. After noise offset hair cells are in an adapted state due to adaptive processes such as neurotransmitter depletion. As a consequence, hair cells can show reduced spontaneous neurotransmitter release, and spontaneous nerve fiber activity will be transiently lowered. Therefore, nerve fibers will fire with increased synchrony when the cochlea is electrically stimulated. When hair cells recover from adaptation, eCAP amplitudes normalize.

We found that higher levels of acoustic noise were necessary to suppress eCAPs with broadband noise and high-frequency noise in animals with a high-frequency hearing loss compared to normal-hearing animals. This finding can be explained by the loss of basal hair cells, which decreases the effect of the high-frequency component in acoustic stimuli on auditory-nerve activity.

Low-frequency and mid-frequency noise could suppress eCAPs in animals with a high-frequency hearing loss, despite the fact that the stimulating electrode was placed in the high-frequency part of the cochlea. Probably electrically and acoustically stimulated regions in the cochlea overlapped due to the fact that relatively high current levels and sound levels were mostly applied. Increasing current levels result in increasing spread of excitation through the cochlea (Van den Honert and Stypulkowski, 1987), while high sound levels induce a basal-ward spread of excitation to high-frequency regions in the cochlea (Pickles, 2008). Both processes can lead to acoustic stimulation of electrically activated regions in the cochlea, thereby suppressing eCAP amplitude.

7.4. Clinical implications

With respect to the effects of electrical stimulation on acoustically evoked CAPs, a few considerations have to be made before extrapolating our results obtained in the anesthetized guinea pig to the human EAS user. First, we used a short and relatively thin platinum wire electrode. Present-day cochlear implant electrode arrays are both thicker and longer and may therefore affect cochlear mechanics

and alter acoustically evoked responses to a larger extent. Furthermore, we applied biphasic current pulses that alternated in polarity after each stimulus presentation. Pulse trains were short (about 10 ms) sequences of pulses of equal amplitude that also alternated in polarity after each stimulus presentation. In cochlear implants amplitude modulated trains of biphasic current pulses are used that do not alternate. Hence, results of our studies may not completely apply to cochlear implant users. Nevertheless, some of our major findings may have clinical relevance.

7.4.1. Effects of electrical stimulation on acoustically evoked auditory nerve responses

The implied dual mechanism of electrical suppression (refractoriness and electrophonics) of acoustically evoked responses suggests two strategies to minimize electro-acoustic interaction in implant users with residual low-frequency hearing.

First, regarding direct neural effects, the electrode configuration applied might have been important. We used stimulating electrodes in the basal turn of the cochlea that will likely have restricted the area of electrical stimulation to the high-frequency region of the cochlea. If the lack of effects on low-frequency acoustic responses was due to this spatial segregation, these findings would plead for the use of short electrodes in EAS strategies.

Second, with respect to electrophonics, it is of interest that we observed electrically evoked, hair cell-mediated mechanical activation in the absence of hair cells in the vicinity of the stimulating electrode. Hence, spatial segregation of electrical and acoustical stimulation by using short electrode arrays used in hybrid implant systems in patients with residual low-frequency hearing may not necessarily prevent interaction of electrophonic responses with acoustical responses. Short pulse widths are recommended in hybrid implants to shift the spectrum of the pulsatile stimuli toward high frequencies in order to limit interaction with acoustically evoked responses to low frequencies. Pulses of short pulse width (80 ms) could be applied to the basal region of the cochlea with hardly any effect on low-frequency evoked responses, especially so in animals with a high-frequency hearing loss. At long pulse widths of 400 ms CAP suppression was pronounced at low frequencies and this could negatively affect residual hearing in EAS users. Furthermore, high pulse rates (e.g. 1000 pps or more) shift the electric spectrum to high frequencies, which could be favourable for the same reasons.

Irrespective of the underlying mechanisms, CAP suppression increased with current level, suggesting that current levels should ideally be kept relatively low to avoid interaction of electrically and acoustically evoked responses.

Last, CAP amplitudes recovered rapidly to normal values when the interval between electric and acoustic stimulus was increased. Within approximately

5 milliseconds after the pulse train, CAPs had near-normal amplitudes. These findings could find use in EAS strategies.

7.4.2. Effects of acoustical stimulation on electrically evoked auditory nerve responses

Regarding acoustic suppression of electrically evoked responses, we found suppression using low- and mid-frequency noise, despite the basal location of the stimulation electrode near the round window. This location corresponds to a characteristic frequency of about 20 – 30 kHz in the guinea pig cochlea (Greenwood, 1990). Since high-frequency regions in the cochlea of EAS users are stimulated electrically while low-frequency regions are still acoustically sensitive, the electro-acoustic interaction we found might be relevant in this population and acoustical stimulation of residual hearing might negatively affect electrical responses. Likely it is best to avoid loud acoustic stimulation to minimize acoustic suppression of electrically evoked responses in hybrid implants.

