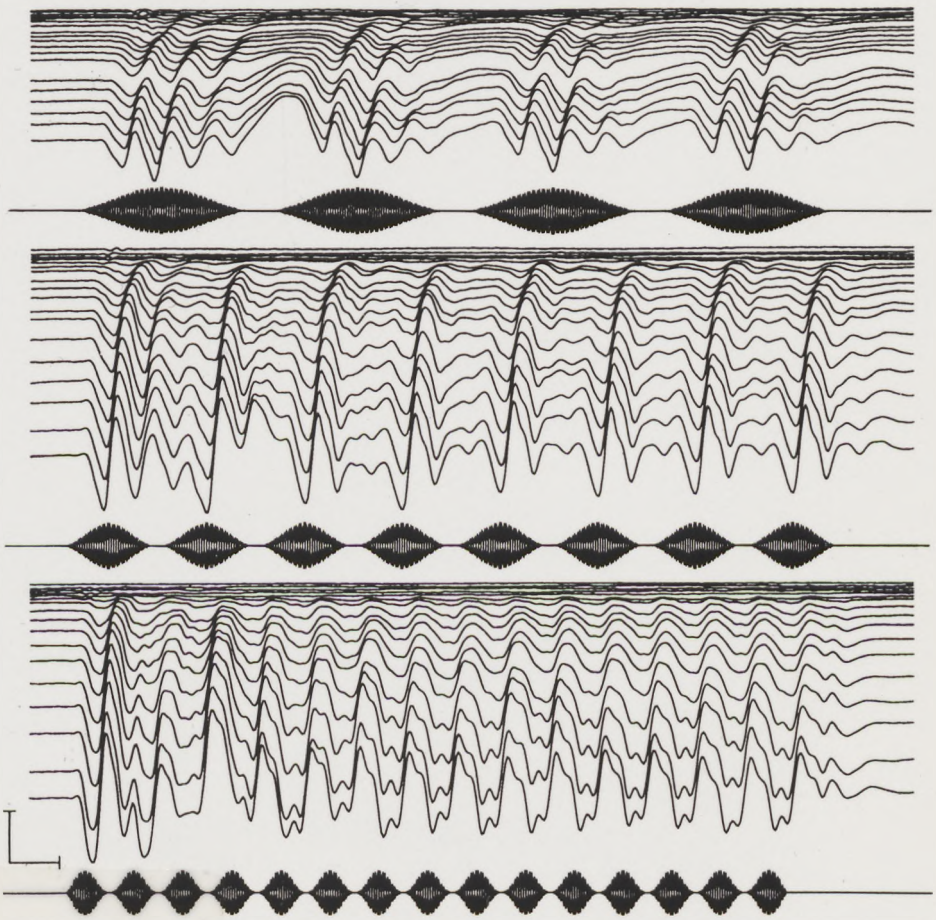
EXCITABILITY OF THE ELECTRICALLY STIMULATED AUDITORY NERVE



M. J. P. Killian

Excitability of the Electrically Stimulated Auditory Nerve

by

Matthijs Killian

cover plot: auditory nerve responses to amplitude modulated 10 kHz electrical signals. horizontal bar: 1 ms; vertical bar: 100 μV

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Excitability of the Electrically Stimulated Auditory Nerve

Exciteerbaarheid van de Elektrisch Gestimuleerde Gehoorzenuw

(met een samenvatting in het Nederlands)

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Promotor: Prof. dr. G.F. Smoorenburg
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De natuur kan men niet bedwingen,

tenzij door zich aan haar wetten te onderwerpen.

Aan mijn ouders,

mijn grootouders

en Matthea

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CONTENTS

CHAPTER 1

Introduction: An overview of psychophysical and physiological literature related to cochlear implants

This thesis presents an electrophysiological study of changes in the excitability of the auditory nerve which are found after sinusoidal electrical stimulation. The aim of this study was to discover to what extent mechanisms at the level of the auditory nerve are involved in the processing of temporal information in cochlear implant users.

In the first section of this introduction part of the history of the development of cochlear implants is described. Psychophysical and electrophysiological literature with emphasis on threshold, dynamic range, pitch perception and temporal information processing in cochlear implant users is presented in sections II and III. Section IV presents an outline of this thesis.

1.1. Historical background

Volta (1800) first gave hope that someday new ears might be given to the deaf. He positioned two metallic rods with rounded tips deeply in his ears and connected them to an apparatus which he called *organe électrique artificiel*, because of its resemblance to the natural electrical organ of the electric ray. When this apparatus was connected to the electrodes he felt a shock in his head, and a moment later he perceived a sound or noise that he found difficult to define. It rather sounded like a kind of crackling or gurgling, like a continuously bubbling paste or material. This noise continued unabated each time the circuit was connected or disconnected. Volta did not repeat these experiments because he thought that the unpleasant sensations in his head indicated that these experiments were dangerous.

Only much later, the first reports appeared dealing with true direct stimulation of the auditory nerve in humans (Andreef *et al.*, 1934; Clark Jones *et al.*, 1940). In 1957, Djourno and Eyries, reported about what seems to be the first 'cochlear implant'. They used a single copper wire inserted into the cochlea of a 50-year-old man who was totally deaf. Later, a second implantation was performed on a girl suffering from total hearing loss after streptomycin therapy. This time they placed the electrode in the round window niche against the round window membrane. Long-term studies showed that neither subject developed open speech discrimination, but the implants were still functioning 4-5 years later (Zollner and Keidel, 1963), while both subjects claimed that the devices helped them greatly with lipreading. The latter report stimulated two groups in the USA to further pursue the possibilities of cochlear implantation: Blair Simmons and his group in San Francisco, and William House and his group in Los Angeles. In 1961, House implanted two subjects with a single gold electrode and tested

them for several weeks. Later in 1961, a multiple electrode system was inserted in one of the subjects but the system had been removed after two weeks in view of a possible allergic reaction (House and Urban, 1973; House, 1976). In 1964, Simmons *et al.* implanted a six-electrode array directly into the modiolus of a 60-year old man. This patient reported auditory sensations when 0.1 ms square waves were used at frequencies from 20-4000 Hz, while his pitch sensations varied according to the electrode stimulated. However, in 1965 the device was removed for fear of infection. Due to criticism from the medical community the clinical work was stopped for several years.

Human studies began again in 1969 when House once more implanted a patient. Other groups (Merzenich, 1975; Banfai *et al.,* 1981; Chouard *et al.,* 1985; Burian *et al.,* 1986) began clinical work during the 1970s and cochlear implants finally achieved a medically accepted and technically realistic status. In 1984 the single-channel 3M/House cochlear implant received approval of the FDA in the United States. Many parallel but independent research efforts have resulted in a great variety of cochlear implant designs (Loeb, 1990; De Foa and Loeb, 1991). Implants differ in number of implantable electrodes (1 to 22), location and orientation of these electrodes (intra- or extracochlear, radial or longitudinal), coupling between processor and implant (a percutaneous plug or transcutaneous inductive coupling) and stimulus processing strategy (a carrier frequency modulated by the acoustic signal, a band-pass filtered acoustic signal or brief pulsatile stimuli, usually at F_0 rate and amplitude-modulated by the acoustic envelope). However, at the moment only the Nucleus cochlear implant, a 22-channel device developed by Clark *et al.* (1984), has received worldwide approval for marketing to licensed practitioners. Other implants are used locally at approved centers, or are designated as investigational implants.

In the Netherlands, the University Hospital of Nijmegen, the Institute for the Deaf in Sint-Michielsgestel and the University Hospital of Utrecht are involved in a cochlear implant program. In Utrecht, the cochlear implant program started in 1982, the first implantation took place in 1985 (Huizing, 1986; Huizing and Smoorenburg, 1986). In that year three postlingually deaf patients received a 3M/House cochlear implant. In the following years four more postlingually deaf patients were implanted with the 3M/House implant. Since 1988 the more advanced Nucleus system is implanted. At the moment 24 postlingually deafened patients have received a Nucleus multichannel cochlear implant in our hospital.

1.2. Psychophysical results in cochlear implant users.

Restoring hearing sensations of deaf patients with a cochlear implant makes them aware and in control of their own voice (Tartter *et al.,* 1989; Svirsky and Tobey, 1991; Perkell *et al.,* 1992; Tye Murray and Kirk, 1993), provides them with environmental signals and can facilitate their communication with other persons through speech, mainly by improving their lipreading abilities (Aran, 1983; Working Group on Communication Aids for the Hearing-Impaired, 1991). Furthermore, cochlear implants may reduce tinnitus sensations (Cazals *et al.,* 1978; Shulman, 1987; Balkany *et al.,* 1987; Hazell *et al.,* 1989; Hazell *et al.,* 1993; Souliere *et al.,* 1992; Dauman *et al.,* 1993). Psychological evaluation of cochlear implant users shows that depression, loneliness, social anxiety and suspiciousness were reduced (Knutson *et al.,* 1991; Cunningham

and Stoeckert, 1992). The psychological outcome, however, was not a simple function of audiological benefit.

The psychophysical performance of cochlear implant users differs greatly from individual to individual (Fourcin *et al.*, 1979). Generally, cochlear implant users who became deaf prelingually perform considerably poorer than postlingually deafened cochlear implant users (Tong *et al.*, 1988; Loeb, 1990; Working Group on Communication Aids for the Hearing-Impaired, 1991; Osberger *et al.*, 1991; Busby *et al.*, 1993). Moreover, speech perception performance with a multichannel device appears to be better than with a single-channel device (Eddington, 1980; Spillmann and Dillier, 1989; Working Group on Communication Aids for the Hearing-Impaired, 1991; Cohen *et al.*, 1993).

1.2.1. Detection threshold

During the first months after implantation the detection thresholds for electrical stimulation may change (Pfingst, 1990a). After this period detection thresholds stay stable over periods of years (Dorman *et al.*, 1992). Detection thresholds seem to be correlated with the amount of sensorineural damage (Pfingst *et al.*, 1981). The range of detection thresholds across subjects is large (20-30 dB), and thresholds appear to be related to the shape, frequency and duration of the stimuli used (Simmons, 1966; Fourcin *et al.*, 1979; Shannon, 1983a; Shannon, 1985; Pfingst, 1988; Pfingst, 1990b; von Wallenberg *et al.*, 1990; Pfingst *et al.*, 1991; Moon *et al.*, 1993). Also, the position and spacing between electrodes have an effect on detection thresholds (Parkin *et al.*, 1985; Lim *et al.*, 1989; Pfingst, 1990b).

1.2.2. Dynamic range

Dynamic ranges found in cochlear implant users are small (6-40 dB) as compared to those for acoustic stimulation in normal-hearing subjects. They decrease with increasing stimulus frequency (Fourcin *et al.*, 1979; Pfingst *et al.*, 1980; Spelman, 1982; Shannon, 1983a). Dynamic ranges depend also on stimulus shape (Fourcin *et al.*, 1979; Shannon, 1985), electrode configuration and condition of the auditory nerve (Pfingst *et al.*, 1980).

Loudness percepts elicited by electrical stimuli appear to be related to stimulus shape, stimulus frequency, stimulus intensity, and separation between stimulus electrodes (Clark *et al.*, 1978; Müller, 1983; Shannon, 1983a; Tong *et al.*, 1983; Shannon, 1985; Tong and Clark, 1986; Pfingst and Rush, 1987; Zeng and Shannon, 1992). Just noticeable loudness difference limens in cochlear implant users range over 3 to 14 % of the dynamic range, as compared to about 1 % of the dynamic range in normal hearing listeners (Fourcin *et al.*, 1979; Pfingst *et al.*, 1983; Shannon, 1983a; Pfingst and Rush, 1987; Tong *et al.*, 1988). Loudness difference limens for electrical stimulation, expressed in dB, are generally smaller than those for comparable acoustic stimulation. Thus, there is some compensation for the limited dynamic range with electrical stimulation (Pfingst *et al.*, 1980).

1.2.3. Pitch perception

Pitch perception in cochlear implant users appeared to be determined by a complex interaction of place of stimulation, rate of stimulation, pulse width and stimulus level (Spelman, 1982;

Shannon, 1983a; Pfingst and Rush, 1987; Townshend *et al.,* 1987; Pfingst and Rai, 1990; Baretto and Pfingst, 1992). Multichannel cochlear implants try to make use of the place principle of frequency coding in the normal ear. Place coding of formant frequencies may provide cochlear implant users with useful information for vowel identification (Tong *et al.,* 1988; Blamey and Clark, 1990; White *et al.,* 1990). Indeed, pitch percepts may change with the position of the activated electrode (Tong *et al.,* 1982; Townshend *et al.,* 1987).

The number of independent channels that can be used for frequency coding depends on the interaction between separate electrodes. Monopolar stimulation produces broader interaction patterns than bipolar stimulation (Shannon, 1983b). The amount of interaction with bipolar stimulation depends on separation of electrode pairs and appears to increase towards the base of the cochlea (Tong and Clark, 1986; Lim *et al.,* 1989). Electrode interactions are weak with non-simultaneous stimulation (Favre and Pelizzone, 1993). Interaction of signals from different electrodes is counteracted in a new coding strategy using continuous interleaved sampling; with this coding technique brief pulses are presented to each electrode in a nonoverlapping sequence (Wilson *et al.,* 1991). Speech perception scores for multichannel cochlear implant users improved significantly using this new coding strategy (Wilson *et al.,* 1991).

Also, the frequency of stimulation has an effect on pitch perception when frequencies below 300-400 Hz are used. Above 300-400 Hz the pitch of an electrical stimulus does not change, and two equally loud stimuli of different frequencies cannot be discriminated (Shannon, 1983a; Tong and Clark, 1985; Townshend *et al.,* 1987). Moreover, cochlear implant users can discriminate sinusoidal signals from rectangular or triangular signals up to 400 Hz (Hochmair *et al.,* 1987), and triangular from trapezoidal signals up to 1000 Hz (Dobie and Dillier, 1985).

Although above 300 to 400 Hz pitches cannot be perceived as such, they may be important for a cochlear implant user. *E.g.*, speech perception in single-channel cochlear implant subjects was shown to degrade when stimuli were low-pass filtered at 900 Hz (Hochmair and Hochmair Desoyer, 1983).

1.2.4. Temporal aspects

The perception of temporal aspects, which is directly related to the subject of this thesis, has received considerable attention in cochlear implant research. Kirk *et al.* (1992) have shown that dynamic vowel cues (temporal changes in the frequency spectrum) may be more important than steady state cues (frequency) for word recognition.

Difference limens for stimulus duration in cochlear implant users seem to be similar to those in normal-hearing subjects (Hochmair *et al.,* 1987; Tong *et al.,* 1988; Busby *et al.,* 1992). The duration of silent gaps detectable by cochlear implant users decreases with stimulus level; moreover, gap duration detection is hardly affected by position or distance between stimulating electrodes, and appears to be similar to gap detection thresholds found in normal-hearing listeners (Dobie and Dillier, 1985; Tong *et al.,* 1988; Moore and Glasberg, 1988; Shannon, 1989; Busby *et al.,* 1992). Silent gaps in periodic stimuli (*e.g.*, pulse trains) are much more readily appreciated than silent gaps in aperiodic stimuli (*e.g.*, noise bands) (Dobie and Dillier, 1985).

Tone decay, a decrease in loudness sensation during the prolonged presence of a steady stimulus, was observed in cochlear implant users stimulated with frequencies above 200-300 Hz

(Shannon, 1983a; Brimacombe and Eisenberg, 1984). The ability to persistently perceive a continuous signal increases with stimulus intensity (Brimacombe and Eisenberg, 1984).
In the University Hospital of Utrecht some users of the 3M/House cochlear implant spontaneously reported that a tone was affected by a preceding one although the inter-tone interval was as long as 500 ms. Time constants for forward masking in cochlear implant users seem to be longer than time constants found in acoustic forward masking (Shannon, 1983a; Cazals *et al.,* 1990). Forward masking with electrical stimulation does not seem to be frequency specific (Shannon, 1983a; Cazals *et al.,* 1990). However, more recent reports suggest that the normalized forward-masking recovery functions for cochlear implant users are similar to those for normal-hearing listeners (Dent and Townshend, 1987; Shannon, 1990a) when the stimulus current strength was put on a *linear* axis (Shannon, 1990a). Also, in subjects with an auditory-brainstem implant the threshold recovery times from forward masking were similar to those of normal-hearing listeners, suggesting the involvement of central mechanisms in forward masking (Shannon and Otto, 1990).
Also, backward masking functions of cochlear implant users seemed to be similar to those of normal-hearing listeners (Dent and Townshend, 1987). Most authors agree that mechanisms responsible for backward masking must be located centrally to the auditory nerve, since auditory-nerve activity induced by an electrical masker will not catch up with the activity induced by a preceding probe.
Simultaneous masking has not been found in cochlear implant users. Actually, the threshold of a probe presented during a masker tends to decrease (Smoorenburg, 1990; Cazals *et al.,* 1990). This phenomenon suggests the detection of beats or modulation in cochlear implant users. At high carrier levels many implant users can detect smaller modulation amplitudes than normal-hearing listeners (Shannon, 1992). Similar sensitivities are found in subjects with a cochlear brainstem implant (Shannon and Otto, 1990).

1.3. Neurophysiological issues

Cochlear-implant related electrophysiological studies measure neural electrical activity evoked by the electrical stimulus. For instance, electrically evoked auditory-brainstem responses (EABR), electrically evoked auditory-nerve compound action potentials (ECAPs) and electrically evoked single auditory-nerve fiber action potentials (spikes) have been recorded.

1.3.1. Status of the cochlea

Generally, no substantial differences show up in electrophysiological measures when they are recorded from normal or deafened cochleas (Hartmann *et al.,* 1984a; Stypulkowski and van den Honert, 1984; van den Honert and Stypulkowski, 1987a; Shepherd and Clark, 1987; Hartmann and Klinke, 1990a). However, in deafened cochleas, most of the auditory-nerve fibers show no spontaneous activity (Hartmann *et al.,* 1984a; Hartmann and Klinke, 1990a), thresholds are a little lower (Black *et al.,* 1983; Hartmann *et al.,* 1984a) and electrophonic effects (*i.e.* activation of fibers by electrically stimulated hair cells) are not observed (Black *et al.,* 1983; Hartmann *et al.,* 1984a; van den Honert and Stypulkowski, 1987a).

Also the vestibular nerve may be activated when the cochlea is electrically stimulated at the round window (Hartmann *et al.,* 1984a; Hartmann *et al.,* 1984b; Bordure *et al.,* 1989). Vestibular effects are also reported in human cochlear implant users (Black, 1977; Black *et al.,* 1978; Black *et al.,* 1980).

1.3.2. Damage

Post mortem histopathological evaluation of cochlear implant users shows that useful auditory sensations may result from only 10 % of the normal spiral ganglion cells. Throughout the extent of electrode insertion the surviving elements of the organ of Corti and the dendrites are usually damaged. The ganglion population, however, is not affected (Linthicum *et al.,* 1991; Fayad *et al.,* 1991).

Hair cell loss causes degeneration of spiral ganglion neurons (Spoendlin, 1979; Webster and Webster, 1981) and the brainstem auditory pathways (Miller *et al.,* 1986). No reversal of these profound effects of deafening on the cochlear nucleus are found as a consequence of chronic intracochlear electrical stimulation (Cazals *et al.,* 1983; Hultcrantz *et al.,* 1991). On the other hand, several investigators have found a trophic effect of electrical stimulation on nerve degeneration (Chouard *et al.,* 1983; Lousteau, 1987; Hartshorn *et al.,* 1991; Leake *et al.,* 1991; Leake *et al.,* 1992) and inferior colliculus activity (Schwartz *et al.,* 1993).

The auditory nerve and the organ of Corti may be damaged by electrical stimuli. The amount of damage can be related to the charge per phase, the frequency and the duration of the stimulus (Sugaya *et al.,* 1975; Yuen *et al.,* 1981; Spelman, 1982; Walsh and Leake-Jones, 1982; Yarowsky *et al.,* 1983; Shepherd *et al.,* 1983; Duckert and Miller, 1984; Dodson *et al.,* 1987; Shepherd and Clark, 1987; McCreery *et al.,* 1992; Ni *et al.,* 1992). Poorly balanced biphasic current pulses cause more damage than well-balanced pulses (Shepherd *et al.,* 1991).

1.3.3. Thresholds

Thresholds found in neurophysiological studies of cochlear implants are consistently higher than thresholds found in psychophysical studies (Pfingst, 1988). EABR thresholds are near behavioral comfort level (Shallop *et al.,* 1991) and depend on the position and separation between stimulation electrodes (Nagel, 1974; Shallop *et al.,* 1990; Abbas and Brown, 1991a; Abbas and Brown, 1991b; Shepherd *et al.,* 1993). The lowest thresholds found for electrostimulation of auditory-nerve fibers are comparable to psychophysical thresholds of cochlear implant users (Hartmann *et al.,* 1984a). The sharp frequency dependence of single auditory-nerve fiber thresholds seen for tonal stimuli is absent for electrical stimuli. Electrically stimulated spiral ganglion neurons showed a broad threshold minimum at between 50 and 200 Hz, regardless of tonotopic location or spontaneous activity (Kiang and Moxon, 1972; Hartmann *et al.,* 1984a; Javel *et al.,* 1987; van den Honert and Stypulkowski, 1987a; Hartmann *et al.,* 1987; Hartmann and Klinke, 1990a; Dynes and Delgutte, 1992). A similar dependence of threshold on electrical stimulus frequency is found for ventral cochlear nucleus neurons (Glass, 1983) and inferior colliculus neurons (Merzenich *et al.,* 1973).

Single auditory-nerve fiber response thresholds decrease exponentially with increasing pulse width (van den Honert and Stypulkowski, 1984; Javel *et al.,* 1987; Parkins and Colombo, 1987).

The most effective pulse width, *i.e.* lowest charge/phase at threshold, is a 100 μs/phase biphasic pulse (Parkins and Colombo, 1987; Colombo and Parkins, 1987). Differences between the auditory-nerve single-neuron strength-duration functions and their psychophysical counterparts (Shannon, 1983a; Pfingst *et al.,* 1985) are best explained as the result of higher neurologic processing (Parkins and Colombo, 1987). Thresholds of electrically stimulated auditory-nerve fibers differ by only about 12 dB for all frequencies (Kiang and Moxon, 1972; van den Honert and Stypulkowski, 1984; van den Honert and Stypulkowski, 1987a; Hartmann and Klinke, 1990b), whereas thresholds of acoustically stimulated auditory-nerve fibers are found over a range of 60 dB (Liberman, 1978). Auditory-nerve fiber thresholds are about 4 dB lower for monopolar stimulation compared to bipolar stimulation (Parkins and Colombo, 1987). A very weak correlation between threshold and spontaneous activity of auditory-nerve fibers has been found (Liberman and Oliver, 1984; van den Honert and Stypulkowski, 1987a).

1.3.4. Dynamic range: growth functions

EABR growth functions depend on the location and the separation between the stimulation electrodes (Shepherd *et al.,* 1993; Miller *et al.,* 1993a), they are steeply sloping, and monotonically related to the stimulus current strength (Charlet de Sauvage *et al.,* 1983; van den Honert and Stypulkowski, 1986; Abbas and Brown, 1988; Brown *et al.,* 1990). EABR growth functions are more gradual for bipolar stimulation than for monopolar stimulation (Abbas and Brown, 1991a). Growth functions of EABRs evoked by stimulation with two electrode pairs, simultaneously in phase, or with inverted phase, are similar but have different sensitivities (Abbas and Brown, 1988). Nonmonotonic EABR growth functions are also reported (Stypulkowski and van den Honert, 1984). Some authors showed that maximum amplitude and slope of EABR growth functions correlate with the number of surviving spiral ganglion cells (Smith and Simmons, 1983; Jyung *et al.,* 1989; Hall, 1990). However, no strong correlation between EABRs and spiral ganglion survival has been found by others (Simmons, 1979; Miller *et al.,* 1983; Stypulkowski *et al.,* 1986). Moreover, EABR response thresholds and growth functions show no strong correlation with the performance on word recognition tests (Abbas and Brown, 1991a).

In general, ECAP growth functions are monotonic and steeply sloping (Charlet de Sauvage *et al.,* 1983; Brown and Abbas, 1990; Brown *et al.,* 1990), but nonmonotonic behavior of peaks present in the ECAP has also been found (Stypulkowski and van den Honert, 1984).

Electrically stimulated single fiber rate-intensity functions are steep, often exceeding 200 spikes/s within 6 dB above threshold (Moxon, 1971; Hartmann *et al.,* 1984a; Javel *et al.,* 1987; van den Honert and Stypulkowski, 1987b), while acoustic rate-intensity curves generally saturate at or below 200 spikes/s at least 20 dB above threshold (Gifford and Guinan, 1983). The dynamic range of electrically stimulated auditory-nerve fibers, the range over which discharge rate increases with increasing stimulus intensity, is about 1 to 6 dB (Kiang and Moxon, 1972; van den Honert and Stypulkowski, 1984; Hartmann *et al.,* 1984b; Javel *et al.,* 1987). This is about 10 times smaller than the dynamic range of acoustically stimulated auditory-nerve fibers. the dynamic range determined with biphasic current pulses increases with increasing pulse rate and is uncorrelated with threshold (Javel *et al.,* 1987). Saturation discharge rates usually equal stimulus pulse rates up to at least 800 to 1000 pulses/s (Javel *et al.,* 1987; Javel, 1990).

Discharge rates of auditory-nerve fibers stimulated by high-frequency sinusoids grow monotonically with stimulus level, up to 700 spikes/s (Dynes and Delgutte, 1992).

1.3.5. Pitch perception

Tonotopic pitch perception with a cochlear implant relies on stimulation of different groups of auditory-nerve neurons with electrodes at different locations. Monopolar stimulation of the cochlea with both intra- and extracochlear electrodes excites fibers throughout the cochlea with little spatial selectivity (van den Honert and Stypulkowski, 1984; van den Honert and Stypulkowski, 1987a; Hartmann and Klinke, 1990a). The spread of excitation can be reduced with bipolar longitudinally oriented electrodes, and a further reduction is found with radially oriented electrodes (Black and Clark, 1980; van den Honert and Stypulkowski, 1987a; Hartmann and Klinke, 1990a). By means of the 2-deoxyglucose technique, cochleotopic selectivity was found at the level of the brainstem and mid-brain nuclei, when the cochlea was stimulated near threshold with bipolar electrodes inside the scala tympani (Ryan *et al.,* 1990; Brown *et al.,* 1992). Snyder et al (Snyder *et al.,* 1990) found that electrical stimulation with an intracochlear bipolar electrode activated a limited sector of inferior colliculus neurons that corresponded with the orderly topographic representation of cochlear place found with normal acoustic stimulation.

With electrical stimulation, all neurons within the electrical field fire synchronously (van den Honert and Stypulkowski, 1984; Parkins, 1987). Synchronization appears to be higher with square waves than with sinusoidal or triangle waveforms (van den Honert and Stypulkowski, 1987b). With increasing stimulus intensity, single-fiber response latencies become shorter, and synchronization indices increase, independent of the characteristic frequency of the auditory-nerve fiber (Hartmann *et al.,* 1984a; Javel *et al.,* 1987; Parkins, 1989; Hartmann and Klinke, 1990a). Auditory-neuron activity (Hartmann and Klinke, 1990a; Dynes and Delgutte, 1992), and also cochlear nucleus neuron activity (Glass, 1984) are found to be highly synchronized with electrical stimuli up to 12.8 kHz. Strong synchronization is particularly evident for frequencies below 500 Hz, and at these frequencies there is a large effect of stimulus intensity on synchronization of activity (van den Honert and Stypulkowski, 1987b; Parkins, 1989). Some nerve fibers were found to exhibit phase locking to both phases of a sinusoidal electrical stimulus of low frequency ($\leq$ 200 Hz) (van den Honert and Stypulkowski, 1987b; Parkins, 1989).

1.3.6. Temporal aspects

Several authors have investigated temporal response properties of the electrically stimulated auditory nerve.

Auditory-nerve fibers respond to an electrical pulse with long ($\geq$ 2 ms) and short ($\leq$ 2 ms) latency responses (Moxon, 1971; Kiang and Moxon, 1972; Hartmann *et al.,* 1984a; van den Honert and Stypulkowski, 1984; Javel *et al.,* 1987; van den Honert and Stypulkowski, 1987b; Parkins, 1989; Javel, 1990). These latency differences are supposedly related to different spike initiating zones, with short-latency responses arising from a central initiating zone of the ganglion cell, and long-latency responses arising from a more peripheral origin. The very-long-latency responses evoked in intact cochleas are probably of an electrophonic origin or due to polarization of the inner hair cells.

Refractoriness of the auditory nerve was determined from EABRs (Abbas and Brown, 1991b; Kasper *et al.,* 1992), ECAPs (Charlet de Sauvage *et al.,* 1983; Stypulkowski and van den Honert, 1984) and electrically stimulated auditory-nerve fibers (Hartmann *et al.,* 1984a). The absolute refractory period ranged from 0.3 to 1 ms, while the relative refractory period extended to at least 5 ms.

EABR amplitudes can be reduced during and after continuous stimulation with electrical stimuli at high levels and frequencies (Meyer *et al.,* 1984; Shepherd and Clark, 1987; Cannon *et al.,* 1990; Miller, 1991).

Spike-frequency adaptation, a decrease in discharge rate during stimulation, has been found in electrically stimulated auditory-nerve fibers (Moxon, 1971; Hartmann *et al.,* 1984a; van den Honert and Stypulkowski, 1987b; Javel *et al.,* 1987; Parkins, 1989; Javel, 1990; Dynes and Delgutte, 1992). Adaptation was particularly present when the fibers were stimulated at high frequencies and moderate or high current strengths. Perhaps summed effects of the relative refractory period could be a factor contributing to adaptation (Chimento and Schreiner, 1991). Fast adaptation as seen with acoustical stimuli is not present in electrically stimulated auditory-nerve fibers (Parkins, 1989). Sustained electrical stimulation at high frequencies and at high levels may lead to a stop in firing due to depolarization block (Javel *et al.,* 1987).

Forward masking in normal hearing at longer masker-probe intervals reveals the perceptual component of recovery from adaptation after stimulation (Plomp, 1964), which is supposed to be due to depletion of neurotransmitter in the hair-cell/auditory-neuron synapse (Eggermont, 1975; Smith, 1977; Norris *et al.,* 1977; Harris and Dallos, 1979; Smith and Brachman, 1982). Adaptation at the auditory-nerve level, induced by electrostimulation, might be involved in forward masking in cochlear implant users (Shannon, 1983a; Javel, 1990; Chimento and Schreiner, 1991). However, it has been suggested that adaptation of auditory-nerve fibers stimulated by high-frequency electrical stimuli plays only a minor role in psychophysical forward masking in cochlear implant users, since adaptation is not found to be very consistent among electrically stimulated auditory-nerve fibers (Dynes and Delgutte, 1992).

1.4. Objective and outline of this study

As mentioned above, the location of the physiological mechanism responsible for psychophysical forward masking in cochlear implant users is not clear.

The aim of this thesis was to investigate whether physiological mechanisms at the level of the auditory nerve could be involved in psychophysical forward masking in cochlear implant users. For this purpose, the electrically stimulated guinea-pig cochlea was used as a model of the human cochlear implant user. Changes in excitability of the auditory nerve were studied after sinusoidal electrical stimuli. In Chapters 2 and 3 excitability was determined from ECAP amplitude changes. Chapter 4 reports spike counts reflecting the excitability of single auditory-nerve fibers.

Chapter 2 describes the general properties of ECAPs recorded from the cochlea and evoked by 5 ms, 15 kHz sinusoidal electrical probes. Changes in excitability were determined subsequent to the presentation of 16 kHz sinusoidal electrical maskers with a duration of 100 ms. Both intact cochleas and cochleas with partially or totally destroyed hair cells were used to assess the

involvement of processes at the hair cell level in forward masking. The frequencies used in these experiments correspond to the frequencies used in a psychophysical forward masking experiment with 3M/House cochlear implant users, which is also described in Chapter 2. The results suggest that mechanisms at the auditory-nerve level are involved in forward masking in 3M/House cochlear implant users. Furthermore, long term fatiguing effects induced by trains of maskers are described.

In Chapter 3 the effect of masker frequency on post-masker excitability was investigated in order to examine whether the post-masker excitability changes reported in Chapter 2 were not linked to the high-frequency of the masker. In this study the apical part of the intact modiolus was stimulated with a brief pulsatile probe, while the ECAP was recorded more basally, directly from the auditory nerve. With this approach only the auditory nerve is stimulated and compound activity of well synchronized auditory-nerve fiber activity is registered. Moreover, slow potential changes showing up immediately after the end of the maskers are studied in Chapter 3.

Chapter 4 describes a study of single auditory-nerve fiber discharges evoked by 10 kHz sinusoidal stimuli. Temporal changes in post-masker excitability inferred from discharge counts evoked by a 25 ms, 10 kHz probe were studied for several masker frequencies. Furthermore, we investigated the possibility that temporal information processing in cochlear implant users is disturbed by spontaneous discharges of auditory-nerve fibers after termination of an electrical stimulus.

CHAPTER 2

A correlate of forward masking in the compound action potential response of the guinea pig VIIIth nerve to electrical stimulation

M.J.P. Killian, S.F.L. Klis and G.F. Smoorenburg, Submitted to Hearing Research

2.1. Abstract

A psychophysical masking study was carried out in 3M/House cochlear implant users. Threshold of a 5 ms, 15 kHz test stimulus (probe) was determined in temporal relation to a 500 ms, 16 kHz masker. Forward masking (probe follows masker) and backward masking (probe precedes masker) were found to extend over 100-200 ms periods. An experimental study, carried out in guinea pigs, was designed to investigate whether these psychophysical results can be attributed to adaptation mechanisms located at VIIIth nerve level. The study was based on electrically evoked VIIIth nerve compound action potentials (ECAPs), using a masking paradigm comparable to the one used in the psychophysical study.

Trains of 50 maskers with inter-masker-intervals of 509 ms appeared to induce a fatigue effect that could influence the recovery measurements. Fatigue stabilized within about 1 to 3 minutes when masker trains were repeated with intervening silent intervals of 10.5 seconds. The amplitude changes of ECAPs elicited by probes at several masker-probe delays for a range of probe currents were determined within steady fatigue states induced by different masker current strengths. The recovery functions obtained from these measurements resembled the forward masking functions found in 3M/House cochlear implant users. No correlate of psychophysical backward masking was found at the VIIIth nerve level.

To examine whether hair cells were involved in fatigue and ECAP recovery functions, experiments were carried out in intact cochleas and cochleas without hair cells. Results were essentially the same in the different preparations. The results suggest that processes at the level of the VIIIth nerve could, at least partly, account for forward masking found in 3M/House cochlear implant users. Backward masking must be attributed to mechanisms located centrally to the VIIIth nerve.

2.2. Introduction

When we started our cochlear implant program in 1985, some users of the 3M/House cochlear implant spontaneously reported that a tone was affected by a preceding one although the inter-tone interval was about 500 ms. In order to clarify the temporal aspects of this interaction we conducted a psychophysical masking study. The threshold of a 5 ms, 15 kHz sinusoidal probe

was measured in temporal relation to a 500 ms, 16 kHz sinusoidal masker using an adaptive method (Levitt, 1971). Masker and probe differed in frequency to eliminate phase effects during simultaneous masking. The frequencies corresponded closely to the carrier frequency used in the 3M/House device. Forward and backward masking were found to extend over 100 to 200 ms. During simultaneous masking the threshold was decreased. This paper addresses possible involvement of the VIIIth nerve in the above non-simultaneous masking effects.

The physiological mechanism responsible for non-simultaneous masking found in the 3M/House cochlear implant users was poorly understood. It has been suggested that the mechanisms responsible for forward masking in cochlear implant users are mainly located centrally to the auditory nerve (Shannon, 1990a; Shannon and Otto, 1990; Dynes and Delgutte, 1992). On the other hand, several authors (Javel, 1990; Chimento and Schreiner, 1991) suggested that adaptation processes at auditory-nerve level might be involved in forward masking. Finally, since cochlear implant users cannot discriminate pitches of electrical stimuli above 300 Hz (Shannon, 1983a), we should realize that detection of the probe will solely be based on temporal cues. Lack of discrimination between the 15 kHz probe and the 16 kHz masker might have contributed to the masking effect found psychophysically.

To clarify whether mechanisms at the level of the VIIIth nerve contribute to forward masking, we designed a neurophysiological study in which electrically evoked compound action potentials (ECAPs) of guinea pigs were determined in a masking paradigm comparable to the psychoacoustic one used in the 3M/House cochlear implant users. In the normal cochlea, the recovery functions of acoustically evoked compound action potentials corresponded more closely to psychophysically obtained forward masking functions (Relkin and Smith, 1991) than the recovery functions obtained from auditory-nerve fibers (Relkin and Turner, 1988). Therefore, we assumed that ECAP recovery functions would be an appropriate measure to determine whether VIIIth nerve activity corresponds to the forward masking functions obtained in cochlear implant users.

Monaural forward and backward masking were found in normal hearing listeners (Elliott, 1962; Elliott, 1967; Zwislocki, 1978), in listeners with sensorineural hearing loss (Nelson and Freyman, 1987), in cochlear implant users (Shannon, 1983a; Dent and Townshend, 1987; Shannon, 1990b; Shannon, 1990a; Smoorenburg, 1990; Cazals *et al.,* 1990) and in auditory-brainstem implant users (Shannon and Otto, 1990). In normal hearing listeners forward and backward masking at very short masker-probe-intervals (<10 ms) can be explained by temporal overlap of the cochlear displacement pattern (Elliott, 1962; Duifhuis, 1973; Carlyon, 1988). The origin of backward masking in normal hearing listeners, extending over more than 10 ms, was assumed to be located centrally to the auditory nerve (Wilson and Carhart, 1971; Weber and Green, 1978). Mechanisms responsible for backward masking in cochlear implant users must be located centrally to the VIIIth nerve, since VIIIth nerve activity induced by the masker cannot catch up with the VIIIth nerve activity induced by the preceding probe.

Forward masking in normal hearing at longer masker-probe delays reveals the perceptual component of recovery from adaptation after stimulation (Plomp, 1964), which was supposed to be due to depletion of neurotransmitter in the hair-cell/auditory-neuron synapse (Eggermont, 1975; Smith, 1977; Norris *et al.,* 1977; Harris and Dallos, 1979; Smith and Brachman, 1982).

However, Javel (1990) and Chimento and Schreiner (1991) suggested that also the spiral ganglion neuron contributed to adaptation and recovery from adaptation.
Since forward and backward masking functions of cochlear implant users were similar to those of normal hearing listeners, Dent and Townsend (1987) supposed mechanisms responsible for forward and backward masking to be located at or centrally to the auditory nerve. For the same reason, and because Javel (1990) found only little adaptation in electrically stimulated auditory-nerve fibers, Shannon (1990a) supposed mechanisms responsible for non-simultaneous masking to be primarily located centrally to the auditory nerve. In addition, similarities between the forward and backward masking functions of auditory-brainstem implant users and those of normal hearing listeners indicated that non-simultaneous masking mechanisms were primarily located centrally to the auditory nerve (Shannon and Otto, 1990).
For short time delays (1-2 ms), forward masking in cochlear implant users can be understood on the basis of refractory properties of individual auditory-nerve fibers (Chimento and Schreiner, 1991). However, forward masking in cochlear implant users extending over 100 ms and more might still have a component related to recovery from adaptation at the auditory-nerve level. Although not pronounced, neuronal adaptation was found in electrically stimulated auditory-nerve fibers (Moxon, 1971; van den Honert and Stypulkowski, 1987b; Javel *et al.,* 1987; Parkins, 1989; Javel, 1990; Dynes and Delgutte, 1992). Firing rates induced in electrically stimulated auditory-nerve fibers increased with increasing stimulus frequency (Javel *et al.,* 1987; Javel, 1990). Thus, more adaptation is expected to occur in nerve fibers stimulated with high-frequency electrical stimuli. Therefore we decided to investigate whether adaptation mechanisms located at the VIIIth nerve level were involved in forward masking found in 3M/House cochlear implant users.
Before we could investigate masking, an investigation into basic properties of ECAPs had to be performed. We first investigated ECAPs for normal cochleas. Subsequently, in order to exclude the possible effects of the presence of hair cells we stimulated cochleas with partially destroyed hair cells and cochleas from which the organ of Corti was removed.
The first experiments dedicated to the investigation of masking confronted us with a problem. Pilot studies showed that trains of maskers may induce a state of fatigue, *i.e.* incomplete recovery from adaptation after presentation of the masker. Harris and Dallos (1979) encountered the same problem when suprathreshold acoustic masker stimuli were presented with short inter-masker-intervals. In this study we accepted, for practical purposes, a certain degree of fatigue. However, we carefully checked that the state of fatigue did not change during series of recovery measurements in a single condition. This state is denoted by the steady-fatigue-state.

2.3. Materials and Methods

2.3.1. Animals and preparation

Experiments were performed on 24 healthy albino guinea pigs (Hsd/Cpb, Dunkin Hartley, 250-500 g). The care for and use of the animals were approved by the Animal Care and Use Committee of the Faculty of Medicine, Utrecht University under number FDC89007, GDL20008. Animals were kept at room temperature with air humidity of 50 $\pm$ 10 %. They were housed in

standard macrolon cages at a 12 h light-dark cycle, and fed ad libitum with a commercial diet (Hope Farms no. 3104). The animals were divided into four groups: a group of 6 animals with intact cochleas (NORM), a group of 7 animals chronically treated with the ototoxic drug Kanamycin (KANA; 3 weeks, 300 mg/kg/day) , a group of 4 animals with cochleas from which the perilymph and parts of the membranous labyrinth were removed by suction using a modified pasteur pipette (SUCT) and a group of 7 animals with cochleas that were partially destructed by removing the bony capsule and the membranous labyrinth, leaving the modiolus intact (DEST). These 4 groups were selected to check whether inner or outer hair cells contributed to ECAPs and masker-induced changes in ECAP.

All animals received 0.1 ml Thalamonal (Janssen Pharmaceutica, 0.25 mg droperidol + 0.05 mg fentanyl/ml) per 100 g body weight before surgery. They were anaesthetized with a gas mixture consisting of oxygen and nitrous-oxide (1:2) and ± 1 % of Halothane. Tracheostomy was performed and the animal was placed in a stereotaxic frame and artificially ventilated with the above mentioned gas mixture. Temperature was monitored by means of a rectal probe and kept near 38 °C. Heart rate was monitored. To prevent dehydration a physiological saline solution (4-6 ml) was given intraperitoneally about every 5 hours. The bulla was exposed from the ventral side, and the cochlea was reached by breaking away small pieces from the bulla with forceps.

Before electrostimulation experiments were started, response thresholds for acoustic stimuli (1000 to 8000 Hz tone bursts) were investigated electrophysiologically. All animals in this study had normal, acoustically evoked, compound action potentials, except animals from the KANA group. The latter group showed a threshold increase of 46.2 ± 17.4 dB at 2, 4 and 8 kHz, and an increase of 17.5 ± 13.6 dB at 1 kHz.

After checking the acoustically evoked compound action potentials, the stapes was cut loose from the malleoincudal complex. The malleoincudal complex and the tympanic membrane were removed. The stapedius muscle was destroyed. Some bony parts of the bulla, dorso-laterally to the round window, were drilled away to make the round window easily accessible. In the DEST animals the bony wall of the cochlea was removed with forceps before the membranous labyrinth was taken away with tissue paper.

2.3.2. Stimulation and recording

Two Pt/Ir-ball electrodes (diameter: ± 0.5 mm), one placed on the apex and one on the round window, served as stimulation electrodes. In animals of the NORM, KANA and SUCT group a small hole was made in the apex and the round window in order to reduce the input resistance between the stimulating electrodes. In the DEST-group we placed the electrodes on the helicotrema and the first turn of the cochlea, respectively. The impedance between the two stimulation electrodes ranged from 5 to 10 kΩ at 1 kHz.

For differential recording two Ag-ball electrodes (diameter: ± 0.5 mm) were used. One electrode was placed on the cochlea, or on the rim of the bulla near the cochlea, and recorded ECAPs contaminated by stimulus artefact. The indifferent electrode was placed on the wall of the bulla at some distance from the cochlea to record primarily stimulus artefact. Electrode positions were chosen so as to maximize the ratio of ECAP amplitude to stimulus artefact, and to record stable ECAPs. A stainless-steel clip, placed between jaw and neck muscles, served as ground

electrode. Fig. 2.1 depicts the stimulation and recording electrode positions in animals with intact cochleas.

Stimulation and recording were controlled by a PDP-11/23 minicomputer system connected to a digital timer. The electrical probe (15 kHz, 5 ms sine wave) and the electrical masker (16 kHz, 100 ms sine wave) were generated by two separate Rockland programmable generators (model: 5100 frequency synthesizer). Each Rockland generator was connected to a programmable Rockland high-pass filter (model: 816, 48 dB/octave roll-off). The output of the two Rockland filters was connected to two separate programmable attenuators. The outputs of the two attenuators were mixed and passed through an analog switch which alternated the polarity of both stimuli. The mixed signal was connected to an isolated constant current source with a bandwidth of 20 kHz. The output of the constant-current source was directly connected to the stimulation electrodes.

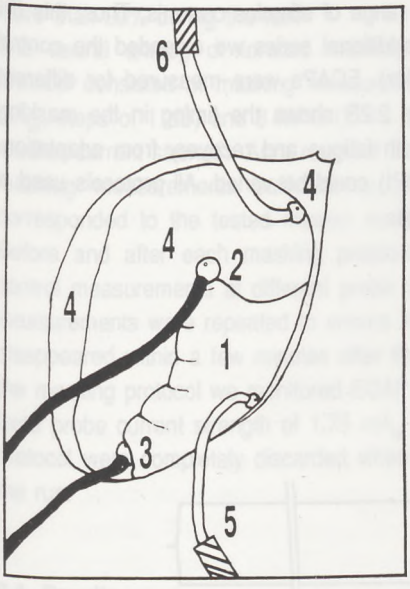

Fig. 2.1 *Schematic drawing of a preparation in the group with intact cochleas (NORM). 1. Cochlea; 2. Apex with Pt/Ir-ball stimulation electrode; 3. Round window with Pt/Ir-ball stimulation electrode; 4. Ventrally opened bulla; 5. Silver-ball recording electrode connected to positive input of differential amplifier; 6. Silver-ball recording electrode connected to negative input of differential amplifier.*

A major problem of recording the physiological response during electrostimulation was contamination of the response by the electrical stimulus. Three techniques were combined to suppress this stimulus artefact.

1. Differential recording: As mentioned before, signals were recorded by two optimally placed recording electrodes and differentially amplified (Princeton Applied Research 113; 1 Hz - 10 kHz).

2. Filtering: Additional reduction of the stimulus artefact was obtained by passing the recorded signal through a Rockland low-pass filter (cut off at 5 kHz, 48 dB/octave). The 15 and 16 kHz stimulus signals were reduced while the ECAPs stayed intact. To minimize the effect of the low-frequency side band of the 15 and 16 kHz tone bursts produced at onset and offset, we first

filtered masker and probe using the two Rockland high-pass filters mentioned above (cut off at 15 kHz, 48 dB/octave). For lower probe frequencies, used occasionally, the cut off frequencies were similar to the probe frequency.

3. Alternating polarity: Finally the stimulus artefact was minimized by alternating the stimulus polarity during the averaging process.

Low signal-to-noise ratios were enhanced by averaging the recordings. The average of 50 ECAPs and the corresponding stimulus conditions were stored on disc for off-line analysis on a personal AT-computer.

2.3.3. Protocol and data analysis

In this paper an ECAP recording always represents the average of 50 ECAPs. Stimulus timing is presented in Fig. 2.2. Fig. 2.2A shows the timing of a control measurement, in which a train of 50 probes was presented at an inter-probe-interval (IPI) of 209 ms. With this IPI no adaptation of individual ECAPs was observed within the complete range of stimulus currents. Thus, this IPI was well suited for the control measurements. In an additional series we extended the control measurements to lower probe frequencies (8 to 16 kHz). ECAPs were measured for different probe current strengths (0.20 mA_{pp} to 2.80 mA_{pp}). Fig. 2.2B shows the timing in the masking condition. These measurements were used to study both fatigue and recovery from adaptation. Inter-masker-interval (IMI) and masker-probe-interval (MPI) could be varied. All protocols used a silent interval of 10.5 seconds between ECAP recordings.

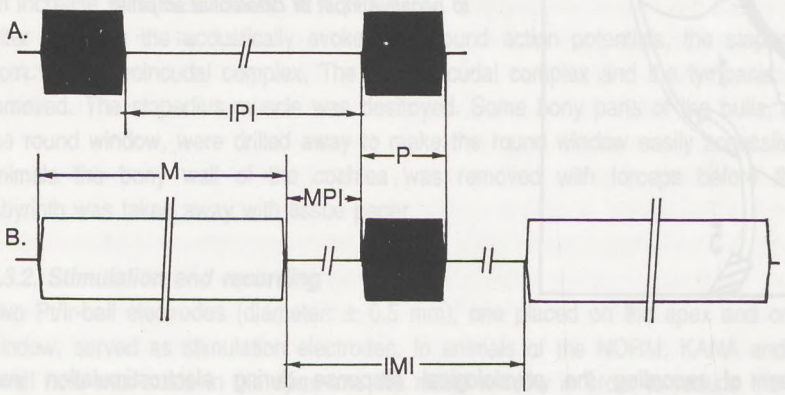

Fig. 2.2 *Schematics of stimulus timing. Fig.* ***2.2A*** *timing of a control measurement; 5 ms, 8-16 kHz probes (P) presented at a fixed inter-probe interval (IPI) of 209 ms. Fig.* ***2.2B*** *timing of a nonsimultaneous masking measurement; 5 ms, 15 kHz probes presented in the presence of 100 ms, 16 kHz maskers (M), masker-probe interval (MPI) and inter-masker interval (IMI) could be varied.*

Two *fatigue* protocols were designed to study the fatigue state during repeated masking measurements. In the first protocol fatigue was tested for 5 different masker current strengths (0.64 mA_{pp} to 2.54 mA_{pp}, in steps of 3 dB) and 3 different probe current strengths (1.39 mA_{pp},

1.75 mA_{pp} and 2.20 mA_{pp}), using a fixed IMI of 509 ms and a fixed MPI of 484 ms. The ECAPs were compared to ECAPs evoked by the control measurements. This fatigue protocol consisted of alternately 10 control and 10 masking measurements. After 5 cycles the protocol was terminated by 15 control measurements. A run of this protocol thus totalled to 115 ECAP recordings.

In the second protocol IMI was not fixed. We tested fatigue for 3 IMIs (1009 ms, 2009 ms or 3009 ms) and 3 corresponding MPIs (984 ms, 1984 ms or 2984 ms, respectively), using a fixed probe current of 1.75 mA_{pp} and a masker current fixed at 1.80 mA_{pp}. This fatigue protocol also consisted of alternately 10 control and 10 masking measurements. After 3 cycles it was terminated by 10 control measurements, the protocol totalling to 70 ECAP recordings.

The stability of ECAP-amplitude during the run of a fatigue protocol was checked visually by plotting ECAPs of control measurements on top of one another. A fatigue measurement was completely discarded when ECAP amplitudes of the control measurements had changed by more than 20% during the run.

The neural analog of forward masking was investigated using the *masking* protocol. This protocol consisted of masking measurements at 13 probe current strengths (0.55 mA_{pp} to 2.20 mA_{pp}, steps of 1 dB) and 8 MPIs (2 ms to 500 ms), using an IMI of 509 ms and a certain fixed masker current strength. As a result of the introductory fatigue study, we included 3 to 5 dummy masking measurements sufficient to put the VIIIth nerve in the steady-fatigue-state that corresponded to the tested masker current strength (0.64 mA_{pp} to 2.54 mA_{pp}, steps of 3 dB). Before and after each masking protocol (including the full set of conditions) we conducted control measurements at different probe current strengths (0.25 mA_{pp} to 2.20 mA_{pp}). The control measurements were repeated to ensure that they were not affected by fatigue. Generally fatigue disappeared within a few minutes after the masking measurements had been terminated. During the masking protocol we monitored ECAP amplitude stability by a masking measurement using a fixed probe current strength of 1.75 mA_{pp} and a fixed MPI of 484 ms. The results of a masking protocol were completely discarded when ECAP amplitudes changed by more than 20% during the run.

2.4. Results

2.4.1. Psychophysical masking in a 3M/House cochlear implant user

The results of the psychophysical masking experiment for one of the 3M/House cochlear implant users are presented in Fig. 2.3. The 500 ms, 16 kHz masker was presented about halfway the dynamic range. Threshold of a 5 ms, 15 kHz probe was determined in temporal relation to the masker. The results showed that probe threshold was increased during a 100 ms interval preceding the masker (backward masking) and about a 200 ms interval following the masker (forward masking). During the masker, the threshold was considerably lower than the masked threshold found for probes presented just before and after the masker. Similar results were obtained in other 3M/House cochlear implant users.

2.4.2. Stability and shape of the ECAPs

All ECAP waveforms exhibited a primary negative peak followed by a positive peak; these will be called N_1 and P_1, respectively. Fig. 2.4 depicts ECAPs recorded at the end of an experiment just before the animal was sacrificed by terminating the oxygen supply and increasing the Halothane supply. Several minutes after the oxygen supply had been stopped the animal died and ECAPs disappeared. The absence of ECAPs in the dead animal demonstrates that these responses represent a physiological process restricted to living animals. The figure also shows that ECAPs were almost free from stimulus-artefact. The N_1P_1 amplitude of the ECAP was taken to be representative of VIIIth nerve activity. In the following this amplitude will simply be called ECAP amplitude. N_1 and P_1 were identified visually.

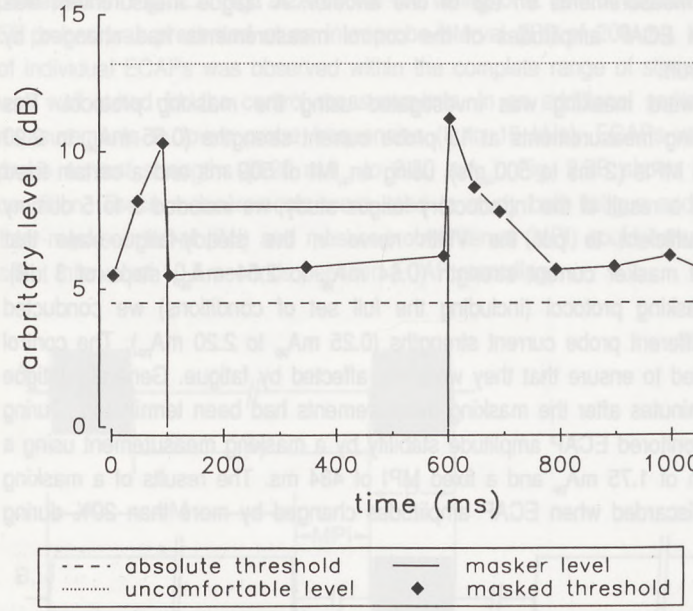

Fig. 2.3 *Psychophysical masking measurement in a 3M/House cochlear implant user. Threshold of a 5 ms, 15 kHz sinusoidal probe is depicted with respect to a 500 ms, 16 kHz sinusoidal masker presented at a level halfway the dynamic range.*

Fig. 2.5 shows representative ECAPs recorded during runs of the fatigue protocol carried out in one animal of each treatment group. Each trace is a superposition of 10 individual ECAPs recorded during control measurements at the beginning (n=5) and at the end (n=5) of a fatigue protocol. Clear coincidence of the 10 ECAPs is observed. Thus, the ECAPs were stable during these fatigue protocol runs (57 minutes).

In most ECAPs, P_1 was followed by several peaks in the waveform. These peaks varied among individual animals. Their shapes were not related to treatment groups. The position of the

stimulation and recording electrodes was the main factor responsible for ECAP shape. Sometimes a small peak interfered with the P_1, but it had rarely an effect on the determination of the N_1P_1 amplitude. The highest peak was taken in cases were P_1 determination was ambiguous.

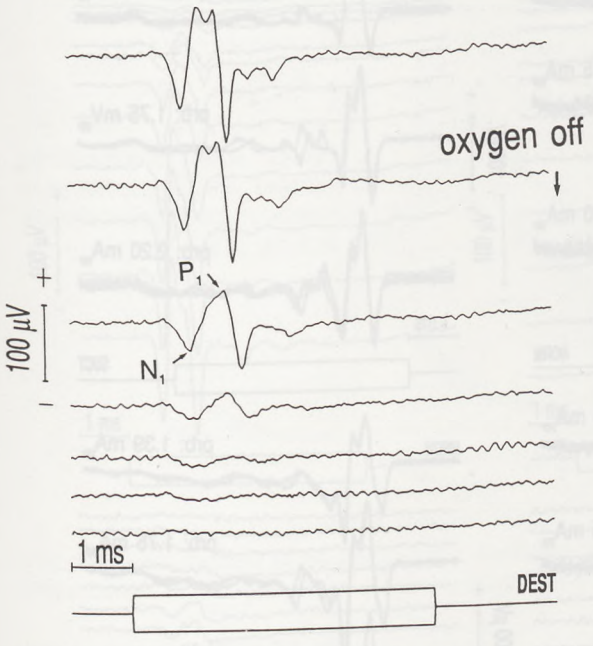

Fig. 2.4 ECAPs elicited by a probe of 1.75 mA_{pp}, recorded at the end of an experiment. ECAPs disappeared when oxygen supply had stopped. N_1 and P_1 can be distinguished. The probe (5 ms, 15 kHz) is indicated at the bottom of the figure.

2.4.3. Relations between ECAPs and probe current strength

Fig. 2.6 depicts representative examples of ECAPs elicited in the 4 treatment groups at different probe currents. The figure shows that amplitudes increased and latencies decreased with increasing probe current strength. As mentioned before there were no fundamental differences between the ECAP waveforms found for the 4 animal groups, but in the DEST animals ECAPs could already be distinguished at lower probe currents.

Fig. 2.7 depicts ECAP amplitudes of the 4 different treatment groups as a function of probe current. The number of recordings used for each curve is presented in parentheses behind the group identification. The ECAP amplitudes were monotonically related to probe current. Saturation of the N_1P_1 was not observed at the highest current strengths used in this study. The 15 kHz sinusoid is known to be a rather ineffective electrical stimulus. Thus, saturation level is not yet reached at a probe current of 2.20 mA_{pp}. The low response threshold for the DEST-group mentioned above is also apparent from Fig. 2.7. The difference with respect to the response thresholds for the other groups is probably related to differences between the positions of the stimulation electrodes with respect to the nerve fibers. In the DEST group the stimulation

electrodes were closer to the auditory neurons. Moreover, the possibility that low-resistant perilymph shortcircuits the stimulus current is smaller in this group.

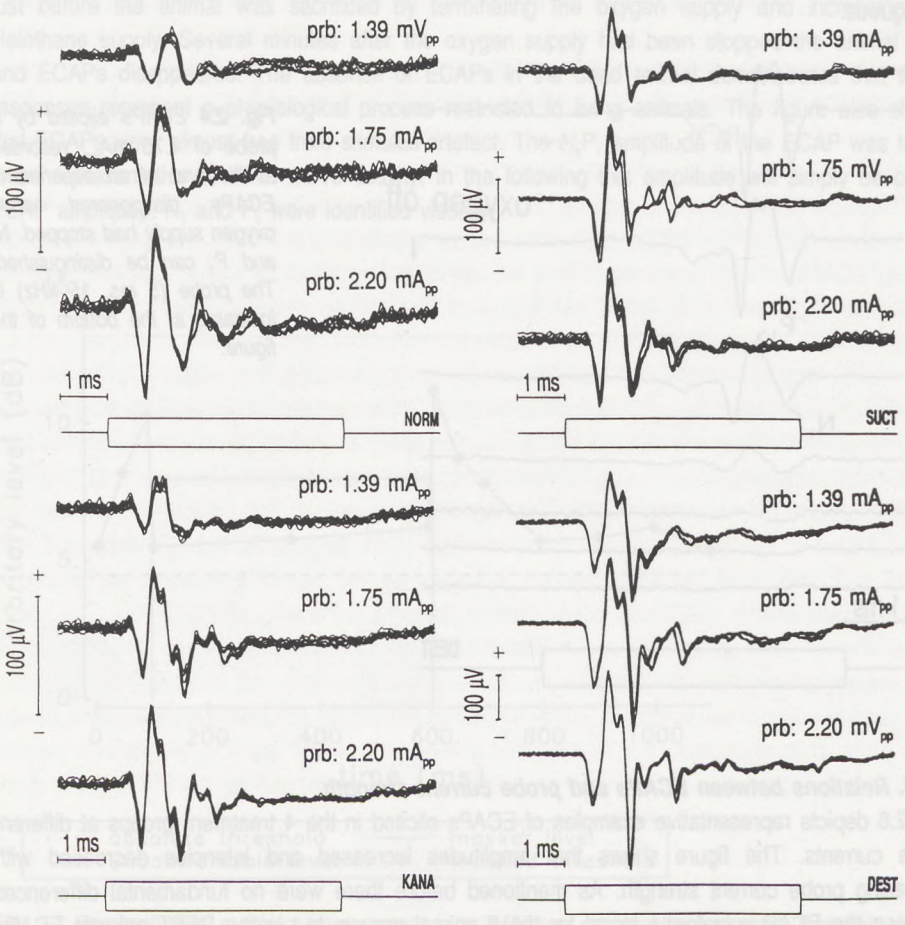

Fig. 2.5 *Representative ECAPs recorded in one animal of each treatment group. Each trace is a superposition of 10 ECAPs, 5 recorded at the beginning and 5 recorded at the end of a fatigue protocol. Similar plots were used for checking of signal consistency.*

2.4.4. Relations between ECAP, probe frequency and electrode position

Fig. 2.8 presents ECAPs elicited by probes of 8 kHz (left plots) and 16 kHz (right plots) using the same probe current strength. All ECAPs were recorded in one animal, before and after destruction of the cochlea. More peaks appeared in ECAPs evoked by 8 kHz probes compared to the ECAP waveforms evoked by 16 kHz probes. Note the scaling difference for ECAPs recorded from the rim of the bulla and those recorded from the basal turn of the cochlea. The

ECAPs recorded from the basal turn of the cochlea had about 10 times larger amplitudes and a simpler waveform. The ECAPs recorded from the rim of the bulla (NORM and DEST) showed a clear resemblance although they had been recorded in different conditions.