Effects of noise were small around eCAP threshold. Given the finding that eCAP thresholds might approximate the maximum comfort level in implant users (Brown et al., 1998), suppressive effects of acoustic noise on electrically evoked responses may be small in EAS users. In addition, suppression of electrically evoked gross potentials such as eCAPs might actually be a reflection of mechanisms beneficial to hearing, as explained below. Desynchronization of firing of auditory nerve fibers in response to electrical stimulation decreases eCAP amplitudes. A decrease of the eCAP amplitude might reflect reduced electrically evoked activity in the auditory nerve. Additionally, it might be a reflection of a more desynchronous activation of individual auditory nerve fibers underlying the eCAP response of the auditory nerve. A certain amount of desynchronization of auditory nerve fiber activity is believed to actually increase hearing function (Hong and Rubinstein, 2006). In CI users for example, it has been demonstrated that modest levels of acoustic or electric noise lowers sound detection thresholds, and increases frequency discrimination and envelope detection (Chatterjee and Robert, 2001; Zeng, 2002). Hence, eCAP suppression might actually reflect a more accurate coding of electrically evoked activity in the auditory nerve. High noise levels can nevertheless be disruptive to hearing function, by masking electrically evoked auditory-nerve responses.

We found evidence that eCAP recordings in some animals with residual low-frequency hearing using a forward masking paradigm resulted in responses contaminated by electrophonic responses evoked by the masker pulse. Such a response might not be representative to the actual cochlear sensitivity to electrical stimulation. Forward masking techniques are also applied in implant systems to record electrically evoked auditory nerve activity. Given the present results, eCAP recordings in hybrid implants users with residual low-frequency hearing might be confounded by hair-cell mediated responses to the masker pulse. We

found no signs of contamination of the early peaks of eABRs (< 1 ms) using the same forward masking paradigm as used for eCAP recordings. Apparently epCAPs are represented to a lesser extent in eABRs and might therefore be a useful alternative for eCAPs to record electrically evoked auditory-nerve activity in EAS users. Since acoustic noise prevented contamination of the eCAP, noise could be used to eliminate any electrophonic responses during eCAP recordings.

The temporal characteristics of eCAP suppression and recovery might be relevant for EAS strategies. Suppression of eCAPs declined rapidly during the first few milliseconds of noise presentation which might be adopted in hybrid implant stimulation strategies. Following noise offset a transient increase in eCAP amplitude was observed, after which amplitudes normalized. The transient increase and normalization of eCAP amplitudes occurred within several milliseconds, which might be of relevance as well.

7.4.3. Isoflurane and nitrous oxide anesthesia

Some remarks can be made on isoflurane anesthesia, although not directly related to the central issue of this thesis. Anesthesia with isoflurane and nitrous oxide suppressed the amplitude and increased the latency of CAPs and ABRs. These findings might be of interest, since isoflurane is used as a general anesthetic during surgical procedures, including head and neck surgery (Crawford et al., 2009). During neuro-otologic surgery such as tumour resection, surgical trauma to the auditory nerve and auditory brainstem must be minimized. CAP and ABR can be recorded intraoperatively to assess the integrity of the auditory pathway (Newman and Sandridge, 2007). Surgical trauma or metabolic abnormalities affecting the auditory nerve or the brainstem result in increased latencies and reduced amplitudes of CAP or ABR waveforms. Early detection of altered CAP and ABR waveforms can therefore provide the opportunity for immediate corrective intervention (Kileny et al., 1988). We showed that isoflurane affects the CAP and ABR in a way resembling the effects of surgical trauma or abnormal metabolism of the auditory nerve or brainstem. This has to be taken into account when CAPs or ABRs are monitored intra-operatively, since adapting the concentration of isoflurane might affect CAP or ABR amplitude and latency.

7.4.4. Concluding remarks concerning EAS strategies

In conclusion, low current levels and short pulse widths are advisable in EAS strategies to preserve acoustical responses. Indirect evidence suggests that high pulse rates and short electrodes may be best to minimize interaction of electrical stimulation on acoustically evoked cochlear responses. Acoustic responses recover within milliseconds after electrical stimulation which might find use in future EAS strategies. Regarding electrically evoked responses, loud acoustic stimuli suppress electrically evoked auditory nerve activity, which is probably

best to avoid in hybrid implants. Nevertheless, (desynchronizing) effects of low-level acoustic noise may actually be beneficial to electric hearing. The findings that suppression of electrical auditory nerve responses decreased rapidly within a few milliseconds during noise presentation might be useful in future EAS strategy design. Last, the transient increase of the eCAP amplitude after noise offset might be important for EAS strategies.

Abbreviations

ABR	auditory brainstem response
CAP	compound action potential (acoustically evoked)
CF	characteristic frequency
CI	cochlear implant
eABR	electrically evoked ABR
EAI	electric-to-acoustic interval
eCAP	electrically evoked compound action potential
epCAP	electrophonically evoked compound action potential
IHC	inner hair cell
OHC	outer hair cell
SGC	spiral ganglion cell

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Nederlandstalige samenvatting

Ernstig tot zeer ernstig perceptief gehoorverlies wordt veroorzaakt door sensorineurale schade in het binnenoor (de "cochlea"). In de meeste gevallen bestaat deze schade uit het verlies van zintuigcellen (de "haarcellen") in de cochlea. Doordat de haarcellen niet meer functioneren heeft een normaal hoortoestel niet meer het beoogde effect. Mensen met een zeer ernstig sensorineuraal gehoorverlies worden daarom bij voorkeur behandeld door middel van cochleaire implantatie. Een cochleair implantaat, of kortweg CI, omzeilt de beschadigde haarcellen door direct de gehoorzenuw te stimuleren met behulp van elektrische stroompulsen.

De eerste klinisch toegepaste cochleaire implantaten werden in 1984 geplaatst in het House Ear Institute. Deze implantaten bestonden uit een enkele elektrode en dienden vooral als hulpmiddel bij liplezen en om de gebruiker op de aanwezigheid van geluid te attenderen. Sindsdien zijn CI technologieën aanzienlijk verbeterd. Zo zijn bijvoorbeeld het aantal elektrodes in de huidige implantaten uitgebreid. Door deze ontwikkelingen zijn moderne implantaten in staat om het spraakverstaan aanzienlijk te verbeteren en sommige CI gebruikers zijn zelfs in staat gesprekken te voeren over de telefoon. Deze bemoedigende resultaten hebben ertoe geleid dat de indicatie criteria voor implantatie zijn versoepeld.

Door de versoepeling van de audiometrische criteria komt een relatief grote groep van ernstig slechthorenden met een "steil" verloop van gehoorverlies nu in aanmerking voor een CI. Het gehoorverlies in deze groep kenmerkt zich door een groot verlies in het hoogfrequente gebied. Gehoordrempels van laagfrequente geluiden daarentegen, zijn veel minder, of zelfs in het geheel niet aangedaan. Het verlies van de hoge tonen kan echter zo ernstig zijn dat het spraakverstaan in deze groep mensen ernstig beperkt is, ondanks het gebruik van een hoortoestel. Deze mensen kunnen geholpen worden door het hoogfrequente deel elektrisch te stimuleren, terwijl het laagfrequente deel met (of zelfs zonder) een hoortoestel akoestisch gestimuleerd kan worden.

Het is gebleken dat gebruik van dit laagfrequente restgehoor voordelig is voor het spraakverstaan met een CI. Dit geldt in het bijzonder voor situaties die bekend staan als problematisch voor slechthorenden, zoals spraakverstaan in omgevingen met achtergrond ruis (bijvoorbeeld tijdens feestjes, of in het restaurant). Door de gebleken voordelen van gecombineerde elektro-akoestische stimulatie (EAS) zijn recentelijk zogenaamde hybride implantaten ontwikkeld, waarin een CI gecombineerd is met een conventioneel hoortoestel in een enkel apparaat.

Gezien het belang van restgehoor voor spraakverstaan met een cochleair implantaat is het belangrijk inzicht te verkrijgen in de wisselwerking tussen elektrisch en akoestisch opgewekte responsies in de cochlea. Dit proefschrift beschrijft een viertal onderzoeken die deze interacties beschrijven. De belangrijkste onderzoeksvragen zijn wat de gevolgen zijn van elektrische stimulatie op akoestisch opgewekte responsies in de auditieve zenuw en wat de gevolgen zijn van akoestische stimulatie op elektrisch opgewekte responsies in deze zenuw. Om deze vragen te beantwoorden hebben wij elektrofysiologische experimenten opgezet waarbij de potentialen in de cochlea van de cavia gemeten zijn. Vooral de akoestisch opgewekte samengestelde actiepotentiaal (in het Engels compound action potential, of kortweg CAP) en elektrisch opgewekte samengestelde actiepotentiaal (kortweg eCAP) waren hierbij van belang. De CAP en eCAP representeren synchrone responsies van vele zenuwvezels. In de beschreven experimenten hebben wij de responsies opgewekt met elektro-akoestische stimulatie vergeleken met responsies opgewekt door alleen akoestische stimulatie of elektrische stimulatie.

Hoofdstuk 2 start met de evaluatie van het gebruikte diermodel. Doordat het noodzakelijk was de cochlea vrij te prepareren, werden alle metingen beschreven in dit proefschrift uitgevoerd in cavia's onder anesthesie. De anesthesie bestond uit de inhalatievloeistof isofluraan verdampt in een mengsel van lachgas en zuurstof. De effecten van isofluraan en lachgas op CAPs zijn nog niet eerder beschreven.