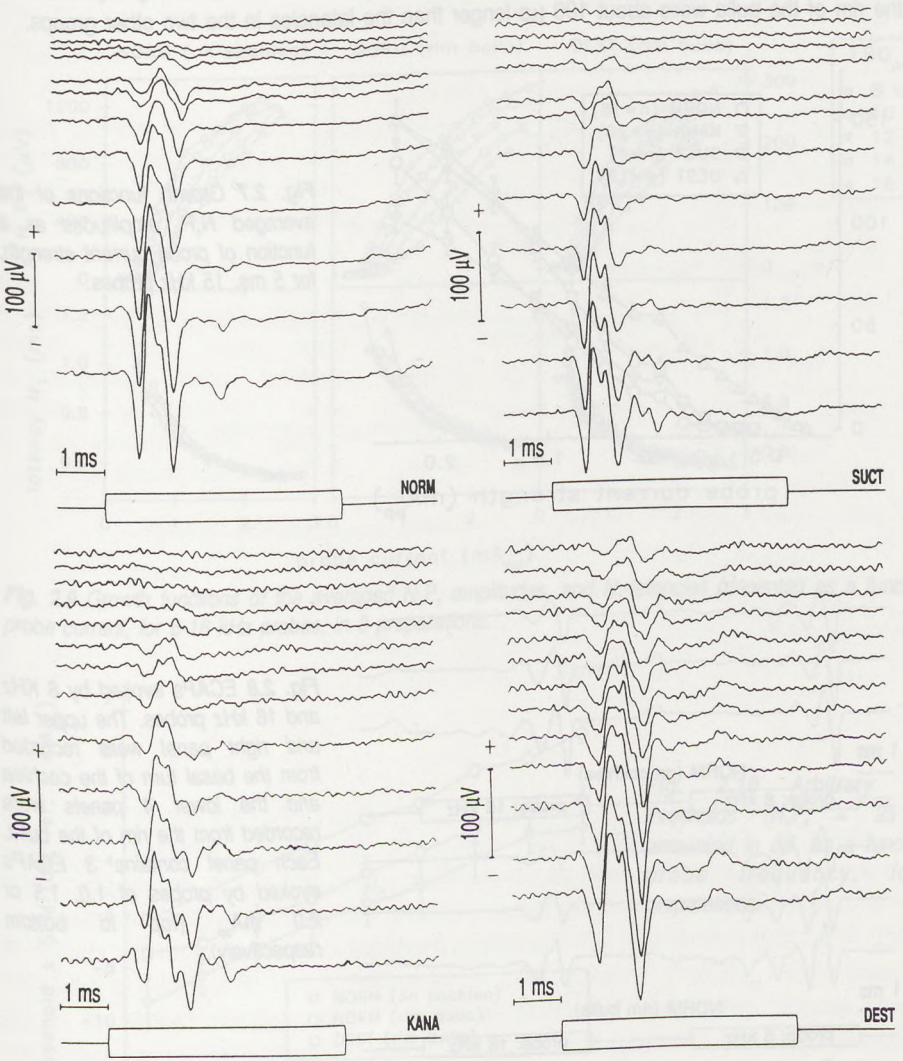

***Fig. 2.6** Plots of ECAPs evoked by several probe current strengths (top to bottom: 0.55 mA_{pp} to 2.20 mA_{pp}, in steps of 1 dB) for animals of the different treatment groups.*

Fig. 2.9 presents the ECAP amplitudes and N_1 latencies as a function of probe current for probes of different frequencies in the 8 to 16 KHz range. Note the different ordinal scale for

amplitudes of ECAP recorded from the basal turn of the cochlea and from the rim of the bulla. ECAP amplitudes decreased with increasing probe frequency. Saturation of ECAP amplitudes is apparent in the DEST group, mainly at the lower probe frequencies. N_1-latencies decreased with probe current, similar for all probe frequencies used. N_1-latencies in the NORM group recorded from the rim of the bulla were about 100 μs longer than the latencies in the two other groups.

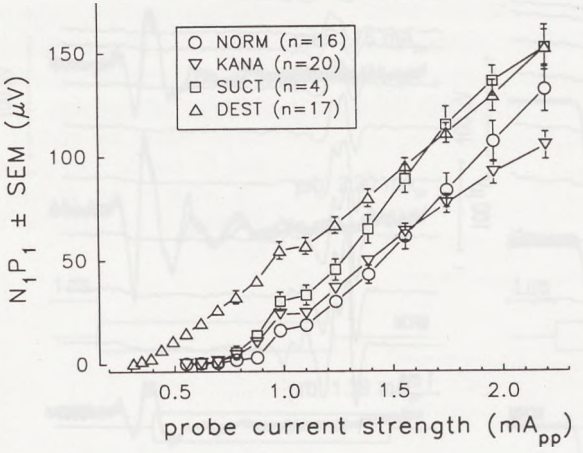

Fig. 2.7 Growth functions of the averaged N_1P_1 amplitudes as a function of probe current strength, for 5 ms, 15 kHz probes.

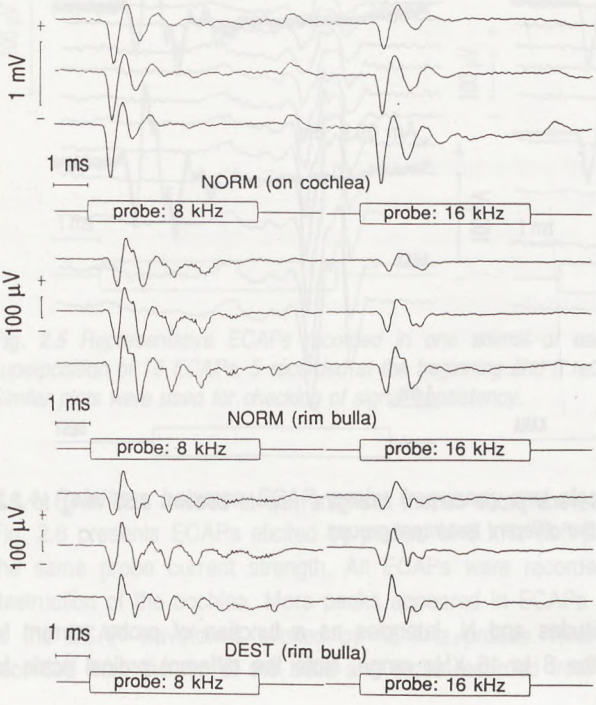

Fig. 2.8 ECAPs evoked by 8 KHz and 16 kHz probes. The upper left and right panel were recorded from the basal turn of the cochlea and the lower 4 panels were recorded from the rim of the bulla. Each panel contains 3 ECAPs evoked by probes of 1.0, 1.5 or 2.0 mA_{pp} (top to bottom respectively).

In order to facilitate comparison with psychophysical threshold data we chose an arbitrary threshold of N_1P_1 amplitude = 25 μV. Fig. 2.10 depicts these ECAP thresholds as a function of probe frequency. Thresholds increased with increasing frequency with a slope of about 6 dB/octave. Similar slopes were found for N_1P_1 thresholds, arbitrarily set at 50, 100 or 200 μV.

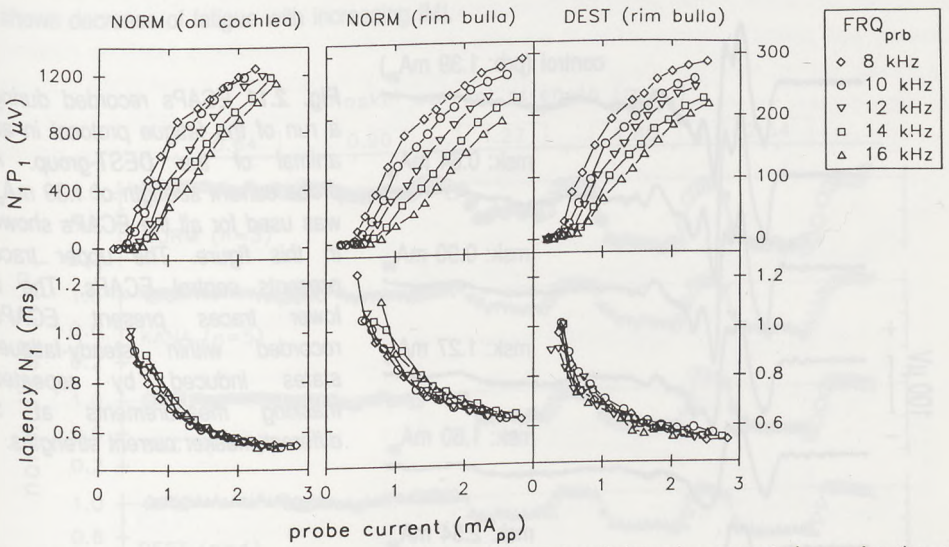

Fig. 2.9 Growth functions of the averaged N_1P_1 amplitudes, and N_1-latencies presented as a function of probe current, for 8-16 kHz probes, in 3 preparations.

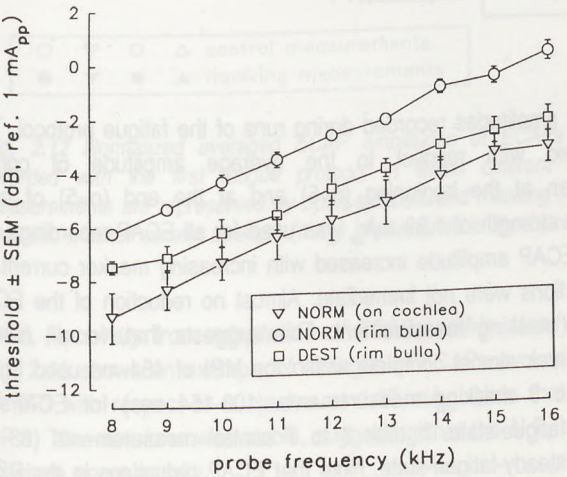

Fig. 2.10 Arbitrary ECAP thresholds (N_1P_1 = 25 μV), presented in dB, as a function of probe frequency, for 3 preparations.

2.4.5. Fatigue of the recorded ECAPs induced by trains of maskers

Fig. 2.11 presents ECAP-recordings of a fatigue experiment in an animal of the DEST group. One probe current strength of 1.39 mA_{pp} was used for all the ECAPs shown in this figure. The

upper trace is a superposition of six control ECAPs. Five of these ECAP registrations were taken just before masking measurements were started, and one ECAP was recorded at the end of the fatigue protocol. Each of the 5 traces below the control ECAPs depict superpositions of 5 ECAP recordings which demonstrate the steady-fatigue-states for a range of masker currents.

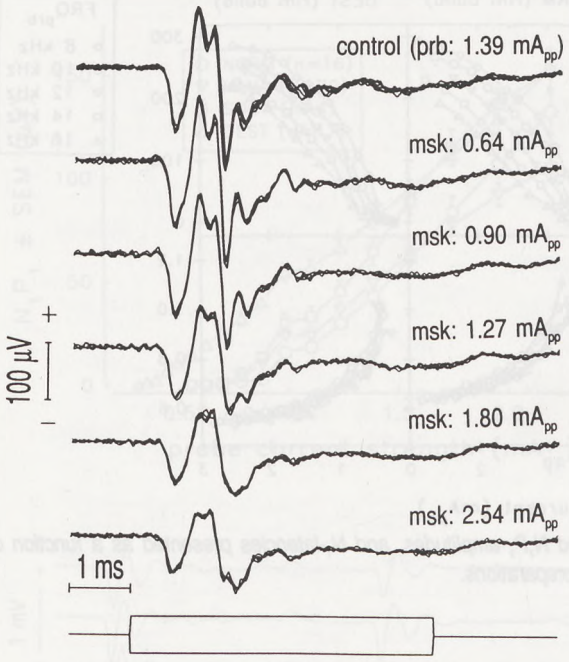

Fig. 2.11 *ECAPs recorded during a run of the fatigue protocol in an animal of the DEST-group. A probe current strength of 1.39 mA$_{pp}$ was used for all the ECAPs shown in this figure. The upper trace presents control ECAPs. The 5 lower traces present ECAPs recorded within steady-fatigue-states induced by repeated masking measurements at 5 different masker current strengths.*

Fig. 2.12 depicts the average ECAP amplitudes recorded during runs of the fatigue protocol. The ECAP amplitudes were normalized with respect to the average amplitude of control measurements (open symbols) taken at the beginning (n=5) and at the end (n=5) of each fatigue protocol run. A probe current strength of 1.39 mA$_{pp}$ was used for all ECAP-recordings.

Fig. 2.12 shows that reductions in ECAP amplitude increased with increasing masker current. At any masker current amplitude reductions were not immediate. Almost no reduction of the ECAP amplitude occurred during the first masking measurement. This suggests that, for all current strengths, recovery from adaptation was almost complete within the MPI of 484 ms used in the fatigue protocol. It took about 2 to 3 masking measurements (103-154 sec) for ECAPs to stabilize at the level called steady-fatigue-state. It took 3 to 7 control measurements (63-148 sec) to reach full recovery from the steady-fatigue-state. Note that ECAP reductions in the DEST group tended to be smaller.

Fig. 2.13 summarizes the normalized ECAP amplitude reductions within the steady-fatigue-states induced by maskers at 5 current strengths for 3 probe currents and 4 treatment groups. The upper plot shows that the amplitudes of ECAPs evoked by the three probe currents were not

affected within steady-fatigue-states induced by maskers at 0.64 mA_{pp}. The lower four plots show that ECAP amplitudes were reduced at higher masker currents. The reductions of the NORM group tend to be larger than the reductions in all other groups, while those in the DEST group tend to be the smallest.

The effects of increasing IMI on induced steady fatigue states is shown in Fig. 2.14. The figure shows decrease of fatigue with increasing IMI.

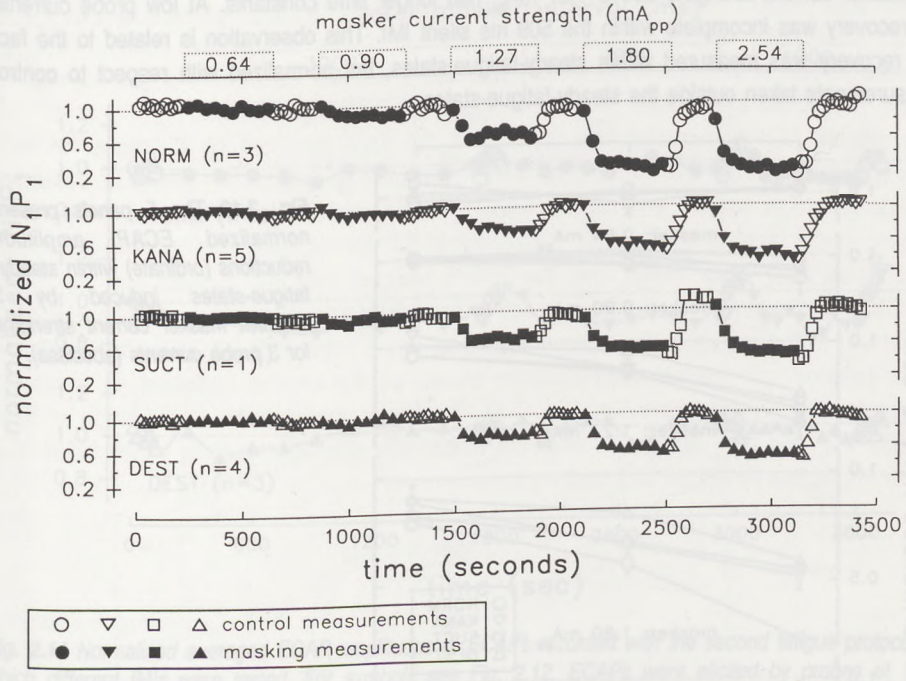

Fig. 2.12 *Normalized averaged ECAP amplitudes of ECAPs evoked by probes at 1.39 mA_{pp} and recorded with the first fatigue protocol in which different masker currents were tested. Control measurements are represented by open symbols and masking measurements are represented by closed symbols. Masker current strength (mA_{pp}) is presented in the boxes on top of the figure.*

2.4.6. Recovery from adaptation of ECAPs within steady-fatigue-states

Fig. 2.15 shows an example of changes in ECAPs evoked by a 1.96 mA_{pp} probe, as a function of MPI with respect to a masker at 1.80 mA_{pp}. Note that amplitudes were decreased at short MPIs. The waveform was stable, suggesting that the N_1P_1 amplitude is an unequivocal descriptor of whole nerve activity.

ECAP amplitudes recorded during the masking protocol were normalized with respect to the amplitudes from control measurements outside the steady-fatigue-state. Recovery-from-adaptation curves were determined in the four treatment groups. Fig. 2.16 shows recovery from adaptation curves obtained at 5 different masker currents in animals of the NORM group. The normalized

ECAP amplitude is presented as a function of MPI for 8 probe current strengths (see lower corner right). As might be expected for recovery from adaptation functions, ECAP amplitude reductions decreased with increasing MPI. Comparison of the different panels shows that the time course and the magnitude of these reductions increased with increasing masker current, and decreased with increasing probe current. For the lowest two masker current strengths, only the lowest two probe current strengths show some recovery from adaptation. For these currents recovery was almost complete within 10 ms after masker offset. The recovery functions obtained for masker current strengths above 1.27 mA_{pp} had longer time constants. At low probe currents the recovery was incomplete within the 509 ms silent IMI. This observation is related to the fact that recovery was measured within steady-fatigue-states, but normalized with respect to control measurements taken outside the steady-fatigue-states.

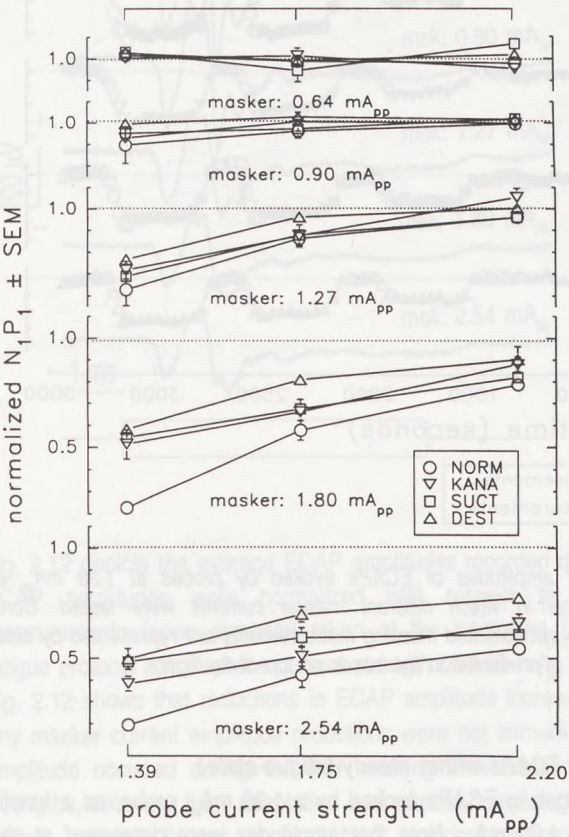

Fig. 2.13 *The 5 panels present normalized ECAP amplitude reductions (ordinate) within steady-fatigue-states induced by 5 different masker current strengths for 3 probe currents (abscissa).*

For the four treatment groups, Fig. 2.17 shows recovery from adaptation functions obtained at a masker current strength of 1.27 mA_{pp}. The recovery functions in the NORM, KANA and SUCT

group were similar. The recovery functions in the DEST group were less affected by the masker than the recovery functions obtained in the three other treatment groups.
The recordings carried out with probe currents of 1.39, 1.75 and 2.20 mA_{pp} at MPIs of 500 ms should correspond to the results for the steady-fatigue-states induced by corresponding masker currents in Fig. 2.13. The fact that this correspondence is imperfect illustrates the variability of experiments performed in different animals.

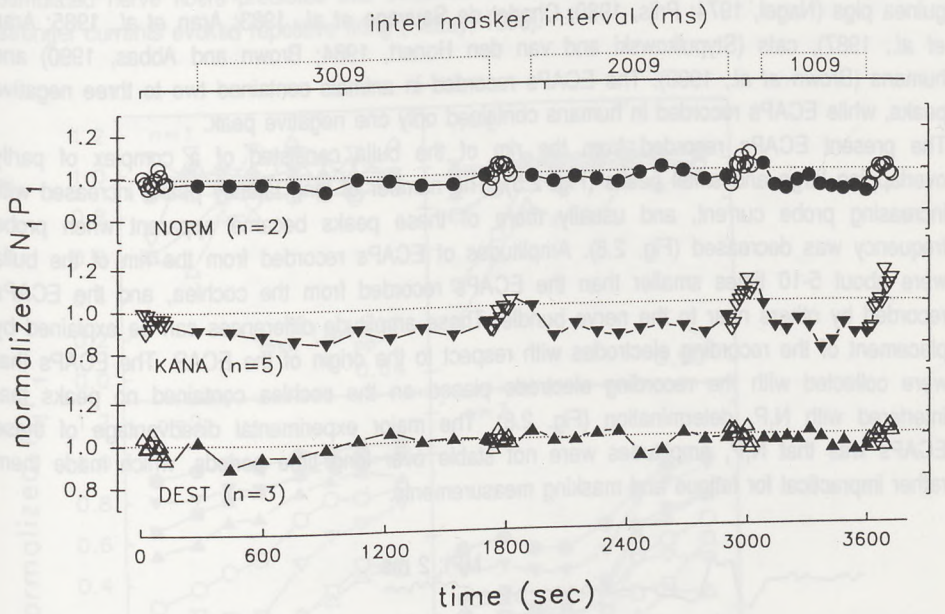

Fig. 2.14 *Normalized averaged ECAP amplitudes of ECAPs recorded with the second fatigue protocol in which different IMIs were tested. For symbols see Fig. 2.12. ECAPs were elicited by probes at 1.74 mA_{pp}, and a masker at 1.80 mA_{pp}. IMIs are presented in the boxes on top of the figure.*

2.4.7. Recovery functions of ECAP response thresholds

For comparison of physiological data and psychophysical data an arbitrary ECAP threshold was defined at N_1P_1 amplitude = 20 μV_{pp}. Thresholds of probes presented after the masker were determined and compared with thresholds obtained with control measurements carried out before and after each run of the masking protocol. Threshold differences obtained in the four treatment groups were combined to create Fig. 2.18. The highest threshold increase was found shortly after masker offset. Except for the highest two masker currents, threshold increase diminished monotonically as a function of MPI. For a masker at 0.64 mA_{pp} recovery seems already complete at about 10 ms. At 0.90 mA_{pp} recovery is complete at about 50-100 ms after masker offset. At the highest three masker currents recovery was incomplete within 500 ms.

2.5. Discussion

2.5.1. ECAP properties

2.5.1.1. Morphology and origin of ECAP peaks

The ECAPs recorded in the present study were evoked by 5 ms, 8 to 16 kHz sinusoids. Other investigators recorded auditory-nerve ECAPs elicited by mono- and biphasic current pulses in guinea pigs (Nagel, 1974; Prijs, 1980; Charlet de Sauvage *et al.,* 1983; Aran *et al.,* 1985; Aran *et al.,* 1987), cats (Stypulkowski and van den Honert, 1984; Brown and Abbas, 1990) and humans (Brown *et al.,* 1990). The ECAPs recorded in animals contained two to three negative peaks, while ECAPs recorded in humans contained only one negative peak.

The present ECAPs recorded from the rim of the bulla consisted of a complex of partly overlapping large and small peaks (Fig. 2.5). The number of long latency peaks increased with increasing probe current, and usually more of these peaks became apparent when probe frequency was decreased (Fig. 2.8). Amplitudes of ECAPs recorded from the rim of the bulla were about 5-10 times smaller than the ECAPs recorded from the cochlea, and the ECAPs recorded by others near to the nerve bundle. These amplitude differences can be explained by placement of the recording electrodes with respect to the origin of the ECAP. The ECAPs that were collected with the recording electrode placed on the cochlea contained no peaks that interfered with N_1P_1 determination (Fig. 2.8). The major experimental disadvantage of these ECAPs was that N_1P_1 amplitudes were not stable over long time periods, which made them rather impractical for fatigue and masking measurements.

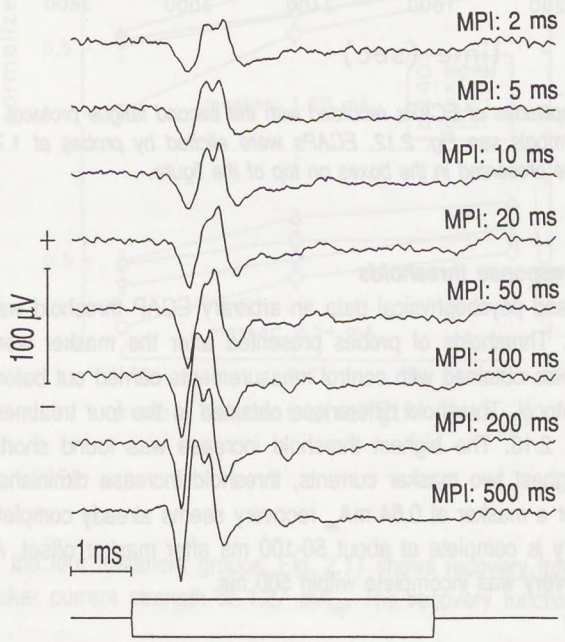

Fig. 2.15 *A representative example of ECAPs recorded during a recovery from adaptation experiment in an animal of the DEST-group. ECAPs were recorded at different MPIs at a probe current of 1.96 mA_{pp} and a masker current of 1.80 mA_{pp}.*

The multiple peaks in the presently recorded ECAPs might have originated from repetitive firing during the 5 ms, 8-16 kHz electrical probes. Parkins (1989) reported that single auditory-nerve fibers stimulated by a 20 ms high-frequency (2500 Hz) electrical stimulus fired repetitively with a constant inter-spike-interval that decreased when stimulus current was increased. At low currents only one spike was elicited at the beginning of the stimulus, while multiple peaks appeared in post-stimulus-time-histograms at higher currents. Also, a model of high-frequency electrically stimulated nerve fibers predicted that threshold currents led to a single action potential, while stronger currents evoked repetitive firing (Rattay, 1986).

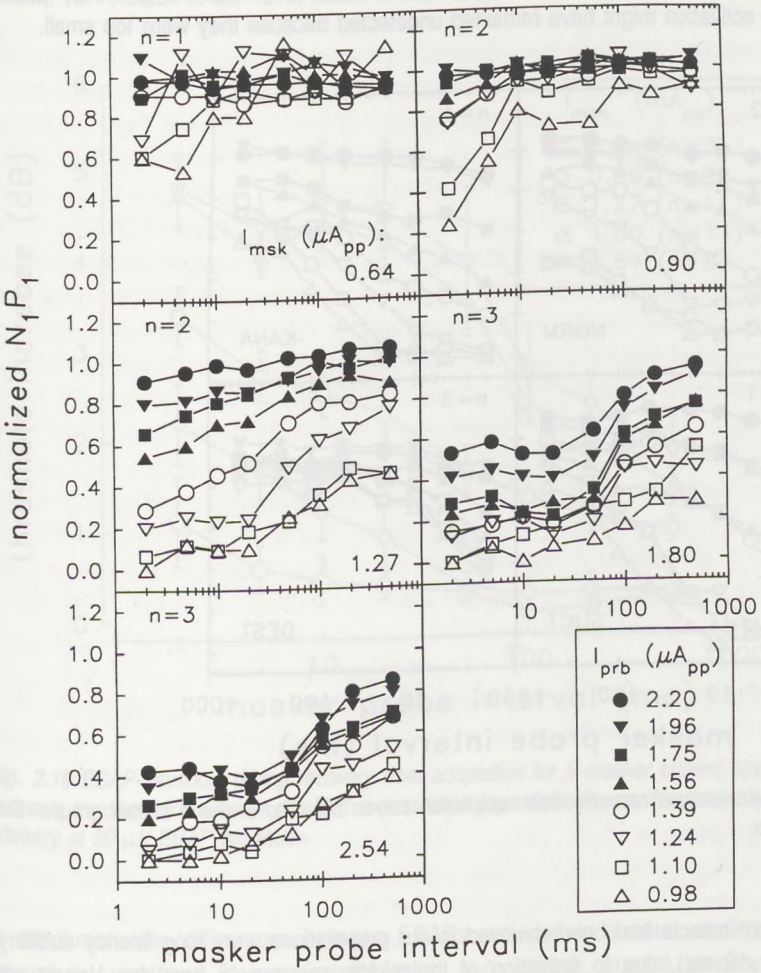

Fig. 2.16 *Normalized averaged recovery from adaptation curves for 5 different masker current strengths and 8 different probe current strengths in animals of the NORM-group. The used masker current strength is shown in the lower right corner of each panel. The number of experiments used for averaging is shown in the upper left corner of each panel.*

Peaks with relatively long latencies (>1.5 ms) showed up in ECAPs when neurons were excited electrophonically by transmitter release from inner hair cells (Moxon, 1971; Kiang and Moxon, 1972; Simmons and Glattke, 1972; van den Honert and Stypulkowski, 1984; van den Honert and Stypulkowski, 1987b; Javel *et al.*, 1987; Javel, 1990). However, we found no differences between ECAPs elicited in normal cochleas an cochleas with destroyed hair cells. Also, Stypulkowski and Van den Honert (1984) found ECAPs in normal hearing cats and neomycin-treated cats to be similar. Moreover, activity patterns of single auditory-nerve fibers (Hartmann *et al.*, 1984a) and electrically evoked auditory-brainstem responses (EABRs) (Shepherd and Clark, 1987) were found to be similar in intact and destroyed or neomycin-deafened cochleas. Although these results suggest that hair cells do not contribute to the responses, long latency peaks elicited by hair cell activation might have remained undetected because they were too small.

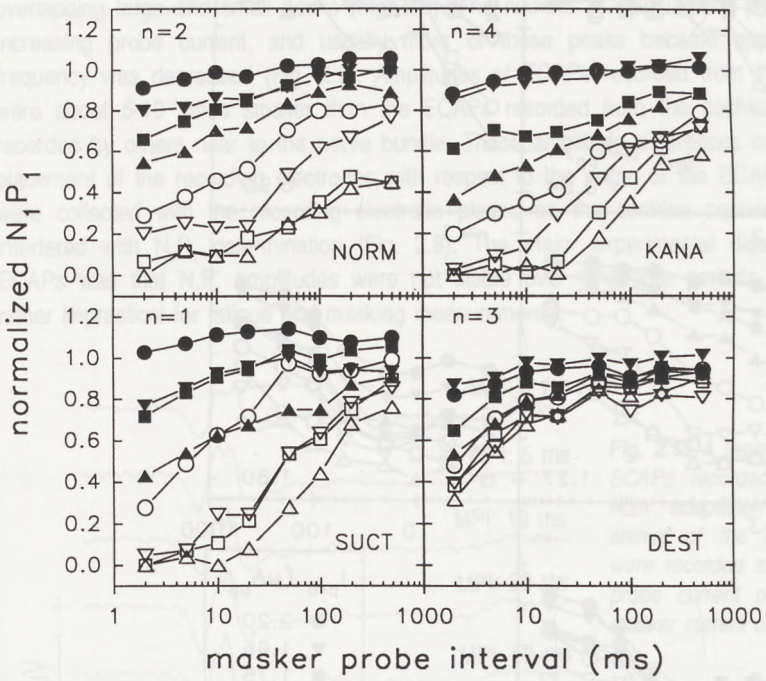

Fig. 2.17 *Normalized averaged recovery from adaptation curves obtained in the 4 animal groups. See Fig. 2.16 for symbols.*

Since the middle ear muscle had been removed in our preparations, very long latency auditory-nerve responses (>10 ms), due to activation of the middle ear muscle (van den Honert and Stypulkowski, 1984; Hartmann *et al.*, 1984a; van den Honert and Stypulkowski, 1987b), were never observed.

Sources outside the cochlea were most likely involved in our ECAPs. Plausible sources are the vestibular nerve, the facial nerve and brain stem nuclei (*e.g.*, cochlear nuclei). Both the vestibular nerve and the facial nerve could have been activated with the presently used probe currents and stimulation configuration. Hartmann *et al.* (1984a; 1984b) found that thresholds of vestibular afferents were significantly lower than those of auditory-nerve fibers, while high currents induced muscle contractions in the area of the facial nerve. We observed facial muscle contractions when current strength exceeded 1.5 mA_{pp}. Bordure *et al.* (1989) showed that brain stem potentials evoked by round-window electrical stimulation contained both auditory and vestibular potentials. Vestibular effects in human cochlear implant users have also been reported (Black, 1977; Black *et al.*, 1978; Black *et al.*, 1980).

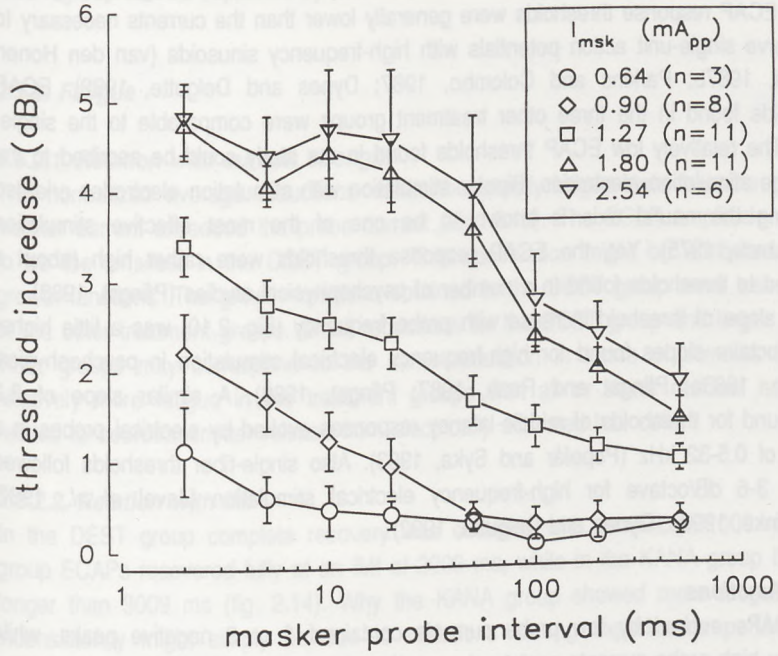

Fig. 2.18 *ECAP-threshold during recovery from adaptation for 5 masker current strengths. The ordinate presents threshold increase in dB. MPI is presented logarithmically on the abscissa. Threshold was set arbitrary at 20 μV ECAP amplitude.*

In conclusion, the differences between ECAPs recorded in various studies were most likely caused by variations in experimental setup (*e.g.*, stimuli, current ranges, stimulation sites, recording sites, animal model, status of the VIIIth nerve, etc.). The multiple peaks in our ECAPs recorded from the cochlea most likely originated from repetitive firing of auditory-nerve fibers,

while ECAPs recorded from the rim of the bulla contained peaks probably originating from sources outside the cochlea (*e.g.*, the cochlear nucleus).
The difference between the N_1 and P_1 peaks was used for further investigation. The width of both the N_1 and P_1 phases did not change substantially with increasing probe current strengths, implicating reasonable synchronization of neural activity at all probe current strengths. Therefore we suppose that the ECAP amplitude is representative of the quantity of firing of VIIIth nerve fibers, activated by the onset of the probe. Furthermore, the chance that these short-latency peaks were contaminated by activity coming from other sources with longer latencies (*e.g.*, cochlear nuclei) was small.

2.5.1.2. Response thresholds

The lowest ECAP response thresholds were obtained in animals from the DEST group (Fig. 2.7). In this group the ECAP response thresholds were generally lower than the currents necessary to evoke auditory-nerve single-unit action potentials with high-frequency sinusoids (van den Honert and Stypulkowski, 1987b; Parkins and Colombo, 1987; Dynes and Delgutte, 1992). ECAP response thresholds found in the three other treatment groups were comparable to the single-fiber thresholds. The relatively low ECAP thresholds found in our study could be ascribed to the configuration of the stimulation electrodes. Bipolar stimulation with stimulation electrodes oriented longitudinally along the neural axis is known to be one of the most effective stimulation configurations (Ranck, 1975). Yet, the ECAP response thresholds were rather high (about a factor 3) compared to thresholds found in a number of psychophysical studies (Pfingst, 1988).
The 6 dB/octave slope of threshold increase with probe frequency (Fig. 2.10) was a little higher than the 2-3 dB/octave slopes found for high-frequency electrical stimulation in psychophysical studies (Shannon, 1983a; Pfingst and Rush, 1987; Pfingst, 1988). A similar slope of 3.2 dB/octave was found for thresholds of middle-latency responses evoked by electrical probes in a frequency range of 0.5-32 kHz (Popelár and Syka, 1993). Also single-fiber thresholds followed slopes of about 3-6 dB/octave for high-frequency electrical stimulation (Javel *et al.*, 1987; Hartmann and Klinke, 1990a; Dynes and Delgutte, 1992).

2.5.1.3. Growth functions

In this study ECAPs evoked by low probe currents contained 1 or 2 negative peaks, while ECAPs evoked by high probe currents contained more than 2 negative peaks (Figs. 2.5, 2.6 and 2.8).
Stypulkowsky and Van den Honert (1984) described a non-monotonic amplitude relation between the first two negative peaks in ECAPs elicited by biphasic current pulses. This non-monotonicity depended on the location of the recording electrodes, and was supposed to have originated from two different stimulation sites, one at the dendrites and one at the axons. We never observed clear non-monotonic behavior of major peaks present in the ECAPs. Actually, the latency difference between the first two major peaks of the present ECAPs was larger than the latency differences described by Stypulkowsky and Van den Honert. This suggests another origin of the major peaks in our ECAPs (*e.g.*, repeated firing). Possibly, our first major peak consisted of two overlapping peaks, corresponding to the two peaks reported by Stypulkowsky and van den

Honert. The minor nonmonotonicity present in the N_1P_1 amplitude growth curves at a probe current of about 1 mA_{pp} may represent the nonmonotonic amplitude behavior found by Stypulkowsky and Van den Honert.
The ECAP amplitude was monotonically or sigmoidally related to probe current (Figs. 2.7,2.9). Saturation is apparent at low probe frequencies and pronounced in the DEST group. Thus, whether or not saturation was found seemed to depend on the effectiveness of the stimulus. The growth functions of ECAPs elicited by biphasic current pulses obtained in human implant users (Brown *et al.*, 1990) and in cats (Brown and Abbas, 1990), were also monotonic and steeply sloping. The decrease of N_1-latency with probe current was similar for all tested probe frequencies (Fig. 2.9). Decrease of N_1-latency with probe current is attributed to shortening of the time to reach spike-threshold at higher probe currents. The relatively short N_1-latencies in the DEST group can be explained by more effective stimulation in this group.

2.5.2. Fatigue

2.5.2.1. Relation with current strength
The normalized average reductions within the steady-fatigue-state became pronounced when masker current exceeded the probe current (Fig. 2.13). The ECAP amplitude reductions tended to be the smallest in the DEST group. This observation might be attributed to differences in growth functions. The growth functions obtained in the DEST group were less steep than those of the other treatment groups. Small reductions for the DEST group and larger reductions for the other groups may correspond to the same reduction in terms of stimulus current. However, relatively more fatigue in the treatment groups with all or some residual hair cells could be related to neurotransmitter release from electrically activated hair cells.

2.5.2.2. Relation with IMI
In the DEST group complete recovery was obtained with an IMI of 1009 ms. In the NORM group ECAPs recovered fully at an IMI of 2009 ms, while in the KANA group IMIs needed to be longer than 3009 ms (fig. 2.14). Why the KANA group showed more fatigue is unclear. This inconsistency might simply be due to variations among experiments performed on different animals.

2.5.2.3. Temporal ECAP amplitude changes
The number of masking measurements necessary to induce a steady-fatigue-state and the number of control measurements necessary for full recovery increased only a little with increasing masker current (Fig. 2.12) and increasing probe current (not shown). Thus, the amount of time needed for fatigue induction (103-154 sec) and fatigue recovery (63-148 sec) did not strongly depend on the applied stimulus currents. In most cases the ECAP amplitudes were not reduced during the first masking measurement of a fatigue test at any masker level. So, recovery seemed to be complete within the used MPI period of 484 ms. In unpublished pilot studies we found that ECAP amplitudes of control measurements were decreased when they

were obtained immediately after a single masking measurement (as used in the fatigue protocol) that by itself had not changed the ECAP amplitude (the latter is also visible in Fig. 2.12). So, a single masking measurement could induce fatigue that only showed up in subsequent measurements. It was actually for this reason that we started to measure ECAP recovery functions within the steady-fatigue-states. Another observed feature of fatigue that will only be mentioned here is that recovery from fatigue seems to be stimulated by the presentation of probes.

2.5.2.4. Related studies

Several authors have described long term changes (up to days) of electrically evoked neural potentials after electrical stimulation of both the cochlea (Meyer *et al.,* 1984; Shepherd and Clark, 1987; Cannon *et al.,* 1990; Miller, 1991) and the auditory brainstem (McCreery *et al.,* 1992).

Cannon *et al.* (1990) found that the response threshold of the guinea-pig electrically evoked middle-latency response increased about 100% for up to 4 hours, after 30 minutes of electrical stimulation, with a 1 kHz sinusoidal stimulus, at a level (200-300 μA_{rms}) below the histopathological threshold of electrical stimulation. A temporary threshold shift of electrically evoked middle-latency responses was found after moderately intense electrical stimulation (Miller, 1991).

Continuous stimulation with 200 μs/phase biphasic current pulses at 0.8 mA (64 $\mu C/cm^2$ gecm/phase) produced only minimal changes in EABR input-output functions. At higher stimulus frequency (> 200 pulses per second) there were progressively increasing reductions that recovered rapidly (within 90 sec) and were followed by a sensitization period during which EABRs were slightly larger (Shepherd and Clark, 1987). Similar stimuli at 1.8 mA (144 $\mu C/cm^2$ geom/phase) markedly reduced EABR input-output functions for periods up to 12 h when high stimulus frequencies were used, while low-frequency stimuli only temporarily reduced the input-output functions. Reductions were ascribed to adaptation and metabolic stress (Yarowsky *et al.,* 1983; McCreery and Agnew, 1983).

The electrical excitability of cochlear nucleus neurons near the site of electrical stimulation was reduced for several days after 4 h of stimulation at 3.6 nC/phase (McCreery *et al.,* 1992). It was supposed that this depression derived largely from the induced neuronal activity.

2.5.2.5. Physiological mechanisms

Temporary threshold shift is a measure of fatigue in the acoustically stimulated cochlea. Hair cells seemed to be involved in this fatigue, since the temporary shifts in response threshold of acoustically evoked compound action potentials could be prevented by electrical stimulation of efferent fibers leading to the outer hair cells (Rajan and Johnstone, 1983; Rajan, 1988a; Rajan, 1988b; Rajan and Johnstone, 1988a; Rajan and Johnstone, 1988b; Rajan, 1990). Theoretically, these efferent fibers could have been involved in the fatigue found in this study in animals with hair cells. However, it is very unlikely that efferent nerve fibers are involved since Kiang and Moxon (1972) found virtually no effect of efferent stimulation on single auditory-nerve fiber responses evoked by an electrical pulse. We suppose that the mechanisms responsible for

fatiguing effects found in this study were mainly located at the VIIIth nerve level, because results found in the NORM and DEST groups were similar.
Oxygen-free radicals produced within the cochlea during electrical stimulation (Sillman *et al.*, 1989) could be responsible for damage related to fatigue. However, fatigue did not induce irreversible VIIIth nerve pathology, since ECAP amplitudes and response thresholds generally returned to previous control values. Also, it is unlikely that fatigue was induced by ischemia since electrical stimulation of the cochlea increases cochlear blood flow (Sillman *et al.*, 1989; Sillman *et al.*, 1989). Metabolic stress induced by high-frequency electrical stimuli (Yarowsky *et al.*, 1983) could have introduced fatiguing effects. However, fatigue could be a phenomenon analogous to spreading depression which is caused by repetitive electrical stimulation of the brain and accompanied by epileptiform activity, increase in extracellular K^+ and a transient loss of membrane permselectivity (Nicholson *et al.*, 1978; McCreery and Agnew, 1983; Hablitz and Heinemann, 1989).
Although there is no direct evidence for slowly inactivating K^+ channels in auditory-nerve fibers, fatigue could have an origin comparable to spike frequency adaptation in amphibian sensory fibers, which was supposed to be due to slowly inactivating K^+ channels (Krylov and Makovsky, 1978).
Another possibility is that ECAP amplitudes decreased because individual spike amplitudes contributing to the ECAP decreased. Javel (1987) found that auditory-nerve fibers ceased firing within 2 to 5 seconds when sustained pulsatile stimuli were presented at high-frequencies (>600Hz) and high current strengths. The cease of response was accompanied by the shrinkage and ultimate disappearance of the action potential. The loss of response was supposed to be due to a depolarization block at the neuronal membrane (Ranck, 1975).

2.5.3. Masking and ECAP recovery studies

2.5.3.1. Backward masking

As already stated in the Introduction backward masking in cochlear implant users must be located centrally to the auditory nerve. The results of this study confirm this view by showing that amplitudes of ECAPs evoked before a masker (IMI=509, MPI=500) were not decreased. We suppose that psychoacoustical backward masking in implant users may be partly due to poor discrimination between masker and probe, because they cannot be discriminated on the basis of pitch differences. On the other hand, if psychoacoustical backward masking represents an authentic phenomenon, it might have an origin somewhere in the central auditory system at a level where the masker interferes with a not yet completely processed probe.

2.5.3.2. Forward masking

Harris and Dallos (1979) showed that in the acoustically stimulated cochlea both adaptation and recovery from adaptation were directly related to the level of excitation elicited by the masker. Because adaptation was found in electrically stimulated auditory-nerve fibers (Moxon, 1971; Javel *et al.*, 1987; van den Honert and Stypulkowski, 1987b; Parkins, 1989; Javel, 1990; Dynes

and Delgutte, 1992), we assumed that acoustically induced adaptation of the auditory-nerve is not solely restricted to the hair-cell/auditory-nerve synapse, but that it might have a component originating from the spiral ganglion cells and the auditory-nerve fibers (Prijs, 1980; Javel, 1990; Chimento and Schreiner, 1991). Moreover, tone decay (a measure of adaptation) in cochlear implant users stimulated at high frequencies (Shannon, 1983a) suggests adaptation. Adaptation, however, is limited. Brimacombe and Eisenberg (1984) found that single-channel cochlear implant users are able to perceive a continuous signal even when stimulus intensities are increased to the highest acceptable levels. Shannon (1983a) suggested that the long time constants (> 400 ms) of forward-masking functions in cochlear implant users were due to the higher firing rates (and thus more adaptation) reached with electrical stimulation. The high carrier frequency used in the 3M/House cochlear implant might induce high neuronal firing rates (accompanied by long recovery periods). However, Dynes and Delgutte (1992) suggested that large adaptation of auditory-nerve fibers stimulated by high-frequency electrical stimuli plays only a minor role in psychophysical forward masking in cochlear implant users, since they found such adaptation not to be very consistent among electrically stimulated auditory-nerve fibers.

More recently, Shannon (1990a) showed that forward masking curves in cochlear implant users resembled those of normal hearing listeners when the amount of masking is expressed in relative μA's. He used a 1 kHz sine wave or trains of pulses (500 to 1600 Hz) at a comfortable-to-loud level as masker, in Symbion and Nucleus cochlear implant users, respectively. The amount of masking decreased linearly as a function of the logarithm of signal delay from masker offset and extended over a period of more than 500 ms. In other studies, comparable forward (Shannon, 1983a; Dent and Townshend, 1987; Shannon and Otto, 1990; Cazals *et al.,* 1990) and also backward masking (Dent and Townshend, 1987) functions were found. In addition, forward masking in cochlear implant users does not seem to be frequency specific (Shannon, 1983a; Cazals *et al.,* 1990). Probably, masker intensity is a more important factor.

We found that the normalized ECAP amplitude reductions during recovery increased with increasing masker current and decreasing probe current (Figs. 2.16, 2.17). This is in agreement with data of Shannon (1983a), who found that threshold increments were decreased when masker level was reduced. The time ECAPs needed to recover from masker stimulation increased with increasing masker current and decreasing probe current.

A striking nonmonotonicity in the recovery functions is the ECAP amplitude drop at MPI ≈ 50 ms. This drop is clearly apparent at high masker currents, and also seems to be present in the forward masking functions obtained by Shannon (1983a; 1990a). The mechanism responsible for this drop is still unclear.

The presently obtained ECAP response threshold recovery functions extended over 10 to more than 500 ms, depending on the tested masker currents (Fig. 2.18). Our results suggest that forward masking functions obtained in cochlear implant users may be related to recovery functions at the VIIIth nerve level. They also indicate that both the magnitude and the time course of forward masking functions of cochlear implant users would decrease with decreasing masker current. Whether this behavior appears in forward masking functions of cochlear implant users remains to be investigated. Moreover, this behavior would contrast with acoustical forward

masking, in which the time constant stays stable over a wide masker intensity range (Plomp, 1964).

2.5.4. Safety of used stimulus currents

The highest stimulus currents used in this study were rather high compared to currents used in cochlear implant users. These current strengths may induce neural damage. Most studies relate damage risk to charge per phase (generally less than 40 $\mu C/cm^2$ geometrical electrode surface is supposed to be safe). Yet, damage seems to be related not only to charge per phase but also to frequency and duration of the stimulus (Shepherd and Clark, 1987). Decrease of the damage threshold with stimulus frequency was supposed to have an electrophoretic origin (Duckert and Miller, 1984). This study was carried out with high-frequency stimuli that were never presented continuously. For this reason we suppose that in our study risk of damage was small. Even after long stimulus sessions ECAP amplitudes returned to their initial value.

2.5.5. Clinical implications

The present results suggest that neural fatigue will occur in 3M/House cochlear implant users. Fatigue may build up within a few minutes during stimulation and may then reach a steady-state from which it will recover within a few minutes. As was described by others, recovery from fatigue may also extend over hours (Cannon *et al.*, 1990; Miller, 1991). At present there are no truly parametric psychophysical studies concerned with fatiguing effects in cochlear implant users (Working Group on Communication Aids for the Hearing-Impaired, 1991). Results of this study suggest that more attention should be paid to fatigue in cochlear implant users. Special attention should be focussed on the time constants of fatigue and steady-fatigue-states induced by different coding strategies. When fatigue shows up in cochlear implant users it could have important implications for the adjustment of loudness in the processor. Furthermore, results of the present study suggest that forward masking in 3M/House cochlear implant users has an origin at the VIIIth nerve level. Tone decay studies in cochlear implant users (Shannon, 1983a) suggest that both fatigue and forward masking might be more pronounced with high-frequency stimulation than with low-frequency stimulation. It was already noticed during the 1940s that high-frequency electrical stimulation of a nerve leads to response failure followed by a recovery period (Bugnard and Hill, 1935a; Bugnard and Hill, 1935b; Cattell and Gerard, 1935; Bugnard and Hill, 1935c). Thus, fatigue and forward masking might be related to the high-frequency stimulus used in the 3M/House cochlear implant processor. Fatigue and adaptation should be considered in present developments of higher-frequency stimulation (*e.g.*, continuous interleaved sampling, (Wilson *et al.*, 1991)).

CHAPTER 3

Changes in excitability of the auditory nerve following sinusoidal electrical stimulation

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3.1. Abstract

Previous research (Chapter 2) showed that the amplitude of an electrically evoked auditory-nerve compound action potential (ECAP) does not fully recover within 500 ms after a high-level, 100 ms, 16 kHz sinusoidal electrical masker stimulus. The long recovery period may be related to the high frequency of the electrical masker. Firing rates induced by an electrical stimulus follow the stimulus frequency up to about 1 kHz. Thus, we may expect recovery functions to change with masker frequency assuming that they are related to the firing rates induced by the masker. In this study recovery functions were obtained by measuring ECAP amplitude after 300 ms maskers of 50 Hz to 10 kHz. Unexpectedly, we found increased ECAP amplitudes for certain masker frequencies. The excitability increments lasted for several hundreds of ms after low-frequency electrical maskers (50 Hz to 800 Hz). The masker frequency after which post-masker excitability increments were found increased with increasing masker intensity. Excitability was mostly reduced after masker frequencies above 800 Hz. Reductions could last for more than 1000 ms.

In addition to changes in ECAP amplitude, we found a slow afterpotential. The slow potential change following the masker was negative and lasted for about 100 ms. The amplitude of the afterpotential reached a maximum value after maskers of about 900 Hz, and increased with masker intensity. Afterpotentials could not be linked to the excitability changes.

Mechanisms that might be involved in the excitability changes were investigated by checking literature concerned with comparable neural systems. The present knowledge suggests that decreased excitability could be related to hyperpolarization induced by a Na^+/K^+-pump, while enhanced excitability might be related to depolarization brought about by accumulation of extracellular K^+.

3.2. Introduction

The physiological mechanisms responsible for psychophysical forward masking in cochlear implant users are poorly understood. In normal hearing, forward masking is thought to be related to recovery from adaptation (Plomp, 1964; Shannon, 1976). Adaptation is most likely due to depletion of neurotransmitter in the hair-cell/auditory-neuron synapse (Eggermont, 1975; Smith,

1977; Norris *et al.*, 1977; Harris and Dallos, 1979; Smith and Brachman, 1982). However, some authors have suggested that adaptation has a component originating from the spiral ganglion (Javel, 1990; Chimento and Schreiner, 1991). One implication of the latter suggestion is that the VIIIth nerve could be involved in forward masking found in cochlear implant users.

In 3M/House cochlear implant users we have previously shown that the psychophysical threshold for a 5 ms, 15 kHz electrical probe stimulus recovers within 200 ms after a 500 ms, 16 kHz electrical tone burst presented at a moderate level. In the guinea pig the electrically evoked VIIIth nerve compound action potential (ECAP) showed recovery intervals from 20 to more than 500 ms depending on the level of a 100 ms, 16 kHz electrical tone burst (Chapter 2). These recovery functions were found for preparations in which the hair cells were present and in which they were completely removed. From these results we have concluded that mechanisms located at the VIIIth nerve level are, at least partly, responsible for psychophysical forward masking in 3M/House cochlear implant users.

Recent psychophysical studies have shown that normalized forward-masking recovery functions of cochlear implant users (Shannon, 1990a) and cochlear brainstem implant users (Shannon and Otto, 1990) are similar to those of normal-hearing listeners. According to Shannon (1990a) and Shannon and Otto (1990) this suggests that the site of the physiological mechanism responsible for psychophysical forward masking is primarily retrocochlear.

In an earlier study, Shannon (1983a) reported that forward masking recovery functions of cochlear implant users did not show complete recovery within 400 ms after a masker. At that time, Shannon noted that this time period was rather long compared to that of normal-hearing listeners. He suggested that this should be attributed to the electrical masker stimulus inducing an extremely high excitation level of the auditory nerve. This idea was based on the notion that, in the acoustically stimulated auditory-nerve fiber, both adaptation and recovery from adaptation are directly related to the level of excitation evoked by the masker stimulus (Smith, 1977; Harris and Dallos, 1979). Indeed, the rate-intensity functions obtained from acoustically stimulated auditory-nerve fibers saturate at firing rates of about 200 spikes/second, at least 20 dB above their threshold (Gifford and Guinan, 1983), while the rate-intensity functions of electrically stimulated auditory-nerve fibers reach firing rates as high as 1000 spikes/second, within 6 dB of threshold (Riach *et al.*, 1962; Moxon, 1971; Hartmann *et al.*, 1984a; van den Honert and Stypulkowski, 1987b; Javel *et al.*, 1987; Dynes and Delgutte, 1992).

At moderate levels firing rates of electrically stimulated auditory-nerve fibers closely correspond to the stimulus frequency for frequencies up to 800 Hz (Hartmann *et al.*, 1984a; Hartmann *et al.*, 1984b; van den Honert and Stypulkowski, 1987b; Hartmann and Klinke, 1990b). At saturation level the firing rate follows stimulus rate even up to 1000 pulses/second (Javel *et al.*, 1987; Javel, 1990). At stimulus frequencies above 1000 Hz spike frequency decreases substantially during high-level stimulation (adaptation) (van den Honert and Stypulkowski, 1987b; Javel *et al.*, 1987; Dynes and Delgutte, 1992), while occasionally some adaptation occurred at lower frequencies (Hartmann *et al.*, 1984a). The 16 kHz electrical masker stimulus used in our previous study (Chapter 2) might have driven auditory-nerve fibers up to high firing rates accompanied by pronounced adaptation. This adaptation might have induced long recovery periods.

In view of the above results and in accordance with the results of recovery from adaptation studies on acoustically evoked single auditory-nerve fiber activity (Smith, 1977; Harris and Dallos, 1979), we may expect that both the time course and the magnitude of the recovery functions will decrease with decreasing electrical masker frequency. In order to test this hypothesis we have measured the recovery of responsiveness of the auditory nerve to short probe tones after electrical stimulation with sinusoidal maskers of different frequencies and durations.

The term recovery suggests restoration of responsiveness to a probe tone after a period of diminished responsiveness. In this paper we will show that for certain stimulus conditions post-masker auditory-nerve responsiveness to probe tones may even exceed the responsiveness shortly before the next masker. For this reason we will use the neutral term post-masker excitability instead of recovery from adaptation.

In our previous study we have measured ECAPs evoked by a 15 kHz sinusoidal probe stimulus from the rim of the bulla (Chapter 2). The waveform of these ECAPs is prone to be influenced by sources outside the cochlea (Chapter 2). In this study we have ensured that we primarily recorded auditory-nerve activity, by stimulating only the apical part of the auditory nerve, and recording ECAPs directly from the auditory nerve.

Using sinusoidal probes including an onset transient, synchronization of neural responses may easily be affected by, for example, the state of adaptation. In order to minimize the effect of desynchronization on ECAP amplitude and, thus, to optimize the relation between ECAP amplitude and whole auditory-nerve activity we used probes consisting of brief (20 μs/phase) balanced biphasic rectangular current pulses. The amplitudes of these ECAPs optimally represent the quantity of active fibers, presuming that the amplitudes of contributing units are comparable. Moreover, closely spacing the two stimulation electrodes at the apical site ensures minor latency differences of single fiber action potentials contributing to the ECAPs. To verify the results of our previous study we carried out a pilot study in which ECAPs were evoked by 5 ms, 10 kHz sinusoidal probes.

In addition to the post-masker changes in excitability reflected in ECAP amplitudes, we will present recordings of slower potentials that showed up after termination of the electrical maskers. The mechanisms responsible for the changes in post-masker excitability will be discussed in relation to these afterpotentials.

3.3. Materials and Methods

3.3.1. Animals and dissection of the cochlea

Experiments were performed with female albino guinea pigs (Dunkin Hartley, 250-500 g). Care for and use of the animals were approved by the Animal Care and Use Committee of the Faculty of Medicine, Utrecht University under number FDC89007, GDL20008. Animals were kept at room temperature with air humidity of 50 ± 10 %. They were housed in standard macrolon cages at a 12 h. light-dark cycle, and fed *ad libitum* with a commercial diet (Hope Farms no. 3104).

All animals received 0.1 ml Thalamonal (Janssen Pharmaceutica, 0.25 mg droperidol + 0.05 mg fentanyl / ml) per 100 g body weight before surgery. A maintenance dose of Thalamonal was given when foot or eye-blink reflexes showed up. They were anaesthetized with a gas mixture consisting of oxygen and nitrous-oxide (1:2) and about 1 % of Halothane. After tracheostomy, the animal was placed in a stereotaxic frame and artificially respirated with the above mentioned gas mixture. Temperature was monitored by means of a rectal probe and kept near 38 °C with a DC-powered heating pad. To avoid dehydration to occur, a physiological saline solution (4-6 ml) was given intraperitoneally about every 5 hours. The left bulla was exposed from the ventral side, and the cochlea was reached by breaking away small pieces of the bulla with forceps. The stapes was cut free from the malleoincudal complex. The stapedius and the malleoincudal complex were removed and the tympanic membrane was destroyed. The visible part of the otic capsule and the membranous labyrinth were removed, leaving the modiolus intact (Fig. 3.1). Fluid around the modiolus was drained permanently using celluloid tissues. In order to get direct contact between recording electrode and auditory nerve, a small opening was made in the bony wall of the basal turn of the modiolus.

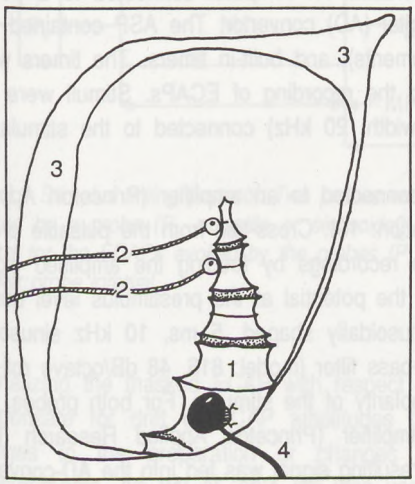

Fig. 3.1 *Preparation of the cochlea. 1. Modiolus; 2. Two Pt/Ir ball stimulation electrodes on the apical turns of the modiolus; 3. Ventrally opened bulla; 4. Ag/AgCl ball recording electrode placed on basal turn of modiolus.*

3.3.2. Histology

To assess the extent of structural damage to the nervous innervation and modiolar microvasculature that might have occurred during preparation and subsequent electrical stimulation in situ, a number of dissected modioli (left ears) were processed for lightmicroscopical examination. The cochleas were divided into two groups: (1) dissected modioli, electrically stimulated for 4 hours; and (2) dissected modioli, left *in situ* for 4 hours without electrical stimulation (sham-treatment). The corresponding right ears served as controls.

Fixation and further tissue processing were described by De Groot *et al.* (1987). The dissected modioli were embedded *in toto* in Spurr's low-viscosity resin. Semithin (1 µm) sections were cut with glass knives on a Reichert Jung 2050 microtome, collected on glass slides and stained with

1% methylene blue, 1% azur II in 1% sodium tetraborate. Sections were examined and photographed with a Zeiss Axiophot photomicroscope.

3.3.3. Stimulation and recording

3.3.3.1. Electrodes

Two Pt/Ir-ball (d= 0.5 mm) stimulation electrodes were placed on the 2 most apical turns of the modiolus (Fig. 3.1). The impedance between the 2 stimulation electrodes ranged from 5 to 10 kΩ at 1 kHz. The tip of the recording electrode was a cintered Ag/AgCl-ball (d= ± 1 mm). It was placed on the opening made in the basal turn of the modiolus. An Ag/AgCl wire electrode, placed in the neck muscle, served as reference electrode. By placing the stimulation electrodes apically and the recording electrode basally we obtained good stimulus-artefact suppression.

3.3.3.2. Electronic equipment

Stimulation and recording were controlled by a personal AT-computer connected to a 16 bit audio signal processor (ASP) and an analog-to-digital (AD) convertor. The ASP contained two digital signal processors (TMS-32010, Texas Instruments), and built-in timers. The timers were used to control both the timing of the stimuli and the recording of ECAPs. Stimuli were fed through an isolated constant-current source (bandwidth: 20 kHz) connected to the stimulation electrodes.

The recording and the reference electrode were connected to an amplifier (Princeton Applied Research 113; bandwidth: DC - 100 kHz; amplification: 10). Cross-talk from the pulsatile probe to the recording electrode was removed from the recordings by feeding the amplified signal through a hold circuit. This hold circuit maintained the potential at the prestimulus level during the pulsatile probe. Stimulus artefact of the cosinusoidally shaped, 5 ms, 10 kHz sinusoidal probe was completely removed by a Rockland low-pass filter (model: 816, 48 dB/octave roll-off, cut-off frequency: 5 kHz), and by alternating the polarity of the stimulus. For both probes, the response was further amplified by a second amplifier (Princeton Applied Research 113; bandwidth: DC - 100 kHz, amplification: 100). The resulting signal was fed into the AD-convertor (sampling rate 50 kHz) of a CED-1401 laboratory interface. Signal-to-noise ratio was enhanced by averaging the recordings. The average of 6 to 20 ECAPs and the corresponding probe conditions were stored on disc for off-line analysis.

3.3.3.3. Data analysis

All ECAP waveforms exhibited a primary negative peak, followed by a positive peak, denoted N_1 and P_1, respectively. N_1 and P_1 were automatically identified under visual control on a personal computer. The N_1P_1 amplitude of the ECAP was taken to be representative of auditory-nerve activity. In the following this amplitude will be simply called the ECAP amplitude. The masked ECAP was normalized by dividing the amplitude of the masked ECAP, evoked by P_m, by the amplitude of the matching control ECAP, evoked by P_c (see Fig. 3.2).

3.3.3.4. Experimental variables

In the pilot studies we used a 5 ms, 10 kHz sinusoidal probe with 0.2 ms cosinusoidally shaped onset and offset. In the main studies the probe consisted of a balanced biphasic rectangular current pulse (20 μs/phase).

Growth functions of ECAP amplitude in relation to probe current strength were obtained by presenting probes without maskers with an inter-probe-interval (IPI) of 303 ms. The ECAPs evoked by the pulsatile stimuli were measured for two polarities: IP-pulse (initially positive at apical electrode) and IN-pulse (initially negative at apical electrode). The most effective pulsatile probe (IP-pulse) was used in the masking protocols. The timing protocol used for the masking measurements is presented in Fig. 3.2. Two probes were presented in each inter-masker-interval (IMI). The ECAP, evoked by a probe presented 10 ms before the next masker (P_c in Fig. 3.2) served as reference (control ECAP) for the ECAP evoked by the probe (P_m) presented after the masker-probe-interval (MPI). MPI was the independent variable which was systematically varied.