Isofluraan bleek CAP amplitudes te verkleinen en CAP drempels te verhogen. Effecten waren het grootst wanneer CAPs opgewekt werden met hoogfrequente tonen. Effecten van isofluraan bleken afhankelijk van de dosis en waren doorgaans statistisch significant als concentraties van 2.5% of meer gebruikt werden. Ondanks deze effecten zijn onze metingen waarschijnlijk weinig beïnvloed. Een meting onder bepaalde EAS condities en bijbehorende controle metingen werden namelijk uitgevoerd in hetzelfde dier en met tijdsverschillen die doorgaans niet meer dan enkele minuten bedroegen. Daarom waren de effecten van isofluraan tussen de verschillende condities nagenoeg identiek. Niettemin kan isofluraan de wisselwerking tussen elektrische en akoestisch opgewekte responsies wel beïnvloed hebben. Daarnaast is de signaal-tot-ruis verhouding mogelijk verminderd door het gebruik van isofluraan.

Hoofdstuk 3 beschrijft de effecten van elektrische stimulatie op akoestisch opgewekte CAPs in normaalhorende cavia's. De stimulatie elektrodes werden op het hoogfrequente deel van de cochlea aangebracht. Elektrodes werden op de buitenkant van de cochlea aangebracht om zo schade aan het binnenoor tot een minimum te beperken. De aangeboden elektrische stimuli bestonden uit korte pulstreinen en waren gelijksoortig aan stimuli die worden toegepast in een CI. CAPs werden opgewekt met korte toonstoten die meestal kort na de elektrische stimulus aangeboden werden. Dit is een vorm van een voorwaarts maskeringsparadigma. De data van deze experimenten beschrijven de effecten van elektrische stimulatie

op akoestisch opgewekte CAPs in gezonde, onbehandelde cochlea's.
CAP amplitudes bleken kleiner wanneer elektrische stimuli werden aangeboden. Deze suppressie was gering wanneer CAPs opgewekt werden met laagfrequente tonen en werd sterker naarmate de toonfrequentie hoger werd. CAP suppressie werd ook sterker als de stroomsterkte van de elektrische pulstrein verhoogd werd. Bij hoge stroomsterktes werden laagfrequent (0.5 of 1 kHz) opgewekte CAPs ook onderdrukt. Suppressie nam af met geluidsniveau en ook wanneer het interval tussen elektrische pulstrein en toonstimulus vergroot werd. De onderliggende mechanismen achter CAP suppressie worden nader onderzocht in hoofdstuk 5. Concluderend vonden wij dat het hoogfrequente deel van de cochlea elektrisch gestimuleerd kan worden, zonder dat dit nadelige effecten hoeft te hebben op CAPs opgewekt op lage frequenties.

In hoofdstuk 4 wordt het vervolgonderzoek beschreven naar de effecten van elektrische stimulatie op akoestisch opgewekte CAPs. In deze studie is getracht om de omstandigheden in EAS gebruikers zoveel mogelijk te benaderen. Daartoe hebben we een diermodel ontwikkeld voor hoogfrequent gehoorverlies door cavia's te behandelen met de twee ototoxische middelen kanamycine en furosemide. De dosis kanamycine was relatief laag, wat resulteerde in hoogfrequent gehoorverlies (CAP drempels waren verhoogd) met beperkte verliezen op lage frequenties. Histologisch bleek dat er haarcelverlies was in het hoogfrequente deel van de cochlea en praktisch niet in het laagfrequente deel. Deze dieren werden na 2 of 10 weken gemeten. Na 2 weken was er geen degeneratie van de gehoorzenuw waarneembaar, terwijl na 10 weken er een duidelijke afname was van spirale ganglioncellen in de cochlea. Spirale ganglioncellen zijn de zenuwcellen van de gehoorzenuw en zijn verantwoordelijk voor de signaaloverdracht van de haarcellen naar de hersenen. Bij langdurig verlies van de haarcellen gaan de spirale ganglioncellen eveneens te gronde. In tegenstelling tot hoofdstuk 3 zijn de elektrische stimuli in deze studie aangeboden met behulp van een intracochleaire elektrode in het hoogfrequente (basale) deel van de cochlea, om zodoende een CI elektrode na te bootsen. Met dit diermodel voor hoogfrequent gehoorverlies hebben wij de effecten van elektrische stimulatie op CAPs onderzocht wanneer deze opgewekt werden met laagfrequente tonen.

Eerst hebben we bepaald wat het effect was van intracochleaire elektrische stimulatie in normaalhorende dieren. De resultaten waren grotendeels gelijk aan de resultaten van de experimenten waarbij extracochleair gestimuleerd werd. Zo waren effecten op de CAP klein als deze opgewekt werden op lage frequentie, terwijl CAPs sterk onderdrukt werden op hoge frequenties en vooral op lage geluidsniveaus. Er werden echter ook verschillen gevonden. Het voornaamste verschil was dat er een duidelijkere afhankelijkheid bestond tussen geluidsniveau en de effecten op de CAP amplitude op lage frequenties wanneer intracochleair gestimuleerd werd. De afname van de CAP amplitude was het grootst op hoge geluidsniveaus, terwijl amplitudes op lage niveaus enigszins vergroot waren. De suppressie van de CAP op lage frequenties kan verklaard worden doordat het