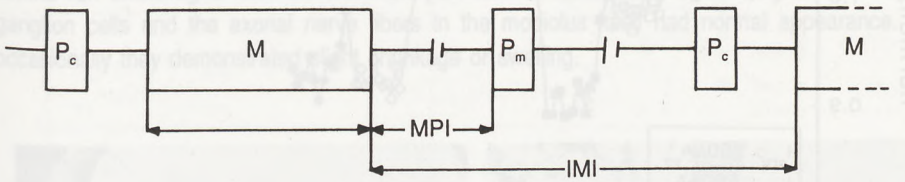

Fig. 3.2 Stimulus timing protocol. Two probes were presented in one inter-masker interval (IMI). ECAPs evoked by a probe (P_c, pulsatile or sinusoidal) presented 10 ms before the next masker served as control for the ECAPs evoked by the probes (P_m) presented earlier after the masker. M: masker; MPI: masker probe interval.

Normalizing the masked ECAP with respect to its corresponding control ECAP enabled us to compensate for drift of ECAP amplitudes that might have occurred as a consequence of changes in the preparation or changes in the excitability state induced by repeated measurements. An IMI of 2304 ms was used in all the masking protocols. On the basis of previous experiments we expected almost full recovery within this IMI (Chapter 2). However, as a measure of precaution, we ensured that post-masker excitability functions would be measured within steady-excitability-states as measured by the response to P_c (cf. steady-fatigue-state in Chapter 2). This was done by presenting three dummy masking measurements at the time when the masker stimulus was changed. We have to mention here that, in spite of the long IMIs used, small changes in excitability at the time of the control probe (P_c) did occur when high masker currents were used. These changes depended on masker frequency (Fig. 3.3). Usually, the excitability increased during presentation of the lower masker frequencies and decreased during presentation of higher masker frequencies. The post-masker excitability functions obtained with the present normalization method would have changed in slope, but not in trend, when the amplitudes of masked ECAPs had been normalized with respect to the amplitudes of ECAPs obtained without maskers, as was done in Chapter 2.

In the main body of measurements we used maskers of 300 ms duration. Masker onset and offset were cosinusoidally shaped with rise/fall times of 20 ms. Eight masker frequencies (50 Hz to 10 kHz) and 10 MPIs (4 ms to 2048 ms) were used. Masker and probe current were kept constant during a series of measurements. We tested at three masker currents (250 μA_{pp}, 500 μA_{pp} and 1000 μA_{pp}) and four probe currents (250 μA_{pp}, 500 μA_{pp}, 1000 μA_{pp} and 2000 μA_{pp}). This masking protocol was also used in the pilot study.

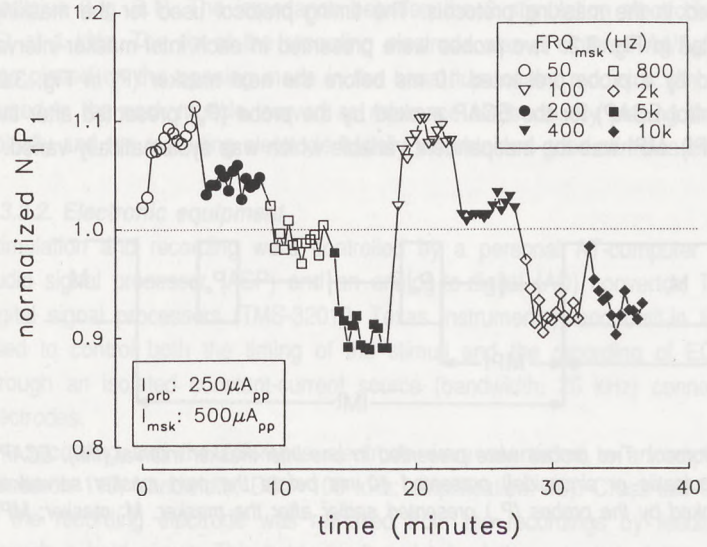

Fig. 3.3 *An example of changes in excitability at the time of P_c in relation to masker frequency. The ordinate represents N_1P_1 amplitudes of ECAPs evoked by P_c which were normalized with respect to an ECAP recorded without masker. The abscissa represents the time of registration during execution of the main protocol.*

In some additional experiments we extended the number of MPIs from 10 to 62 in about the same range from 5 to 2000 ms. Also, we added a masker duration of 50 ms. Three masker frequencies (100 Hz, 1 kHz and 5 kHz) were tested. Maskers were shaped with rise/fall times of 5 ms. In these additional experiments the masker current was kept at 400 μA_{pp}, while the probe current was kept at 500 μA_{pp}.

3.3.3.5. Post-masker potential measurements

The recorded potentials showed negative shifts, slow with respect to the ECAPs, within 100 ms after masker offset. These afterpotentials disappeared when the animal had died. When the animal was made anoxic for a brief period of time the afterpotential also disappeared, while it returned when the oxygen supply was turned back on. This implies that the afterpotential represents a metabolic physiological response with possible relevance to excitability. Therefore,

we determined the baseline levels at the tail of the ECAP waveform. These baseline levels of the control ECAPs were subtracted from the baseline levels of the masked ECAPs. The baseline differences were plotted as a function of MPI. Afterpotential measurements were carried out as a function of masker frequency and masker current.

3.4. Results

3.4.1. Histology

There were no obvious differences in morphological appearance between the electrically-stimulated and sham-treated modioli. In all modioli studied, both the organ of Corti and the basilar membrane were absent in nearly all turns. In the basal turns the basilar membrane was occasionally present, but the organ of Corti was always missing. The osseous spiral lamina and the spiral limbus remained intact. The myelinated nerve fibers (dendrites) in the osseous spiral lamina ran undamaged from the habenula perforata to the spiral ganglion (Fig. 3.4). The spiral ganglion cells and the axonal nerve fibers in the modiolus itself had normal appearance. Only occasionally they demonstrated slight shrinkage or swelling.

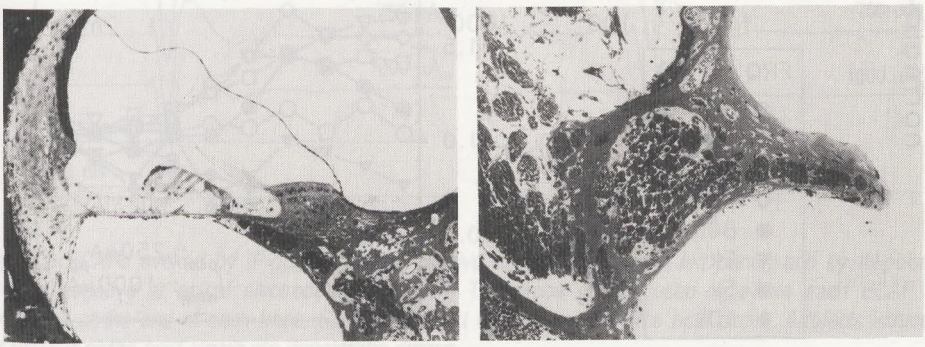

Fig. 3.4A *(left) Organ of Corti in the apical turn of a normal cochlea (x 70). Fig.* ***3.4B*** *(right) Apical organ of Corti from a dissected and stimulated cochlea, demonstrating the intact osseous spiral lamina with the spiral limbus. In the osseous spiral lamina the dendrites run undamaged from the habenula perforata to the spiral ganglion. All nerve fibers and spiral ganglion cells are intact (x 70).*

3.4.2. Pilot studies: 5 ms, 10 kHz sinusoidal probe

The results obtained for the standard masking protocol in one animal are shown in Fig. 3.5. Normalized ECAP amplitudes are presented as a function of MPI, for 8 masker frequencies. The results for maskers of opposite polarity were essentially the same for all experiments carried out in this study. For this reason we present only the results averaged over the two masker polarities. The three panels in Fig. 3.5 present post-masker excitability functions for several masker and probe currents. Fig. 3.5 shows that the normalized magnitudes of the changes in

ECAP amplitude decrease with increasing probe current, and increase with increasing masker current. Surprisingly we found that ECAP amplitudes may increase after particular maskers. Post-masker excitability increments were found predominantly with low-frequency maskers, while high-frequency maskers reduced post-masker excitability.
A close look at the time course of the post-masker data shows that in most cases they do not simply follow a gradually changing function of MPI (*e.g.*, Fig. 3.5 shows a local peak at about 30 ms MPI). This suggests that several mechanisms may be involved in post-masker excitability.

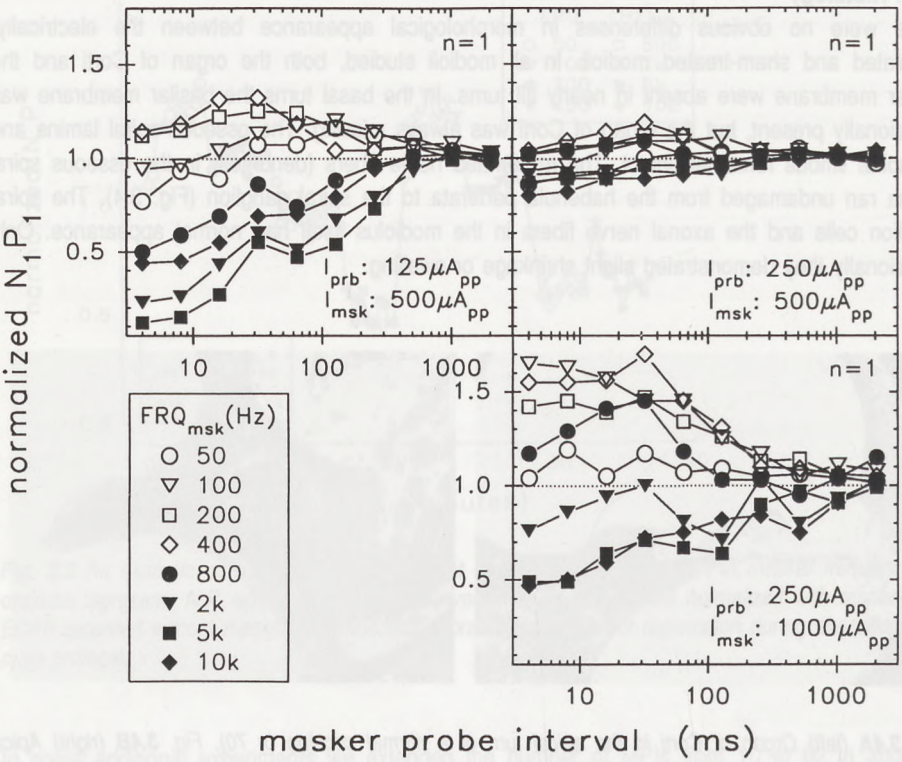

Fig. 3.5 *Post-masker excitability functions obtained for 8 masker frequencies. The normalized amplitudes of ECAPs elicited by 5 ms, 10 kHz probes are presented as a function of the logarithmically plotted MPIs. These data were obtained in one animal.*

3.4.3. Main studies: 20 μs/phase balanced biphasic pulse probe

3.4.3.1. ECAP waveform and growth functions

The ECAPs evoked by biphasic current pulses have generally one narrow large negative peak (N_1) followed by a wider positive peak (P_1). At high current levels P_1 is always followed by two or

three minor oscillations. A representative example of ECAPs elicited at several probe currents is presented in Fig. 3.6. Spikes preceding the ECAPs (arrows) are artefacts induced by releasing the hold circuit. ECAPs presented in the left panel were evoked by IP-pulses, those in the right panel by IN-pulses. ECAPs elicited by the IP-pulses have the shortest N_1-latencies and mostly larger ECAP amplitudes.

The amplitudes of ECAPs evoked by IN-pulses and IP-pulses are presented as a function of probe current in the upper panel of Fig. 3.7. The ECAP amplitudes are sigmoidally related to the probe current, and start to saturate at a probe current of about 1500 μA_{pp}. The lower panel of Fig. 3.7 presents N_1-latency as a function of probe current. The N_1-latency decreases exponentially with pulse current.

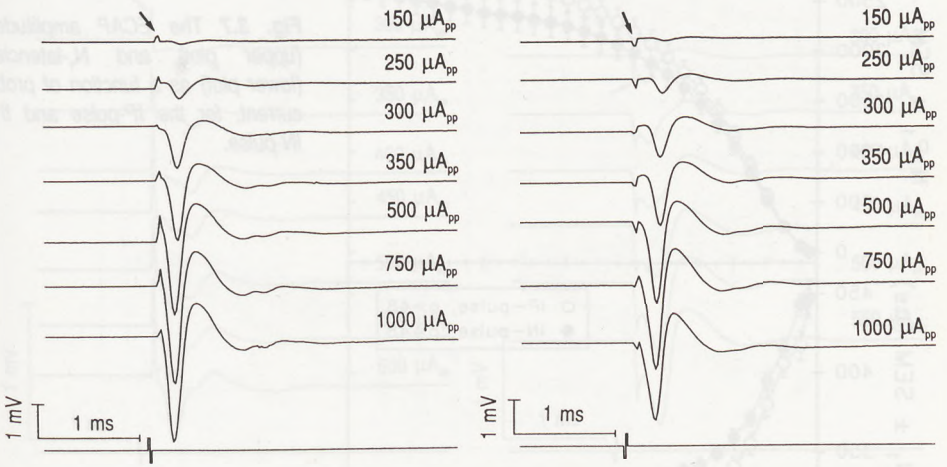

Fig. 3.6 *ECAPs evoked by IP-pulses (initially positive at apical electrode, left panel) and by IN-pulses (initially negative at apical electrode, right panel). The probe current used to evoke each ECAP is presented at the end of each trace. Stimulus artefact was removed by the hold circuit. Artefacts induced by release of the hold circuit are denoted by arrows.*

Occasionally, a bimodal waveform showed up in the N_1-peak of the ECAPs. Four examples are presented in Fig. 3.8. The upper left panel presents an example of the most frequently found pattern (8 out of 48 animals). Starting at low currents (300 μA_{pp}), ECAPs exhibit a negative peak with a relatively long latency. A second negative peak with a shorter latency shows up when the probe current is increased, and the waveform becomes bimodal. At even higher probe currents the long-latency peak may merge with the short-latency peak. This pattern showed up only when the IP-pulse was used to elicit ECAPs. In 2 of the 48 animals we have found the opposite pattern using the IN-pulse. In this case a long-latency peak takes over from a short-latency peak (upper right panel). Two bimodal peak patterns that showed up when ECAPs were elicited by probes at high current levels are presented in the two lower panels of Fig. 3.8. The lower left panel presents a bimodal pattern with a stable short-latency peak and a long-latency peak which

latency increases with probe current strength (1 out of 48 animals). It should be noted that the onset of the short-latency peak is partly affected by the hold circuit. The lower right panel presents a bimodal pattern in which a long-latency peak overrides a short-latency peak when probe current is increased (2 out of 48 animals). This bimodal pattern is similar to the one presented in the upper right corner for lower probe current strengths.
For bimodal ECAPs, in which peaks were not affected by the spike artefact, we always used the most negative peak to determine N_1P_1 amplitude. No discontinuities in the peak patterns of bimodal ECAPs were found in post-masker excitability studies. Both peaks seemed to be equally affected by the masker.

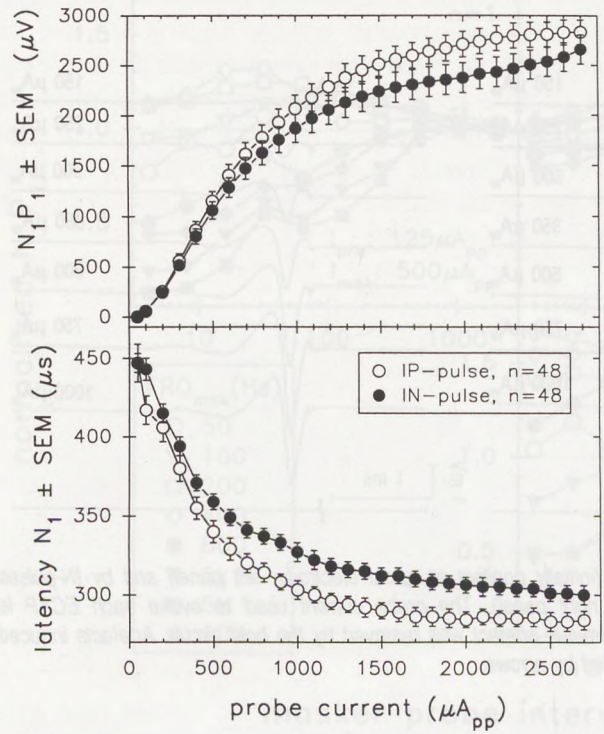

Fig. 3.7 *The ECAP amplitudes (upper plot) and N_1-latencies (lower plot) as a function of probe current, for the IP-pulse and the IN-pulse.*

3.4.3.2. Post-masker excitability changes: effect of masker frequency

Fig. 3.9 shows averaged post-masker excitability functions for 300 ms maskers. Post-masker excitability was found to be either decreased or increased when a probe current of 500 μA_{pp} was used. In essence, these post-masker excitability functions are comparable to those obtained with a 5 ms, 10 kHz sinusoidal probe (Fig. 3.5). Post-masker excitability was generally decreased when a probe current of 1000 μA_{pp} was used, except for one experiment in which we found an ECAP amplitude increment after maskers at frequencies of 50 Hz to 400 Hz.
The normalized ECAP amplitude changes obtained at MPIs of 4, 8 and 16 ms were averaged for the 8 tested masker frequencies, the 3 tested masker currents and the 4 tested probe

currents. The results are representative of the magnitude of the change in excitability immediately after masker offset and are presented in Fig. 3.10. Increments in post-masker excitability showed up for the lower masker frequencies (50 Hz to 400 Hz), when low probe currents (upper two panels) were used. Post-masker excitability increments increased with increasing masker current. The range of masker frequencies inducing post-masker excitability increments showed some shift toward higher masker frequencies with increasing masker current. Reductions in post-masker excitability were found over the whole range of applied masker frequencies, when high probe currents were used (lower 2 panels). Only small post-masker excitability changes were found when probe current exceeded the masker current.

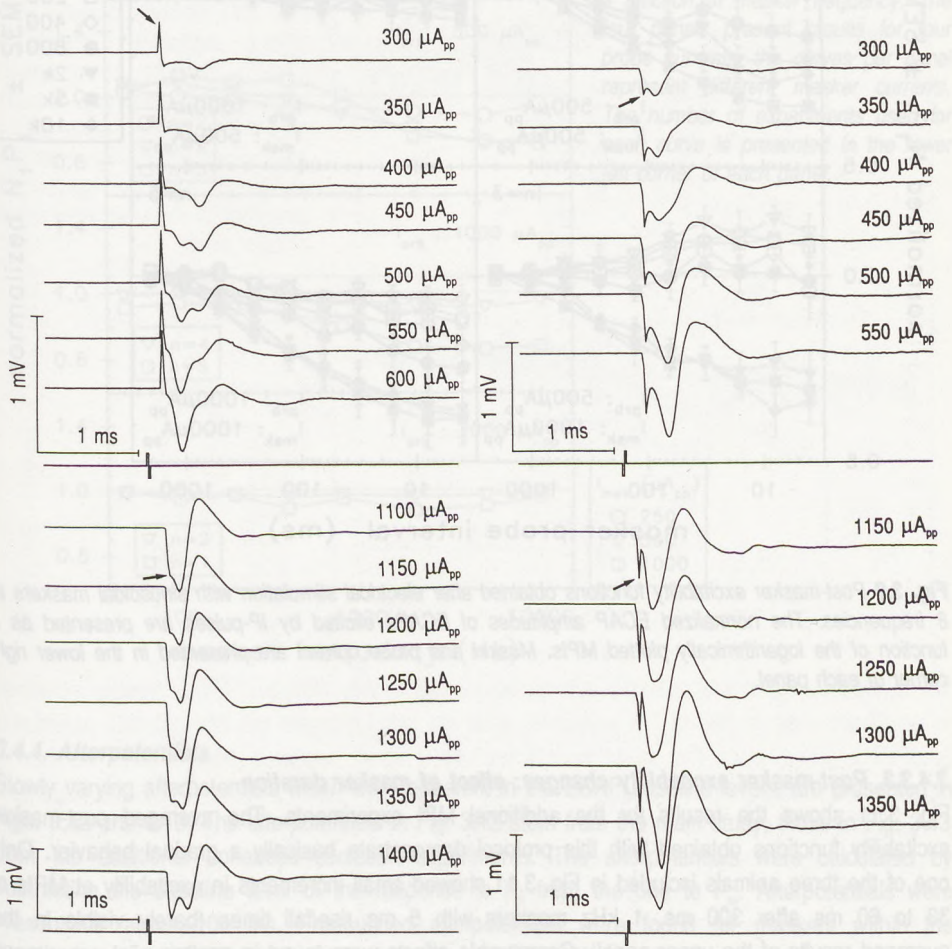

Fig. 3.8 *Examples of ECAPs in which the initial negative phase was bimodal (left panels: IP-pulses; right panels: IN-pulses). Probe currents are presented at the end of each trace.*

Most of the averaged post-masker excitability functions of Figs. 3.5 and 3.9 show a gradual change with MPI. However, some individual post-masker excitability functions showed less gradual changes over the measured time course. After maskers of 800 Hz and 2 kHz, a slight increase in excitability often seemed to be present at an MPI of 32 ms. In order to more accurately determine the time course of post-masker excitability, we extended the number of MPIs in additional experiments.

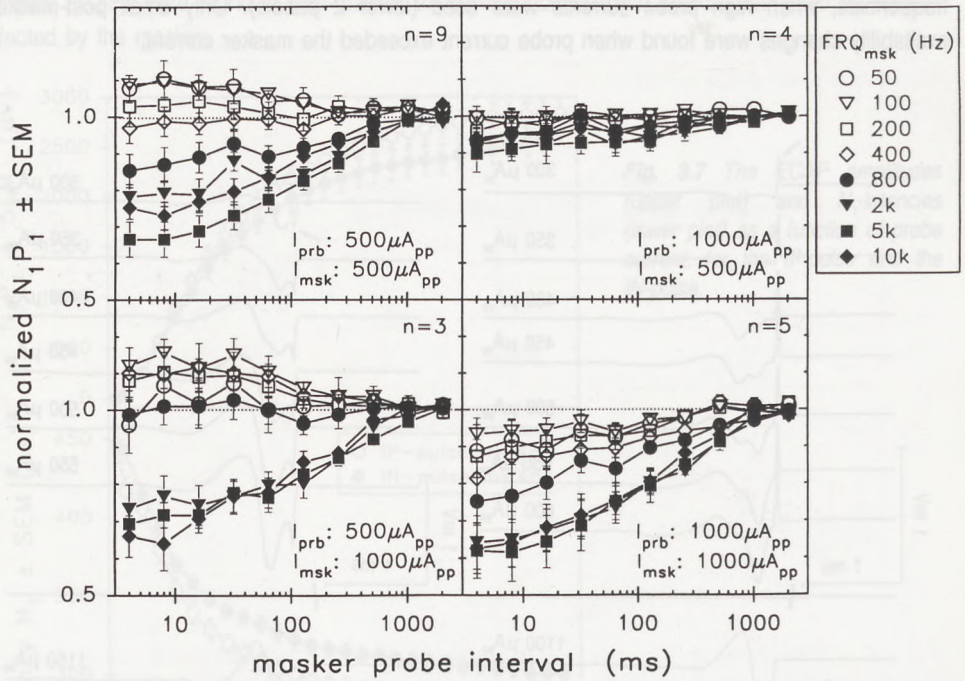

Fig. 3.9 Post-masker excitability functions obtained after electrical stimulation with sinusoidal maskers at 8 frequencies. The normalized ECAP amplitudes of ECAPs elicited by IP-pulses are presented as a function of the logarithmically plotted MPIs. Masker and probe current are presented in the lower right corner of each panel.

3.4.3.3. Post-masker excitability changes: effect of masker duration

Fig. 3.11 shows the results for the additional MPI experiments. The averaged post-masker excitability functions obtained with this protocol demonstrate basically a gradual behavior. Only one of the three animals included in Fig. 3.11 showed small increments in excitability at MPIs of 30 to 60 ms after 300 ms, 1 kHz maskers with 5 ms rise/fall times (barely visible in the averaged results of the upper panel). Comparable effects were found in another pilot experiment. Together, however, these detailed measurements suggest that we did not miss rapid changes because of possibly insufficient sampling density in Figs. 3.5 and 3.9.

Fig. 3.11 also presents effects of masker duration. The magnitude of the change in post-masker excitability decreased with decreasing masker duration. In accordance with the previous results, the magnitude of the post-masker excitability reductions is larger for the 5 kHz masker than for the 1 kHz masker over the whole range of MPIs.

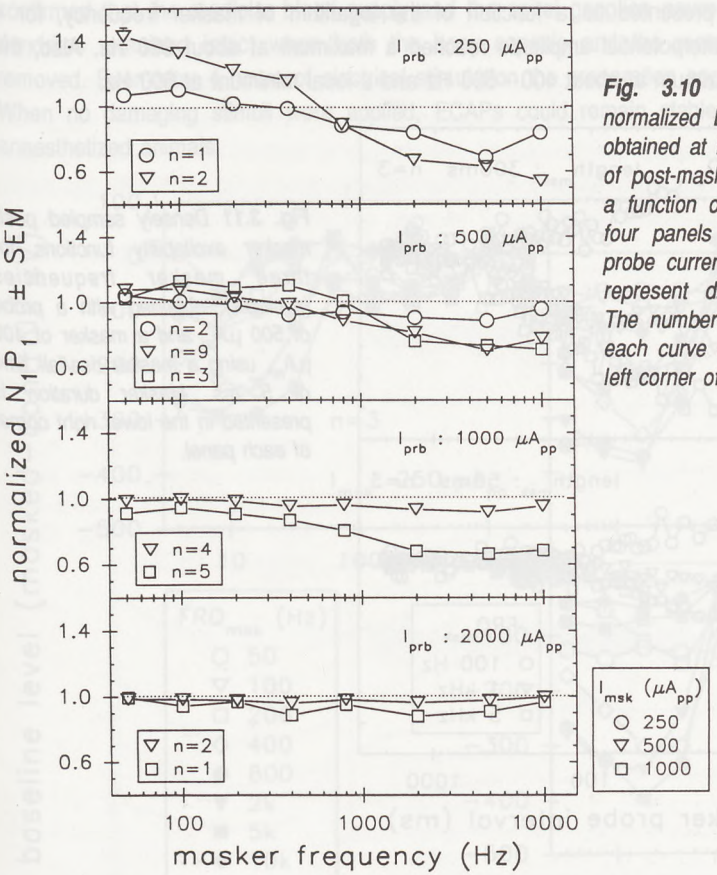

Fig. 3.10 *Magnitude (average of normalized ECAP amplitude changes obtained at MPIs of 4, 8 and 16 ms) of post-masker excitability change as a function of masker frequency. The four panels present results for four probe currents, the curves per panel represent different masker currents. The number of experiments used for each curve is presented in the lower left corner of each panel.*

3.4.4. Afterpotentials

Slowly varying afterpotentials which were apparent in the ECAP baseline levels, are presented in Figs. 3.12 and 3.13. The afterpotentials in Fig. 3.12 stem from the main study, those in Fig. 3.13 from the additional enhanced-resolution experiments. The afterpotentials were calculated by subtracting the baseline level of the response to P_c from the one to P_m. Afterpotentials were present for about 100 ms. Pronounced afterpotentials were found for maskers within the frequency range of 400 Hz to 2 kHz (see Figs. 3.12, 3.13). Usually, pronounced afterpotentials were followed by a small oscillation before they returned to baseline level. Fig. 3.13 shows that the afterpotential diminishes when masker duration was reduced. Fig. 3.14 presents actual recordings of potentials recorded without probe, after maskers of different frequencies. The

afterpotentials reached a minimum at about 30 to 50 ms following termination of the masker. The latency of the minimum decreased with increasing masker frequency. The amplitude of the afterpotential was defined as the difference between the minimum in the waveform and the subsequent maximum, or the baseline level when no subsequent maximum was present. Fig. 3.15 shows the oscillating character of the afterpotential. In Fig. 3.16 the average amplitudes of the afterpotentials are presented as a function of the logarithm of masker frequency, for 4 masker currents. The afterpotential amplitude reached a maximum at about 900 Hz. Also, the results show a local maximum at about 400 - 500 Hz and a local minimum at 600 Hz.

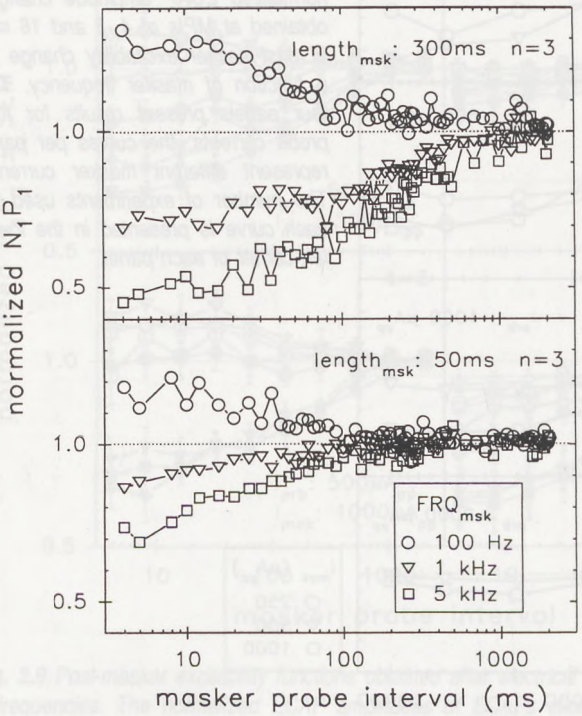

Fig. 3.11 *Densely sampled post-masker excitability functions for three masker frequencies (symbols), obtained with a probe of 500 μA_{pp} and a masker of 400 μA_{pp}, using a masker rise/fall time of 5 ms. Masker duration is presented in the lower right corner of each panel.*

We noticed during the recording of afterpotentials that initially individual afterpotentials increased in amplitude when an IMI of 1 s was used. After the initial increase the afterpotential amplitudes reached a steady-state that could be followed by a small decrease after a large number of stimulus presentations. No amplitude changes were observed when an IMI of 10 s was used.
In principle, the sloping baseline will influence the measurement of ECAP amplitude. ECAP amplitude might have been underestimated when the potential was decreasing and overestimated when the potential was increasing. However, this effect was small (< 1%) since ECAP amplitude was much greater than the change in baseline potential over the 0.5 ms ECAP N_1P_1 time interval. For this reason we did not correct the ECAP amplitudes with respect to the slow changes in baseline potential.

3.5. Discussion

3.5.1. Evaluation of the presently used animal model

The preparation of the guinea pig modiolus used in the present study is a suitable model for neurophysiological research on electrostimulation of the auditory nerve. Histological examination confirmed that the modiolar blood supply and the spiral ganglion neurons with their axons and dendrites remained intact when both the bony capsule and the membranous labyrinth were removed. Even after 4 hours of electrical stimulation the preparation appeared normal (Fig. 3.4). When no damaging stimuli were applied, ECAPs could remain stable for up to 10 hours in anaesthetized animals.