hoogfrequente aandeel in de zenuwrespons onderdrukt werd. Het is namelijk bekend dat bij hoger wordende geluidsniveaus, tonale stimuli steeds grotere gebieden in de cochlea stimuleren en deze spreiding van excitatie vindt vooral plaats richting de hoogfrequente gebieden. Omdat hoogfrequent opgewekte CAPs sterk werden onderdrukt, zullen CAPs opgewekt op lage frequenties en hoge geluidsniveaus eveneens onderdrukt zijn geweest. De geringe vergroting van de CAP op lage frequenties en laag geluidsniveau werd ook niet waargenomen met extracochleaire stimulatie. Deze vergroting van de CAP kan mogelijk te maken hebben gehad met mechanismen waarbij het centrale zenuwstelsel betrokken is.

Dieren met een laagfrequent gehoorverlies vertoonden geen onderdrukking van de CAP op lage frequenties. Dit was verklaarbaar op grond van de afwezigheid van hoogfrequent gehoor. Hierdoor viel het hoogfrequente aandeel van de CAPs opgewekt op hoge geluidsniveaus weg en kon dus ook niet meer onderdrukt worden door elektrische stimulatie.

In hoofdstuk 5 wordt getracht het onderliggende mechanisme achter de onderdrukking van akoestisch opgewekte CAPs door elektrische stimulatie te ontrafelen. Het is namelijk bekend dat elektrische stimuli de auditieve zenuw op twee manieren kunnen prikkelen. Ten eerste kunnen elektrische stimuli de gehoorzenuw prikkelen door een directe wisselwerking van elektrische stroom op de gehoorzenuwcellen. Daarnaast kunnen elektrische stimuli ook zogeheten elektrofone responsies opwekken in de gehoorzenuw. Elektrofone activiteit wordt opgewekt doordat elektrische stimuli mechanische trillingen veroorzaken in de cochlea die vervolgens de haarcellen kunnen stimuleren zoals normale akoestische stimuli dat ook kunnen doen. Er zijn aanwijzingen dat de frequentiesamenstelling van elektrische stimuli bepalend zijn voor de gebieden die in de cochlea gestimuleerd worden via elektrofone processen. In dit hoofdstuk wordt getracht elektrofone responsies van directe neurale effecten te onderscheiden door de frequentiesamenstelling van de elektrische stimuli te wijzigen.

Wat betreft elektrofonie vonden we sterke aanwijzingen dat elektrofonie een rol speelde in CAP suppressie. Wanneer de frequentie-inhoud van de elektrische stimulus gewijzigd werd door een verandering van de pulsbreedte, veranderde de frequentieafhankelijkheid van CAP suppressie ook. Deze verandering kon verklaard worden door de verandering in het frequentiespectrum van de elektrische stimulus. Bij korte pulsen werden CAPs namelijk vooral onderdrukt wanneer deze opgewekt werden met hoogfrequente tonen, terwijl bij brede pulsbreedtes de CAP suppressie op hoge tonen afnam ondanks een hogere ladingsinjectie. Echter, bij zeer lange pulsen steeg de suppressie van CAPs opgewekt op hoge frequenties. Deze bevinding hebben wij verklaard door een toegenomen CAP onderdrukking door directe elektrische activering van neurale elementen in de cochlea bij zeer hoge ladingsinjecties. Deze resultaten tezamen laten zien dat zowel directe neurale activering als elektrofone responsies een rol gespeeld hebben in de waargenomen CAP suppressie.

Hoofdstuk 6 beschrijft de resultaten van experimenten waarin de effecten

van akoestische ruis op elektrisch opgewekte zenuwresponsies gemeten werden. Voor deze experimenten werd ook gebruik gemaakt van het caviamodel voor hoogfrequent gehoorverlies. Allereerst wordt de relatie gelegd tussen eCAP amplitude en histologische tellingen van haarcellen en spirale ganglioncellen in afwezigheid van akoestische ruis. Daarna worden de effecten beschreven van akoestische ruis op de eCAP amplitude en gerelateerd aan de mate van gehoorverlies in de dieren.

Verlies van haarcellen bleek de eCAP amplitude te vergroten, terwijl verlies van spirale ganglioncellen de eCAP amplitude juist verkleinde. De afwezigheid van haarcellen verhoogde waarschijnlijk de synchroniciteit van elektrisch opgewekte vuringen in gehoorzenuwcellen, wat een vergroting van de eCAP amplitude kan verklaren. Haarcellen genereren namelijk een spontaan, asynchroon vuurgedrag in auditieve zenuwvezels. Verlies van spirale ganglioncellen resulteert in een degeneratie van de auditieve zenuw en leidt daardoor tot afname van de eCAP amplitude.