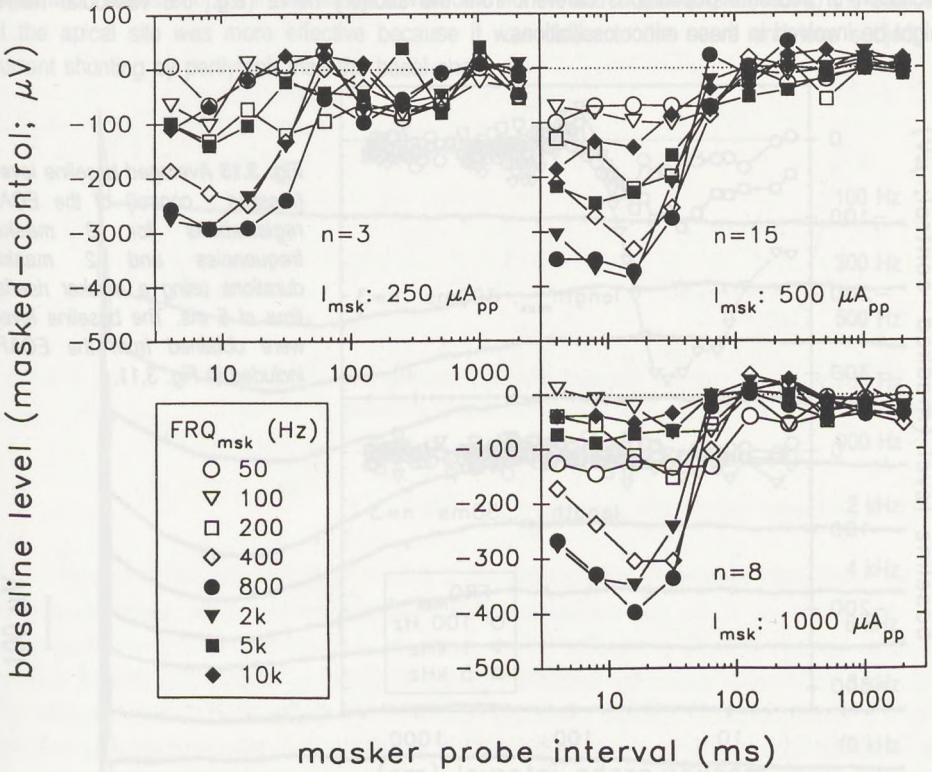

Fig. 3.12 Averaged baseline levels (masked - control) of the ECAP registrations for 8 masker frequencies at 3 masker currents (I_{msk}). Baseline levels were obtained from the ECAPs used for Fig. 3.9. and 3.10.

3.5.2. ECAP characteristics

All recorded ECAPs contained a short-latency negative peak (N_1) followed by a positive peak (P_1). The N_1-peaks of ECAPs evoked by pulsatile probes were about twice as narrow as those

obtained with 5 ms, 10 kHz sinusoidal probes. This indicates that synchronization of auditory-nerve fiber activity is optimized by using a pulsatile probe instead of the sinusoidal probe.
Most ECAPs evoked by pulsatile probes contained some minor oscillations following P_1. By and large, these ECAPs closely resemble the ECAPs recorded by other investigators using either mono- or biphasic current pulses in guinea pigs (Nagel, 1974; Prijs, 1980; Charlet de Sauvage *et al.*, 1983; Aran *et al.*, 1985; Aran *et al.*, 1987), cats (Stypulkowski and van den Honert, 1984; Brown and Abbas, 1990) and humans (Brown *et al.*, 1990). Since the present preparation contained no functional hair cells we may exclude the suggestion that the minor oscillations had an electrophonic origin (Prijs, 1980; Charlet de Sauvage *et al.*, 1983). Nagel (1974) suggested that these minor oscillations represented repeated discharges of auditory-nerve fibers. Moreover, excitation of neuronal populations different from the auditory nerve (*e.g.*, the vestibular nerve) might be involved in these minor oscillations.

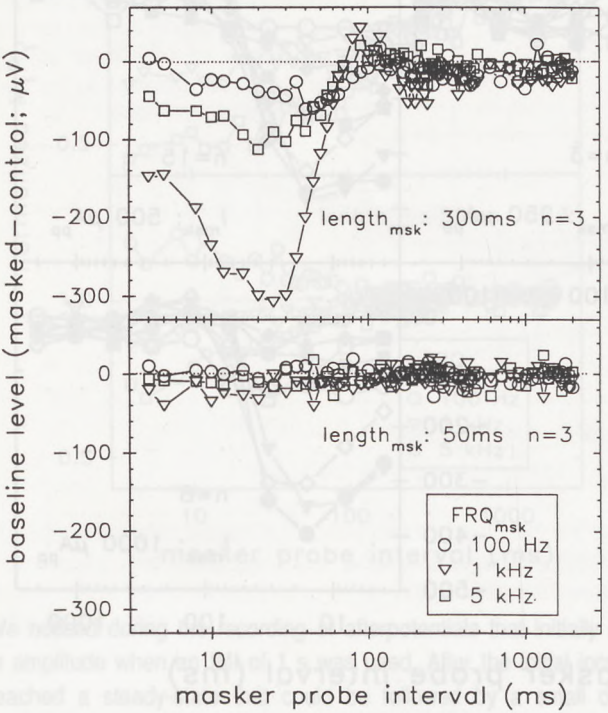

Fig. 3.13 *Averaged baseline levels (masked - control) of the ECAP registrations for 3 masker frequencies and 2 masker durations using a masker rise/fall time of 5 ms. The baseline levels were obtained from the ECAPs included in Fig. 3.11.*

At higher probe currents latency and amplitude of ECAPs evoked by the biphasic current pulses of opposite polarity (IP and IN-pulse) differed by about 20 μs and 350 μV, respectively (Fig. 3.7). The difference of 20 μs corresponds to the pulse width. The result suggests that the apex-positive, base-negative phase of the biphasic current pulse is the more effective one. Also, Miller *et al.* (1993a) found that latencies of wave I of EABRs differed 20 to 30 μs when the stimulus, a brief (20 μs/phase) biphasic current pulse, was inverted in polarity. It is difficult to compare our

effective polarity with theirs because they used a radially oriented electrode configuration at the apical turns.
In this study the biphasic pulse was most effective when the initial phase of the pulse was anodic apically (IP-pulse). It was shown by van den Honert and Stypulkowski (1987a) that auditory-nerve fibers responded to monophasic pulses of lower current strengths when the cathode of a basally located, longitudinally oriented electrode pair was located at the apical site. This discrepancy is most likely related to the different locations of stimulation electrodes used in both studies. Cathodic stimulation is more effective than anodic stimulation (Ranck, 1975; Rubinstein and Spelman, 1988). In our study the most effective stimulus was the one with the cathode located close to the base where the fiber density is higher and the distance to the registration site is shorter. In the study of van den Honert and Stypulkowski (1987a) the cathode at the apical site was more effective because it was closer to the nerve and less subject to current shunting by perilymph than the basal one.

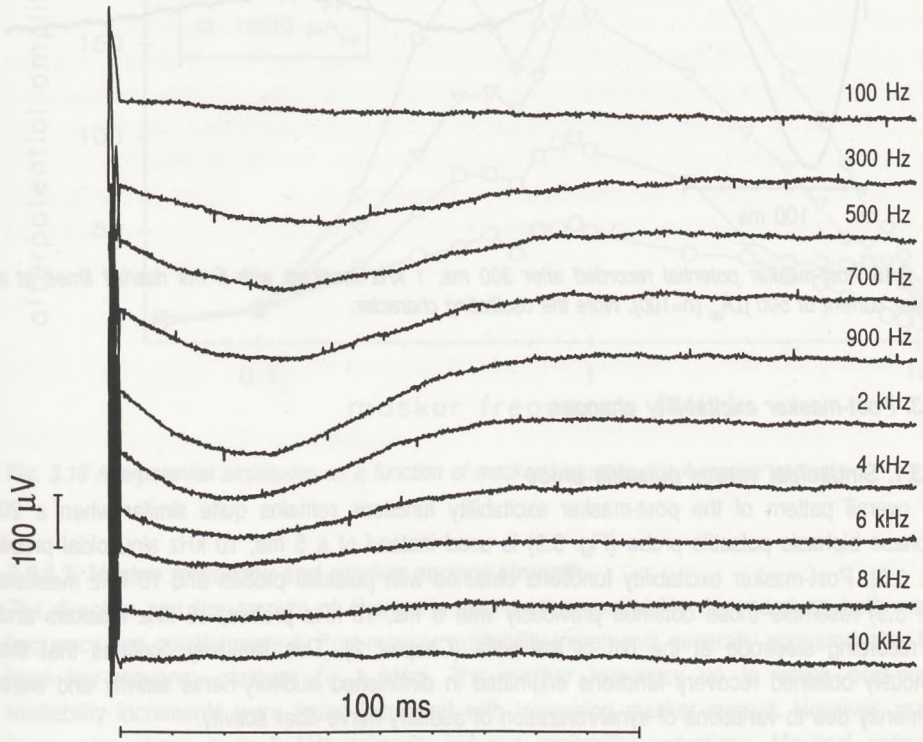

Fig. 3.14 Afterpotentials obtained with maskers of different frequencies presented at a current strength of 500 μA_{pp}. The impulse at the beginning of each trace is a stimulus artefact.

Occasionally a bimodal phase showed up in the ECAP waveform (Fig. 3.8). In separate preparations always either IP or IN stimuli evoked the bimodal response. The origin of our

bimodal ECAPs is unclear. The most frequently found bimodal pattern (Fig. 3.8, upper left corner) had similar amplitude and latency behavior as the bimodal pattern found by Stypulkowski and van den Honert (1984). Stypulkowski and van den Honert (1984) attributed the long-latency peak to stimulation of the peripheral process of the neuron (*e.g.*, the dendrites), while the short-latency peak was attributed to stimulation of the axon. Data from electrically evoked auditory-brainstem responses of Miller *et al.* (1993b) were in agreement with their hypothesis. Our bimodal ECAPs could have a similar origin as supposed by Stypulkowski and van den Honert; however, an origin in excitation of spiral ganglion neurons innervating different parts of the cochlea cannot be excluded, because we found several different bimodal response patterns at several intensity ranges.

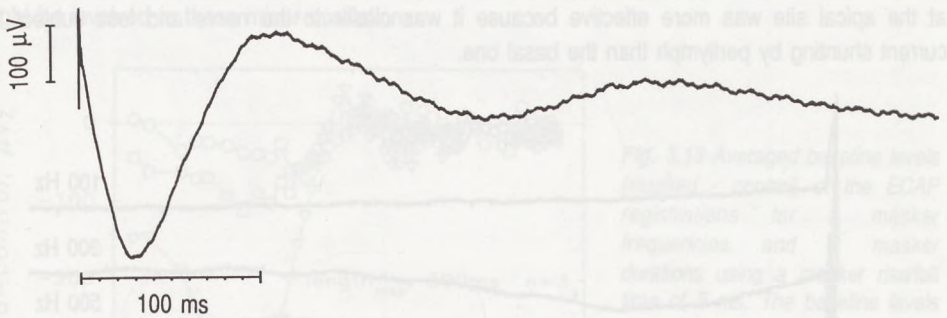

Fig. 3.15 *Post-masker potential recorded after 300 ms, 1 kHz maskers with 5 ms rise/fall times at a masker current of 500 µA_{pp} (n=100). Note the oscillating character.*

3.5.3. Post-masker excitability changes

3.5.3.1. Sinusoidal versus pulsatile probe

The overall pattern of the post-masker excitability functions remains quite similar when a 20 µs/phase biphasic pulsatile probe (Fig. 3.9) is used instead of a 5 ms, 10 kHz sinusoidal probe (Fig. 3.5). Post-masker excitability functions obtained with pulsatile probes and 10 kHz maskers (Fig. 3.9) resemble those obtained previously with 5 ms, 15 kHz probes, 16 kHz maskers and the recording electrode at the rim of the bulla (Chapter 2). This similarity confirms that the previously obtained recovery functions originated in diminished auditory-nerve activity and were not merely due to variations of synchronization of auditory-nerve fiber activity.

3.5.3.2. Probe current strength

Changes in post-masker excitability, as measured from ECAP amplitudes, were largest when low probe currents were used. When high probe currents were used ECAP amplitudes were reduced or unaffected for all masker frequencies. Increments in ECAP amplitude, as found at lower probe currents, were not found at high probe currents, probably because of saturation of the ECAP amplitudes at high probe currents.

The ECAP amplitude increments induced by the maskers might be due to increments of the amplitudes of individual single-fiber action potentials (spikes) contributing to the ECAP and/or to activation of more fibers. The first possibility seems quite unlikely. If the amplitude of individual single-fiber action potentials grew, then we would expect to find also ECAP amplitude increments at saturation levels, assuming that saturation is determined by recruitment of all fibers. Therefore, the second possibility of ECAP amplitude increments due to recruitment of additional fibers is the more likely one.

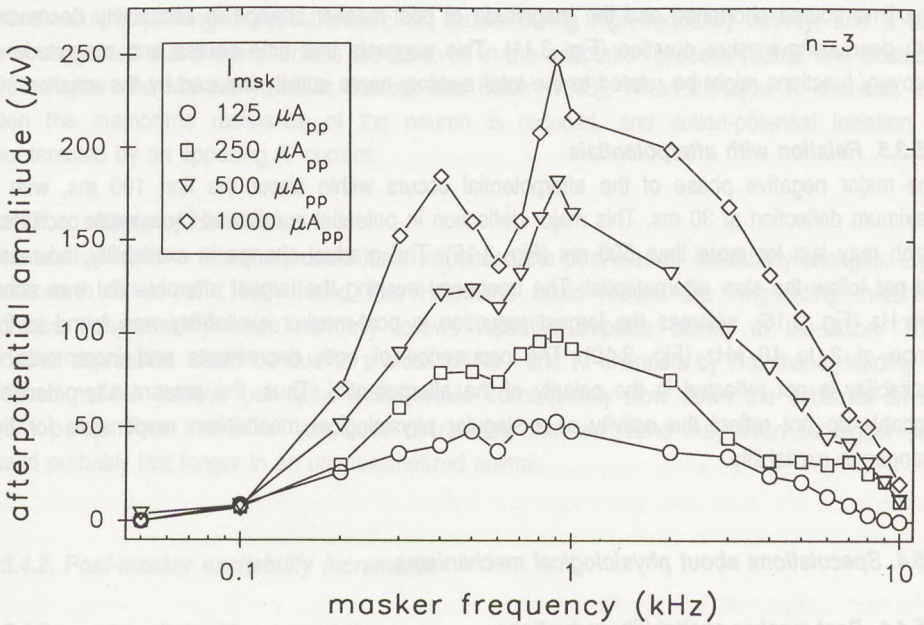

Fig. 3.16 Afterpotential amplitudes as a function of masker frequency, for 4 masker currents.

3.5.3.3. Masker frequency and masker current strength

The direction and the amount of change in post-masker excitability depended on both masker frequency and masker current. Post-masker excitability increments generally appeared after high-level low-frequency maskers (< 1 kHz). The masker frequency up to which post-masker excitability increments were found increased with increasing masker current. However, masker frequencies above 1 to 2 kHz primarily induced excitability reductions. Maximal reductions tended to occur after maskers in the frequency range of 2 to 10 kHz (Fig. 3.10).

The effect of masker frequency could be a result of effective masker level. Thresholds for electrostimulation with sinusoids are minimal at about 100 Hz, and increase about 10-fold at 10 kHz (Kiang and Moxon, 1972; Hartmann and Klinke, 1990a; Hartmann and Klinke, 1990b), while firing rates with 100 Hz stimuli saturate below the maximal firing rate induced by a 1 kHz stimulus. If we correct masker levels according to the threshold levels we may, for example,

compare a 400 Hz masker at 500 μA_{pp} to an 800 Hz masker at 1000 μA_{pp}. Comparing the excitability changes at a probe level of 1000 μA_{pp} in Fig. 3.10 shows that reduction with the 800 Hz masker is about tree times bigger than that with the 400 Hz masker. This indicates that the post-masker excitability changes with frequency may not be a first-order intensity effect, but masker frequency itself, and probably also phase duration, are important factors in post-masker excitability.

3.5.3.4. Masker duration

The time course shortened and the magnitude of post-masker change in excitability decreased with decreasing masker duration (Fig. 3.11). This suggests that time course and magnitude of recovery functions might be related to the total auditory-nerve activity induced by the masker.

3.5.3.5. Relation with afterpotentials

The major negative phase of the afterpotential occurs within about the first 100 ms, with a maximum deflection at 30 ms. This major deflection in potential is followed by a weak oscillation which may last for more than 500 ms (Fig. 3.15). The gradual change in excitability, however, did not follow the slow afterpotential. The frequency evoking the largest afterpotential was about 900 Hz (Fig. 3.16), whereas the largest reduction in post-masker excitability was found in the range of 2 to 10 kHz (Fig. 3.10). The occurrence of both decrements and increments in excitability is not reflected in the polarity of the afterpotential. Thus, the present afterpotentials probably do not reflect the activity of a singular physiological mechanism responsible for the changes in excitability.

3.5.4. Speculations about physiological mechanisms

3.5.4.1. Post-masker excitability reductions

3.5.4.1.a. refractory period

Post-masker excitability reductions immediately after the masker may be related to the refractory period of auditory-nerve fibers. Depressed excitability during the refractory period is most likely due to inactivation of Na^+-channels and the high K^+-conductance following a single-fiber action potential (Hodgkin and Huxley, 1952). The absolute refractory period of the auditory nerve varies from 0.3 to 1 ms, while the relative refractory period was found to extend from 3 ms to at least 5 ms (Charlet de Sauvage *et al.*, 1983; Stypulkowski and van den Honert, 1984; Hartmann *et al.*, 1984a; Parkins, 1989; Abbas and Brown, 1991b; Kasper *et al.*, 1992). The relative refractory period of a nerve fiber increases when a neuron is firing at a high rate. Summed effects of the relative refractory period with repetitive firing may have contributed to post-masker excitability reductions up to, at most, about 20 ms. Therefore, other mechanisms must be involved in the changes in excitability at longer post-masker intervals.

3.5.4.1.b. afterhyperpolarization

Post-masker excitability reductions at longer MPIs might be related to afterhyperpolarization induced by an electrogenic Na^+/K^+-pump that is activated by intracellular Na^+, accumulated during neural activity (Straub, 1961; Schoepfle and Katholi, 1973; Raymond, 1979; Bostock and Grafe, 1985; Carley and Raymond, 1987; Gordon *et al.*, 1990). Afterhyperpolarization could also be induced by slow K^+-channels (Eng *et al.*, 1988). In mammalian myelinated nerve fibers slow K^+-channels were found at the node of Ranvier and also, at lower densities, at internodal locations (Baker *et al.*, 1987; Röper and Schwarz, 1989; Waxman and Ritchie, 1993). These channels activate during prolonged depolarization, as occurs during high-frequency activity, and it has been suggested that these channels are involved in the adaptation process (Baker and Bostock, 1989; Krylov and Makovsky, 1978; Bostock and Baker, 1988). When the slow K^+-channels are open the membrane resistance of the neuron is reduced, and action-potential initiation is counteracted by an opposing K^+-current.

3.5.4.1.c. effects of Halothane

Anaesthetics used in this study could have influenced the post-masker excitability changes. *E.g.*, Butterworth IV *et al.* (1989) found that Halothane could reduce the long-lasting threshold increase ('depression') which normally follows repetitive impulse activity in the axon. This reduced depression would be due to a block of Na^+- and K^+-channels by Halothane, leading to reduced net ion transfer per spike which would consequently slow down the substrate-driven Na^+/K^+-pump. Thus the ECAP amplitude decrements that we found may even be bigger and would probably last longer in an unanaesthetized animal.

3.5.4.2. Post-masker excitability increments

3.5.4.2.a. extracellular K^+-accumulation

The post-masker excitability changes found in this study could be related to extracellular K^+-accumulation, brought about by the outward K^+-current during the repolarization phase of an action potential. Kocsis *et al.* (1983) have shown that excitability of the non-myelinated parallel fibers of the rat cerebellar cortex increased with small increases in extracellular K^+, while greater increases in extracellular K^+ led to reduced excitability and eventually conduction block. Furthermore, they have shown that extracellular K^+ accumulated in the vicinity of neighboring non-activated fibers, and that these fibers displayed an increase in excitability. Thus, post-masker excitability increments could be related to small increments in extracellular K^+-concentration, while post-masker excitability reductions could be related to greater increases in extracellular K^+-concentrations. However, in this study excitability increments appeared when maskers in the frequency range from 50 to 800 Hz were presented at high current strength, while the same masker frequencies did not induce any change or post-masker excitability reductions when presented at lower current strength (Fig. 3.10). Extracellular K^+-concentration will increase with stimulus intensity. Therefore it is unlikely that post-masker excitability reduction is due to high levels of extracellular K^+. In addition, if post-masker excitability reductions were

caused by high extracellular K^+-concentrations, we would expect an excitability increase to follow the reduction because the extracellular K^+-concentration decreases due to diffusion and re-uptake. The recovery functions, however, never showed an increase of excitability following the decrease; within IMIs used we found complete recovery from reduced excitability without a period of increased excitability. Thus, in the present preparation extracellular K^+-concentration does not seem to reach the conduction-block level. However, the extracellular K^+-concentration might very well be responsible for the increments in excitability.

3.5.4.2.b. K^+-accumulation in relation to masker frequencies

High-frequency electrical stimuli might activate a more spatially restricted region of fibers than low-frequency electrical stimuli (Rubinstein and Spelman, 1988). Thus, the high-frequency probes used in this study might have activated fibers that are located in the center part of the region that is actually activated by the low-frequency maskers. This may explain the finding that post-masker excitability increments were found primarily with high-level low-frequency maskers. For low-frequency maskers there will be no rapid diffusion of extracellularly accumulated K^+ out of the center region activated by the probe, because the masker will have elevated the extracellular K^+-concentration in the surrounding region. Moreover, high-frequency electrical stimuli are more prone to induce conduction block (Connors *et al.,* 1982). Consequently there would be less K^+-accumulation during high-frequency maskers.

3.5.4.3. Afterpotentials

Slow potential changes of the auditory nerve after termination of an electrical stimulus of several hundreds of ms have not been reported previously. They might be related to negative afterpotentials recorded in other nerve fibers (Gasser, 1935; Gasser, 1937).

3.5.4.3.a. relation with masker frequency

The afterpotential-amplitude masker-frequency relationship (Fig. 3.16) could be related to the discharge rates evoked by the masker. The maximum at 900 Hz might be related to the highest sustained frequency-following discharge occurring at this frequency. The local maximum at 400-500 Hz may originate with multiple discharges within one sinusoidal period (Hartmann *et al.,* 1984a; Parkins, 1989). Above 400 Hz multiple discharges within one sinusoidal period will diminish because of the refractory period. Loss of the frequency-following response above 900 Hz does not explain the decrease in the amplitude of the afterpotential; the firing rate may assume a steady level. The decrease in amplitude might be related to the fact that high-frequency electrical stimuli require higher current strengths to activate a fiber (Hartmann and Klinke, 1990a). Furthermore, high-frequency maskers would have stimulated a spatially restricted region (Rubinstein and Spelman, 1988).

3.5.4.3.b. inward rectification

Since the deflection of the afterpotential is negative, it might be related to a voltage and time dependent inward rectifying ion channel (Waxman and Ritchie, 1993) as found in rat spinal root axons (Baker *et al.,* 1987) and rat optic nerve fibers (Eng *et al.,* 1990). This channel is

permeable to both Na^+ and K^+ and is slowly activated (after 30 ms) by hyperpolarization. Its activation mediates a depolarization that brings the membrane potential towards its normal resting potential (Eng *et al.,* 1990). The inward rectifying current would help to maintain the membrane potential at appropriate levels during periods of intense firing when hyperpolarization due to activation of outwardly rectifying channels or Na^+/K^+-pump activity would be prominent (Baker *et al.,* 1987; Eng *et al.,* 1990). The inward rectifying current would thus help to counteract the reduced excitability brought about by hyperpolarization. However, the role of inward rectification is not fully understood. It has been suggested that the inward rectifier might participate in ionic homeostasis at the node of Ranvier (Waxman and Ritchie, 1993). According to this hypothesis, during high-frequency activity, K^+ would exit the axon via the slowly activating K^+-channels. The resulting increase in extracellular K^+ would be buffered via uptake (together with Cl^- ions) by astrocyte or Schwann cell processes (Ritchie, 1992). After stimulation K^+ and Cl^- would be returned to the extracellular space, which would still leave a requirement for re-uptake by the axon. As already mentioned in section 3.5.4.1.b. there would be a Na^+/K^+-pump mediated hyperpolarization after a period of high activity (Gordon *et al.,* 1990). Strong hyperpolarization could have activated an inward rectifying current, permitting a K^+ flow into the axoplasm. This flow together with the pump-mediated K^+-flux into the axoplasm would replace the ions lost during activity (Waxman and Ritchie, 1993).

3.5.5. Relation to psychophysics and clinical implications

We have found post-masker excitability increments after high-level low-frequency maskers, while psychophysical studies of forward masking in cochlear implant users report threshold increments (Shannon, 1983a; Dent and Townshend, 1987; Shannon, 1990a), even after 300 Hz sinusoidal maskers at comfortable to loud levels (Shannon, 1983a; Shannon, 1990a). This discrepancy between the electrophysiological and psychophysical studies might have originated with the high current strengths used in the electrophysiological studies compared to the psychophysical ones (Pfingst, 1988). Moreover, in our pilot studies with 5 ms, 10 kHz probes, excitability increments showed up in ECAP N_1P_1 amplitudes, while the following peaks were usually reduced in amplitude. The ECAP onset responses might not be representative of the psychophysical thresholds obtained with probes of 5 ms or longer.

Apart from the increments in excitability, most conditions showed reductions in excitability with a time course resembling the time course of psychophysical forward masking functions (Shannon, 1983a; Dent and Townshend, 1987; Shannon, 1990a; Shannon and Otto, 1990). The overall small change in excitability within the first 16 ms following termination of the masker (Figs. 3.5, 3.9, 3.11), correspond to the small threshold changes during the first 20 ms found by Shannon (1983a). Shannon (1983a) also reported that the amount of forward masking in cochlear implant users increased with increasing masker intensity. We generally found a similar relationship when high-frequency maskers or high probe levels were used.

By and large, our post-masker excitability functions suggest involvement of the auditory nerve in forward masking in cochlear implant users; they do not concur with the suggestion that forward masking is a sensation with a primarily centrally located origin (Shannon, 1990a; Shannon and Otto, 1990). This insight might be relevant for future designers of cochlear implant processors.

We advise them to be cautious with high-rate electrical stimuli (Wilson *et al.*, 1991) because they might be fatiguing and might disturb temporal information processing.
The present data suggest that forward masking in cochlear implant users depends on masker frequency, masker intensity and masker duration. In future psychophysical forward masking studies all these parameters have to be investigated. Furthermore, our data suggest that the 16 kHz stimulus used in the 3M/House cochlear implant might not be an optimal choice in view of temporal information processing. Also, our results suggest that avoiding long time constants in forward masking would require frequencies below 500-1000 Hz when sinusoidal stimuli are used.
This study was carried out in guinea pigs with intact auditory-nerve fibers. The status of the myelin around the remaining auditory-nerve neurons of a cochlear implant user could have a great impact on the time course of psychophysical forward masking functions. We may expect more adaptation and longer recovery periods when myelin is loose or absent (Bostock and Grafe, 1985). This suggests that forward masking might be related to the etiology of deafness and that temporal information processing might differ among cochlear implant users. Better knowledge of the physiological mechanisms will aid engineers in developing cochlear implant processors tuned to the status of the auditory nerve of the individual user.

CHAPTER 4

Changes in excitability of single auditory-nerve fibers following sinusoidal electrical stimulation

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4.1. Abstract

Changes in the excitability of single auditory-nerve fibers were tested after stimulation with 300 ms sinusoidal electrical stimuli (maskers) of different frequencies (100 Hz to 10 kHz). Reductions in excitability seemed to be largest after maskers in the frequency range from 1 to 10 kHz. The reductions in excitability after 300 ms, 10 kHz maskers generally increased with increasing masker current and decreasing probe current and lasted for more than 300 ms when high-level maskers were used. Adaptation or depolarization block during the 10 kHz masker was not a prerequisite for post-masker excitability reduction to occur.

Excitability reduction after 100 Hz maskers was small, while there was a slight tendency for the excitability reduction to decrease with increasing masker current. The single-fiber post-masker excitability functions resembled those obtained previously from changes in the electrically-evoked compound action potential (ECAP) amplitude (Chapters 2 & 3). This suggests that the changes in ECAP amplitude reflect the number of single units contributing to the ECAP.

4.2. Introduction

In our clinic, some users of the 3M/House cochlear implant spontaneously reported that perception of a tone was affected by a preceding one although the inter-tone interval was as long as 500 ms (Chapter 2). One possible explanation for this phenomenon might be that auditory-nerve fibers keep discharging after termination of the electrical stimulus. However, there appear to be no animal experiments showing this post-stimulus firing. Yet, slow changes in potential, occurring after electrical stimulation of the guinea-pig auditory nerve (Chapter 3), suggest ion movements which might be related to auditory-nerve fiber activity after termination of the electrical stimulus. For this reason we studied single-fiber auditory-nerve activity in relation to electrical stimulation.

It has been suggested that the mechanism responsible for forward masking in cochlear implant users is mainly located centrally to the auditory nerve (Shannon, 1990a; Shannon and Otto, 1990; Dynes and Delgutte, 1992). However, electrophysiological studies in the guinea pig showed reduction of the amplitude of the electrically-evoked auditory-nerve compound action potential (ECAP) for post-masker intervals of hundreds of ms (Chapters 2 & 3). This result

suggested that psychophysically measured forward masking might have a correlate at the level of the auditory nerve. In addition, one of these studies (Chapter 3) showed enhancement of the ECAP amplitude after electrical stimulation with high-level low-frequency maskers.
Shannon (1983a) has suggested that forward masking in cochlear implant users might be related to the high firing rate and accompanying adaptation, induced by the electrical masker. On the other hand, Dynes and Delgutte (1992) have suggested that adaptation occurring during high-frequency electrical stimulation is only of minor importance as a mechanism to explain psychophysical forward masking in cochlear implant users. Therefore the present study includes the measurement of fiber activity during and after 10 kHz electrical tone bursts.

4.3. Material and Methods

4.3.1. Animals and dissection of the cochlea

Experiments were performed on 7 healthy female albino guinea pigs (Hsd/Cpb, Dunkin Hartley, 250-500 g). The care for and use of the animals were approved by the Animal Care and Use Committee of the Faculty of Medicine, Utrecht University under number FDC89007, GDL20008. Details of animal anaesthesia and preparation have been described before (Chapter 3). A short summary is given below.
The animal was placed in a stereotaxic frame mounted on an air suspension. The left bulla was exposed from the ventral side and opened. The visible part of the otic capsule and the membranous labyrinth were removed, leaving the modiolus intact. In order to be able to position a microelectrode, a small opening was made in the bony wall of the basal turn of the modiolus.

4.3.2. Stimulation and recording

4.3.2.1. Electrodes

Two Pt/Ir-ball (d= 0.5 mm) stimulation electrodes were placed on the surface of the 2 most apical turns of the modiolus. The impedance between the 2 stimulation electrodes ranged from 5 to 10 kΩ at 1 kHz. Single neuron responses were recorded with 3 M KCl filled glass micropipettes, pulled on a Brown-Flaming micropipette puller. The impedance of the microelectrodes ranged from 20 to 40 MΩ. The microelectrode was mounted on a hydraulic microdrive (Narishigi, model 11). The tip of the recording electrode was placed above a small opening in the bony wall of the basal turn of the modiolus, and advanced in steps of 1 to 3 μm into the auditory nerve.

4.3.2.2. Electronic equipment and data collection

Stimulation and recording were controlled by a personal AT-computer connected to a 16 bit audio signal processor (ASP) and a CED-1401 laboratory interface. The ASP contains two digital signal processors (TMS-32010, Texas Instruments), and timers. The timers were used to control

both the timing of the stimuli and the recording of spikes. Stimuli were fed through an isolated constant-current source (bandwidth: 20 kHz) connected to the stimulation electrodes.
The microelectrode was connected to a pre-amplifier (WPI, Electro 705). A stainless-steel clip, placed between jaw and neck muscles, served as reference electrode. Stimulus artefact of the 10 kHz electrical stimuli was reduced by a Rockland low-pass filter (model: 816, 48 dB/octave roll-off, cut-off frequency: 3 kHz). The signal was further amplified by a second amplifier (Princeton Applied Research 113; bandwidth: 300 Hz - 3 kHz, amplification: 100 to 5000). The resulting signal was displayed on an oscilloscope, and fed into a spike discriminator (CED 1401-18 event conditioner). Spikes were recognized when their rising phase passed a preset threshold level, and when signal-to-noise or signal-to-artefact ratio was above 3:1.
Generally, the auditory neurons were not spontaneously active. When spontaneously active fibers were found they hardly reacted to electrical stimulation. Probably most of the spontaneously active fibers were stimulated mechanically by the electrode. A 300 ms, 10 kHz electrical signal presented at a moderate level (about 100 - 200 μA above threshold) with a 2 s inter-stimulus-interval was used as a search stimulus. When a neuron was found, the threshold level of the spike discriminator was fixed before automated measurement protocols were started. The occurrence of spikes was determined over a 1 s time period divided into 1000 bins of 1 ms each. The resulting data arrays plus the corresponding stimulus conditions were stored on disc for off-line analysis. Spike counts per bin were always averaged over all stimulus presentations. Trials were discarded when the standard deviation of the spike counts elicited by the control probe exceeded 25 % of its average spike count.
A major problem in electrophysiological experiments on electrostimulation is contamination of the physiological signal by the stimulus signal: the stimulus artefact. Therefore we used a low-pass filter at 3 kHz; well below the probe frequency of 10 kHz. Moreover, probe onset and offset were shaped with rise/fall times of 5 ms each to avoid frequency splatter below the 3 kHz cut-off frequency. In addition, we restricted the recording of spikes during the masker to the masker at 10 kHz. All maskers in this study had a cosinusoidally shaped onset and offset of 20 ms.

4.3.2.3. Experimental variables

Recordings were made in about 120 different fibers. In some fibers the input-output relation for 10 kHz maskers was obtained by presenting maskers from 100 to 1000 μA_{pp} in steps of 100 μA. The probe stimulus was always a 25 ms, 10 kHz sinusoidal tone burst with rise/fall times of 5 ms. The probe levels were set to evoke 3 to 5 spikes during one probe presentation. Probe levels were chosen close to threshold level because our previous ECAP studies (Chapters 2 & 3) had indicated that post-masker excitability changes were most apparent when low-level probes were used. The stimulus protocol used in this study resembled the one used in our previous ECAP study (Chapter 3). A control probe was placed immediately preceding the 300 ms masker, and the test probe was presented after the masker at variable masker-probe intervals (MPIs). A fixed inter-masker-interval (IMI) of 2000 ms was used. Each stimulus configuration was presented 5 to 10 times, because most single fibers were kept for only several minutes. Post-masker excitability was tested at 5 MPIs (5, 25, 100, 300 and 500 ms) for several

masker frequencies (100 Hz to 10 kHz). This protocol and the input-output protocol, measuring activity during the 10 kHz masker were also used to search for afterdischarges.

4.4. Results

4.4.1. Activity during the 10 kHz masker

Spontaneously active fibers were rarely found. Threshold was reached when a not-spontaneously active fiber had fired at least once during one of the stimulus presentations. The response threshold for 10 kHz maskers, averaged over all units recorded in the 7 animals, was 292 μA_{pp} (sd: 196 μA_{pp}). Thresholds varied considerably among animals and among fibers. Differences among animals were probably due to differences in preparation (*e.g.*, placement of stimulation and registration electrodes). Near threshold there was often an onset-response followed by less sustained firing; most of the spikes appear within the first 50 ms after the 20 ms rise-time of the masker. Occasionally, the threshold response consisted of a few spikes or bursts of spikes randomly spread over the stimulus period. Usually, the firing rate increased and the onset latency decreased with increasing masker level. At the higher masker levels we observed increasing spike-frequency adaptation during stimulation, and subsequently discharge block.

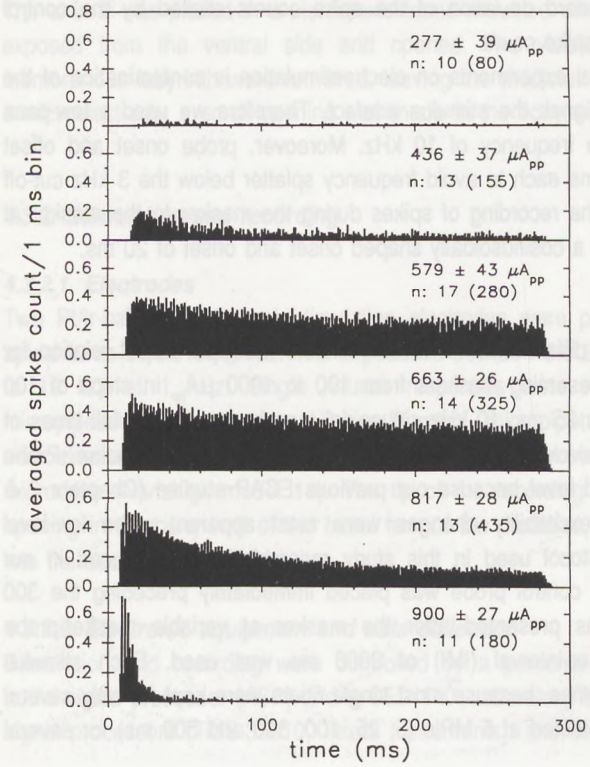

Fig. 4.1 *Peri-stimulus time histograms (PSTHs) presenting spike probability during 300 ms, 10 kHz sinusoidal electrical stimuli with 20 ms rise/fall times in a representative animal. PSTHs from different fibers were pooled according to the first bin in which a spike appeared. The averaged current strength ± SEM per latency class is presented in the upper right corner of each panel together with the number of fibers used for each latency class and the number of stimulus presentations in parentheses.*

Since we used a rise-time of 20 ms, the latency of the first spike in the peri-stimulus time histograms (PSTHs) decreased considerably with increasing stimulus intensity. This property gave us a means to get an impression of the overall features of the auditory-nerve fiber response to a range of intensities of a 300 ms, 10 kHz electrical stimulus without having to take into account the large variability in threshold level of the individual fibers. We gathered all PSTHs of a representative animal and sorted them according to the bin in which the first spike appeared. The sorted PSTHs were divided into 5 latency-classes and the average masker current strength was calculated for each class (Fig. 4.1). In this way, an overall image of the change in shape of the PSTH with increasing stimulus strength was acquired. PSTHs with long onset response latencies represent near-threshold responses (upper two panels Fig. 4.1). The PSTHs in the lower two panels of Fig. 4.1 share the same onset-response latency. These two PSTHs were obtained by separating the individual PSTHs into two groups: a group in which firing had ceased at the end of the masker (lower panel) and a group in which firing was still present at masker termination.

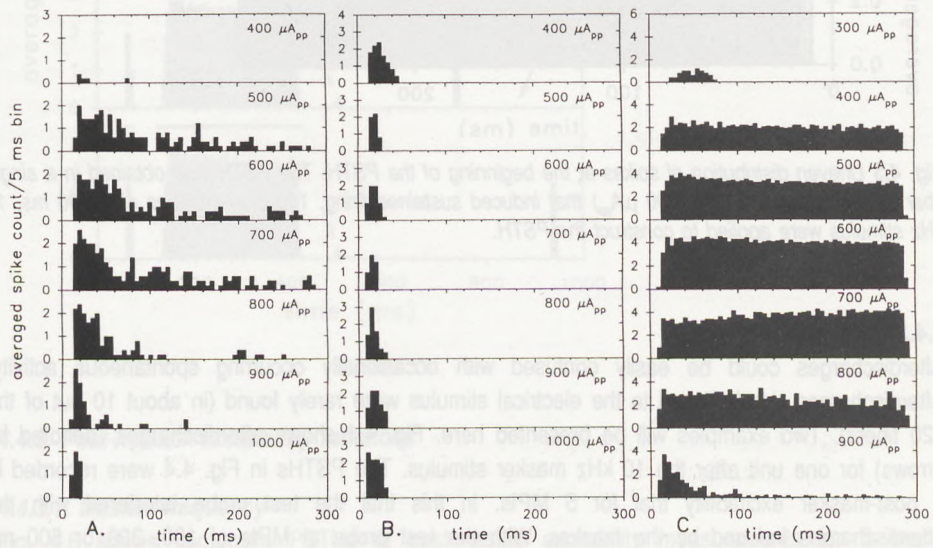

Fig. 4.2 *Spike activity during the masking stimulus as a function of current strength in three individual fibers. Fig.* ***4.2A*** *presents a fiber in which adaptation is present over a range of currents and* ***4.2B*** *presents a fiber in which discharge block was found at all current strengths. Fig.* ***4.2C*** *presents a fiber in which firing rate slightly increased during a 700 μA$_{pp}$, 10 kHz stimulus.*

Pooling of data across fibers and stimulus levels, as described above, obscures differences in behavior among individual fibers. Not all fibers showed the overall behavior depicted in Fig. 4.1. In about 25 % of the fibers we found no range of stimulus currents evoking a constant firing rate during the presence of the stimulus (Figs. 4.2A). In one fiber we found post-onset discharge block over the whole range of tested stimulus currents (Fig. 4.2B), and occasionally a fiber was

found in which the firing rate seemed to increase during stimulation at a particular stimulus level (Fig. 4.2C, 700 μA_{pp}).
The PSTH shown in Fig. 4.3 was obtained by stimulating a single fiber 100 times with a 300 ms, 10 kHz stimulus at 300 μA_{pp}. During the first 30 ms of stimulation the spikes appear fairly locked to specific time bins, while after this period the spikes are more randomly spread.

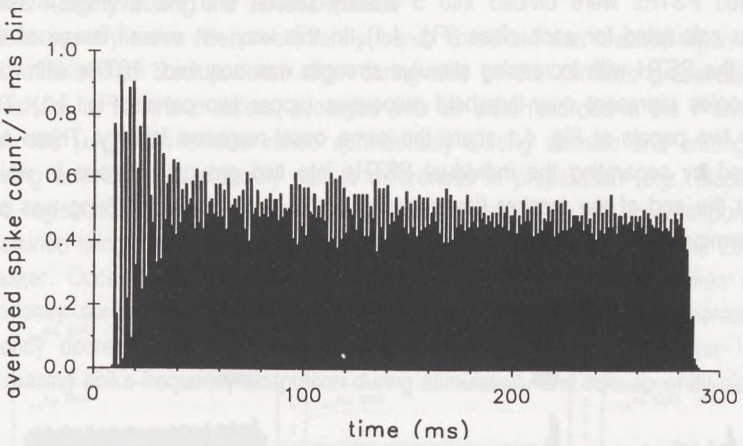

Fig. 4.3 Uneven distribution of spikes at the beginning of the PSTH. The PSTH was obtained in a single fiber at a stimulus intensity (300 μA_{pp}) that induced sustained firing. 100 presentations of a 300 ms, 10 kHz stimulus were applied to construct the PSTH.

4.4.2. Afterdischarges

Afterdischarges could be easily confused with occasionally occurring spontaneous activity. Afterdischarges clearly linked to the electrical stimulus were rarely found (in about 10 out of the 120 fibers). Two examples will be presented here. Fig. 4.4 shows afterdischarges (denoted by arrows) for one unit after the 10 kHz masker stimulus. The PSTHs in Fig. 4.4 were recorded in a post-masker excitability trial for 5 MPIs. In this trial the test probe interfered with the afterdischarges induced by the masker. With the test probe at MPIs of 100, 300 or 500 ms (lower 3 panels) the afterdischarges appear within a fixed interval of 55 to 80 ms after the masker. The appearance of afterdischarges is delayed when the test probe is placed at an MPI of 5 and 25 ms, while the test probe at an MPI of 25 ms induced more spread of the afterdischarges. Note some spontaneous discharging after test probes at MPIs of 100 and 300 ms. Afterdischarges obtained in another unit and following a 100 Hz masker are shown in Fig. 4.5. In this case the afterdischarges seemed to be spread in accordance with an decaying function starting immediately after the masker. Activity during the 100 Hz masker could not be measured accurately because of stimulus artefact problems associated with this masker frequency. We did not find any indication of a relation between afterdischarge pattern and masker frequency.

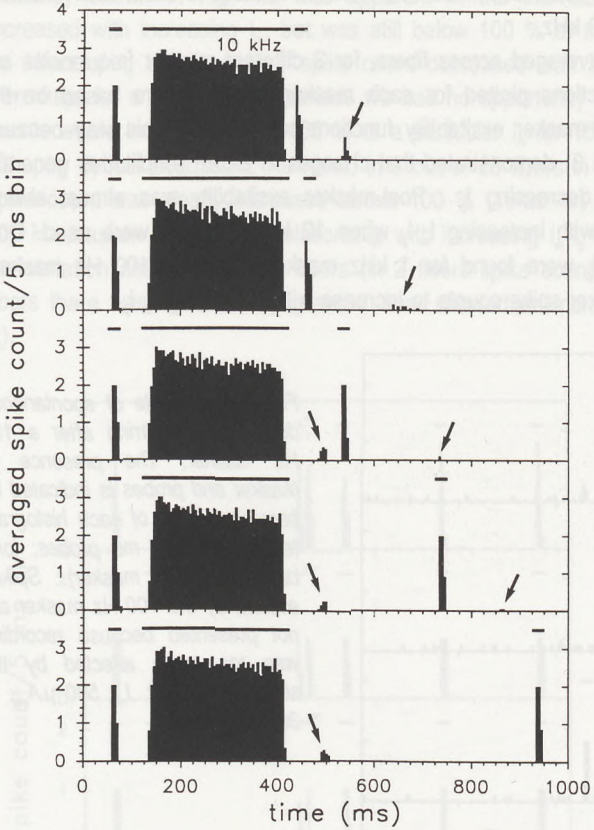

Fig. 4.4 Example of spontaneous discharges (indicated by arrows) after a 10 kHz masker. The presence of masker and probes is indicated by bars at the top of each histogram (short bars: 25 ms probes, long bar: 300 ms masker). I_m: 300 μA_{pp}; I_p: 200 μA_{pp}.

4.4.3. Post-masker excitability functions

4.4.3.1. Masker frequency

Fig. 4.6 shows composite PSTHs of post-masker excitability measurements for 5 masker frequencies in one single fiber. Each PSTH was constructed by adding PSTHs measured at different MPIs, thereby taking the averaged number of spikes fired during the control probe and the masker at 10 kHz only. Activity during the other masker frequencies was not included because of stimulus artefact problems. One probe current strength (I_p: 200 μA_{pp}) and one masker current strength (I_m: 250 μA_{pp}) were used in these trials.

In Fig. 4.7 the excitability changes, expressed in % of the spike counts for control probes, at MPIs of 5 and 25 ms were averaged and plotted as a function of masker frequency, together with the results of another set of trials (I_p: 250 μA_{pp} and I_m: 500 μA_{pp}) obtained in the same fiber. The figure shows that, in this fiber, post-masker spike count reductions were maximal after

maskers of 5 kHz. Studies in other fibers confirmed that maximal reductions occur after maskers in the frequency range from 1 to 10 kHz.

Post-masker excitability functions, averaged across fibers, for 3 different masker frequencies are plotted in Fig. 4.8. The three functions plotted for each masker frequency are based on the difference between I_m and I_p. Post-masker excitability functions were pooled this way because our previous studies (Chapters 2 & 3) demonstrated that changes in ECAP amplitudes generally increased with increasing I_m and decreasing I_p . Post-masker excitability was almost always reduced. Spike counts decreased with increasing I_m-I_p when 10 kHz maskers were used. Only minor changes with increasing I_m-I_p were found for 1 kHz maskers. For the 100 Hz maskers there was a tendency for post-masker spike counts to increase with increasing I_m-I_p.

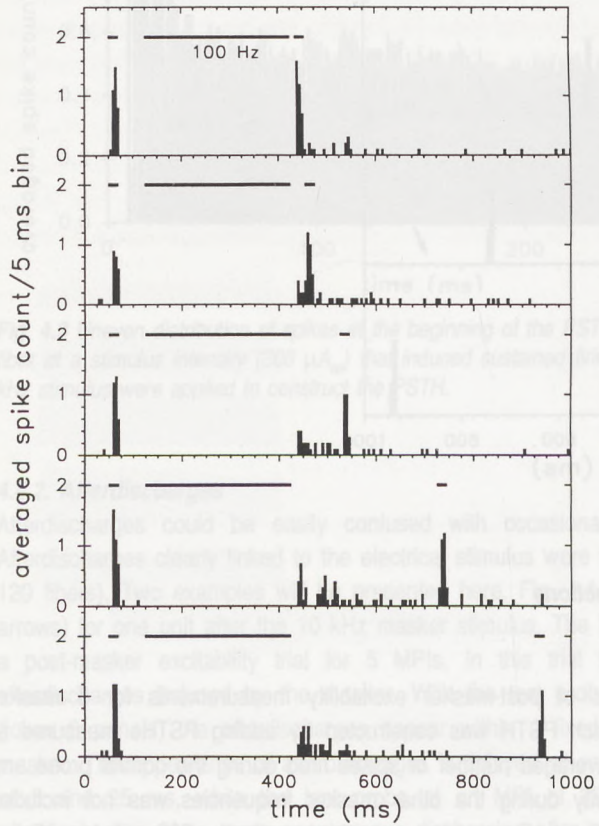

Fig. 4.5 Example of spontaneous discharges occurring after a 100 Hz masker. The presence of masker and probes is indicated by bars at the top of each histogram (short bars: 25 ms probes; long bar: 300 ms masker). Spikes evoked by the 100 Hz masker are not presented because recording was too much affected by the stimulus artefact. I_m: 500 μA_{pp}; I_p: 300 μA_{pp}.

The relation between post-masker excitability and I_m was further assessed by evaluating post-masker excitability functions in individual fibers obtained for several I_m, a fixed I_p and a fixed masker frequency. The relationship for 10 kHz maskers shown in Fig. 4.8 was basically confirmed in individual fibers (in 9 out of 12 fibers spike count decreased with increasing I_m [*e.g.*, Fig. 4.9], while in 3 fibers no clear change in spike count with increasing I_m was found [*e.g.*, Fig. 4.10]). Changes in spike count with I_m after 100 Hz maskers were generally small but the

tendency shown in Fig. 4.8 was apparent in the individual fibers (in 3 fibers spike count increased with increasing I_m, but was still below 100 % at the highest I_m, in 4 fibers there was no effect of I_m and in 1 fiber spike count decreased with increasing I_m). Because most fibers were kept for only short time periods we had no opportunity to systematically test whether spike counts would increase above 100 % at a particular I_m for 100 Hz maskers, as our ECAP study (Chapter 3) had suggested. However, in 3 out of 26 fibers in which 100 Hz maskers were tested spike count was clearly increased above 100 % (10-20 %) up to an MPI of 300 ms after the 100 Hz masker. The lack of relationship with increasing I_m-I_p for 1 kHz maskers might be due to the variation among individual fibers (in 2 fibers spike count decreased with increasing I_m, in 2 fibers there was no effect of I_m and 1 fiber showed an increase in spike count with increasing I_m).

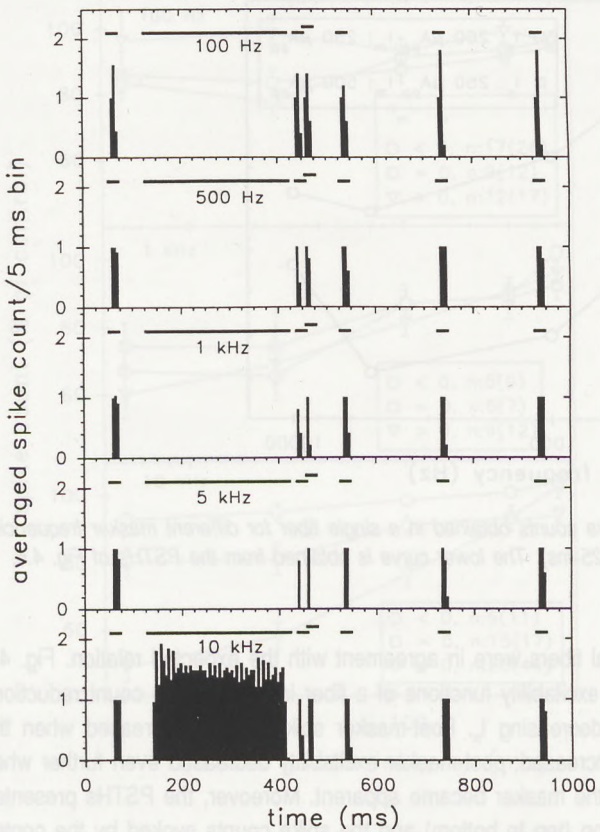

Fig. 4.6 *PSTHs obtained for maskers of different frequencies in a single fiber. The PSTHs obtained for 5 MPIs and one I_m were pooled together in a single PSTH for each masker frequency. I_m: 250 μA_{pp}; I_p: 200 μA_{pp}. Due to stimulus artefact problems the spikes evoked by the 100 Hz to 5 kHz maskers are not presented.*

4.4.3.2. Relation to firing during 10 kHz masker

As mentioned above, probe-evoked spike counts after termination of the 10 kHz masker decreased with increasing I_m. Extrapolating from the overall input/output relation shown in Fig.

4.1, we might expect that up to the level where discharge block or adaptation occurs, post-masker spike counts would decrease with increasing firing rates induced by the masker. Spike counts would even further decrease when adaptation or depolarization block during the masker becomes apparent. However, taking all post-masker excitability trials with 10 kHz maskers and dividing them into latency classes in accordance with the overall input/output relation did not reveal the expected relation (*i.e.* decreasing post-masker probe evoked spike counts with the overall input/output relation). We did not even find the expected relation when we eliminated a possible effect of effective probe level, by using only those trials in which the range of probe-evoked spike counts was limited. Furthermore, we did not find a clear relationship between post-masker spike count and discharge rate or adaptation during the 10 kHz maskers.

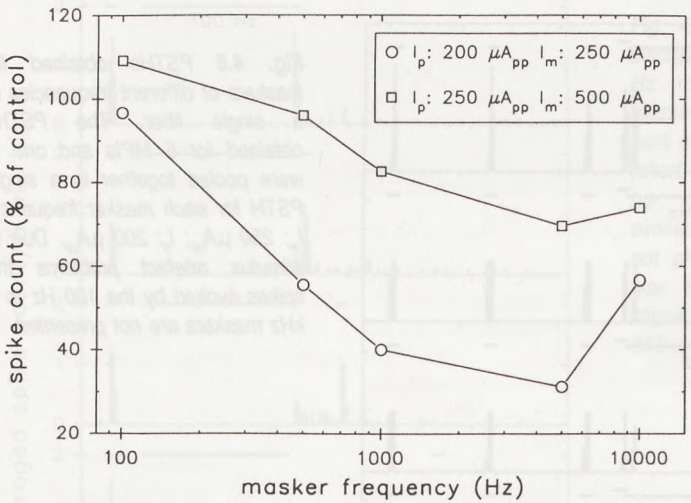

Fig. 4.7 *Normalized post-masker spike counts obtained in a single fiber for different masker frequencies and averaged over two MPIs (5 and 25 ms). The lower curve is obtained from the PSTHs of Fig. 4.6.*

However, recordings from individual fibers were in agreement with the expected relation. Fig. 4.9 presents PSTHs and post-masker excitability functions of a fiber in which spike count reductions increased with increasing I_m and decreasing I_p. Post-masker spike counts decreased when the firing rate evoked by the masker increased; post-masker excitability decreased even further when spike-frequency adaptation during the masker became apparent. Moreover, the PSTHs presented in Fig. 4.9 were taken in succession (top to bottom) and the spike counts evoked by the control probe (I_p= 300 μA_{pp}) in the upper three PSTHs tended to decrease with increasing I_m. This reduction may represent a fatiguing (Chapter 3) or long-term adaptation effect, induced by repeated masker presentations. In contrast, Fig. 4.10 presents a fiber in which post-masker spike counts did not change consistently with increasing I_m, although a clear input/output relation (increasing firing-rate and subsequent depolarization-block) was apparent.

4.5. Discussion

4.5.1. Activity during the masker

The large threshold variations found in this study are consistent with other reports (van den Honert and Stypulkowski, 1984; van den Honert and Stypulkowski, 1987a; Hartmann and Klinke, 1990b; Dynes and Delgutte, 1992). Near threshold most fibers responded only during the first 100 ms of the stimulus, after the 20 ms rise time. Parkins (1989) also found that near threshold electrically stimulated auditory-nerve fibers responded with an onset response. At higher stimulus levels a sustained response is found during stimulus presentation, while an onset response could still be identified. At the highest stimulus levels adaptation and eventually depolarization block occurred.

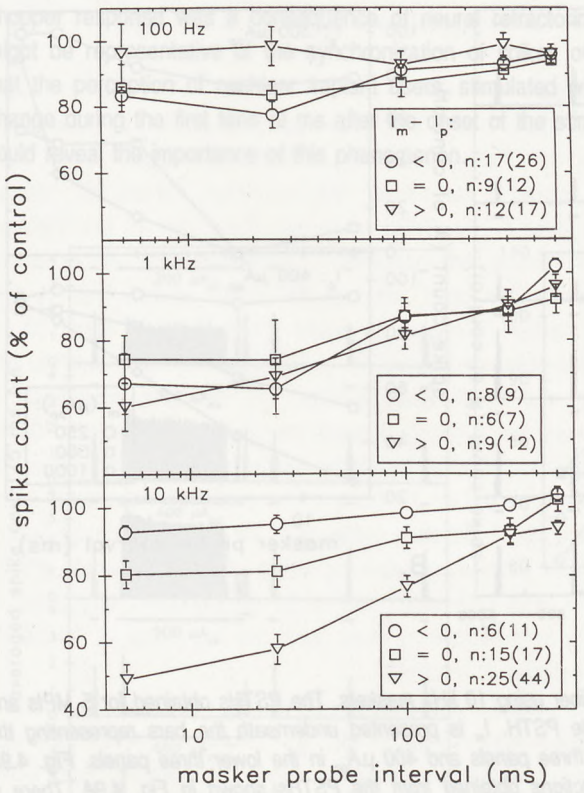

Fig. 4.8 Normalized post-masker spike counts obtained for three different masker frequencies and 5 MPIs. The 3 curves in each panel are based on the difference between masker and probe current strength (I_m-I_p). The number of fibers used for each curve is presented together with the number of trials in parentheses.

The input/output relation shown in Fig. 4.1 is representative for most fibers, but as shown in Fig. 4.2 not all neurons exhibited this input/output relation. For instance, the increasing firing-rate during stimulation, as found in the fiber shown in Fig. 4.2C, implies that in particular conditions excitability might actually increase during stimulation. In about 25 % of the neurons, measured over a full range of masker currents, no sustained firing during stimulation was found (e.g., Figs. 4.2A and 4.2B). The neuron presented in Fig. 4.2B responded only during the initial phase of

the stimulus, so that even at the lowest current strength discharge block seems obvious. The neuron presented in Fig. 4.2A exhibited a range of currents in which spike-frequency adaptation over the whole stimulus period was evident. Dynes and Delgutte (1992) also reported adaptation in more than 50 % of cat auditory-nerve fibers, stimulated with 1 s high-frequency electrical sinusoids. They obtained time constants of adaptation by fitting an exponentially decaying function superimposed on a baseline discharge rate corresponding to the stimulus intensity. They noted that for a given fiber the time constant of adaptation was constant over a small range of tested stimulus intensities (from threshold to 3 dB above threshold level). The present results indicate that adaptation and/or depolarization block might appear in almost all fibers when the level of a high-frequency electrical stimulus is set at 6 to 20 dB above threshold.

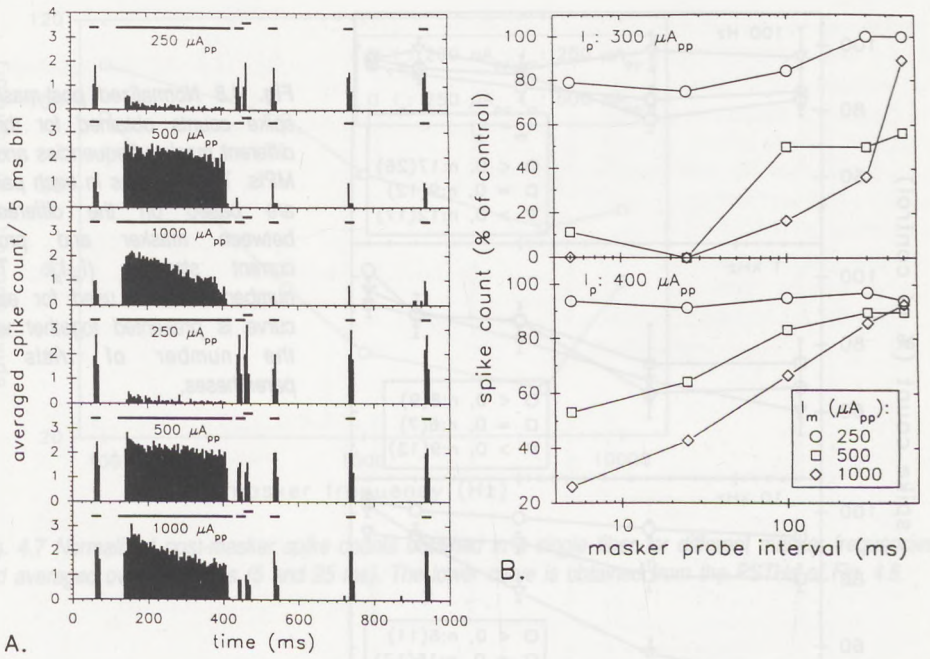

Fig. 4.9A *PSTHs obtained in a single fiber using 10 kHz maskers. The PSTHs obtained for 5 MPIs and one I_m were joined together in a single PSTH. I_m is presented underneath the bars representing the masker. I_p was 300 μA_{pp} in the upper three panels and 400 μA_{pp} in the lower three panels.* ***Fig. 4.9B*** *Normalized post-masker excitability functions obtained from the PSTHs shown in Fig. 4.9A. There is some discrepancy between the PSTHs of Fig. 4.9A and these excitability functions because the excitability functions have been normalized with respect to their corresponding control activity whereas in Fig. 4.9A control activity is pooled over all PSTHs with varying MPIs.*

In two fibers we found that, although total discharge block was apparent, the fiber discharged during the last 10 to 20 ms of the cosinusoidally shaped offset of the stimulus. This could imply

that discharge block induced by high-level 10 kHz stimuli is not maintained when the block-provoking stimulus level is lowered. Discharge block might be involved in tone decay in cochlear implant users; a fast release from discharge block when the stimulus intensity is lowered, could be involved in the decrease in tone decay found for amplitude-modulated electrical stimuli in cochlear implant users (Shannon, 1983a).

The initial temporal response pattern shown in the PSTH of Fig. 4.3 resembles the uneven pattern of the electroneural response to a 20 ms, 2500 Hz electrical stimulus found by Parkins (1989), and to a 50 ms, 6400 Hz electrical stimulus found by Hartmann and Klinke (1990b). Parkins ascribed this uneven pattern to the refractory status of the neuron. At the end of the stimulus the temporal response is more randomly spread, which might be related to temporal jitter in the refractory period. Moreover, the total response pattern resembles the 'Chopper'-type response to an 800 Hz pulsatile stimulus described by Javel (1990). Javel suggested that the chopper response was a consequence of neural refractoriness. The PSTH of this single fiber might be representative of the synchronization of spikes over multiple units. This would imply that the perception of cochlear implant users, stimulated with a high-frequency stimulus, might change during the first tens of ms after the onset of the stimulus. Future psychophysical studies could reveal the importance of this phenomenon.