Vervolgens werd het effect getest van akoestische ruis op de eCAP amplitude. Verschillende typen ruis werden toegepast, waarbij breedbandige ruis gefilterd werd met verschillende filters, namelijk een hoogdoorlaatfilter (> 5.7 kHz), een bandfilter (1.4 – 5.7 kHz) en een laagdoorlaatfilter (< 1.4 kHz). Over het algemeen waren alle typen ruis in staat de eCAP amplitude te onderdrukken in normaalhorende dieren. Onderdrukking van eCAPs was het sterkst bij hoge stroomsterktes en hoge geluidsniveaus.

In dieren met een hoogfrequent gehoorverlies had akoestische stimulatie minder effect op de eCAP amplitude in het geval van breedbandige en hoogfrequente ruis, dan in normaalhorende dieren. Intermediair frequente en laagfrequente ruis waren even effectief in normaalhorende cavia's en in dieren met hoogfrequent gehoorverlies. Deze resultaten zijn te verklaren door het hoogfrequente gehoorverlies. De bevinding dat laagfrequente ruis eCAPs kon onderdrukken in dieren met een hoogfrequent gehoorverlies wijst erop dat elektrisch en akoestisch gestimuleerde gebieden overlapten, ondanks dat de stimulatie elektrode in het hoogfrequente gebied van de cochlea geplaatst was. Dit is waarschijnlijk veroorzaakt doordat in de meeste experimenten relatief hoge stroomsterktes en hoge geluidsniveaus toegepast werden. Hoge stroomsterktes activeren grote delen van de cochlea en hoge geluidsniveaus leiden tot een verspreiding van akoestische activering naar hoogfrequente gebieden in de cochlea. Hierdoor zijn naar alle waarschijnlijkheid elektrisch gestimuleerde gebieden ook akoestische gestimuleerd, wat heeft geleid tot eCAP onderdrukking.

Ruis was vooral effectief in het onderdrukken van de eCAP wanneer deze opgewekt werd net na aanvang van de ruispresentatie. De eCAP suppressie daalde naarmate de eCAP later opgewekt werd in de ruisstimulus. Amplitudes van eCAPs opgewekt net na stopzetten van de ruis waren kortstondig vergroot. Deze temporele aspecten kunnen verklaard worden doordat bij aanvang van de ruis alle zenuwvezels synchroon geactiveerd worden, wat tot gevolg heeft dat

elektrische stimuli weinig additionele vezels kunnen activeren (eCAP suppressie). Naarmate de ruis langer gepresenteerd wordt daalt de synchroniciteit van akoestische activatie, waardoor de eCAP groter wordt. Net na stopzetten zijn de haarcellen uitgeput, wat leidt tot een tijdelijk verlaagde spontane activering van zenuwcellen door de haarcellen. Hierdoor kunnen elektrische stimuli tijdelijk responsies veroorzaken met een grote mate van synchroniciteit, resulterend in verhoogde eCAP amplitudes.

Hoofdstuk 7 bevat een samenvatting en algemene discussie van de resultaten en werkt toe naar de klinische betekenis van dit onderzoek. Wat betreft de klinische toepasbaarheid van onze resultaten moet met een aantal elementen in onze experimentele aanpak rekening worden gehouden, afgezien van het feit dat de metingen werden uitgevoerd in cavia's onder anesthesie en niet in humane EAS gebruikers. Allereerst werd ten behoeve van elektrische stimulatie gebruik gemaakt van een dun platina draadje in de basis van de cochlea. Huidige cochleaire implantaten zijn dikker en langer, wat de cochleaire mechanica waarschijnlijk anders beïnvloed dan de door ons gebruikte draadelektrode. Daarnaast hebben wij elektrische stimuli gebruikt die lijken op die van een CI, maar ze verschilden in het feit dat ze een constante amplitude hadden gedurende een meting. Daarbij werden stimuli in polariteit gealterneerd tijdens het meetproces om elektrische stimulusartefacten te onderdrukken. In CI's worden echter amplitude gemoduleerde pulstreinen toegepast die niet alterneren. Niettemin hebben een zijn een aantal van onze belangrijkste bevindingen klinische relevant.

Ten eerste hebben we aanwijzingen gevonden dat akoestisch opgewekte CAPs onderdrukt konden worden door zowel responsies opgewekt door direct elektrische neurale activering, als door elektrofone mechanismen. Middels deze bevinding kunnen we twee mogelijke strategieën aandragen om deze wisselwerking te minimaliseren. Ten aanzien van directe neurale activering was mogelijk de elektrode configuratie van belang; de stimulatie elektrodes waren in het hoogfrequente deel van de cochlea geplaatst, terwijl het restgehoor in het laagfrequente deel aanwezig was. Deze bevinding wijst op minimale wisselwerking tussen elektrische en akoestische responsies wanneer gebruik gemaakt zou worden van korte elektrodes. Korte implantaten speciaal ontwikkeld voor hybride CI's zijn al in gebruik. Ten aanzien van elektrofonie concluderen wij dat het gebruik van korte pulsen wellicht beter is dan brede, doordat deze nauwelijks haarcellen stimuleren in het laagfrequente gebied. Korte pulsen met een duur van 80 ms konden in het hoogfrequente gebied van de cochlea aangeboden worden met weinig effect op laagfrequent opgewekte CAPs. Dit gold in het bijzonder voor dieren met een hoogfrequent gehoorverlies. Lange pulsen van 400 ms hadden daarentegen ook een onderdrukkende werking op laagfrequent opgewekte responsies. Gezien de bevinding dat het frequentiespectrum van belang is ten aanzien van CAP suppressie, zijn hoge pulsrepetities (van bijvoorbeeld 1000 pulsen per seconde of meer) waarschijnlijk aanbevelenswaardig, doordat deze stimuli weinig energie hebben in het laagfrequente gebied.

Ongeacht het onderliggende mechanisme verminderde de suppressie van CAPs als lagere stroomsterktes gebruik werden. Derhalve zijn lage stroomsterktes aanbevelenswaardig.

Als laatste hebben we gevonden dat CAP suppressie zeer snel verminderde gedurende de eerste paar milliseconden nadat de elektrische stimulatie was afgelopen. Mogelijk kan hiervan gebruik gemaakt worden in EAS strategieën.

Ten aanzien van de akoestische suppressie van elektrisch opgewekte responsies vonden we een onderdrukking van de eCAP amplitude door laagfrequente ruis in cochlea's met haarcelbeschadiging in het hoogfrequente gebied. Deze suppressie trad op ondanks dat de stimulatie-elektrode in het hoogfrequente deel van de cochlea geplaatst was. Deze situatie kan ook optreden in EAS gebruikers, doordat in deze gevallen ook het hoogfrequente deel van de cochlea elektrisch gestimuleerd wordt, terwijl het laagfrequente deel nog gevoelig is voor akoestische stimulatie. Derhalve kunnen responsies op elektrische stimuli mogelijk nadelig beïnvloed worden door restgehoor.

Effecten van ruis waren echter klein rond de eCAP drempel en namen toe met stroomsterkte. Mogelijk zijn de hoge bovendrempelige stroomsterktes niet representatief voor de gemiddelde EAS gebruiker. Daarnaast kan een verkleining van elektrisch opgewekte samengestelde potentialen zelfs gunstige effecten op het gehoor weerspiegelen. Samengestelde potentialen zoals wij die gemeten hebben representeren namelijk een synchrone activering van vele zenuwvezels door elektrische stimulatie. Zoals boven reeds vermeld kan haarcelactivering de synchroniciteit verlagen van de auditieve zenuw. Er wordt verondersteld dat een zekere mate van desynchronisatie gunstig kan zijn op de informatieverwerking in de auditieve zenuw. Derhalve kan ruis aangeboden op een laag geluidsniveau zelfs voordelig werken op het spraakverstaan van CI gebruikers.

Wat betreft de temporele aspecten van eCAP suppressie door simultaan aangeboden ruis vonden we dat de suppressie het sterkst was direct na aanvang van de ruisstimulus, waarna de suppressie snel afnam. Net na het beëindigen van de ruis presentatie waren amplitudes kortdurend groter, waarna binnen enkele milliseconden de eCAP amplitudes normaliseerden. Deze temporele aspecten kunnen van belang zijn voor toekomstige stimulatie paradigma's in hybride implantaten.

Als laatste kunnen aan de hand van onze bevindingen met isofluraan anesthesie een aantal conclusies getrokken worden, hoewel deze los staan van het centrale thema van EAS. Anesthesie met isofluraan en lachgas onderdrukte de amplitude en verlengde de latentie van CAPs en ABRs. Deze bevinding kan relevant zijn, omdat isofluraan ook in de mens gebruikt wordt als narcosemiddel voor algehele anesthesie. Gedurende neuro-otologische ingrepen zoals het verwijderen van tumoren in het gebied van de auditieve zenuw en de hersenstam moet eventuele schade aan deze structuren geminimaliseerd worden. Methodes om de functionaliteit van het auditieve systeem in de gaten te houden gedurende dit soort riskante operaties zijn CAP en ABR metingen. Schade aan de auditieve

zenuw of hersenstam uit zich veelal door verlenging van de pieklatentie en verkleining van piekamplitudes. Op deze manier kan schade aan het auditieve systeem bijtijds geregistreerd worden en kunnen maatregelen getroffen worden om verdere schade te beperken. Isofluraan bleek in onze experimenten de latentie van de CAP en de ABR te verlengen en de amplitude van beiden te verkleinen. Dit betekent dat dit ook zou kunnen gebeuren gedurende operaties in mensen. Het is daarom nuttig om hierop bedacht te zijn wanneer CAP en ABR metingen beoordeeld worden in de operatiekamer.