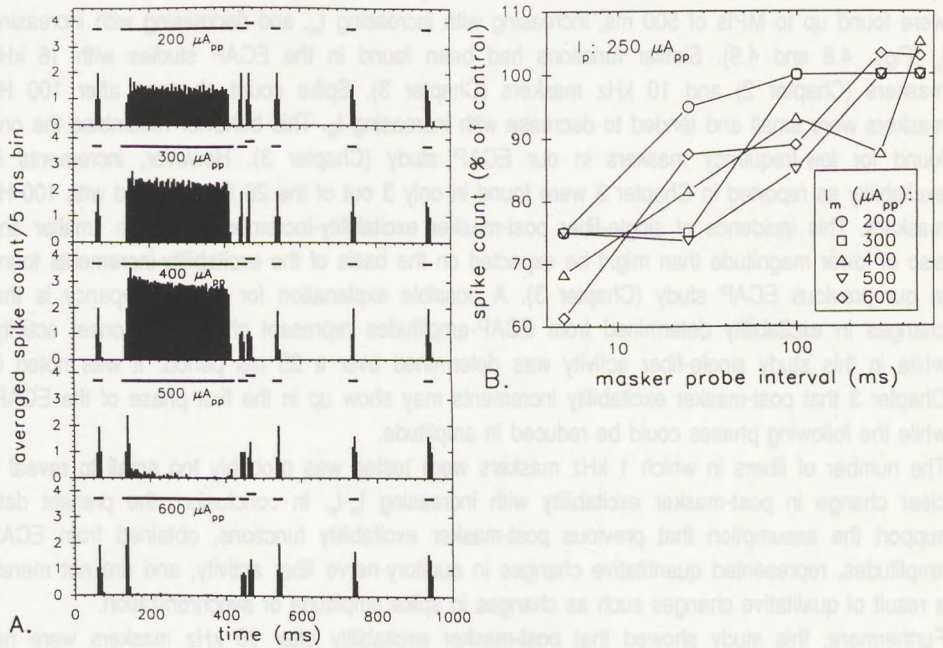

Fig. 4.10A *left plot: PSTHs obtained in a single fiber using 10 kHz maskers. The PSTHs obtained for 5 MPIs and one* I_m *were joined together in a single PSTH.* I_m *is presented underneath the bars representing the masker.* I_p *was 250* μA_{pp}*. Fig.* ***4.10B*** *right plot: Normalized post-masker excitability functions obtained from the PSTHs shown in Fig. 10A.*

4.5.2. Discharges after electrical stimulation

As far as we know, this is the first study reporting discharges of auditory-nerve fibers after termination of the electrical stimulus. Several types of afterdischarges were found during the forward masking study. Afterdischarges were most obvious when they clustered within a limited time interval after the masker (e.g., Fig. 4.4). Afterdischarges were scarce, and it was difficult to distinguish them from an occasional spontaneous spike when they were randomly spread after the masker. However, the timing of the discharges shown in Fig. 4.5 was unequivocally linked to the stimulus. Sometimes a relation with stimulus current was apparent because afterdischarges were found only within a specific range of current strengths. The mechanism responsible for discharging after electrostimulation is unknown, but we suggest that depolarization induced by extracellular potassium-ion accumulation (Kocsis *et al.,* 1983) or inward rectifying currents (Baker *et al.,* 1987; Eng *et al.,* 1990) might be involved. Further investigations using longer stimulus durations should reveal the significance of afterdischarges in cochlear implant users.

4.5.3. Post-masker excitability changes

The post-masker excitability functions found in this single-fiber study resemble the ECAP post-masker excitability functions obtained in Chapter 2 and 3. As found in Chapter 3, the post-masker spike count reductions were related to the masker frequency (Figs. 4.6 and 4.7) with an optimum between 1 and 10 kHz, at about 5 kHz. Spike count reductions after 10 kHz maskers were found up to MPIs of 500 ms, increasing with increasing I_m, and decreasing with increasing I_p (Figs. 4.8 and 4.9). Similar functions had been found in the ECAP studies with 16 kHz maskers (Chapter 2) and 10 kHz maskers (Chapter 3). Spike count changes after 100 Hz maskers were small and tended to decrease with increasing I_m. This behavior resembles the one found for low-frequency maskers in our ECAP study (Chapter 3). However, increments in excitability as reported in Chapter 3 were found in only 3 out of the 26 fibers tested with 100 Hz maskers. This incidence of single-fiber post-masker excitability-increments is much smaller and also of lower magnitude than might be expected on the basis of the excitability-increments found in our previous ECAP study (Chapter 3). A possible explanation for this discrepancy is that changes in excitability determined from ECAP-amplitudes represent changes in onset activity, while in this study single-fiber activity was determined over a 25 ms period. It was noted in Chapter 3 that post-masker excitability increments may show up in the first phase of the ECAP, while the following phases could be reduced in amplitude.

The number of fibers in which 1 kHz maskers were tested was probably too small to reveal a clear change in post-masker excitability with increasing I_m-I_p. In conclusion, the present data support the assumption that previous post-masker excitability functions, obtained from ECAP amplitudes, represented quantitative changes in auditory-nerve fiber activity, and are not merely a result of qualitative changes such as changes in spike amplitude or synchronization.

Furthermore, this study showed that post-masker excitability after 10 kHz maskers were not necessarily related to firing rate and accompanying adaptation, but rather to I_m. In this respect these data support the suggestion made by Dynes and Delgutte (1992) that adaptation itself, occurring during the masker, seemed not to be an important mechanism responsible for psychophysical forward masking in electrostimulation (Shannon, 1983a; Shannon, 1990a).

For a discussion about possible mechanisms involved in post-masker excitability changes we refer to Chapter 3.

SUMMARY AND CONCLUSIONS

Currently more than 6000 deaf individuals in the world have received a cochlear implant. Sound perception via a cochlear implant differs from normal hearing. Psychophysical research elucidates the perceptual differences and similarities between normal hearing and cochlear implant hearing. It was noticed that cochlear implant users implanted at the University Hospital Utrecht had problems with the perception of temporally separated sound patterns. For that reason a psychophysical forward-masking study was carried out in some 3M/House cochlear implant users. Both forward and backward masking were found, while simultaneous masking was completely absent. The study described in this thesis originated from this masking study.
The main goal of this study was to investigate to what extent masking, and particularly forward masking, might have a correlate at the level of the auditory nerve. Beforehand it was assumed that backward masking could not have a correlate at this peripheral level, while lack of simultaneous masking was found to be related to the fact that auditory-nerve activity follows the intensity modulations of the beating electrical stimulus (unpublished results and poster presented at the 1991 Conference on Implantable Auditory Prosthesis, Asilomar, California). In this thesis forward masking at the level of the auditory nerve has been investigated in an animal model by using sinusoidal electrical maskers, and measuring changes in electrically evoked neural activity after these maskers.

Chapter 1 gives an overview of historical and current psychophysical and electrophysiological literature published in relation to cochlear implant research.

Chapter 2 describes the psychophysical masking study that was carried out in 3M/House cochlear implant users. The threshold of a 5 ms, 15 kHz test stimulus (probe) was determined in temporal relation to a 500 ms, 16 kHz masker. Forward masking (probe follows masker) and backward masking (probe precedes masker) were found to extend over 100-200 ms periods. An experimental study, carried out in guinea pigs, was designed to investigate whether these psychophysical results can be attributed to adaptation mechanisms located at the VIIIth nerve level. The study was based on electrically evoked VIIIth nerve compound action potentials (ECAPs), using a masking paradigm comparable to the one used in the psychophysical study.
Trains of 50 maskers with inter-masker-intervals of 509 ms appeared to induce a fatigue effect that could influence the recovery measurements. Fatigue stabilized within 1 to 3 minutes when masker trains were repeated with intervening silent intervals of 10.5 seconds. The amplitude changes of ECAPs elicited by probes at several masker-probe delays for a range of probe currents were determined within steady fatigue states induced by different masker current strengths. The recovery functions obtained from these measurements resembled the forward

masking functions found in 3M/House cochlear implant users. No correlate of psychophysical backward masking was found at the VIIIth nerve level.
To examine whether hair cells were involved in fatigue and ECAP recovery functions, experiments were carried out in intact cochleas and cochleas without hair cells. Results were essentially the same in the different preparations. The results suggest that processes at the level of the VIIIth nerve could, at least partly, account for forward masking found in 3M/House cochlear implant users. Backward masking must be attributed to mechanisms located centrally to the VIIIth nerve.

In Chapter 3 the effect of masker frequency was investigated. As shown in Chapter 2 the amplitude of an ECAP does not fully recover within 500 ms after a high-level, 100 ms, 16 kHz sinusoidal electrical masker stimulus. The long recovery period may be related to the high frequency of the electrical masker. Firing rates induced by an electrical stimulus follow the stimulus frequency up to about 1 kHz. Thus, we may expect recovery functions to change with masker frequency assuming that they are related to the firing rates induced by the masker.
In this study recovery functions were obtained by measuring ECAP amplitude after 300 ms maskers of 50 Hz to 10 kHz. Unexpectedly, we found increased ECAP amplitudes for certain masker frequencies. The excitability increments lasted for several hundreds of ms after low-frequency electrical maskers (50 Hz to 800 Hz). The masker frequency after which post-masker excitability increments were found increased with increasing masker intensity. Excitability was mostly reduced after masker frequencies above 800 Hz. Reductions could last for more than 1000 ms.
In addition to changes in ECAP amplitude, we found a slow afterpotential. The slow potential change following the masker was negative and lasted for about 100 ms. The amplitude of the afterpotential increased with masker intensity, and reached a maximum value after maskers of about 900 Hz. A relation between the afterpotentials and the change in excitability could not be established.
Mechanisms that might be involved in the excitability changes were investigated by checking literature concerned with comparable neural systems. The present knowledge suggests that decreased excitability could be related to hyperpolarization induced by a Na^+/K^+-pump, while enhanced excitability might be related to depolarization brought about by extracellular accumulation of K^+.

In Chapter 4 we investigated excitability changes at the level of single auditory-nerve fibers. Spike counts evoked by 25 ms, 10 kHz sinusoidal electrical probes were used as a measure for single-fiber excitability. Changes in the excitability of single auditory-nerve fibers were tested after stimulation with 300 ms sinusoidal electrical maskers of different frequencies (100 Hz to 10 kHz). The reductions in excitability seemed to be largest after maskers in the frequency range from 1 to 10 kHz. The reductions in excitability after 300 ms, 10 kHz maskers generally increased with increasing masker current and decreasing probe current and lasted for up to more than 300 ms when high-level maskers were used. Adaptation or discharge block during the 10 kHz masker was not a prerequisite for post-masker excitability reduction to occur.

Excitability reduction after 100 Hz maskers was small, while there was a slight tendency for excitability reductions to decrease with increasing masker current. The single-fiber post-masker excitability functions resemble to those obtained in Chapter 2 and 3 from changes in ECAP amplitude. This suggests that the changes in ECAP amplitude reflect the number of single units contributing to the ECAP.
In addition to the post-masker changes in excitability, we found some auditory-nerve fibers spontaneously discharging after termination of the electrical masker stimulus.

Conclusions:
1. The cochlea of the guinea pig in which only the modiolus is left intact is a suitable model for neurophysiological investigations of the short-term effects of electrostimulation on the auditory nerve.
2. The excitability of the auditory nerve might be changed by electrostimulation. Reductions in excitability occur particularly after high-frequency (>1 kHz), high-intensity sinusoidal electrical stimuli, while after low-frequency, high-intensity sinusoidal electrical stimuli the excitability may be enhanced. Changes in excitability after a 300 ms electrical stimulus may last for up to more than 1 s and they return gradually back to baseline level.
3. The changes in excitability of the auditory nerve after electrostimulation indicate that mechanisms at the auditory-nerve level are involved in forward masking in cochlear implant users. The present results suggest that forward masking in cochlear implant users has a correlate at the auditory-nerve level, in particular after high-intensity, high-frequency electrical stimulation.
4. Continuous and intermittent stimulation of the auditory nerve with high-intensity, high-frequency electrical stimuli might induce long-term adaptation or fatigue in this nerve.
5. There is a slow change in potential at the auditory-nerve level evolving immediately after termination of an electrical stimulus. The potential becomes more negative with a maximum deflection between 20 and 50 ms after termination of the stimulus.
6. Auditory-nerve fibers may discharge spontaneously after an electrical stimulus.

Suggestions for future research:
1. In Chapter 3 we have discussed some conceivable physiological mechanisms that could have been involved in the post-stimulus change in excitability and the afterpotential found in this study. It will be of particular interest to further examine the physiological significance of these postulated mechanisms. This could be done by using intracellular studies, pharmacological tools and ionselective electrodes. A better understanding of the physiological mechanisms underlying the excitability changes would help us to develop a model that successfully predicts electrically induced changes in excitability. Such a model may be of great importance to those who formulate and implement the coding strategies of the cochlear implant signal processors.
2. Pulsatile electrical stimulation of the auditory nerve has to be the subject of future neurophysiological studies because this stimulus type is commonly used in current cochlear implants. Furthermore, activity induced by short biphasic electrical stimuli can be better predicted, which may give a better estimate of the relationship between activity induced by the

stimulus and the post-stimulus changes in excitability. Preliminary results, obtained in our laboratory, indicate that excitability enhancements were primarily related to the phase duration of one phase of the waveform of an electrical pulse and not the frequency of a pulse train.

3. Psychophysical investigations of forward masking in cochlear implant users should elaborate the significance of masker frequency, masker intensity and masker duration. Furthermore, the psychophysical significance of fatigue or temporary threshold shift in cochlear implant users has to be investigated, certainly since new coding strategies tend to use higher stimulus frequencies.

SAMENVATTING EN CONCLUSIES

Momenteel zijn er op de gehele wereld meer dan 6000 mensen voorzien van een elektrische binnenoorprothese. Met de binnenoorprothese is het mogelijk om mensen met bepaalde vormen van doofheid of zware slechthorendheid tot op zekere hoogte weer te laten horen. Sinds de jaren tachtig worden er in het Academisch Ziekenhuis Utrecht en het Academisch Ziekenhuis Nijmegen binnenoorprothesen geïmplanteerd.
Met een binnenoorprothese wordt de gehoorzenuw elektrisch gestimuleerd. Een binnenoorprothese is opgebouwd uit een aantal basiscomponenten. Een microfoon vangt omgevingsgeluiden op en stuurt deze door naar een processor die het geluid omzet in elektrische signalen. Deze elektrische signalen worden naar een uitwendige spoel geleid die met behulp van een magneet achter de gehoorschelp op het mastoïd wordt gefixeerd. Inwendig bevindt zich in een in het slaapbeen uitgeboorde holte een inwendige spoel. Via inductie wordt het elektrische signaal door de uitwendige spoel aan de inwendige spoel overgedragen. De elektrode(s) waarmee de gehoorzenuw wordt gestimuleerd zijn aangebracht op of in het binnenoor, en zijn verbonden met de inwendige spoel.

Hoofdstuk 1 beschrijft kort hoe Volta in 1800 voor het eerst bij zichzelf elektrisch gehoorsensaties opwekte. Meer dan 100 jaar later ontstaat de eerste echte binnenoorprothese, een ontwikkeling die heeft geleid tot de huidige geavanceerde binnenoorprothesen. Verder wordt er in dit hoofdstuk een overzicht gegeven van de psychofysische en fysiologische literatuur met betrekking tot de binnenoorprothese. Er wordt onder andere aandacht geschonken aan perceptiedrempels, dynamisch bereik, perceptie van toonhoogte en perceptie van temporele aspecten zoals maskering.

In Hoofdstuk 2 wordt een elektrofysiologische studie beschreven die direct gebaseerd is op een psychofysische maskeerstudie onder een aantal gebruikers van de 3M/House binnenoorprothese. De psychofysische studie vond zijn oorsprong in het feit dat gebruikers van de 3M/house binnenoorprothese vaak moeite hebben met het na elkaar waarnemen van twee afzonderlijke tonen. De maskeerstudie bepaalde de perceptiedrempel voor een korte teststimulus vlak voor, en vlak na een langere stimulus (maskeerder). Er werd gebruik gemaakt van 5 ms, 15 kHz testtonen en 500 ms, 16 kHz maskeerders. Voorwaartse maskering (verhoogde drempel na de maskeerder) hield aan tot langer dan 300 ms na het einde van de maskeerder, en terugwaartse maskering (verhoogde drempel voor de maskeerder) duurde tot langer dan 100 ms voor het begin van de maskeerder.
Het is aannemelijk dat de fysiologische oorsprong van terugwaartse maskering centraal ten opzichte van de gehoorzenuw moet worden gezocht. De oorsprong van voorwaartse maskering

daarentegen zou mogelijk geheel of gedeeltelijk op het niveau van de gehoorzenuw kunnen plaatshebben.
Door gehoorzenuwactiviteit te meten in een maskeerparadigma dat vergelijkbaar was met het stimulusparadigma gebruikt tijdens de psychofysiche maskeerstudie kon worden nagegaan in hoeverre processen op het niveau van de gehoorzenuw betrokken zouden kunnen zijn bij voorwaartse maskering in gebruikers van de 3M/House binnenoorprothese. Om gehoorzenuwactiviteit bij elektrostimulatie te kunnen meten werd de gehoorzenuw van de cavia gebruikt als model voor de gehoorzenuw van de binnenoorprothesegebruikers. Samengestelde actiepotentialen van de gehoorzenuw, opgewekt met 5 ms, 15 kHz elektrische stimuli, werden gemeten in de buurt van het slakkehuis. De amplitude van deze actiepotentialen diende als maat voor de exciteerbaarheid van de gehoorzenuw.
Om de samengestelde actiepotentiaal nauwkeurig te kunnen bepalen is het noodzakelijk de stimulus herhaald aan te bieden en de gemeten signalen te middelen. Vooronderzoek toonde aan dat herhaald aanbieden van 100 ms, 16 kHz maskeerders de exciteerbaarheid langdurig deed inzakken. Om verwarring met voorwaartse maskering te voorkomen werden allereerst de eigenschappen van dit vermoeidheidsverschijnsel bestudeerd. Het bleek dat het vermoeidheidsverschijnsel stabiliseerde wanneer reeksen van maskeerders herhaald werden aangeboden met een vaste stille tussenliggende periode. Het vermoeidheidsniveau bleek direct gerelateerd te zijn aan de intensiteit van de maskeerder en het stille interval tussen de afzonderlijke maskeerders (inter-maskeerder-interval: IMI). Om praktische redenen werden de maskeerstudies verricht tijdens stabiele vermoeidheidstoestanden die werden opgewekt door gebruik te maken van 100 ms maskeerders en een IMI van 509 ms. Voorwaartse maskering op gehoorzenuwniveau werd bepaald door amplitudes van de actiepotentialen te meten voor een reeks van maskeerder-teststimulus-intervallen (MTI), een reeks maskeerderintensiteiten en een reeks teststimulusintensiteiten.
Om vergelijking van de elektrofysiologische gegevens met de psychofysische gegevens te vergemakkelijken werd gebruik gemaakt van een arbitraire actiepotentiaaldrempel (d.w.z. het stimulusniveau waarbij een vooraf gestelde actiepotentiaalamplitude werd bereikt). De op deze manier verkregen voorwaartse maskeerfunkties op gehoorzenuwniveau waren vergelijkbaar met die van de gebruikers van de 3M/House binnenoorprothese.
Binnenoorprothesegebruikers hebben veelal geen functionele haarcellen meer. Om het effect van de aanwezigheid van haarcellen op vermoeidheisverschijnselen en voorwaartse maskering te onderzoeken werden zowel cavia's met intacte binnenoren, als cavia's met binnenoren waarin de haarcellen geheel of gedeeltelijk waren vernietigd gebruikt. De aanwezigheid van haarcellen had slechts een geringe invloed op de vermoeidheidsverschijnselen en de voorwaartse maskeerfunkties. Hieruit kon worden geconcludeerd dat mechanismen op het niveau van de gehoorzenuw betrokken zijn bij voorwaartse maskering in gebruikers van de 3M/House binnenoorprothese.

Hoofdstuk 3 beschrijft een elektrofysiologische studie waarin het effect van de frequentie van de maskeerder nader werd bestudeerd. Met deze studie wilden we onderzoeken of de frequentie van sinusvormige maskeerders van invloed was op voorwaartse maskering op

gehoorzenuwniveau. Mogelijk waren de maskeerfunkties uit Hoofdstuk 2 slechts geldig voor hoogfrequente (16 kHz) maskeerders. Op grond van de eigenschappen van een zenuw mochten we verwachten dat de frequentie van de maskeerder van invloed zou kunnen zijn.
Gebruikmakend van de ervaring opgedaan tijdens de hierboven beschreven experimenten werden een aantal veranderingen in de opstelling, het preparaat, de stimuli en de signaalanalyse aangebracht. In deze studie werd het binnenoor van de cavia zonder buitenste benige wand en vliezig labyrint, en dus zonder haarcellen, gebruikt. In dit preparaat konden stimulatie- en afleidelektrodes zodanig worden geplaatst dat specifiek de gehoorzenuw kon worden gestimuleerd, terwijl direct van de gehoorzenuw kon worden afgeleid. Als teststimulus werd een kortdurende gebalanceerde bifasische impuls (20 μs/fase) gebruikt. Deze korte stimulus garandeert een goede synchronisatie van de activiteit van afzonderlijke gehoorzenuwvezels, waardoor met de amplitude van de samengestelde actiepotentiaal een goede maat wordt verkregen voor het aantal actieve gehoorzenuwvezels. Maskeerders waren 300 ms lang en een reeks van maskeerderfrequenties (50 Hz tot 10 kHz) werd getest. Om vermoeidheidsverschijnselen te minimaliseren werd een IMI van 2304 ms gebruikt. Twee teststimuli werden aangeboden binnen ieder IMI. Actiepotentialen opgewekt met teststimuli vlak voor maskeerders dienden als referentie voor de actiepotentialen die opgewekt werden met teststimuli op variabele MTIs. Ook in deze studie diende de amplitude van de actiepotentialen als maat voor de exciteerbaarheid van de gehoorzenuw.
Over het algemeen was na hoogfrequente (>800 Hz) maskeerders de exciteerbaarheid gedurende langer dan een halve seconde verminderd. Buiten verwachting werd na laagfrequente maskeerders gedurende honderden ms een verhoogde exciteerbaarheid van de gehoorzenuw gevonden. Deze verhoogde exciteerbaarheid werd vooral gevonden na maskeerder van hoge intensiteit. Zowel de verhoogde als de verlaagde exciteerbaarheid na de maskeerder keerden geleidelijk terug naar het controleniveau.
In Hoofdstuk 3 wordt tevens een verschuiving van de basispotentiaal beschreven die direct na het einde van de maskeerder inzette in negatieve richting. Amplitudes van deze potentiaalveranderingen werden gemeten als functie van de maskeerderfrequentie (50 Hz tot 10 kHz). De amplitude van deze potentiaalverandering was maximaal voor maskeerders van ongeveer 900 Hz met een lokaal maximum in de buurt van 400 Hz. Wat de oorsprong is van de napotentiaal is onduidelijk. Wel suggereren onze data dat deze napotentiaal in direct verband staat met de door de maskeerder opgewekte activiteit.
In de discussie wordt een aantal speculaties ten aanzien van de fysiologische oorsprong van de veranderingen in exciteerbaarheid en de napotentiaal nader uitgewerkt.

Hoofdstuk 4 beschrijft een elektrofysiologische studie op het niveau van individuele gehoorzenuwvezels. Onderzocht werd of exciteerbaarheidsveranderingen op het niveau van de gehele gehoorzenuw (Hoofdstuk 3) ook plaats vinden op het niveau van individuele gehoorzenuwvezels. Tevens werd onderzocht of het inzakken van de exciteerbaarheid na 10 kHz maskeerders in verband kon worden gebracht met adaptatieverschijnselen gedurende deze maskeerder. Omdat de verstoorde perceptie van temporele patronen bij binnenoorprothese-

gebruikers zou kunnen samenhangen met spontaan vuren na beëindiging van een stimulus hebben we tevens gekeken naar spontaan navuren van vezels na een elektrische stimulus.
In deze studie werd hetzelfde preparaat gebruikt als in Hoofdstuk 3. Actiepotentialen van individuele gehoorzenuwvezels (spikes) werden met behulp van een microelektrode afgeleid, en met behulp van een window discriminator geteld. Het gebruikte stimulusprotocol kwam grotendeels overeen met dat van Hoofdstuk 3. Als teststimulus werd een 25 ms, 10 kHz elektrisch signaal gebruikt. De intensiteit van de teststimulus was altijd vlak boven de responsdrempel. Het aantal spikes opgewekt met deze teststimulus diende als maat voor de exciteerbaarheid van de gehoorzenuwvezel. Maskeerders van 300 ms en verschillende frequenties (100 Hz tot 10 kHz) werden getest voor verschillende maskeerderintensiteiten.
Studies beschreven in Hoofdstuk 2 en 3 hadden laten zien dat exciteerbaarheidsveranderingen afhankelijk waren van zowel teststimulus- als maskeerderintensiteit. Omdat responsdrempels van verschillende vezels nogal varieerden hebben we in deze studie de maskeerfunkties van de individuele fibers gesorteerd op basis van het verschil tussen maskeerderintensiteit en teststimulusintensiteit.
Gevonden werd dat ook op het niveau van de gehoorzenuwvezel de exciteerbaarheid was verminderd na hoogfrequente maskeerders. De exciteerbaarheid nam verder af naarmate het verschil tussen maskeerderintensiteit en teststimulusintensiteit toenam. Verhoging van de exciteerbaarheid na 100 Hz maskeerders werd slechts sporadisch gevonden. Over het algemeen werden slechts geringe veranderingen in exciteerbaarheid gevonden na 100 Hz maskeerders. Wel was er een tendens dat de exciteerbaarheid na 100 Hz maskeerders toenam met het verschil tussen maskeerderintensiteit en teststimulusintensiteit. Een belangrijk verschil tussen deze studie en de eraan voorafgaande studies is dat voor deze studie de totale activiteit gedurende een lange teststimulus werd gebruikt als maat voor de exciteerbaarheid, terwijl in de voorafgaande studies de exciteerbaarheid werd gemeten aan de hand van de amplitude van het begin van de respons. Bovendien waren in Hoofdstuk 3 aanwijzingen gevonden dat alleen de beginrespons van de samengestelde actiepotentialen opgewekt met 5 ms, 10 kHz teststimulus was vergroot na 100 Hz maskeerders, terwijl de daaropvolgende amplitudes waren ingezakt.
In de literatuur wordt over het algemeen aangenomen dat voorwaartse maskering in direct verband kan worden gebracht met adaptatie gedurende de maskeerder. Sommige auteurs suggereerden echter dat een dergelijk verband niet noodzakelijk is. Uit onze studie bleek dat adaptatie gedurende 10 kHz maskeerders niet van essentieel belang was voor de verminderde exciteerbaarheid na deze maskeerders.
Spontaan navuren werd slechts sporadisch gevonden, en is daarom waarschijnlijk niet het belangrijkste mechanisme dat verantwoordelijk is voor voorwaartse maskering.

Conclusies:
1. De zorgvuldig vrijgeprepareerde modiolus van de cavia is een goed bruikbaar proefdiermodel ter bestudering van korte-termijneffecten van elektrostimulatie op de gehoorzenuw.
2. De exciteerbaarheid van de gehoorzenuw kan veranderen wanneer deze elektrisch wordt gestimuleerd. Over het algemeen vermindert de exciteerbaarheid van de gehoorzenuw wanneer deze wordt gestimuleerd met hoogfrequente (>1 kHz) sinusvormige elektrische stimuli van hoge

intensiteit. Laagfrequente sinusvormige elektrische stimuli van hoge intensiteit daarentegen kunnen de exciteerbaarheid van de gehoorzenuw verhogen. De veranderingen in exciteerbaarheid na een 300 ms elektrische stimulus kunnen langer dan 1 s aanhouden en keren geleidelijk terug naar controleniveau.
3. Veranderingen in de exciteerbaarheid van de gehoorzenuw na een elektrische stimulus suggereren dat mechanismen op het niveau van de gehoorzenuw betrokken zijn bij voorwaartse maskering in gebruikers van een binnenoorprothese. De huidige resultaten geven aan dat voorwaartse maskering in binnenoorprothesegebruikers in verband kan worden gebracht met gehoorzenuwactiviteit. In het bijzonder zullen hoogfrequente elektrische maskeerders van hoge intensiteit voorwaartse maskering veroorzaken.
4. Continue of onderbroken stimulatie van de gehoorzenuw met hoogfrequente elektrische stimuli kan vermoeidheidsverschijnselen veroorzaken in deze zenuw.
5. Onmiddellijk na het stoppen van een elektrische stimulus vindt er een langzame verschuiving in negatieve richting van de basispotentiaal plaats. Deze verschuiving bereikt een maximum 20 tot 50 ms na het einde van de stimulus.
6. Gehoorzenuwvezels kunnen spontaan vuren na een elektrische stimulus.

Suggesties voor verder onderzoek:
1. In Hoofdstuk 3 zijn een aantal fysiologische mechanismen besproken die mogelijk betrokken zijn bij de potentiaalveranderingen en veranderingen in exciteerbaarheid na een elektrische stimulus. Met behulp van farmacologische methoden en ionselectieve elektrodes zouden deze hypothetische mechanismen kunnen worden geverifieerd. Een betere kennis ten aanzien van de fysiologie van de elektrisch gestimuleerde gehoorzenuw stelt ons in staat om een model ervan te ontwikkelen waarmee succesvol veranderingen in exciteerbaarheid kunnen worden voorspeld. Zo'n model zou van groot belang kunnen zijn voor diegenen die coderingsstrategieën voor de processor van de binnenoorprothese formuleren en implementeren
2. In toekomstige neurofysiologische studies dienen de effecten van impulsvormige elektrische stimuli op de gehoorzenuw nader te worden onderzocht, omdat deze stimulusvorm algemeen gebruikt wordt in de gangbare binnenoorprothesen. Verder kan met behulp van korte bifasische elektrische impulsen de activiteit van de gehoorzenuw beter worden voorspeld waardoor we mogelijk meer te weten komen over de relatie tussen opgewekte activiteit en veranderingen in exciteerbaarheid na een elektrische stimulus. Voorlopige resultaten met impulsvormige stimuli in ons laboratorium laten zien dat de verhoging van de exciteerbaarheid vooral samenhangt met de faseduur van een impuls en niet zozeer met de frequentie van een serie van impulsen.
3. In psychofysisch onderzoek naar voorwaartse maskering in binnenoorprothesegebruikers moet de frequentie, de intensiteit en de duur van de elektrische maskeerder worden betrokken. Bovendien dient het voorkomen van tijdelijke drempelveranderingen of vermoeidheids-verschijnselen in binnenoorprothesegebruikers nader te worden onderzocht, zeker nu er een tendens is om hogere stimulusfrequenties te gebruiken in nieuwe coderingsstrategieën

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Curriculum vitae

Matthijs Killian is op 3 februari 1962 geboren te Rotterdam.
Nadat in 1980 het Atheneum-B examen behaald was aan het Bisschoppelijk College te Sittard ging hij datzelfde jaar Biologie studeren aan de Universiteit Utrecht.
Voor het hoofdvak Moleculaire Neurobiologie (prof. dr. W.H. Gispen) werd een onderzoeksstage gelopen bij het Rudolf Magnus Instituut voor farmacologie (dr. I.J.A. Urban), alwaar *in vivo* electrofysiologisch onderzoek werd verricht aan hippocampale neuronen (Urban *et al.*, 1986a, 1986b, 1990, Killian *et al.*, 1986). Wegens het succes van deze stage mocht worden deelgenomen aan een uitwisselingsprogramma met de Universiteit van Toronto, Canada (prof. dr. L.A. Grupp). In Toronto werd bij de afdeling farmacologie de effecten van Angiotensine II op alcohol drinkgedrag bij ratten bestudeerd (Grupp *et al.*, 1988). Voor het bijvak Ethologie (prof. dr. J.A.R.A.M. van Hooff) werd een onderzoeksstage gelopen bij de afdeling sociale insecten (mw. dr. M.J.A.M. Duchateau). Correlaties tussen gedrags- en fysiologische parameters in aardhommel kolonies werden onderzocht. Als derde bijvak werd de module psychopathologie gevolgd bij de vakgroep Klinische Psychologie (drs. E.J.G. Meijer). De eindscriptie werd zowel voor Moleculaire Neurobiologie als Klinische Psychologie geschreven en behandelde de fysiologie en farmacologie van REM slaap in relatie tot geheugen en de betekenis van dromen.
Na deze studie werd de militaire dienst vervuld bij de DMGZ te Utrecht. De belangrijkste taak bestond uit preventie, voorlichting en het opstellen van richtlijnen binnen het leger inzake het AIDS vraagstuk (Killian and Bruins, 1989).
In 1989 werd aangevangen met het in dit proefschrift beschreven onderzoek.

Publikaties:

I.J.A. Urban and M.J.P. Killian (1986a). Two pharmacologically different actions of arginine^{8}-vasopressin in the rat hippocampal neurons revealed by microiontophoresis. *Proc. 27th Dutch Fed. Meeting Groningen, Abstr 403*

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