Concluderend adviseren wij lage stroomsterktes en korte pulsbreedtes voor toepassing in EAS strategieën om cochleaire responsies op laagfrequente akoestische stimuli minimaal te beïnvloeden. Indirect bewijs wijst erop dat hoge pulsrepetities en korte elektrodes mogelijk ook bijdragen aan een minimalisering van de wisselwerking tussen elektrische stimulering en akoestisch opgewekte responsies. Het gegeven dat akoestische responsies binnen enkele milliseconden herstellen na de presentatie van elektrische stimuli, kan mogelijk toepassing vinden in EAS strategieën. Wat betreft akoestische onderdrukking van elektrische stimuli is het aanbevelenswaardig het aanbod van luide akoestische stimuli te voorkomen in hybride implantaten. De bevindingen dat suppressie van elektrisch opgewekte zenuwresponsies gedurende ruisstimuli snel afneemt en dat de eCAP tijdelijk vergroot is na een akoestische stimulus kunnen relevant zijn voor toekomstige EAS strategieën.

Gebruikte afkortingen

CAP	akoestich opgewekte samengestelde actiepotentiaal
CI	cochleair implantaat
EAS	gecombineerde elektrisch en akoestische stimulatie
eCAP	elektrisch opgewekte samengestelde actiepotentiaal

Dankwoord

De laatste regels van mijn proefschrift! Deze wil ik wijden aan de mensen die het onderzoek in dit proefschrift mogelijk gemaakt hebben. Allereerst wil ik professor Grolman bedanken voor het optreden als promotor. Het is lang onzeker geweest wie mijn promotor zou worden en het was een grote geruststelling dat u deze taak op u wilde nemen. Ondanks alle drukte tijdens de eerste maanden van uw hoogleraarschap hebt u de tijd genomen om als promotor op te treden en hebt u zelfs mijn contract met drie maanden verlengd. Professor Grolman, ik wil u daarvoor hartelijk bedanken.

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Curriculum Vitae

Christiaan Stronks werd op 10 januari 1977 geboren in Oranjestad, Aruba en groeide op in Aalten in Nederland. Na afronding van het Middelbaar en Hoger Algemeen Voortgezet Onderwijs (1989 - 1996) op het Christelijk College Schaersvoorde begon hij aan een studie Biochemie aan het Hoger Laboratorium Onderwijs (1996 – 2000) aan de Hoge School van Arnhem en Nijmegen. Als afstudeerstage deed hij onderzoek naar H^+,K^+-ATPase en Na^+,K^+-ATPase chimaera op de afdeling Biochemie aan de Radboud Universiteit Nijmegen. Na een jaar werkzaam te zijn geweest als analist, waarbij hij eiwit-eiwit interacties onderzocht van het Aquaporine-2 op de afdeling Celfysiologie aan de Radboud Universiteit Nijmegen (2000 – 2001), begon hij aan de studie Biologie aan de Radboud Universiteit Nijmegen (2001 – 2005). Als eerste stage deed hij onderzoek naar de regulatie van de elektrische membraanactiviteit door de calcium-sensing receptor in melanotrope cellen op de afdeling Dierfysiologie aan de Radboud Universiteit. Als afstudeerstage onderzocht hij de effecten van GABAerge stoffen op het elektro-encefalogram in absence epileptische ratten op de afdeling Biologische Psychologie aan de Radboud Universiteit. Aansluitend werd een promotieonderzoek gestart op de afdeling Keel-, Neus- en Oorheelkunde van het Universitair Medisch Centrum Utrecht (2005 – 2010), waarvan de resultaten in dit proefschrift beschreven zijn. Thans is hij werkzaam als postdoctoraal onderzoeker op de afdeling Ophthalmology van de Johns Hopkins universiteit in Baltimore in Maryland, alwaar hij participeert in een onderzoek naar elektrisch opgewekte responsies in de retina van mensen met een retina implantaat.

Curriculum Vitae

Christiaan Stronks was born in Oranjestad, Aruba, and raised in Aalten, the Netherlands. After finishing secondary education at the Christelijk College Schaersvoorde in Aalten and Graafschap College in Doetinchem (1989 – 1996), he obtained his bachelor in Biochemistry at the college for professional education (1996 – 2000). A student-internship was completed on chimera of the H^+,K^+-ATPase en Na^+,K^+-ATPase at the department of Biochemistry at the Radboud University Nijmegen. After working for a year as a technician on a project on protein-protein interactions of Aquaporin-2 at the department of Cell Physiology at the Radboud University Nijmegen (2000 – 2001) he obtained his masters in Biology at the Radboud University of Nijmegen (2001 – 2005). The first student internship concerned a study on the regulation of the membrane activity of melanotrope cells by the calcium-sensing receptor at the department of Animal Physiology at the Radboud University. The final internship was completed at the department of Biological Psychology at the Radboud University for which the effects of GABAergic drugs on the electroencephalogram were investigated in a rat model for absence epilepsy. He started as a Ph.D. student at the department of Otorhinolaryngology at the University Medical Center Utrecht (2005 – 2010). The results of this study are described in this thesis. At present he is a post-doctoral researcher at the department of Ophthalmology at the Johns Hopkins University in Baltimore (Maryland), where he is involved in a research project on electrically evoked responses in retinal implant users.

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